CHAPTER 4
THEORETICAL ANALYSIS

4.1 INTRODUCTION TO BIOREMEDIATION

Bioremediation is the use of microorganisms for the degradation of hazardous chemicals in soil, sediments, water, or other contaminated materials. Often the microorganisms metabolize the chemicals to produce carbon dioxide or methane, water and biomass. Alternatively, the contaminants may be enzymatically transformed to metabolites that are less toxic or innocuous. It should be noted that in some instances, the metabolites formed are more toxic than the parent compound. For example, perchloroethylene and trichloroethylene may degrade to vinyl chloride. There are at least five critical factors that should be considered when evaluating the use of bioremediation for site clean up. These factors are:

i. Magnitude, toxicity, and mobility of contaminants: It is imperative that the site be properly investigated and characterized to determine the (a) horizontal and vertical extent of contamination; (b) the kinds and concentrations of contaminants at the site; (c) the likely mobility of contaminants in the future, which depends in part on the geological characteristics of the site.

ii. Proximity of human and environmental receptors: Whether bioremediation is the appropriate cleanup remedy for a site is dependent on whether the rate and extent of contaminant degradation is sufficient to maintain low risks to human or environmental receptors.
iii. Degradability of contaminants: The biodegradability of a compound is generally high if the compound occurs naturally in the environment (e.g., petroleum hydrocarbons). Often, compounds with a high molecular weight, particularly those with complex ring structures and halogen substituents, degrade more slowly than simpler straight chain hydrocarbons or low molecular weight compounds. Whether synthetic compounds are metabolized by microorganisms is largely determined by whether the compound has structural features similar to naturally occurring compounds. The rate and extent to which the compound is metabolized in the environment is often determined by the availability of electron acceptors and other nutrients.

iv. Planned site use: A critical factor in deciding whether bioremediation is the appropriate cleanup remedy for a site is whether the rate and extent of contaminant degradation is sufficient to reduce risks to acceptable levels.

v. Ability to properly monitor: There are inherent uncertainties in the use of bioremediation for contaminated soils and aquifers due to physical, chemical and biological heterogeneities of the contaminated matrix. It is important to recognize that biological processes are dynamic and, given current knowledge, often lack the predictability of more conventional remediation technologies. Thus, it is important to insure that unacceptable risks do not develop in the future. These risks may include migration of contaminants to previously uncontaminated media and the failure of bioremediation to achieve acceptable contaminant concentrations.

Bioremediation is one of the widely used methods where in microorganisms more than any other class of organisms has a unique ability to interact both chemically and physically with a huge range of both man made
and naturally occurring recalcitrant compounds leading to a structural change or complete degradation of target molecule. Bioremediation is defined as the process whereby organic wastes are biologically degraded under controlled conditions to an innocuous state. It uses naturally occurring bacteria and fungi or plants to degrade or detoxify substances hazardous to human health and/or the environment. The microorganisms may be indigenous to a contaminated area or they may be isolated from elsewhere and brought to the contaminated site. Contaminant compounds are transformed by living organisms through reactions that take place as a part of their metabolic processes. Biodegradation of a compound is often a result of the actions of multiple organisms. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate. Bioremediation technologies utilize relatively low-cost, low-technology techniques, which generally have a high public acceptance and can often be carried out on site. Although the methodologies employed are not technically complex, considerable experience and expertise may be required to design and implement a successful bioremediation program, due to the need to thoroughly assess a site for suitability and to optimize conditions to achieve a satisfactory result, because bioremediation seems to be a good alternative to conventional clean-up technologies.

### 4.2 Metabolic Modes of Bioremediation

The design of bioremediation processes requires determination of the desired degradation reactions to which the target compounds will be subjected. This involves selecting the metabolic mode that will occur in the process. The metabolic modes are broadly classified as aerobic and anaerobic. Aerobic transformation occurs in the presence of molecular oxygen serving as the electron acceptor. This form of metabolism is also known as aerobic
respiration. In aerobic respiration, microbes use O₂ to oxidize part of the carbon in the contaminants to carbon dioxide (CO₂), with the rest of the carbon used to produce new cell mass. In the process O₂ gets reduced resulting in the formation of water. Thus, the major products of aerobic respiration are carbon dioxide, water and an increased population of microorganisms.

Anaerobic reactions occur only in the absence of molecular oxygen and the reactions are subdivided into anaerobic respiration and fermentation. In anaerobic respiration nitrate (NO₃⁻), sulfate (SO₄²⁻), metals such as iron (Fe³⁺) and manganese (Mn²⁺) or even CO₂ can play the role of oxygen, accepting electrons from the degraded contaminant. Thus, anaerobic respiration uses inorganic chemicals as electron acceptors. In addition to new cell matter, the byproducts of anaerobic respiration may include nitrogen gas (N₂), hydrogen sulfide (H₂S), reduced forms of metals, and methane (CH₄) depending on the electron acceptor. The aerobic biodegradability of organic substances (Figure 4.1) can be affected by the change of one of the following factors during the processes (i) removal rate of organic substances (ii) the amount of oxygen consumption (iii) end products and (iv) the activity of microorganisms.

**Figure 4.1 Aerobic Biodegradation of Organic Substances**

Sediment microorganisms are crucial for the biodegradation of organic matter and the cycling of nutrients while these microorganisms are susceptible to toxic pollutants (Eismann and Montuelle. 1999). The
degradation of organic pollutants in aquatic ecosystems is mainly performed by bacteria (Verrhiest et al., 2002). Most of the bacteria in an aquatic ecosystem are bound to sediment particles. The activity of the bacteria at the sediment surface rapidly degrade organic compounds and thereby generate an oxygen gradient. When oxygen is depleted sulphide can be formed which has a strong influence on the partitioning of metals and the degradation of organic compounds in the sediment.

A bioremediation process is based on the activities of aerobic or anaerobic heterotrophic microorganisms. Microbial activity is affected by a number of physicochemical environmental parameters. The factors that directly impact on bioremediation are energy sources (electron donors), electron acceptors, nutrients, pH, temperature, and inhibitory substrates or metabolites. The organic matter serves as a storehouse of carbon and energy as well as a source of other macronutrients such as nitrogen, phosphorous, and sulfur. Bacteria become more dominant in the microbial community with increasing depth in the soil profile as the numbers of other organisms such as fungi or actinomycetes decrease. This is attributed to the ability of bacteria to use alternative electron acceptors to oxygen. Other factors that control microbial populations are moisture content, dissolved oxygen, and temperature.

4.3 FACTORS AFFECTING BIOREMEDIATION

The control and optimization of bioremediation processes is a complex system of many factors. These factors include the existence of a microbial population capable of degrading the pollutants; the availability of contaminants to the microbial population; the environment factors. The rate at which the microorganisms degrade the contaminants is influenced by the nature of the contaminants present: oxygen supply, moisture, nutrient supply,
pH, temperature, the availability of the contaminant to the microorganism (clay soils can adsorb contaminants making them unavailable to the microorganisms). Further it also depends on the concentration of the contaminants (high concentrations may be toxic to the microorganism), the presence of substances toxic to the microorganism (e.g., mercury) and inhibitors to the metabolism of the contaminant. Oxygen level in ex-situ applications is easy to control than in-situ applications and is typically maintained by mechanical tilling, venting and sparging. Water serves as the transport medium through which nutrients and organic constituents pass into the microbial cell and metabolic waste products pass out of the cell. Moisture levels in the range of 20% to 80% generally allow suitable biodegradation in soils.

Nutrients required for cell growth are nitrogen, phosphorous, potassium, sulfur, magnesium, calcium, manganese, iron, zinc and copper. If nutrients are not available in sufficient amounts, microbial activity will deficient in the contaminated environment and thus are usually added to the bioremediation system in a useable from (e.g., as ammonium for nitrogen and as phosphate for phosphorous). Various factors affecting the process of bioremediation are discussed below.

### 4.3.1 Temperature

Temperature of soil influences biodegradation in both direct and indirect ways. Temperature affects microbial activity in the treatment unit. The biodegradation rate will decrease with decreasing temperature. The microorganisms remain viable at temperatures below freezing and will resume activity when the temperature rises. Compost piles require periodic tilting to release self-generated heat. Temperature also affects non-biologically losses of contaminants mainly through the volatilization of contaminants at high
temperatures. The solubility of contaminants typically increases with increasing temperature. However, some hydrocarbons are more soluble at low temperatures than at high temperatures. Additionally, oxygen solubility decreases with increasing temperature. Temperature also can have profound effects on both the soil/sediment matrix and the physicochemical state of the contaminants. Temperature influences soil volume, oxidation-reduction potential and water structure within the soil matrix (Filler., 2001).

4.3.2 pH

pH affects the solubility and consequently the availability of many constituents of the soil/sediments which can affect biological activity. Many metals that are potentially toxic to microorganisms are insoluble at elevated pH. Therefore, elevating the pH of the treatment system can reduce the risk of poisoning the microorganisms. Biodegradation of contaminants is rapid at neutral or near neutral pH values. Fu & Alexander (1994) measured volatilization and biodegradation of styrene in lake water and sediment samples. Dibble & Bartha (1979b) found a soil pH of 7.8 to be optimal for biodegradation of petroleum hydrocarbons. Marek et al., 2001 found that adjustment of soil pH from acidic conditions (pH 4.5) to near neutral conditions (pH 7.4) resulted in doubling of the rate of biodegradation of contaminated soil.

4.3.3 Moisture Content

Biodegradation of contaminants is dependent on adequate supply of water within the soil/sediment environment. Water is necessary not only to meet the physiological requirements of microorganisms but also for the transport of nutrients and metabolic by products to and from the microorganisms and to determine the oxygen status of the soil
microenvironment. Biodegradation of contaminants in soil/sediment systems is optimal at soil moisture content between 30% and 80% of field capacity (Riser - Roberts, 1992).

4.3.4 Energy Sources

One of the primary variables affecting the activity of bacteria is the ability and availability of reduced organic materials to serve as energy sources. Whether a contaminant will serve as an effective energy source for an aerobic heterotrophic organism or not depends on the average oxidation state of the carbon in the material. In general, higher oxidation states correspond to lower energy yields, which thus provide less energy for degradation by the microorganisms. The outcome of each degradation process depends on microbial (biomass concentration population diversity, enzyme activities), substrate (physico-chemical characteristics, molecular structure, concentration), and range of environmental factors (pH, temperature, moisture content, availability of electron acceptors and carbon and energy sources). These parameters affect the acclimation period of the microbes to the substrate. The molecular structure and contaminant concentration have been shown to strongly affect the feasibility of bioremediation and the type of microbial transformation occurring and whether the compound will serve as a primary, secondary or co-metabolic substrate.

4.3.5 Bioavailability

The rate at which microbial cells can convert contaminants during bioremediation depends on the rate of contaminant uptake and metabolic and the rate of transfer to the cell (mass transfer). Increased microbial conversion capacities do not lead to higher biotransformation rates when mass transfer is a limiting factor (Boopathy, 2000). This appears to be the case in most
contaminated soils and sediments. For example, most of the explosives, which contaminate in soil, do not undergo biodegradation process even after 50 years.

The bioavailability of a contaminant is controlled by a number of physico-chemical processes such as sorption and desorption, diffusion and dissolution. The slow mass transfer causes a reduced bioavailability of contaminants in soil to the degrading microbes. Contaminants become unavailable when the rate of mass transfer is zero. The decrease of the bioavailability in course of time is often referred to as aging or weathering which may result from

- Chemical oxidation reactions incorporating contaminants bound to natural organic matter, (viz., humic substances present in soil).
- Slow diffusion to small pores and subsequent adsorption to organic matter and
- The formation of semi-rigid films around non-aqueous-phase liquids (NAPL) with a high resistance toward NAPL-water mass transfer.

The bioavailability problems can be overcome by the use of food-grade surfactants (Boopathy 2000), which increase the availability of contaminants for microbial degradation.

