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## Biodegradation: Process Mechanism

S.B. ESKANDER<sup>1</sup> AND H.M. SALEH<sup>1\*</sup>

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### ABSTRACT

*One of the major environmental problems today is contaminations resulting from various human activities e.g., petroleum industry, petrochemical sector, agriculture sector, nuclear technology. The release of wastes generated due to those activities are of a particular concern in the environment. Most of these contaminants or may be all are hydrocarbons in nature. Hydrocarbon components have been known to belong to the family of carcinogenic and neurotoxic organic pollutants. In addition for the radioactive wastes; the hazard of harmful irradiation can be added. Currently accepted disposal methods after treatment of these wastes or direct burial insecure landfill can become prohibitively expensive. Mechanical and chemical conventional methods generally used for treating these contaminants have limited effectiveness and also cost. Biotechnology has a potential to play an immense role in the development of treatment processes for hazardous wastes. The bioprocesses for treating of the hazardous contaminants is a promising technology, since it is cost effective and can lead to complete removal of hazard and their mineralization. Biodegradation is nature's way of recycling wastes, or breaking down organic matter into nutrients that can be used by other organisms. By harnessing these natural forces of biodegradation, some types of environmental contaminants can be reduced and completely removed. This article presents an overview for biodegradation process of some hazardous wastes e.g. oil, plastics, pesticide and radioactive.*

**Key words:** Biodegradation, Aerobic, Anaerobic, Biotransformation, Oil spills, *Phormidium foveolarum*, *Achnanthes minutissima*, Cyanobacteria, Plastic wastes, Pesticide, Actinomycete, Radioactive wastes, *Desulfovibrio* spp., *Pleurotus pulmonarius*, Blue green algae, Fukushima.

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## INTRODUCTION

RCRA defines a hazardous waste as a solid waste (including liquids and gases) which may:

- Cause or significantly contribute to an increase in serious illness and/or in mortality; or
- Pose a substantial hazard to human health or the environment when improperly managed. By definition, wastes are hazardous if they are
  1. “Listed” (specifically named) or
  2. If they exhibit any of four hazardous waste characteristics (ignitability, corrosivity, reactivity, and toxicity).

Mixtures of a solid waste and a “listed” hazardous waste are also considered hazardous<sup>[1]</sup>.

Reactive wastes are chemically unstable and react violently with air or water. Ignitable wastes burn at relatively low temperature and may cause immediate fire hazard. Corrosive wastes include strong acidic or alkaline substances that can destroy material and living tissue upon contact, by chemical reaction. Infection wastes include used bandages hypodermic needles and other materials from hospitals or biological research facilities.

Radioactive waste comes from a number of sources. The majority of those wastes originate from the nuclear fuel cycle and nuclear weapons reprocessing. The other sources include medical, research, agricultural and industrial wastes, as well as naturally occurring radioactive materials (NORM) that can be concentrated as a result of the processing or consumption of coal, oil and gas, and some minerals, in addition to nuclear and radiation accidents and incidents. Some radioactive wastes can persist in the environment for many thousands of years before fully decaying<sup>[2]</sup>.

The whole world must be charged with protecting the Global’s land, air, and water resources. Under a mandate of international environmental laws, all nations strives to formulate and implement actions leading to a compatible balance between human activities and the ability of natural systems to support and nurture life. To meet this mandate, research programs should providing data and technical support for solving environmental problems today and building a science knowledge base necessary to manage our ecological resources wisely, understand how pollutants affect our health, and prevent or reduce environmental risks in the future.

The principle worldwide problem is waste disposal, especially; with billions of tones of these hazardous waste generated every year. Landfill used to be one of the main routes for disposal, but landfill capacity is now diminishing. Many new methods for reducing solid wastes have been studied. In several

cases, storage has been compromised, leading to contamination of trillions of gallons of groundwater and millions of cubic metres of contaminated soil and debris. The costs of cleaning up these sites are estimated to be in excess of a trillion dollars in the US.

Degradation refers to the gradual breakdown of components of a material as a result of external influences such as heat, light, mechanical force, chemical reagent, microorganisms, etc. According to the preconditions or mechanism of degradation, it can be divided into several types: biodegradation, photo-degradation, oxidative degradation and water-degradation.