4.3.6 Bioactivity and Biochemistry

The term bioactivity is used to indicate the operating state of microbiological processes. Improving bioactivity implies that at the system conditions are adjusted to optimize biodegradation (Blackburn & Hafker, 1993). In nature, the ability of organisms to transfer contaminants to both simpler and more complex molecules is very diverse. In light of the current limited ability to measure and control biochemical pathways in complex
environments, favorable and unfavorable biochemical conversions are evaluated in terms of whether individual groups of parent compounds are converted to measurable metabolites.

4.3.7 Properties of the Contaminant

The nature of the contaminants will dictate whether biological processes should even be considered for bioremediation. The first step is therefore to evaluate possible bioengineering application to understand the characteristics of the contaminants. The following properties are used to characterize a contaminant and dictate its potential migration pathways once it is released into the environment (Lyman et al., 1992). The density of a substance is important to know whether a contaminant distributes itself between the solid and solution phases of water or saturated/unsaturated soil is dependent on several physical and chemical properties of both the contaminant and the soil. The parameter $K_{ow}$ is used to symbolize the tendency of the contaminant to be adsorbed and it can be described as the ratio of the amount of chemical adsorbed per unit weight of organic carbon in the soil to the concentration of the contaminant in solution at equilibrium. The extent of adsorption not only will influence the mobility of the contaminants but also effects volatilization, photolytic degradation, hydrolysis and biodegradation. Solubility in water is a measure of the degree to which the contaminant will dissolve in water and is a function to temperature. The presence of dissolved salts or minerals in water also influences the solubility. Naturally occurring dissolved organic material also can affect the solubility of the contaminants. Solubility in various solvents also depends to a considerable degree on whether one liquid is soluble in another, solid in a liquid and gases in liquids. This parameter is important to consider at sites where there is more than one contaminant present.
4.3.8 Carbon and Energy Source

In order for the microorganisms to sustain, biodegradable organic compounds must be present to serve as the carbon and energy source. The mechanisms by which microorganisms carry out biodegradation are primary or secondary utilization and co-metabolism, which may depend on the concentration of the contaminant.

4.3.9 Electron Acceptors

In most instances, bioremediation of contaminated soils depends on the activities of aerobic (oxygen requiring) organisms. Although correct research into anaerobic processes may lead to the development of anaerobic or anoxic soil remediation processes, these systems are not practical at this point. Therefore the presence of an adequate supply of oxygen within the soils is essential for bioremediation. In general, a ratio of approximately 2 to 3 lb of oxygen per pound of petroleum hydrocarbon degraded is necessary to ensure adequate oxygenation for biodegradation (Riser-Roberts, 1992). Microorganisms gain energy by catalyzing energy producing chemical reactions that involve breaking chemical bonds and transferring electrons away from the contaminant. The type of chemical reaction is called an oxidation-reduction reaction: the organic contaminant is oxidized. Correspondingly the chemical that gains the electrons is reduced. The contaminant is called the electron donor while the electron recipient is called the electron acceptor. The energy gained from these electron transfers is then ‘invested’, along with some electrons and carbon from the contaminant to produce more cells. These two materials—the electron donor and acceptor—are essential for cell growth and are together called the primary substrates. Oxygen and nitrate are the electron acceptors in aerobic and anoxic
environments respectively. Sulfate, carbon dioxide or reduced organics act as electron acceptors in anaerobic environments.

**4.3.10 Nutrients**

Although the microorganisms are present in contaminated soil, they cannot necessarily be there in the numbers required for bioremediation of the site. Their growth and activity must be stimulated. Biostimulation usually involves the addition of nutrients and oxygen to help indigenous microorganisms. These nutrients are the basic building blocks of life and allow microbes to create the necessary enzymes to break down the contaminants. All of them will need nitrogen, phosphorous, and carbon (Table 4.1). Carbon is the most basic element of living forms and is needed in greater quantities than other elements. In addition to hydrogen, oxygen, and nitrogen it constitutes about 95% of the weight of cells. Phosphorous and sulfur contribute with 70% of the remainders. The nutritional requirement of carbon to nitrogen ratio is 10:1, and carbon to phosphorous is 30:1.

**Table 4.1 Nutritional Requirements of Microorganisms**

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Element</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Carbon</td>
<td>50</td>
</tr>
<tr>
<td>2</td>
<td>Nitrogen</td>
<td>14</td>
</tr>
<tr>
<td>3</td>
<td>Oxygen</td>
<td>20</td>
</tr>
<tr>
<td>4</td>
<td>Hydrogen</td>
<td>8</td>
</tr>
<tr>
<td>5</td>
<td>Phosphorous</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>Sulphur</td>
<td>1</td>
</tr>
<tr>
<td>7</td>
<td>Potassium</td>
<td>1</td>
</tr>
<tr>
<td>8</td>
<td>Sodium</td>
<td>1</td>
</tr>
<tr>
<td>9</td>
<td>Calcium</td>
<td>0.5</td>
</tr>
<tr>
<td>10</td>
<td>Magnesium</td>
<td>0.5</td>
</tr>
<tr>
<td>11</td>
<td>Chloride</td>
<td>0.5</td>
</tr>
<tr>
<td>12</td>
<td>Iron</td>
<td>0.2</td>
</tr>
<tr>
<td>13</td>
<td>others</td>
<td>0.3</td>
</tr>
</tbody>
</table>

(Source : Stainer et al)
Nutrients such as nitrogen (which can be present as ammonium, nitrite, nitrate, or organic nitrogen) and phosphorus which are taken by microorganisms for metabolic reactions. In some situations, trace meals (potassium, iron, molybdenum, zinc) may not be present in sufficient quantities in the soil and may need to be added. Nitrogen is necessary for the synthesis of cellular proteins and cell wall components while phosphorous is necessary for nucleic acids, cell membranes and ATP. Nitrogen and phosphorous are frequently limiting in soil environments. Preferred form of nitrogen for growth of soil microorganisms is the form of ammonium ions (Alexander, 1995).

4.3.11 Toxic Materials

A toxic material in the context of biodegradation is the one that inhibits the rate at which microbiological activity occurs. Whether or not a material is toxic depends on the concentration, the characteristics of the material, the ability of the microorganisms to adapt to its presence and time of exposure.

4.3.12 Adequate Contact

In order for the biodegradation of contaminant to occur, there must be sufficient interaction between the contaminated matrix and the microorganisms. Adequate contact must be provided between the contaminants, the microorganisms performing the biodegradation process and the added amendments (nutrients, electron donor, pH adjusters). For in-situ treatment systems, this will be greatly affected by the hydrogeological characteristics of the site. In case of slurry system, better contact with the pollutant is facilitated because of mixing.
4.4 ENVIRONMENTAL REQUIREMENTS FOR BIOREMEDIATION

Optimum environmental conditions for the degradation of contaminants are reported in Table 4.2

**Table 4.2 Optimum Environmental Conditions for Degradation of Contaminants**

<table>
<thead>
<tr>
<th>S. No</th>
<th>Parameters</th>
<th>Conditions required for microbial activity</th>
<th>Optimum values for biodegradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Moisture</td>
<td>25-28% of water holding capacity</td>
<td>30-90%</td>
</tr>
<tr>
<td>2</td>
<td>pH</td>
<td>5.5-8.8</td>
<td>6.5-8.0</td>
</tr>
<tr>
<td>3</td>
<td>Oxygen content</td>
<td>Aerobic, minimum air filled pore space of 10%</td>
<td>10-40%</td>
</tr>
<tr>
<td>4</td>
<td>Nutrient content</td>
<td>N and P for microbial growth</td>
<td>C:N:P = 100: 10:1</td>
</tr>
<tr>
<td>5</td>
<td>Temperature</td>
<td>15-45</td>
<td>20-30</td>
</tr>
<tr>
<td>6</td>
<td>Contaminants</td>
<td>Not too toxic</td>
<td>5-10% dry weight of soil</td>
</tr>
<tr>
<td>7</td>
<td>Heavy metals</td>
<td>Total content 2000 ppm</td>
<td>700 ppm</td>
</tr>
<tr>
<td>8</td>
<td>Type of soil</td>
<td>Low clay or silt content</td>
<td>Contaminated soil</td>
</tr>
</tbody>
</table>

4.5 MICROBIAL POPULATIONS FOR BIOREMEDIATION PROCESSES

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. The main requirements are energy source and a carbon source. Because of the adaptability of microbes and other biological systems, these can be used to degrade or remediate environmental hazards. Microorganisms are subdivided into aerobic and anaerobic.
4.5.1 Aerobic Microorganisms

Aerobic microorganism survives in the presence of oxygen. Examples of aerobic bacteria recognized for their degradative abilities are Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, and Mycobacterium. These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

4.5.2 Anaerobic Microorganisms

Anaerobic microorganism survives in the absence of oxygen. Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and chloroform.

Ligninolytic fungi: Fungi such as the white rot fungus Phanaerochaete chrysosporium have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or com cobs. Methylotrophs: Aerobic bacteria that grow utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatics trichloroethylene and 1,2-dichloroethane. An overview of the microbiological aspects of the application of microorganisms is given in Table 4.3. Microbes such as fungi grow in a filamentous form towards the contaminant. It is possible to enhance the mobilization of the contaminant utilizing surfactants.
Table 4.3 Various organic pollutants subjected aerobic and anaerobic degradation mechanisms

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Class of contaminants</th>
<th>Specific examples</th>
<th>Aerobic</th>
<th>Anaerobic</th>
<th>More potential sources</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorinated solvents</td>
<td>Trichloroethylene</td>
<td></td>
<td>+</td>
<td>Drycleaner chemical manufacture</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Perchloroethylene</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>Polychlorinated biphenyls (PCB’s)</td>
<td>4-Chlorobiphenyl</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>4,4-Dichlorobiphenyl</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Chlorinated phenols</td>
<td>Pentachlorophenol</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>BTEX</td>
<td>Benzene Toulene Ethyl benzene Xylene</td>
<td></td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Poly Aromatic Hydrocarbons (PAHs)</td>
<td>Naphthalene Anthracene Fluorene Pyrene Benzo(α)Pyrene</td>
<td></td>
<td>+</td>
<td></td>
</tr>
</tbody>
</table>

4.6 BIOREMEDIATION METHODS

Three types of bioremediation are predominant in the industry today. They are natural attenuation, biostimulation, and bioaugmentation.

4.6.1 Natural Attenuation

Natural attenuation is a collection of biological, chemical and physical processes that occur naturally resulting in the containment transformation or destruction of undesirable chemicals in the environment. In contrast to biostimulation, monitored natural attenuation (MNA), if effective, provides significant benefits in terms of cost and effort. It can also be termed as intrinsic remediation, bioattenuation and intrinsic bioremediation. In this case, the contaminants are left on site and the naturally occurring processes are
left to clean up the site. Processes include combination of both biotic and abiotic mechanisms like sorption, volatilization, dilution, and dispersion coupled with biodegradation (Vega et al., 2007).

4.6.1.1 Abiotic processes

Adsorption reactions or processes involving organic chemicals and soil fractions are governed by: (a) the surface properties of the soil fractions, (b) the chemistry of the pore water, and (c) the chemical and physical-chemical properties of the pollutants. In general, organic chemical compounds develop mechanisms of interactions which are somewhat different from those given previously for inorganic contaminants. If the transport of organic chemicals in soils is considered, interactions between the contaminant and soil surfaces are important in predicting the retention capacity of the soil and the bioavailability of the contaminant. The interaction mechanisms are influenced by soil fractions, the type of and size of the organic molecule, and the presence of water. As in the case of inorganic contaminant-soil interactions, the existence of surface active fractions in the soil such as soil organic matter (SOM), amorphous non-crystalline materials and clays, can significantly enhance oil retention in soils because of large surface areas, high surface charges and surface characteristics.