## **BIODEGRADATION**

A very broad sense, in nature, there is no waste because almost everything gets recycled. In the microbiological sense, “biodegradation” means the decaying of all organic materials that is carried out by life forms comprising mainly bacteria, fungi, protozoa and other organisms. Through this, biologically natural process, hazardous toxic contaminants are transformed into less toxic or non-toxic substances. The secondary metabolites, intermediary molecules or any degradation products from one organism can become the nutrient for others, substantiate carbon source and energy and they are further working-on breaking down the remain organic matter. By harnessing microbial communities, the natural “forces” of biodegradation, reduction of wastes and clean-up of most of environmental contaminants can be achieved. Depending on the nature of the micro-organisms, they may eat a part of the organic molecule only, destroying the intact parent substance in a process known as “primary biodegradation”, or they may eat it completely in a process known as “ultimate biodegradation”. The energy-producing part of the metabolic activity consumes oxygen, resulting in the immediate formation of carbon dioxide, water and mineral salts in a process known as “mineralisation”.

## **Definitions**

### ***Biodegradation***

“Transformation of a substance into new compounds through biochemical reactions or the actions of microorganisms such as bacteria”<sup>[3]</sup>.

### ***Biodegradation***

A process by which microbial organisms transform or alter (through metabolic or enzymatic action) the structure of chemicals introduced into the environment”<sup>[4]</sup>.

**Biodegradation**

Breakdown of a substance catalyzed by enzymes *in vitro* or *in vivo*. This may be characterized for purpose of hazard assessment as:

1. Primary alteration of the chemical structure of a substance resulting in loss of a specific property of that substance.
2. *Environmentally acceptable*: Biodegradation to such an extent as to remove undesirable properties of the compound. This often corresponds to primary biodegradation but it depends on the circumstances under which the products are discharged into the environment.
3. Ultimate complete breakdown of a compound to either fully oxidized or reduced simple molecules (such as carbon dioxide/methane, nitrate/ammonium, and water). It should be noted that the products of biodegradation can be more harmful than the substance degraded<sup>[5]</sup>

**Biodegradation**

“Is a biologically catalyzed reduction in complexity of a chemical”. In the case of organic compounds biodegradation, frequently, although not necessarily, leads to the conversion of much of O, C, N, P, S elements in the original compounds to the inorganic products<sup>[6]</sup>.

**Biodegradation**

“Is a process leading a change in the chemical composition and structure of contaminant caused by biological activity leading to naturally occurring metabolite end products”<sup>[7]</sup>.

**Aerobic biodegradation:** “Is the breakdown of organic contaminants by microorganisms when oxygen is present”. Aerobic biodegradation also is known as aerobic respiration<sup>[8]</sup>.

**Aerobic biodegradation:** “Is the degradation of compounds by microorganisms in the presence of oxygen”. In aerobic biodegradation, microorganisms convert oxygen to water in the process of transforming other components to simpler products<sup>[9]</sup>.

**Aerobic respiration:** “Is the process whereby microorganisms use oxygen as an electron acceptor to generate energy”<sup>[10]</sup>.

**Aerobic respiration:** “The process of destroying organic compounds with the aid of oxygen (O<sub>2</sub>) is called aerobic respiration”. In aerobic respiration, microbes use O<sub>2</sub> to oxidize part of the carbon in the contaminant to carbon dioxide (CO<sub>2</sub>), with the rest of the carbon used to produce new cell mass. In this process the O<sub>2</sub> gets reduced, producing water. Thus, the major

byproducts of aerobic respiration are carbon dioxide, water, and an increased population of microorganisms<sup>[10]</sup>.

**Anaerobic biodegradation:** “Is the degradation of compounds by microorganisms in the absence of oxygen”<sup>[10]</sup>.

**Anaerobic respiration:** “Is the process whereby microorganisms use a chemical entitle other than oxygen as an electron acceptor”. Common ‘substitutes’ for oxygen are nitrate, sulfate, and iron<sup>[9]</sup>. In anaerobic respiration, nitrate ( $\text{NO}_3^-$ ), sulfate ( $\text{SO}_4^{2-}$ ), metals such as iron ( $\text{Fe}^{3+}$ ) and manganese ( $\text{Mn}^{4+}$ ), or even  $\text{CO}_2$  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 ( $\text{N}_2$ ), hydrogen sulfide ( $\text{H}_2\text{S}$ ), reduced forms of metals, and methane ( $\text{CH}_4$ ), depending on the electron acceptor<sup>[9]</sup>.

**Anaerobic processes:** Anaerobic processes are used for treating wastewater with high concentrations of biodegradable organic materials, such as concentrated domestic wastewater, biosolids, animal manure slurry, and food processing wastes. The three biochemical reactions that characterize anaerobic processes are:

**Hydrolysis:** “Enzyme mediated transformation of complex organic compounds into simple compounds”.

**Acidogenesis:** “Is the bacterial conversion of simple compounds into substrates for methanogenesis (acetate, formate, hydrogen, carbon dioxide)”.

**Methanogenesis:** “Is the bacterial conversion of methanogenic substrates into methane and carbon dioxide”<sup>[11]</sup>.

**Electron acceptor:** “Is a chemical entity that accepts electrons transferred to it from another compound”. It is an oxidizing agent that, by virtue of its accepting electrons, is itself reduced in the process<sup>[12]</sup>.

**Electron acceptor:** “Are inorganic compounds that can accept electrons and can allow complete oxidations of organic substrates are called electron acceptors”. The most common electron acceptors used by microorganisms in natural environments are oxygen, nitrate, ferric iron, sulfate, and carbon dioxide<sup>[13]</sup>.

**Electron donor:** “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, the technical term for losing electrons; correspondingly, the chemical that gains the electron 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 are then 'invested,' along with some electrons and carbon from the contaminant, to produce more cells<sup>[10]</sup>.

**Biotransformation:** "Is a chemical conversion of a substance that is mediated by living organisms or enzyme preparations derived from there<sup>[14]</sup>.

However, there have been problem in producing a standard definition for Biodegradation and still differences between definitions and sometimes there can be confusion between degradation mechanisms. On the other hand, there is a conformity that the process proceed through microorganism *i.e.*, "living system".

The term microorganism covers a vast variety of life form, Bacteria, Blue-green cyano bacteria, Algae, Lichens and Fungi together with Protozoa. An important feature of microbial life is the possibility to degrade any naturally occurring compounds. This is called "microbial omnipotence". Expection to this rule are a few man made compounds such as highly polymerized materials (resins, plastics), halogenated compounds and others. Those are called "Xenobiotica", because they are strange to the living world and organisms had little time to adapt to these compounds and develop degradative enzymes<sup>[15]</sup>.

## REQUIREMENTS FOR BIODEGRADTION<sup>[16]</sup>

Biodegradation is an important attenuation mechanism of contaminants concentration in groundwater and soil. Biodegradation when it occurs, it limits pollutants migration and reduces contaminants mass in the subsurface. Biodegradable plumes are generally less widespread than that non-biodegrading counterparts and transported at slower relative rate. Biodegradation is a biochemical reaction which is mediated by microorganisms. In general, an organic compound is oxidized (losses electrons) by an electron acceptor which in itself is reduced (gain electrons). Under aerobic conditions, or toxic environmental conditions, oxygen acts as the electron acceptor. The oxidation of the organic compounds coupled to the reduction of molecular oxygen and this is termed as aerobic respiration. When oxygen is not present (anaerobic conditions) microorganisms can use organic chemicals or inorganic anions as alternative electrons acceptors. Anaerobic biodegradation can occur under fermentative, denitrifying, iron-reducing, sulphate-reducing or methanogenic conditions. Microbial investigations of the subsurface have revealed that most aquifers support a microbial population. Typical microbial population numbers ranges from  $1 \times 10^5$  to  $1 \times 10^7$  cell per gram dry weight. Relatively high numbers of microorganisms have been detected in both contaminated and pristine aquifers of varying depth and geological composition. The picture that

emerges from microbiological studies is that subsurface microorganisms tend to be small, capable response to the influx of nutrients and primarily attached to solid surface. There are six requirements for biodegradation they are simply:

1. The presence of the appropriate microorganisms,
2. An energy source such as organic moiety that is used by the organisms for cell maintenance and growth,
3. A carbon source,
4. An electron acceptor such as  $O_2$ ,  $NO_3^-$ ,  $SO_4^{2-}$ ,  $CO_2$
5. Nutrients as nitrogen, phosphorus, calcium, magnesium, iron, trace elements, and
6. Acceptable environmental conditions *e.g.*, Appropriate temperature, pH, salinity levels.