Volatilization may be an important attenuation mechanism for volatile organic contaminants in the sediments. Freshly spilled petroleum products such as gasoline can exhibit high rates of volatilization that can occur from the free phase or dissolved phase. The distribution of organic chemical pollutants between soil fractions and pore water is generally known as partitioning. By this, chemical pollutants are partitioned such that a portion of the pollutants in the pore water (aqueous phase) is removed from the aqueous phase. This is because precipitation of the heavy metals will also serve to
remove the heavy metals from solution. The abiotic reactions and transformations are sensitive to at least two factors: (a) the physico-chemical properties of the pollutant itself, and (b) the physico-chemical properties of the soil (i.e. soil fractions comprising the soil). Similar to inorganic contaminants, abiotic chemical reactions with organic compounds occur and include (a) hydrolysis, (b) formation of a double bond by removal of adjacent groups and (c) oxidation / reduction, or dehydrohalogenation reactions. Chemical mass transfer is responsible for partitioning of contaminants in the fate and transport of contaminants. Reduction-oxidation reactions can also play an important role in the fate of the contaminants. Assessment of retention or retardation processes is responsible for determining the partitioning and the attenuation of contaminants within the soil.

4.6.1.2 Biotic processes

Microorganisms are key to the biological treatment of contaminants including bacteria, protozoa, fungi, algae and viruses (Mulligan et al, 2001). Understanding the types of chemicals that can be biodegraded or transformed and the pathways of conversion is important as well as the toxicity and availability of several chemicals since this will serve as the foundation of knowledge required for determining the potential for natural attenuation. Determination of the capacity for bioattenuation has not received a great deal of attention in assessment of the natural attenuation of organic chemical pollutants. Substrates can become less bioavailable via interaction with negatively charged clay particles and organic material (Alexander, 1994). Sorption and sequestration can be influenced by pH, organic matter content, and temperature and pollutant characteristic and bioavailability of electro acceptors.
4.6.2 Biostimulation

If natural degradation does not occur or if the degradation is too slow, the environment has to be manipulated in such a way that biodegradation is stimulated and the reaction rates are increased. The measures to be taken, called biostimulation which include supplying the environment with nutrients such as nitrogen and phosphorus, with electron acceptors such as oxygen and with substrates such as methane, phenol, and toluene. Thus the concentrations of these chemicals during biostimulation should be carefully monitored. Addition of nutrients such as nitrogen fertilizers has therefore been observed to enhance biotransformation (Walworth et al., 2007). In general, microbes have an average C:N ratio of about 5:1 to 10:1 within their biomass. Therefore, the general rule of thumb for biodegradation of or to cell mass is to add nitrogen and phosphorus at a ratio of carbon to nitrogen to phosphorus of 100:10:1 (Norris & Matthews, 1994). Also, several studies on the effects of
biostimulation with mainly N-P-K or oleophilic fertilizers have reported positive effects (Margesin and Schinner, 2001). In most bioremediation techniques significant amounts of two nutrients- nitrogen (N) and phosphorous (P) are necessary for the metabolism of the biodegrading microorganisms. The concentration of the organic contaminant is so high in the soils/sediments that intrinsic N and P cannot produce sufficient biomass to biodegrade the contaminant quickly. However, nutrient amendment may be ineffective because some other factor is limiting (Alexander, 1994), the intrinsic nutrient levels are already high (Yu et al, 2005), the nutrients exert inhibitory effects (Morgan and Watkinson, 1992) or the nutrient is consumed as a growth substrate (Carmichale and Pfaender, 1997). Normally, inorganic orthophosphates or polyphosphates provide P while inorganic nitrates or ammonium salts provide nitrogen. Organic nutrients contain an organic group which allows them to adhere to organic contaminants at the organic-aqueous interface, hence facilitating bacterial growth on the organic contaminants. The amount of nutrients to add has traditionally been determined by a C:N:P ratio (in moles) of 100:10:1 (Chen et al., 2003) since this is an accepted elemental composition of a mixed microbial population in soil. Other ratios have been used (Chen et al., 2003) because the carbon in organic contaminants may mineralize into carbon dioxide rather than assimilate into biomass (Dibble and Bartha, 1979) or the organic contaminant and nutrients are simply not bioavailable (Kwok and Loh., 2004).

4.6.3 Bioaugmentation

The third choice in the treatment hierarchy is bioaugmentation, which is a way to enhance the biodegradative capacities of contaminated sites by inoculation of bacteria with the desired catalytic capabilities. This is considered to be an effective approach in the case of very recalcitrant chemicals where natural attenuation or biostimulation does not work.
However, much attention has been paid to the application of bioaugmentation because of its unknown effects on the ecosystem. Since large amounts of degradative bacteria are added to contaminated sites, the effect of the bacteria on both human and environment must be clarified in advance. Moreover, it needs to be confirmed that the injected bacteria have perished after the remediation and thus do not affect the indigenous microbial community for a long period.

Bioaugmentation, the addition of microorganisms to enhance a specific biological activity has been practiced intentionally for years in the remediation of contaminated sites. Bioaugmentation is generally accepted as an efficient technique for soil/sediment bioremediation, as proponents continue to demonstrate possible advantages. The utility of bioaugmentation is supported by studies showing the incompetence of indigenous microorganisms in some cases and the apparent enhanced bioremediation rate after the addition of competent microorganisms (Boon et al., 2001). The reinoculation of soil with indigenous microorganisms directly isolated from the same soil is often included in the term bioaugmentation (Otte et al., 1994).

Among the parameters that appear to be important are the pollutant characteristics e.g. bioavailability (Stegmann et al., 2001), concentration (Crawford., 1996) and microbial toxicity (Baud & Vogel., 1995), the soil physico-chemical characteristics e.g. humidity or water content (Vogel., 1996), organic matter content, clay content and pH, microbial ecology (Ramdan., 1990), microbiology e.g. the presence of co-substrates (Barriault, & Sylvestre., 1993), genetics of the relevant organisms, and enzyme stability and activity (Trombley, 1995), and methodology e.g. inoculation concentration (Pearce et al., 1995), method of inoculation (Romich et al., 1995), the presence / absence of indigenous activity (Liang & McFadand., 1994) and
inoculum heterogeneity (Petrie et al., 1995). The following are the various factors influencing the process of bioaugmentation.

4.6.3.1 Indigenous microorganisms

The adaptation capacity of indigenous microorganisms is currently under study which reveals that this capacity is tremendous and more rapid. As these rapidly adapting microorganisms are going to be the reference for bioaugmentation. Consideration should be given to their applicability to the bioremediation of contaminated soil/sediments. They are often better distributed than added microorganisms, although not necessarily with regard to the target pollutant because both the pollutant and the added microorganisms often enter the soil by similar methods (dumping). One important concept is, thus, related to the distance between the target compound and microorganism. Perhaps added microorganisms are closer to compounds recently added and indigenous microorganisms are closer to older historic pollution, although concepts such as size exclusion (Petrich et al., 1995) might be important determinants of microbe-compound proximity.

4.6.3.2 Compound Characteristics

Pollutant toxicity is often used as justification for bioaugmentation because, conceptually, this toxicity could inhibit the degradative activity of indigenous microorganisms. Compound biodegradability is sometimes related to compound structure and its related physico-chemical characteristics such as solubility and bioavailability (which itself is not intrinsic to the compound, but related to the interactions between the compound, the microorganism(s) and the soil).
4.6.3.3 Physico-chemical and environmental characteristics

Environmental conditions play a pivotal role in determining biological activity, whether of indigenous microorganisms, added microorganisms; or cultured indigenous microorganisms returned to the soil. These conditions fall into two general categories: those that reduce the microbial activity, such as temperature, humidity and ionic strength and also increased cell attachment (Shonnard et al., 1994); and those that restrict the mass transfer (mainly by diffusion) of the compound to the microorganism, such as clay and organic matter content (Godbout et al., 1995). In addition, several aspects of the bioaugmentation process are affected by advective transport, such as permeability. This affects the addition of microorganisms during bioaugmentation as well as the addition of nutrient and electron acceptors during both bioaugmentation and biostimulation. As stated above, emphasis recently has been on the understanding of the movement and fate of added microorganisms during bioaugmentation. The fate of added microorganisms is not unrelated to the ecologically stable microorganism concentration one observes in soils.

Microbial ecology is very important in evaluating both the potential success of bioaugmentation and its possible advantages over biostimulation. Microorganisms are affected by maintenance energy, the production of, and resistance to, antibiotics and toxic metabolites, predation, etc. The advantages of employing mixed cultures as opposed to pure cultures in bioremediation have also been widely demonstrated. It could be attributed to the effects of synergistic interactions among members of the association.
4.6.4 Application of Bioassays as Indicators of Sediment Bioremediation

Bioassays are a useful complement to chemical analyses for evaluation of soil/sediment contamination and bioremediation process. Due to the complexity of the process in contaminated soils/sediments detailed monitoring and the final evaluation of remediation efficiency are essential for process control, as well as environmental safety considerations. The main advantage of bioassays is their integrative character. They provide valuable information about the bioavailability of all toxic compounds present in contaminated soils/sediments which depends on the physical and chemical properties of the soil.

In recent years ecotoxicological tests have been used as supplementary tools to monitor bioremediation of organics both in laboratory and field studies (Thomas and Ward, 1994). The effects based assessment provides more realistic targets for cleanup criteria than non-specific measurements. Soil biological investigations (such as soil respiration, biomass, enzyme activities, microbial counts) can give information on the presence of viable microorganisms as well as on the intensity and on the kind and duration of the effects of pollutants on the metabolic activity of soil, and are thus well suited to measure the impact of pollution on soil health (Leahy and Colwell., 1990). The following are various parameters which indicate the progress of bioremediation.

4.6.4.1 Microbial enumeration

Initial soil/sediment analysis of the total heterotrophic microbial counts specific organic compound degrading microbial counts in the contaminated soil/sediment can provide useful information on soil biological activities and the extent to which the indigenous microbial population capable
of supporting bioremediation. In addition initial microbial assessment of the contaminated soil, monitoring microbial populations during the soil bioremediation is a useful tool for following the microbial growth and activities bioremediation is a useful tool for following the changes and discerning for microbes active in organic degradation has been reported (Song and Bartha, 1990). Bacterial count is usually determined using standard serial dilution and nutrient agar plate counting techniques (Lorch et al., 1995). The plate counts for mesoplillic bacteria are typically incubated at 30°C for 24h before bacterial counts are conducted. The counts are usually expressed as CFUs (Colony Forming Units).

4.6.4.2  Dehydrogenase activity

Biological oxidation of organic compounds is generally a dehydrogenation process, which is catalyzed by dehydrogenase enzymes (Paul and Clark 1989). Therefore, these enzymes play an essential role in the oxidation of organic matter by transferring hydrogen from the organic substrates to electron acceptor. Many different specific enzyme systems are involved in the dehydrogenase activity of the soils. These systems are an integral part of the soil microorganisams and reflect to a great extent the soil biochemical activities. The assay of dehydrogenase in contaminated sediments/soils can be used as the simple method to examine the possible inhibitory effect of the contaminants on the soil microbial activities (Balba et al., 1998). Dehydrogenase activity depends on the total metabolic activities of the soil microorganisms as its values not always reflect the total number of viable microorganisms isolated on a particular medium.
4.6.4.3 Respirometric Tests

Respiration measurements are used frequently as sensitive and easy analyzable microbial parameter for the characterization of soil/sediment samples. The respiration activity is close connected to other microbial parameters such as microbial biomass (Dilly, 2001). In addition, it is used for the assessment of ecotoxic effects in contaminated soils/sediments (Hollender et al., 2003). Margesin et al (2000) used respiration as a monitoring instrument for the decontamination process in soils/sediments.