## FACTORS AFFECTING CONTAMINANT BIODEGRADATION<sup>[15]</sup>

### Biological Factors

#### *Rates of contaminant degradation*

The rate of contaminant degradation is often dependent on the concentration of the contaminant and the amount of “catalyst” present. In this context, the amount of “catalyst” present represents the number of organisms able to metabolize the contaminant as well as the amount of enzyme(s) produced by each cell. Thus, any factor that affects concentration of contaminant, the number of microorganisms present or the expression of specific enzymes by the cells can increase or decrease the rate of contaminant degradation. It should be noted that the rate of degradation is generally not constant and independent of contaminant concentration but rather decreases as the concentration of contaminant decreases. Any factor that affects concentration of contaminant, the number of microorganisms present or the expression of specific enzymes by the cells can increase or decrease the rate of contaminant degradation.

#### *Extent of contaminant degradation*

The extent to which contaminants are metabolized is largely a function of the specific enzymes involved and their “affinity” for the contaminant and the availability of the contaminant. The “affinity” of an enzyme is an inherent property of the enzyme that is determined by its structure. This “affinity” varies among different enzymes and even among enzymes with identical functions produced by different populations of microorganism.



### ***General indicators and microbial physiology***

#### *Carbon: nitrogen: phosphorus (C:N:P) ratios*

Microbial cells are largely comprised of carbon (C), nitrogen (N) and phosphorus (P) at an average C:N:P ratio of 50:14:3. Sufficient amounts of these nutrients must be available in a usable form and in proper proportions for unrestricted microbial growth to occur. It is important to realize that measures of total carbon, nitrogen and phosphorus do not necessarily reflect the amounts that can actually be used by microorganisms for growth as they may be physically or chemically unavailable.

#### *Nutrient availability*

Organic compounds serve as sources of carbon and can be estimated based on concentrations of total organic carbon (TOC). All organic chemicals present in a sample, including compounds that are unavailable or not readily metabolized, are included in measures of TOC. Thus, TOC tends to overestimate the carbon available to microorganisms. In general, the total number of organisms present is proportional to the amount of carbon available (assuming other nutrients are not limiting). Thus, the microbial density of surface soils is greater than subsurface soils which in turn are generally greater than in aquifer sediments.

Provided that carbon is present in sufficient amounts, the total amount of nitrogen available to microorganisms in the form of organic nitrogen, ammonia ( $\text{NH}_4^+$ ), nitrate ( $\text{NO}_3^-$ ), and nitrite ( $\text{NO}_2^-$ ) can significantly influence the rate of contaminant degradation. A C:N ratio of less than 40 suggests that adequate nitrogen is present.

Microorganisms can utilize both soluble inorganic (orthophosphate) and organic forms of phosphorus. Phosphorus limitation can occur when the C:P ratio is more than 120:1 and a C:N:P ratio of 100:10:1 is considered optimal. High concentrations of calcium (Ca) and magnesium (Mg) may precipitate phosphates reducing the amount available for microbial metabolism.

In most soils other nutrients such as sulphur (S), Ca, Mg, potassium (K), as well as trace amounts of metals, are typically found in adequate supply for metabolic needs.

#### *Terminal electron acceptors*

The metabolism of organic contaminants by microorganisms often entails oxidation of the compound and this requires that the electrons derived from these processes be used to reduce other compounds. A wide range of organic and inorganic compounds can be used as terminal electron acceptors by microorganisms (Table 1).

The energy derived by the organism from these respiratory processes varies. Aerobic respiration yields the most energy and methanogenesis the

least (the processes are listed in Table 1 in the order of most to least energy conserved). As a first approximation, the rate of contaminant degradation is roughly proportional to the amount of energy obtained.