Mineralization studies involving measurements of total CO$_2$ production can provide excellent information on the biodegradability potential of organics in contaminated soils. This approach which is considered a preliminary step in the feasibility study provides rapid, relatively unequivocal time course data suitable for testing different biological treatment options, such as effect of nutrient supplementation, microbial inoculation, etc. The test can be useful for confirming active degradation of organics during full scale bioremediation. During the respiration tests, oxygen consumption and/or carbon dioxide evolution rates can be monitored by using either expensive automated equipment or by simple respirometric flask methods, which can handle large number of samples simultaneously, or by simple respirometric flask method, which are commonly used (Pritchard et al., 1991). In the latter case measurement of oxygen consumption can be carried out by the use of a U-tube manometer and barometric control so when oxygen is consumed by aerobic metabolism, a measurable negative pressure develops within the respirometric flask which is directly related to the oxygen partial pressure.
4.6.4.4 Microbial Biomass

Soil microbial biomass which represents collectively the mass of all soil microorganisms considered as a single soil organic matter fraction (Jenkinson, 2004) is one of the best parameters to be tested for this purpose. The biomass is responsible for organic matter decomposition. The soil microbial biomass responds readily to soil disturbance and can provide an effective early warning of the deterioration of soil quality (Zak et al., 1994; Powlson, 1993). However, non-experimental field data on the size of microbial biomass responses are usually difficult to interpret because of the lack of suitable controls. To overcome this problem, Brookes (1993) suggested the use of linked parameters, normalized by an internal control such as: the biomass C expressed as a percentage of total soil organic C (C_{bio}/C_{org}) and the specific respiration rate (i.e., metabolic quotient) of microbial biomass (qCO_2). Anderson and Domsch (1993) originally proposed the use of the metabolic quotient (rate or respiration per unit of biomass) of the soil microbial biomass as an index of changes in microbial biomass activity in response to stress factors or disturbance. The qCO_2 has been shown to be a good indicator of environmental change, such as produced by heavy metals (Brookes and Grath, 1984), substrate addition (Ocio and Brookes, 1990), pH variations, drying and rewetting cycles, although it is still debated if qCO_2 can be considered always and unequivocally an index of microbial stress (Insam et al., 1996). The soil microbial biomass is also the agent of biodegradation, the primary mechanism by which dissolved organic matter is decomposed and organic pollutants are removed from soil (Marschner and Kalbitz, 2003).

Laboratory and field-scale investigations dealing with the transformation of pollutants are required to optimize the bioremediation processes. Data from laboratory-scale experiments provide information on the metabolic pathways involved in the degradation process, the biodegradation
potential pure or mixed cultures, the bioavailability and the ecotoxicity of pollutants. A better understanding of the chemical and biochemical processes involved in the transformation of the pollutants helps in the design of more efficient bioremediation processes. Laboratory and pilot testing are recommended in cases where polluted sites are extremely difficult to treat.

4.7 COMPOSTING

Aerobic composting is the biological decomposition and stabilization of organic substrates in the presence of oxygen under thermophilic conditions created by biologically produced heat (Haug, 1993). The metabolically generated heat is trapped within the compost matrix which leads to elevations in temperature, a characteristic of composting (Williams et al., 1992). Cooperband (2003) has described the composting process as the transformation of raw organic materials into biologically stable humic substances. This definition is based on the end product namely compost. Composting at elevated temperatures can be an effective way to reduce the levels of aerobically biodegradable organic pollutants (Epstein, 1996).

![The Composting Process](image)

Figure 4.3 A Typical Composting Process
4.7.1 Organic Amendments and Bulking Agents

Compost is the resultant product of composting. In the composting of contaminated materials, organic amendments including manure, yard wastes, food processing wastes and animal wastes are often added to supplement the amount of nutrients and readily degradable organic matter and also have high microbial diversity, with total microbial populations which are primary agents for the degradation of organic contaminants in soil, increasing microbial density can accelerate degradation of the contaminants (USEPA, 1998). The ratio of contaminated soil to organic amendments should be determined because an inappropriate ratio may retard or inhibit microbial activity (Thomas et al., 1992). Addition of organic amendments compost can facilitate degradation of organic contaminants because they play a role in supplementing nutrients and carbon source in contaminated soil. Bulking agents are the materials of low density that when added to the composting mixtures lower the bulk density, increase porosity, decrease moisture levels, increase oxygen diffusion and help to form water stable aggregates. Thus, most composting systems utilize bulking agents such as bark chips, wood chips, sawdust, and straw and chopped sugar beet. Bulking agents are added to the compost mixture to increase porosity and serve as source for easily assimilated carbon for biomass growth.

4.7.2 Main Composting Phases

The conventional aerobic composting process passes through four major microbiological phases related to the temperature profile: mesophilic (30-45°C), thermophilic or biooxidative (45-75°C), cooling, and maturation. The initial mesophilic phase is characterized by the activity and growth of mesophilic organisms leading to a rapid increase in temperature (Finstein and Morris. 1975). In the next stage, thermophilic organisms take over the
degradation process and the growth and activity of non-thermo tolerant organisms are inhibited. The final phase including a cooling and maturing period is characterized by the development of a new mesophilic community.

The composting process can also be discussed in terms of two well defined phases, namely, mineralization and humification. Mineralization is a very intensive process involving the degradation of readily degradable organic substances followed by intensive microbial activities producing heat, carbon dioxide and water, along with partially transformed and stabilized organic residuals. When the assimilable organic fraction is exhausted, some of the cells undergo decay by auto-oxidation to provide energy for the remaining cells. The transformation process of the organic substances is completed in the second phase under less oxidative conditions, thus allowing the formation of the humic substances and eliminating the dense toxic compost, eventually formed during the first phase. The humification phase is conducted by specific microorganisms which synthesize the complex tri-dimensional polymers that create the energy substratum for future microbial activities. It is to be noted that during the first phase of composting, it is necessary to provide sufficient oxygen to allow a good start for the microbial transformation as well as to have an appropriate temperature rise, needed to maintain the material hygiene. According to many authors, during the bio-oxidation phase (thermophillic phase) oxygen in the range of 5-15% must be present. On the other hand, the process of humus formation under aerobic and micro-aerobic conditions (less oxidisable) is preferred during the second phase. This is necessary to avoid excessive mineralization of the organic substance.

In the maturing phase, the oxygen requirement is less, biological processes become very slow and the temperature partially reduces. The oxygen percentage in the present phase is of the order of 5% or so.
Considering various stages of humus formation, the bio-oxidation phase of composting used for the degradation of organic substances could be identified. On the other hand, the synthesis phase of humus substances started during the first phase of composting develops and finally finishes in the matured phase of the compost. As a biodegradative process, composting efficiency depends on microbial biomass and activity. The microbes are normally derived from composting mass, old compost (acclimated microbial source) and also commercial bacterial strains.

4.7.3 Microbial Activity during Composting

Composting is a dynamic process carried out by a rapid succession of mixed microbial populations. The main groups of microorganisms involved are bacteria, including actinomycetes, and fungi (Golueke, 1992). Although the total number of microorganisms does not significantly change during composting, the microbial diversity can vary during the different phases of composting (Alkinson et al., 1996a). The precise nature of succession and the number of microorganisms at each composting phase is dependent on the substrate and on the preceding microorganisms in the succession.

Composting is an aerobic process in general, but anaerobic microenvironments may develop. Because of their small size, bacteria have a very high surface/volume ratio, which allows rapid transfer of soluble substrates into the cell. As a result, bacteria are usually far more dominant than larger microorganisms such as fungi. Some bacteria, e.g. Bacillus spp., are capable of producing thick-walled endospores which are very resistant to heat, radiation and chemical disinfection (Haug, 1996).

The first microorganisms to colonize in the compost heap are mesophilic such as mesophilic bacteria, actinomycetes, fungi and protozoa
(Figure 4.4). They grow between 10 and 45°C (Cooperband, 2000) and break down easily degradable components (Hellmann et al., 1997). At the beginning of composting mesophilic bacteria predominate, but after the temperature increases to over 40°C, thermophilic bacteria take over and thermophilic fungi also appear in the compost. When the temperature exceeds 60°C, microbial activity decreases dramatically, but after the compost has cooled mesophilic bacteria and actinomycetes again dominates (McKinley and Vestal, 1985).

Organic fresh matter degradation starts as soon as the compost heap is made, and because of the oxidative action of microorganisms, the temperature increases. Despite a drop in pH at the very beginning of composting, the degradation of acids brings about a pH increase. When the temperature reaches 45-50°C, thermophilic microorganisms replace mesophilic ones (Ishi et al., 2001). The second phase is called the thermophilic phase and can last several weeks. It is the active phase of composting. Most of the complex organic matter is degraded and consequently most oxygen is consumed. Composting at elevated temperatures can be an effective way to reduce the levels of aerobically biodegradable organic pollutants (Epstein, 1996).

After the thermophilic phase this corresponds to a peak of degradation of organic matter, the microbial activity decreases, as does the temperature. This is called the cooling phase. The compost maturation phase begins when the compost temperature falls to that of the ambient air. More specifically mesophilic microorganisms colonize compost and continue to degrade complex organic compounds such as lignin. This last phase is important because humus like substances is produced to form mature compost (Cooperband, 2000). Mineralisation and humification occur simultaneously during composting and are the main processes of the degradation of the organic matter (Figure 4.4). Several gases are emitted during composting: carbon dioxide (CO$_2$), ammonia (NH$_3$), nitrous oxide, methane (CH$_4$),
hydrogen sulphide (H$_2$S), nitrogen oxides (NO$_x$) and volatile organic compounds (VOCs) (Hellmann et al., 1997; Mahimairaja et al., 1995; Tamura et al., 1999).

**Figure 4.4 Schematic View of Composting Process**

### 4.7.4 Biochemical Reactions in Composting

During composting, carbon is transformed into CO$_2$ and integrated into humus like substances as result of humification. Depending on the oxygen concentration within the heap, CH$_4$ can be formed (Josephine and Philippe, 2004). Methanogenesis is a biochemical reaction controlled by methanogenic bacteria, generally produced in soils/sediments or composts where anaerobic conditions prevail (Germon and Henault, 1995).
Nitrogen transformations during composting involve several biochemical reactions that occur during the mineralization process. Most of the nitrogen found in a composting mixture is organic. A small part of the organic nitrogen is mineralized to ammonia by ammonification reactions resulting from microbial activity developed. The ammonia thus formed undergoes different processes depending on the condition of mixture being composted. It may be dissolved (as ammonium) and then immobilized by microorganisms of the mixture, which use it as nitrogen source and transform again to organic nitrogen. Alternatively, it may be volatilized and be given off, as happens when the mixture is at high temperature with a pH of above 7.5. Volatilization is reduced when the static mixture system is used instead of classic turned windrow system. Lastly the ammonium is transformed into nitrate by nitrifying bacteria when the temperature is below 40°C and aeration conditions are favourable. A lack of oxygen leads the organisms to use the nitrate as an nitrogen source which results in denitrification (Sanchez Monedero et al., 2001). During the nitrification process, the nitrifying bacteria lower the pH of medium due to the liberation of hydrogen ions, a process which can be summarized in the following equations.

\[
\text{Nitrosomonas bacteria: } 2\text{NH}_4^+ + 3\text{O}_2 \rightarrow 2\text{NO}_2^- + 4 \text{H}^+ + 2 \text{H}_2\text{O} \quad (4.1)
\]

\[
\text{Nitrobacter bacteria: } 2\text{NO}_2^- + \text{O}_2 \rightarrow 2 \text{NO}_3^- \quad (4.2)
\]

Another step of degradation is the nitrification, which transforms \(\text{NH}_4^+\) into \(\text{NO}_3^-\) (nitrate) by oxidation (aerobic conditions). One of the by-products of nitrification is \(\text{N}_2\text{O}\). Although composting is an aerobic transformation of organic matter, anaerobic conditions can appear and thus, denitrification can also occur (Mahimairaja et al., 1995). Denitrification reduces \(\text{NO}_3^-\) to \(\text{N}_2\text{O}\) and \(\text{N}_2\) (Figure 3.4) in anaerobic conditions, with low pH (volatile acid produced by anaerobic degradation) and in the presence of \(\text{NO}_3^-\).
and carbon. In addition to \( \text{N}_2 \) formation, \( \text{N}_2\text{O} \), NO and under not completely anaerobic conditions.

### 4.7.5 Application of Composting Bioremediation Technologies

Composting matrices and composts are rich sources of xenobiotic-degrading microorganisms which can biotransform pollutants into less toxic substances and/or lock up the pollutants within organic matrix, thereby reducing pollutant bioavailability (Semple et al., 2001). Composting was successfully demonstrated as a bioremediation methodology for biodegrading polycyclic aromatic hydrocarbons (PAHs) (Potter et al., 1999), chlorophenols (Laine and Jorgensen, 1997), polychlorinated biphenyls (Block, 1998), explosives (Gray, 1999), and petroleum hydrocarbons (Namkoong et al., 2002) at the laboratory and/or field scales.

### 4.7.6 Interactions of Organic Pollutants with Soil/Sediment

Degradation and transformation reactions during composting affect both organic matter and organic contaminants present in contaminated matrix. Composts are rich sources of contaminant-degrading microorganisms including bacteria, actinomycetes and ligninolytic fungi, which can transform contaminants into less toxic substances and to a certain extent, mineralize them. A fraction of the contaminants is locked up within the organic matrix by formation of non-extractable residues (NER). The chemical association of organic contaminants and their metabolites with macromolecular organic matter may be based on both sorptive processes (e.g. van der Waals forces, hydrogen bonding, charge transfer) or covalent binding (ester, ether, carbon-carbon bonds). In addition, non-polar contaminants are believed to undergo hydrophobic interactions and entrapment within the sieve-like molecular structure of the humic matrix (Steinberg et al., 1987). The desorption of the
reversible bound contaminants can generally be reduced as a function of time, a process called aging (Hartlieb et al., 2003). Therefore, although entrapment is basically a reversible process, the sequestered chemicals are irreversibly bound under common extraction conditions using aqueous and organic solvents. Thus, dissipation of the chemicals during composting comprises degradation, mineralization and formation of bound residues. Once the contaminants are irreversibly bound to the humic matter, they are no longer analytically detectable without destruction of the matrix. Therefore, no control of compost quality exists, since the allanalytical depletion of the extractable amount of contaminants may not only be due to degradation but also to strong adsorption or binding to the compost matrix. However, while covalently bound contaminants become integral components of the humic substances (Wais, 1998) sequestered residues may be remobilized in the course of humus-turnover. Therefore, depending on the stability of contaminant humus complexes and the nature of binding, composting may either provide a strategy for bioremediation (Wischmann and Steinhart, 1997) or pose a hazard potential to the environment (Figure 4.5). The decision whether the bound residues and their metabolites are "locked up" safely or rather serve as a slow-release-pool under natural conditions, requires understanding of the concept of their stabilization. Therefore it is essential to study the formation of nonextractable residues and their long term stability.

Possible fates of pollutants and their breakdown metabolites entering soil environments include volatilization to air, biodegradation, and transfer to organisms, binding to soil and leaching into groundwater (Cerniglia, 1992). These processes are summarized in Figure 4.6. The fate and behaviour of organic pollutants in soil is governed by many different factors including soil characteristics, chemical properties and environmental factors such as temperature and precipitation. The persistence of certain organic
pollutants in soil has been proposed to be related to compound hydrophobicity (Cerniglia, 1992). Pollutants generally dissipate from soils in a biphasic manner, i.e. a preliminary short period of rapid loss is followed by a subsequent longer period of slower loss (Jones et al., 1996). Pollutant volatility, hydrophobicity and affinity for organic matter govern the relative importance of each phase. In addition to removal/loss processes, intra-soil processing of pollutants also occurs. These processes reduce pollutant bioavailability and promote the formation of non-bioavailable residues with time.

Organic pollutants interaction with soil also occurs through a number of attractive forces, such as dipole± dipole, dipole-induced dipole and hydrogen bonding (Pignatello and Xing, 1996). Sorption is widely accepted as the controlling factor in the ageing process and can occur in a number of ways,
including: sorption onto inorganic soil constituents and sorption onto SOM (Burgos et al., 1996). Pollutant entrapment occurring from diffusion of compounds into spatially remote areas, such as soil macro and micro pores (Steinberg et al., 1987).

4.7.7 Heavy Metals in Composting

Heavy metals in compost are derived from both the starting materials which generally contain high concentrations of these elements and the leaching of metallic wastes carried out by the organic acids produced during the composting process. Heavy metals can be found in compost in different chemical forms, namely as salts, bound onto organic matter and in an adsorbed or exchangeable form. Moreover, the typical chemical forms are in general partitioned between the soluble and insoluble species in relation both...
to the state of the metal in the starting materials and nature and chemistry of composting process (Wong and Selvam., 2005). Loss mechanisms of heavy metals during a composting process depend first of all on their solubility and mobility which implies the possibility of leaching processes occurring. The composting process however is generally marked by an increase in metal concentrations due to evident reduction in composting mass by decomposition.

The determination of total heavy metal content does not provide useful information about the risks of bioavailability, the capacity for remobilisation and the behaviour of the metals in the environment (Hsu and Lo, 2001). While, the chemical forms of a metal or speciation allows the estimation of heavy metal bioavailability and is related to the different natures of the metals, their bonding strength, either in free ionic form or complexed by organic matter, or incorporated in the mineral fraction of the sample. The treatment by composting leads to the development of microbial populations, which cause numerous physico-chemical changes within mixture. These changes could influence the metal distribution through release of heavy metals during organic matter mineralization or the metal solubilization by the decrease of pH, metal biosorption by the microbial biomass or metal complexation with the newly formed humic substances (HS) or other factors (Hsu and Lo, 2001).

Metal mobility and availability can be reduced by raising pH, since cationic ions are less available at high pH conditions. Co composting of solid waste with alkaline materials, such as bauxite residue, clay, and coal fly ash has been carried out with the purpose of reducing availability of heavy metals in compost (Fang and Wong., 1999). Lime is considered as one of the common amendment materials used in composting as it plays a role in heavy metal immobilization.
Assessment of the chemical speciation of heavy metals in composts enables one to evaluate their bioavailability and suitability for land application. Speciation of heavy metal involves the fractionation of its total content into exchangeable, acid extractable (carbonate bound), reducible (Fe-Mn oxides bound), oxidizable (organic bound) and residual forms. The exchangeable and acid extractable fractions are mobile fractions and easily available. The oxidizable and reducible forms will be leached out only under extreme conditions while then residual fraction is almost inert (Shrivastava and Banerjee, 1998).

4.7.8 Composting Process Parameters

As with any other biological process, composting is a complex interaction of many factors. Variations in those factors can improve or degrade effectiveness of the process. The following discussion indicates ideal conditions and the effect on the composting process when changes are made in the controlling parameters.

4.7.8.1 Temperature

It is widely accepted that temperature is an important environmental variable in determining the composting efficiency (Nakasaki et al., 1985). Not only is microbial metabolism highly temperature dependent, but also the population dynamics (e.g., composition and density) of microbes are dramatically influenced by temperature (Figure 4.7). Temperature increase within composting materials is a function of initial temperature, metabolic heat evolution and heat conservation. The achievement of minimum temperature levels is essential to an effective composting process (Finstein and Miller., 1985) and contributes substantially to the high rates of decomposition achieved during processing (De Bertodi et al., 1983). Indeed, temperatures of
composting material below 20°C have been demonstrated to significantly slow or even stop the composting process. Temperatures in excess of 60°C have also been shown to reduce the activity of the microbial community, and above this temperature microbial activity declines as the thermophilic optimum of microorganisms is surpassed (Miller, 1992). MacGregor et al. (1981) found that optimum composting temperatures, based on maximizing decomposition were in the range of 52-60 °C (Figure 4.8).

![Figure 4.7 Changes in Composting Temperature during Composting, (Source: Aparna, 2008)](image)

4.7.8.2 pH

The parameters such as pH, alkalinity and volatile acids are closely inter-related in the composting process. The pH which is easily determined should be monitored regularly. It is desirable to maintain the pH of the process between 6 and 7.5 (depending upon the composting material) which seems to be the optimum range. It is to be noted that during the composting process the
pH undergoes considerable change. In the beginning the formation of carbon dioxide and organic acids causes values of approximately 5-6, whereas as the process progresses the pH value reaches even up to 8-8.5. This is mainly due to the decomposition of nitrogenous compounds as well as elimination of the carbon dioxide. The pH has a marked effect on the microbial population. In the early stages of composting pH is slightly acidic because of the production of organic acids but later the pH increases because of decomposition of nitrogenous compounds that liberates ammonium. The end product has a neutral-alkaline pH (Bernal et al., 1998).

![Figure 4.8 Changes in Temperature and pH during Composting Process](Source: Aparna, 2008)

**4.7.8.3 Moisture Content**

Moisture content of the composting blend is an important environmental variable as it provides a medium for the transport of dissolved nutrients required for the metabolic and physiological activities of
microorganisms (Stentiford, 1987). Very low moisture content values would cause early dehydration during composting which will arrest the biological process thus giving physically stable but biologically unstable composts (Bertoldi et al., 1983). On the other hand, high moisture may produce anaerobic conditions from water logging which will prevent and halt the ongoing composting activities (Schulze, 1962; Tiquia et al., 1996). Many investigators have conducted experiments and identify that 50-60% moisture content is suitable for efficient composting (Tiquia et al., 2000)

4.7.8.4 Contaminated soil / Sediment loading

The economics of remediation composting system will be based on the amount of contaminated soil/sediment pushed through the system during the time period. The higher ratio of contaminated soil in a unit volume of compost, given an equivalent microbial activity the lower the overall cost of the system. This economic incentive must be balanced carefully, however with a possible decrease in compost effectiveness from lower temperature or microbial inhibition. Replacing organic sub states with mostly inert soil may lower compost temperature. Loading the system with excess of inhibitory contaminant will slow microbial activities and result in overall lengthening of composting time or may cause a total process upset. As a result the composting process may not be able to reach or may maintain a stable thermometric state for the desired reaction time due to the lack of organic sub state. In bench scale remediation composting studies experiments should test a range of contaminant concentration and soil loadings to provide some insight into the impact of contaminant concentration and soil loading on the compost mixture under consideration.
4.7.8.5  C/N Ratio

The C/N ratio represents a very good index for the biodegradation of organic substances and affects significantly the microbiological growth. The activities of the heterotrophic microorganisms involved in the process are dependent upon the nitrogen and carbon content. The microorganisms use carbon as the energy source, whereas the nitrogen is used for synthesis means for proteins. During the oxidation reactions that involve the release of carbon dioxide the major portion of the carbon (approximately 2/3) is used by the microorganisms as the energy source, while the remaining portion serves to form protoplasm cells, along with nitrogen, phosphorus, potassium and other micro-elements. Nitrogen in the form of ammonium ions is generally required as a major nutrient. Ammonia is produced from deamination of nitrogenous compounds, hydrolysis of urea and, probably also, from purines and pyrimidines. Considering that living organisms use on average, 30 carbon atoms for each nitrogen atom, it has been observed that the optimum C/N ratio should be in the range of 20-30. Excessive carbon presence slows the microbiological activities, whereas excessive nitrogen, allowing rapid decomposition, causes big nitrogen loss through volatilization. The mixing of lignocellulosic residues (C/N = 100-300) with sludge (C/N = 5-15) allows the re-equilibrium of the ratio and, thus, guarantees optimal conditions for the biological transformation process. The C/N ratio changes during the composting process. Higher C/N ratios require more time for completion of the maturation phase. From experience so far, it can be concluded that a good quality product, in general, has a C/N ratio of the order of 20-30. A C/N ratio higher than these values slows the decomposition of the organic material. A C/N ratio lower than the optimum leads to excessive nitrogen losses. An optimum carbon-to-nitrogen (C:N) ratio should be maintained as microorganisms require C for growth and N for protein synthesis. Low ratios
cause the loss of N as ammonia and give rise to odor problems, whereas higher ratios lengthen the composting process (Huang et al., 2004). Low ratios cause the loss of N as ammonia and give rise to odor problems, whereas higher ratios lengthen the composting process.

4.7.8.6 Particle size

A fairly small initial particle size aids rapid decomposition by providing greater surface area for microbial attack, however, if the particle size is too small, air circulation through the pile is inhibited, free air space in the system decreases and reduces oxygen diffusion. To control the compost feed material, a balance between diffusion transport and oxygen supply is necessary. The optimum rate for particle size is in the range 1.25-5 cm.

4.7.8.7 Aeration

Aeration is defined as the most important factor in composting systems. Aeration in general composting is important in two aspects: oxygen supply to aerobic microorganisms and cooling/drying of the compost by evaporation of water. For non-mechanical aeration systems, oxygen may be transported via molecular diffusion and free convection of air movement due to temperature gradients existed with in compost piles. Oxygen may be supplied by forcing air through the compost matrix or enhance the oxygen by mechanical mixing the compost intermittently to enhance the oxygen transfer. Aeration is also used to dry and subsequently cool the compost temperature through promoting evaporation of water. Approximately 10 to 30 times more aeration is required for drying of 20% solids then that for biological oxygen oxidization (Hogg 1993). The usual procedure in system design is the maintenance of aerobiosis in excess of that required for an efficient and nuisance-free compost process.
4.7.9 Types of Composting

The aerobic composting technologies are windrow (turned pile), aerated static pile and in-vessel (bioreactors), being the former two the most commonly used. The technologies vary in the method of air supply, temperature control, mixing/turning of the material, and the time required for composting. The corresponding capital and operating costs also vary considerably.

4.7.9.1 Composting in piles turned periodically (Windrows)

In this case pile is dimensioned in such a way that it is capable of minimizing the loss of heat (i.e. to maintain the temperature needed both for evaporation of water for the maximum degradation of organic matter, eventually present in the wastes) and its tendency to become more compact. Generally a pile has the dimension given below:
Height = 1-3 meters and Base = 3-6 meters.

The frequency of turning (used to mix the bulk material) depends upon the humidity, porosity and decomposition rate of the substance. The turning frequency rate is generally high in the beginning and is decreased gradually with the increase of stabilization. The turned pile system requires periodic turning of these organic materials to increase the oxygen supply to microorganisms and to homogenizes the materials and reduces particle size, at the same time as it redistributes, microorganisms, moisture and nutrients (Figure 4.9 and 4.10).
4.7.9.2 Composting in static but ventilated piles

In this system, oxygenation is guaranteed by passive or forced air circulation using a set of tubes (Figure 4.11 and 4.12). The tubes with suitable
holes and open end points (placed above the mature compost layer spreaded on the bottom) rise through the waste pile. As no turn over or other such operation is involved during the process, it is therefore very important that the bulk material be mixed well. The method is especially important for the treatment of industrial wastes with considerable olfactory effect or high concentration of nitrogen compounds (wastes from ichthyic industries, slaughtering, etc.).

Figure 4.11 A schematic diagram of the composting in static ventilated Pile (Source: Aparna)

4.7.9.3 Composting using bio-reactors

Stabilization of substrate using composting through reactors involves combinations of different techniques for material handling and forced air ventilation in the matrix. No doubt a wide variety of bioreactors are available but rotating cylinder bioreactors are in use on a wide scale. Such bioreactors with large cylinders (approximately 3 m in diameter and 35 m in length with daily capacity of 50 tonnes at a retention period of 3 days) laid down horizontally (slight inclination) and subjected to slow rotation
movement allow very good mixing and decomposition of the organic feedstock (Figure 4.13).

Figure 4.12 Static Aerated Composting Pile (Source: Aparna)

Figure 4.13 A Schematic Diagram of Composting Reactor (Source: Aparna)
4.7.10 Stability and Maturity Indices in Composting

In order to use the advantages of compost application, the hazard potential of contaminant mobilization has to be investigated since degradation and transformation reactions in the course of composting affect the organic matter quality. Much of research on composting has been focused on the changes in chemical and biological parameters as reliable indicators of compost stability and maturity. Compost stability and compost maturity are two-terms often used to describe the rate of decomposition and transformation of the organic matter (OM) in compost. Stability refers to the level of biological activity of the compost and is dependent on the degree of degradation achieved during the composting process. Maturity refers to the degree of biological, chemical and physical stability of the compost. Compost stability is strongly related to the rate of microbial activity in the compost. Under these conditions the temperature of the compost remains constant and equals that of the ambient. The quality of the organic matter is reflected by the degree of humification which depends on the composting process itself and on the organic matrix used (Adani et al., 2003).

The pollution potential of the composting process depends upon the rate of production and consumption of intermediate compounds formed during the decomposition of large organic molecules. The production of intermediate compounds is unavoidable and not easily controlled due to: (a) the heterogeneous nature of source-separated organics, (b) the degree of decomposition that has inevitably occurred prior to delivery to a centralized compost site and (c) the step-wise destruction of the macromolecules that naturally takes place at compost facilities. Many of the intermediate byproducts are toxic and odour is released into the environment prematurely. Due to the contamination risks associated with immature compost, the adoption of an accurate measure of compost maturity is therefore critical since
mature compost does not produce offensive odours and does not pose a risk to environment (Iannotti et al. 1993). Distinguished compost stability in terms of microbial activity (such as microbial respiration or energy release), while compost maturity was determined by plant growth, compositional content or sensory assessment (such as smell). They suggested that most existing tests can be separated between maturity and stability tests. Maturity tests involve plant growth, while stability tests deal with odour concentrations and respiration rates.

Chemical methods are related to the evolution of organic substrates (Bemal et al., 1998). Various parameters that have been used to assess the quality and maturity of composts include the C: N ratio of the finished product, water soluble carbon, caution exchange capacity, humus content, carbon dioxide evolution from the finished compost (WU and Ma, 2002), and spectroscopic measurements including UV, FTIR and NMR (Smidt et al., 2002; Hsu and Lo, 1999). Germination index which is a measure of phytotoxicity has been considered as a reliable indirect quantification of compost maturity (Cunha Qeda et al., 2002; Benito et al., 2003). Ambiguities and uncertainties surrounding compost stability maturity are in part due to a number of factors that include: (a) the variety of compost systems utilizing mixed microbial populations, (b) the heterogeneous and often unpredictable nature of organic substrates and (c) the variety of applications, potential uses and product compositions.

Stability relates to the rate of decomposition of the OM, as expressed by the biological activity of the compost. This is usually assessed by different measures of the respiration rate or by the self-heating of the compost in standard conditions (Adani et al., 2003). Usually maturity has been evaluated based on chemical parameters. Since the use of the term aims to
relate to compost quality, they believe that it should also reflect optimal utilization potentials, namely, be correlated with plant response. This stability can be used as an indicator of compost maturity in addition to other tests proposed to control compost maturity (Huang et al., 2001).

**Table 4.4 Various Stability and Maturity Parameters in Composting**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>General method</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Chemical analysis</td>
<td>Carbon/Nitrogen ratio, water soluble ions, water soluble organic matter,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>cation exchange capacity, crude fibre analysis.</td>
</tr>
<tr>
<td>2</td>
<td>Physical analysis</td>
<td>Temperature, colour, particle size, water and air content</td>
</tr>
<tr>
<td>3</td>
<td>Microbial assays</td>
<td>Indicator microorganisms, respiration rate, microbial activity and biomass</td>
</tr>
<tr>
<td>4</td>
<td>Plant bioassays</td>
<td>Cress seed germination test in water extracts, rye grass growth in compost</td>
</tr>
<tr>
<td></td>
<td></td>
<td>containing mixtures, seeding development in compost and water extracts</td>
</tr>
<tr>
<td>5</td>
<td>Spectroscopy</td>
<td>Solid state CPMAS, NMR, Infrared-FTIR-DRIFT</td>
</tr>
<tr>
<td>6</td>
<td>Degree of humification</td>
<td>Total substances, functional groups content and ratios of humic, fulvic</td>
</tr>
<tr>
<td></td>
<td></td>
<td>and non humic fractions</td>
</tr>
</tbody>
</table>

Most studies on compost characterization focus on the quantitative variation in the organic and inorganic constituents. The chemical nature of compost OM changes throughout the composting process and can be a useful indication of compost stabilization (Chen, 2003). Therefore compost maturity and stability are key factors during application of composting process. For achieving compost maturity, environmental factors such as temperature,
aeration, moisture and nutrients should be appropriately controlled (Epstein, 1997). C/N is among one of the important factors affecting compost quality.

4.7.10.1 Self heat

Perhaps the least expensive and simplest technique to assess compost maturity is in terms of the self-heating test, which can serve as a direct indication of microbial activity and an indirect indication of remaining readily available organic matter. Compost is placed in an insulated flask and the temperature is monitored over time. A rise in compost temperature to a pre-determined maximum above ambient conditions constitutes immature compost.

4.7.10.2 C:N Ratio

The change in the ratio of the two predominant elements (carbon and nitrogen) within the composting matter has also been considered in terms of stability for as the readily available organic matter. It is oxidized and released as carbon dioxide. There is a general reduction in carbon content over time. Inbar et al (1990) traced the C:N ratio of the solid phase, typically beginning at 40:1 before dropping quickly at first then slowing to finish at about 18:1. Additional thresholds of compost stability include C:N ratios of less than 20 (Saviozzi et al. 1987; Jimenez and Garcia, 1989).

4.7.10.3 Nitrification parameters

Compost maturity can also be defined in terms of nitrification. The ratio between the inorganic forms of nitrogen has been used as criterion for assessing the maturity. When the NH$_4$ concentration decreases and NO$_3$ appear in the composting material it is considered ready to be used as compost (Finstein and Miller, 1985). The ratio of NH$_4$-N to NO$_3$-N has been suggested
as an index of maturity. However the final value of NO₃ reached depends on
the source material and no particular level of NO₃ or its ratio to NH₄ can be
relied upon as an indicator of compost biomaturity (Bernal et al., 1998).

4.7.10.4  Respiration rate

Microbial respiration is considered as a reliable index of microbial
activity (Nannipieri et al., 1990), as well as a good indicator of the processes
of organic matter mineralization, the evolution of the composting process and
the maturity of the final compost (Tiquia et al., 1996). Jorgenson et al (2000)
defines microbial respiration as the consumption of oxygen and the release of
CO₂ by bacteria, fungi, algae and protozoas, including gas interchange by the
metabolism of both aerobic and anaerobic microorganisms. Aspects of
respiration expressed in terms of carbon dioxide production rate or oxygen
consumption rate provides the most accurate assessment of microbial activity
and compost stability. Respiration is now considered as the most suitable
method of determining the stability of organic matrices (U.S. Composting
Council, 1997). Insufficiently mature compost has a strong demand for O₂ and
high CO₂ production rates due to intense development of microorganisms as a
consequence of the abundance of easily biodegradable compounds in the raw
material. For this reason, O₂ consumption or CO₂ production are indicative of
compost stability and maturity (Jorgenson et al., 2000).

4.7.10.5  Biodegradable volatile solids (BVS)

Not all biodegradable (organic) matter is immediately available for
consumption by the composting microbes; therefore measuring the change in
the biodegradable portion of the waste provides an indication of compost
stability. Since the compost process involves the slow transformation of
readily available organic (or volatile) matter to a less digestible, more stable
form, the progress of the compost process can be measured in terms of the reduction in BVS over time in both the solid compost matrix (in terms of volatile solids) and the extract produced from the composting material (in terms of soluble volatile solids).

4.7.10.6 **Humification parameters**

Humic substances are defined as a category of naturally occurring, biogenic, heterogeneous organic substances that can generally be characterized as being yellow to black in colour, of high molecular weight and refractory. They represent a heterogeneous mixture of molecules which in any given soil, sediment, or compost may range in molecular weight from as low as 2000 to over 300,000. Humic substances are operationally divided into humic acids (HA), fulvic acids (FA), and non-humic fraction (NHF) according to their solubility in acid and alkali (Ouatmane et al., 2000). As the compost process is ultimately one of stabilization that incorporates the production of humus the degree of organic reconstitution or humification is frequently considered as the most appropriate indicator of compost stability (Ciavatta et al., 1993).

Humification is the bio-chemical process promoting the chemical re-synthesis and repolymerization of moieties produced from the decomposition of fresh organic material producing aromatic and aliphatic macromolecules. Chen et al (1996) evaluated the stability of a number of composts in terms of humic substances comprised of fulvic acid (FA), humic acid (HA), and the non-humified fraction. In general, there was an increase in humic acid as a percentage of organic matter in the humification ratio. The humic acid concentration increases and depends upon the nature of the substrate. Among the indices of maturity proposed, those most representative of the evolution of the maturity of the compost were presented in particular those indices measuring the evolution of humic acids (HA) compared to fulvic fraction (FF) or fulvic acids (FA). The following parameters for example can be cited,
where the $C_{HA}$, $C_{HS}$, $C_{FF}$, $C_{FA}$ and $C$ are respectively the dissolved organic carbon in the humic acids, in the humic substances, in the fulvic fraction, in the fulvic acids and the total organic carbon in the solid sample of compost.

Jimenez and Garcla (1992) reviewed most of the indices of maturity found in the literature and compared the results using principal component analysis. They found that the $C_{HA}/C_{FA}$ ratio was the best indicator of maturity and humification. The ratio of optical densities of humic acids and fulvic acids at 465 and 665 nm respectively ($E_{465}/E_{665}$) has been considered to reflect the degree of condensation of aromatic nucleus of humus indicating its maturity (Schnitzer et al., 1993).

Table 4.5 Various Humification Indices indicating Stability and Maturity

<table>
<thead>
<tr>
<th>The ratio of humification</th>
<th>$C_{HA}/C_{FF}$</th>
<th>Inbar et al (1990)</th>
</tr>
</thead>
<tbody>
<tr>
<td>The rate of extraction (TE)</td>
<td>$C_{HS}/C$</td>
<td>Morel et al (1979)</td>
</tr>
<tr>
<td>The humic acid/Fulvic acid ratio</td>
<td>$C_{HA}/C_{FA}$</td>
<td>Sugahara and Inoko (1981)</td>
</tr>
<tr>
<td>The Index of Polymerization</td>
<td>$C_{FF}/C_{HS}$</td>
<td>Morel et al (1979)</td>
</tr>
</tbody>
</table>

4.7.10.7 Cation exchange capacity

One of the variables that are frequently determined to estimate the degree of transformation reached by compost during the process of composting is cation exchange capacity (CEC). The humification process produces functional groups influenced by the increased oxidation of organic matter, which leads to rise in cation exchange capacity (CEC), for which it has been used to evaluate maturity.
4.7.10.8 Water soluble carbon

Organic carbon in water extract of immature compost consists of phenolic compounds, sugars, hemicellulose, organic and amino acids, peptides and degradable substances (Chen and Inbar 1993). In mature compost soluble organic carbon is present as humic substances, which are resistant to further degradation thus explaining the increased stability during composting. The water soluble organic C to organic N ratio (sol org C / org N) has been suggested as a maturity index by several research groups (Bernal et al., 1998).

4.7.10.9 Enzymatic Assays

Microbiota play a key role in the composting process, determining most of the changes that take place as well as the quality of the product and the rate of the process. Microbes in the compost pile produce: hydrolytic extracellular enzymes to depolymerize the larger compounds to smaller fragments that are water-soluble and assimilated by microorganisms in the compost. Characterizing and quantifying the enzymatic activity during composting can reflect the dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformations and provide information about the maturity of the resulting compost (Ros et al., 2006). During composting, the starting material is modified by decomposition and humification through a wide variety of biological and biochemical processes. Enzymes play a key role in these transformations. The mineralization of organic N, which involves the release of N from nonpeptide C-N bonds in amino acids and urea, is mediated by enzymes such as amidohydrolases and dehydrogenases. Alkaline and acid phosphatases are important enzymes in organic P mineralization and plant nutrition.
4.7.10.10 Germination index

The degree of maturity can also be revealed by biological methods involving seed germination and root length (Zucconi et al., 1981), since immature composts may contain phytotoxic substances such as phenolic acids and volatile fatty acids. Although a number of tests exist to determine compost stability, few tests truly distinguish the product quality. In addition to providing results in a relatively quick manner, plant growth bioassays such as the rye grass test and phytotoxicity bioassays are commonly used. Zucconi et al (1981) assessed the phytotoxicity of composts in terms of a cress germination bioassay using Lepidium sativum. Compost extracts at various stages of decomposition were incubated for 24 hours at 27°C with cress seeds. A germination index in terms of germination occurrence (high toxicity) and root growth (lower toxicity) was used to compare results.

4.7.10.11 Spectroscopic parameters

 Matured compost consists of stable organic matter, water, minerals, and ash. Consequently the composting process directly involves the changes of the chemical composition and physico-chemical parameters of the compost mixture, and can be controlled by spectral methods. Fourier transform infrared (FT-IR) spectroscopy being a modern nondestructive analytical method more and more often is used for the structure elucidation and quantification of a large variety of organic, inorganic, and biological samples. Two main advantages of IR spectroscopy are: i) the analyzed samples are not subjected to any chemical treatment thus avoiding secondary reactions; ii) all the compounds present in the sample are measured simultaneously, thereby simplifying and speeding up the analysis. Fourier transform infrared
spectroscopy provides information about the chemistry of the waste materials in a general way. Several indicator bands that are referred to functional groups represent components or metabolic products. The metabolic products can be identified by their absorption bands in the infrared spectrum. The infrared spectrum provides fast qualitative assessment of the waste material.

Today FT-IR spectroscopy is more frequently applied in environmental analyses including soil organic matter (Grube et al., 2005), humic and process control fulvic acids (Quatmane et al., 2002), organic wastes and their compost (Smidt et al., 2002). Considering that the IR spectra reflect the chemical composition, preliminary studies of a particular composting mixture are necessary to select the criteria indicating the decomposition processes of the organic matter. For practical applications, spectra representing progressive stages of composting process can serve as reference for process control.

4.8 BIODEGRADATION OF ORGANIC COMPOUNDS

Biodegradation can be defined as the biologically catalyzed reduction in complexity of chemical compounds. In case of organic compounds, biodegradation frequently although not necessarily leads to the conversion of much of the C, N, P, S, and other elements in original compound to inorganic products. Such a conversion of organic substrate to inorganic products is known as mineralization. Thus, in the mineralization of organic C, N, P, S or other elements, CO$_2$ or inorganic forms of N, P, S or other elements are released by the organism and processes that destroy numerous organic molecules of living organisms but the mineralization of synthetic chemicals by biological, of converting synthetic chemicals to inorganic products. Few non-biologicals are converting synthetic chemicals to inorganic products. Few non-biological reactions in nature bring about comparable changes, it is because of
their ability to mineralize anthropogenic compounds that microorganisms play a large role in soils, waters, and sediments.

Many synthetic molecules discharged into these environments are directly toxic or become hazardous following biomagnifications. Because mineralization to result in the total destruction of the parent compound and its conversion to inorganic products, such processes are beneficial. In contrast, non-biological and many biological processes although degrading organic compounds, convert them to other organic products. Some of these products are toxic but others evoke no untoward response. Nevertheless, the accumulation in nature of an organic product is still cause for some concern in as much as a material not currently known to be harmful, may with new techniques or measurements of new toxicological manifestations reveal it to be undesirable.

4.8.1 Microorganisms and Their Biodegradation

Microorganisms carry out biodegradation in many different types of environments of particular relevance for pollutants or potential pollutants are sewage treatment systems, soils, and underground sites for the disposal of chemical wastes, ground water, surface waters, oceans, sediments, and estuaries. Microbial processes in the various kinds of aerobic and anaerobic systems for treating industrial, agricultural, and municipal wastes are extremely important because these treatment systems represent the first point of the discharge of the many chemicals into the environments of importance to humans or other living organisms. Microbial processes have long been known to be important in sewage and wastewater for the destruction of a large number of synthetic compounds. Land spreading of industrial wastes, accidental spills, or sludge disposal, and the degradation of natural materials in soils was recognized even in prehistoric times. In this centaury the disposable
of industrial wastes on or below the surface of the land became widespread before the evidence of surface water pollution became prominent, but the water bodies adjacent to points of chemical disposal contain microbial communities that, are directly affected by the toxicity of the wastes are acclimatized to many of organic compounds. Contaminated dam adjacent to industrial activities and waste disposal sites receive deliberate discharge of chemicals. A variety of pollutants discharged into the dam are retained by the sediments. These sediments contain highly diverse often very active communities of bacteria, fungi and protozoa that directly or indirectly metabolize a multitude of synthetic chemicals.

4.8.2 Conditions for Biodegradation

Several conditions must be satisfied for biodegradation to take place in an environment. These include the following: (a) an organism that has the necessary enzymes to bring about biodegradation must exist. The mere existence of an organism with the appropriate catabolic potential is necessary but not sufficient for biodegradation to occur. (b) Organism must be present in the environment containing the chemical. Although some microorganisms are present in essentially every environment near the earth surface, particular environment may not contain an organism with the necessary enzymes, (c) the chemical must be accessible to the organism having the requisite enzymes. Many chemicals persist even in environments containing the biodegrading species simply because the organism does not have access to the compound that it would otherwise metabolize. Inaccessibility may result from the substrates being in different micro environment from the organism, in a solvent not miscible with water or sorbed to solid surfaces. (d) If the initial enzyme bringing about the degradation is extra cellular, the bonds acted upon by that enzymes must be exposed for the catalyst to function. This is not always the case because of sorption of many organic molecules. (e) should the
enzymes catalyzing the initial degradation be intracellular that molecule must penetrate the surface of the cell to the internal sites where the enzymes acts. Alternatively, the products of an extra cellular reaction must penetrate the cell for the transformation to proceed further. (f) Because the population or biomass of bacteria or fungi acting on many synthetic compounds is initially small, conditions in the environment must be conducive to because microorganisms are frequently are the major and occasionally the sole means for degradation of particular compounds, the absence of a microorganism from particular environment, or its inability to function, often means that the compound disappears very slowly.

4.8.3 Biological Degradation of Environmental Pollutants

The microbial world is characterized by an incredible metabolic and physiological versatility that permits microorganisms to inhibit hostile ecological niches and to exploit compounds as carbon and energy sources, which are unpalatable for higher organisms. This metabolic versatility has led to the notion of microbial infallibility - their ability to degrade and grow at the expense of any organic material and is also the basis of the recycling of recalcitrant organic compounds in the biosphere. The degradation of naturally occurring organic compounds by microorganisms insitu is of critical importance for successful functioning of the biosphere. By enzymatically attacking high molecular weight biomass (such as plant materials) in terrestrial and aquatic ecosystem, microorganisms complete the cycling of carbon begun by photosynthesis.

During the last ten years the increasing knowledge of microorganisms existing at great depth in the soil and their ability to degrade a variety of synthetic organic compounds (Kanaly et al., 2000; Kasai et al., 2001) to inorganic products has contributed to the development of optimal
insitu bioremediation strategies. Efforts have been made to characterize bacterial and their responses to pollutants (Colores et al., 2000) to isolate potential degraders (Cerniglia, 1992) and to identify functional genes involved in particular degradation processes (Laurie Lloyd-Jones et al., 1999). The ability of microbes to degrade toxic chemicals in terrestrial and aquatic environments depends on several factors like pH (Zaidi and Imam, 1999), salinity (Barthoolomew and Pfaender, 1983), temperature (Bauer and Capone, 1985), oxygen availability and nutrients (Zaidi and Imam, 1999).

For the development of treatment protocol for the biodegradation and detoxification of hazardous organics the efficiency of microorganisms in a reactor or the insitu operation procedures have to be optimized. When the compounds are toxic or recalcitrant efficient microorganism may be unavailable or not isolated readily. It is necessary to develop enrichment procedure to isolate a new strain with the desired catabolic potential or to construct strains in laboratory. In the enrichment culture technique the contaminated soil is used as inocula in a culture medium wherein for a particular set of conditions a specific kind of microorganism will predominate because of its typical ability to grow more rapidly than the others. To obtain organisms with degradation capacity, a chemical to be degraded is supplied as a growth limiting source and essential nutrient in a culture medium. Compounds with pollutant potential are used as the limiting carbon source. Sometimes nitrogen, phosphorous or sulphur is used as the growth limiting carbon source for the degradation of nitro aromatics and triazines.

Bioremediation is a strategy by which degradation of contaminants can be enhanced either by stimulation of degradative processes in native microflora or by the addition of pollutant degrading microorganisms (Semprini et al., 1990). There are three principally different routes that result in bacteria (or
other microbes) capable of degradation of a certain compound or group of compounds at a certain site (Romantschuk et al., 2000).

i. The indigenous micro flora has been exposed to the organic contaminant long enough for genetic evolution to create a capacity to degrade the contaminant. This type of evolution takes place constantly but is relatively slow. As a consequence the microbial community possesses the degradative pathways, but may be inefficient because of low cell number or low activity level.

ii. The indigenous microbial flora which is adapted to the local conditions is exposed to a contaminating organic compound. The bacteria acquire genes and degradative pathways from bacterial cells immigrating from elsewhere (Mc.Gowan et al., 1998). Transfer of genetic material can take place through conjugation, transduction or transformation.

iii. The above evolution is also relatively slow but can be enhanced by supplying gene clusters. If no natural gene clusters are available, these may be constructed. Laboratory strains can be used as donors, either to transfer the capacity to ‘wild type’ strains newly isolated from the site or by introducing the donors into the site and letting gene to transfer occur.

Biological treatment using immobilized microbial consortia has been studied by several researchers (Gary Miller et al., 1990) where results showed a good removal efficiency of hazardous organics by the large immobilized bacterial population.

Among the microorganisms bacteria and fungi are especially capable of producing a wide variety of enzymes that can degrade organic compounds and mineralize the substances (Claxton and Houx, 1995). Bacteria
breakdown complex organic compounds through a series of coupled reaction termed catabolism. For respiratory bacteria the central pathways involved in metabolism are glycolysis, Krebs cycle and electron transport system. The overall generalized reaction is

$$\text{CH}_2\text{O} \text{ (complex carbon)} + \text{O}_2 \rightarrow \text{CO}_2 + \text{H}_2\text{O} + \text{Energy}$$  \hspace{1cm} (4.3)

This equation represents the complete conversion of the complex carbon compound (substrate) and water. Energy attained in the form of ATP is used for a variety of cellular activities including the synthesis of new cell components and motility. Microbial degradation of complex organic compounds does not always result in mineralization. Incomplete degradation (transformation) of the compound may occur as a result of microbial activity. Complex mixtures of products also may be produced during the biodegradation of even single compounds. The breakdown products are dependent upon both the microorganisms present and the environmental circumstances (Schenk, 1990). However the particular substance will not be degraded if the relevant microbial strains are absent or fail to grow during the treatment (Alexander, 1995).

Studies of the microbial ecology (community structure, diversity, numbers and activity) of soil and subsurface habitats have largely focused on aerobic microorganisms (Ehrlich et al., 1983; Ghirose and Balkwill, 1984; Federle et al., 1986). Many pure cultures of bacterial strains capable of degrading pollutants have been isolated from these habitats and found to contain an active aerobic micro flora. There have been only a few examples of pure cultures of anaerobic bacteria, which degrade recalcitrant compounds (Quensen et al., 1988). Degradation of other aromatic compounds also has been proved by many authors (Diaz et al., 2001; Hideaki et al., 2001). The structure of a chemical substance highly affects its biodegradability.
Substituents at the benzene ring like methyl groups, halogens, NO$_2$, NH$_2$, SO$_3$H and CN groups have a slowing effect on the degradation compared to non-substituted benzene rings and mono-substituted benzenes are more rapidly degraded than di or tri substituted benzenes (Goulding et al., 1988). Also the position-of the substituent at the ring has an impact on the degradation. Because bacteria degrade aromatic compounds either through ortho or meta cleavage pathways, the effect of the position of the substituent may not be the same in all localities. Generally, highly branched hydrocarbons are degraded much more slowly than unbranched hydrocarbons (Morgan and Watkinson, 2000).

4.8.4 Degradation of Aromatic Compounds

A large variety of aromatic substances participates in the life processes and forms an important part of the natural carbon cycle. In nature aromatic compounds are derivatives of the secondary metabolism of plants, biological and chemical cleavage of lignin and bioconversion of aromatic amino acids. Furthermore many aromatic compounds are produced by the chemical industry for utilization as pesticides insecticides, herbicides, detergents, solvents, wood preservatives etc. Some xenobiotic benzoid structures are relatively recalcitrant requiring the competence of the microbial world for their dissimilation. In nature, many organic compounds are substituted with either halogens or nitro groups.

In nature, many hydrocarbons are substituted with either halogens or nitro groups. It is estimated that about 15,000 chlorinated compounds are currently being used in various industries worldwide. Consequently there is a constant increase in the dissemination of these materials into the environment. It is known that the presence of these substituents on aromatic rings hinder biodegradation. This is because these groups can deactivate the aromatic
nucleus to electrophillic attack by oxygenase or other enzymes by withdrawing electrons from the ring (Steiert and Cardford, 1985). This is deactivating effect increases with the number of halogen or nitro substituents resulting in their greater persistence in the environment.

4.8.4.1 Degradation of chloro aromatic compounds

A surprisingly large number of halogenated organic compounds are produced in nature. As a result there is correspondingly large number of microorganisms able to degrade halogen substituted compounds that bear some similarity to natural substances. Despite the deactivating effects of chlorine substitution a few aerobic microorganisms can degrade chlorinated phenols. Generally with lower chlorinated aromatics (1 or 2 chlorines) microorganisms may open the ring with dioxygenases before removal of chlorines. The reactions are very similar to those acting on non-chlorinated substrates. Often the same enzyme which hydroxylates a non-chlorinated aromatic ring may also hydroxylate a ring containing one chlorine at the side of the attack. The subsequent removal of chlorine is believed to be spontaneous. Highly chlorinated aromatics have to undergo stepwise dechlorination prior to ring fission. Often with highly chlorinated compounds dechlorination may be the rate limiting step in their biodegradation.

Dechlorination can be carried out by both aerobic and anaerobic microorganisms. Susceptibility to degradation for all the halogenated aromatic hydrocarbons depends on the nature of the halogen substitution, the number of substituents, and the position of the substituents. Halogenated aromatic hydrocarbon compounds containing bromine or iodine substitutions are more susceptible to degradation than those containing chlorine substitutions, which in turn are more susceptible than molecules with fluorine substitutions. Susceptibility to degradation generally decreases as the number of
substitutions increases, however even highly chlorinated compounds such as pentachlorophenol has been shown to be susceptible to aerobic degradation. Finally since the dioxygenase enzymes involved in ring cleavage require the presence of two adjacent hydroxyl group-carrying carbon atoms, the stereochemistry of the compound will play a significant role in determining its susceptibility to degradation. Any substitution which blocks potential sites for ring cleavage will decrease the compounds susceptibility to cleavage. Table 4.6 summarizes the most environmentally important groups of halogenated aromatic compounds.

**Table 4.6 Halogenated Aromatic Compounds**

<table>
<thead>
<tr>
<th>S.No.</th>
<th>Class</th>
<th>Examples</th>
<th>Sources</th>
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</thead>
<tbody>
<tr>
<td>1</td>
<td>Chlorobenzoic acids</td>
<td>2,3,6 – Trichlorobenzoic acid</td>
<td>Degradative products of polychlorinated triphenyls, herbicides, plant growth regulators</td>
</tr>
<tr>
<td>2</td>
<td>Chlorobenzenes</td>
<td>Chlorobenzene, Pentachloronitrobenzene</td>
<td>Industrial solvents, diluents for PCBs, paint solvents, by-products of textile dyeing, fungicides</td>
</tr>
<tr>
<td>3</td>
<td>Chlorophenols</td>
<td>2,3,4,6 – Tetrachlorophenol, Pentachlorophenol</td>
<td>Antifungal agents, wood preservatives, degradation products of chlorophenoxy herbicides</td>
</tr>
<tr>
<td>4</td>
<td>Chlorophenoxy and Chlorophenyl herbicides</td>
<td>2,4-D; 2,4,5-T</td>
<td>Herbicides</td>
</tr>
<tr>
<td>5</td>
<td>Phenylamide herbicide</td>
<td>Phenyl Urea</td>
<td>Herbicides</td>
</tr>
<tr>
<td>6</td>
<td>Chlorinated biphenyls</td>
<td>PCBs, Arocolors</td>
<td>Dielectric fluids in capacitors and transformers, gas turbines and hydraulic systems, fire retardants, plasticizers.</td>
</tr>
<tr>
<td>7</td>
<td>Chlorinated dioxins and furans</td>
<td>2,3,78 Tetrochlorodibenzop-dioxin (TCDD)</td>
<td>By-products during the manufacturing of chemicals, pyrolysis of chlorophenol salts, heat exchange fluids and hydraulic fluids, pyrolysis of PCBs and polychlorinated diphenyl ethers.</td>
</tr>
</tbody>
</table>
Among the other chlorocompounds, chlorophenols are another class of toxic compounds that are included in the USEPA list of priority pollutants. They are used in industry primarily as biocides as well as preservatives for wood, glue, paint, vegetable fibres and leather. Chlorophenols may also be formed in the chlorination of surface waters and sludges, sediments, groundwater (due to leaching from contaminated soils), surface water (due to surface runoff or direct industrial waste discharges) and rainfall. Chlorinated phenols are of growing concern as environmental pollutants. The toxicity of chlorinated phenols tends to increase with their degree of chlorination and because few microorganisms can decompose them, the more highly chlorinated phenols tend to accumulate in the environment. The biodegradation of chlorophenols has been studied in both aerobic and anaerobic systems. Under anaerobic conditions chlorine substituents can be removed from the aromatic ring by reductive dechlorination. In this process chlorines are replaced by hydrogen resulting in less toxic and less recalcitrant compounds. Anaerobic processes are reported to be suitable for the dechlorination of low to highly chlorinated phenolic compounds while aerobic systems have a tendency to be more suitable for biodegrading the less halogenated phenolic compounds.

4.8.4.2 Degradation of nitro aromatic compounds

Several nitro aromatics and their derivatives are being used in increasing amounts in a number of industrial operations such as the manufacture of chemical solvents, pesticide production, dyestuffs, drugs, photographic chemicals, varnishes, vulcanizaiton accelerators, antioxidants and other petroleum based industries. Consequently there is constant increase in the dissemination of these materials into the environment. For example purposeful application of pesticides onto fields for pest control, accidental spillage of chemicals during any phase of their use, transport seepage of
chemicals from disposal sites, discharge of chemicals from municipal waste treatment systems and into natural waters are some of the human activities that pollute the environment with these toxic chemicals. Biological degradation is one of the primary routes by which nitro aromatic compounds are broken down in the environment.

Aerobic microorganisms use diverse biochemical reactions to initiate the degradation of nitro aromatic compounds. Reactions that attack the nitro substituents can be grouped into two general categories: either oxidative or reductive (Rieger and Knackmuss, 1995). With mono or dinitro substituted aromatic compounds, the preferred route for their initial degradation is hydroxylation carried out by mono or deoxygenases. These reactions normally result in replacement of the nitro group by an -OH group, with nitrite release. When the number of nitro substituents on the aromatic ring is greater than two, the predominant initial reactions become reductive. These reactions reduce the nitro substituent first to nitroso and then hydroxylamine followed by an amino derivative prior to further processing with the release of ammonium ion. In some strains the aromatic ring rather than the nitro group may be reduced first to generate a hydride-meisenheimer complex. On protonation and rearomatization the nitro group is replaced by a proton and a nitrite is released.

4.9 PRESumptive evidence for bioremediation

No one piece of evidence can unambiguously prove that microbial processes are responsible for an observed decrease in contaminant concentration. Typically, a “weight of evidence” argument must be formulated based on several lines of evidence. The kinds of data consistent with bioremediation include:

i. Accumulation of intermediates formed during the metabolism of contaminants.
ii. An elevated concentration of metabolic by-products formed through microbial activity.

iii. An increased ratio of compounds that are difficult to degrade relative to those that are readily degraded.

iv. Metabolism of contaminants at reasonable rates in laboratory microcosms that contain soils, sediments or water from the site.

v. Depletion of oxygen or nutrients required for microbial growth.

vi. More rapid depletion of added oxygen in the contaminated areas as compared to unimpacted areas.

vii. Decreased contaminant levels following addition of oxygen or nutrients.

viii. An increase in the number of protozoans suggesting there has been an increase in the number of bacteria that they feed on.

ix. Changes in the isotope ratio of inorganic carbon consistent with the conversion of contaminant to carbon dioxide, carbonate, and related compounds.

x. Documentation of the amount of contaminant removed by nonbiological processes using conservative tracers.

xi. Demonstrated conversion of contaminants to metabolites using isotopically labeled contaminants.