**Table 1:** Organic and inorganic compounds that can be used as terminal electron acceptors by microorganisms

<i>Electron acceptor</i>	<i>Chemical symbol</i>	<i>Process</i>
Oxygen	O <sub>2</sub>	Aerobic respiration
Nitrate and nitrite	NO <sub>3</sub> & NO <sub>2</sub>	Aerobic respiration (denitrification)
Ferric iron	Fe <sup>+3</sup>	Anaerobic respiration
Manganese	Mn <sup>+4</sup> & Mn <sup>+2</sup>	Anaerobic respiration
Sulfate	SO <sub>4</sub> <sup>-2</sup> , S <sub>2</sub> O <sub>3</sub> <sup>-2</sup> & SO <sub>3</sub> <sup>-2</sup>	Anaerobic respiration (sulfate reduction)
Organic compounds	Many	Fermentation
Carbon dioxide	CO <sub>2</sub>	Methanogenesis

In aerobic processes, oxygen is the terminal electron acceptor. The rate of contaminant degradation by aerobic processes is often limited by the availability of oxygen, which is supplied by diffusion from the atmosphere or dissolved in and transported by water. The diffusion of oxygen from the atmosphere into soil and water is slow and the solubility of oxygen in water is low (about 10 mg/L at 25°C). Consequently, oxygen may be consumed faster than it is replaced and the system may become anaerobic. The availability of oxygen becomes rate limiting at dissolved oxygen concentrations below about 1 mg/L.

As oxygen is depleted, other organic and inorganic compounds are used as terminal electron acceptors. This often results in a continuum of processes that extend away from the source of contamination with the regions nearest the source being anaerobic (no oxygen present) and extending to regions where oxygen is more readily available. Microbial processes coupled to the reduction of ionic compounds such as iron, manganese and sulfate compounds, can offer significant advantages since these compounds are more water-soluble than oxygen. Moreover, in some environments they are present in high concentration (nitrate in groundwater, for example) or they can readily be added in engineered bioremediation processes.

“As oxygen is depleted, other organic and inorganic compounds are used as terminal electron acceptors.”

### *Soil respirometry*

Soil respirometry provides a measure of oxygen (O<sub>2</sub>) consumption or carbon dioxide (CO<sub>2</sub>) production in soils and is an indication of net aerobic biological activity *in situ*. The determination is typically made by measuring the consumption of oxygen by contaminated soils over time and comparing the rate to that observed with soils from a nearby region that is not contaminated. Increased O<sub>2</sub> use, measured as lower O<sub>2</sub> levels, is taken as an indication of increased respiratory activity that is potentially due to

metabolism of contaminant(s). However, there are numerous problems with the use of respirometry to demonstrate contaminant degradation. Perhaps the most significant is that this method measures oxygen consumption resulting from metabolism of all carbon sources, not just contaminant(s). The additional oxygen consumed as a result of contaminant metabolism may be a relatively small increment of the total if the contaminant represents a small fraction of the total organic carbon present in the sample. Moreover, the method is useful only for monitoring aerobic respiration and does not include anaerobic respiration or other processes that may be involved in contaminant degradation.

### ***Temperature***

Temperature directly influences the rate of biodegradation by controlling the rates of enzyme catalyzed reactions. The rate of biodegradation is decreased by roughly one-half for each 10°C decrease in temperature. Rates of biodegradation are generally exceedingly low at 0°C. Conversely, higher soil temperatures result in higher microbial metabolic activity and higher rates of biodegradation up to a maximum of about 65°C. As a result, the rate of biodegradation may vary seasonally and microbial metabolic activity itself can increase soil temperature.

It should be noted that most research studies reported in the scientific literature have employed temperatures that range from 20–25°C and therefore, the reported rates of contaminant degradation are higher than would be expected in subsurface environments where average soil temperatures at depth are around 10°C<sup>[15]</sup>. A better estimate of rates of natural biodegradation based on literature values is possible when soil temperature is known.

### ***Moisture***

Moisture (water) influences the rate of contaminant metabolism because it influences the kind and amount of soluble materials that are available as well as the osmotic pressure and pH of terrestrial and aquatic systems. As mentioned above, the amount of water in the pore spaces of soil also affects the exchange of oxygen. Under saturated conditions, oxygen can be consumed faster than it is replenished in the soil vapor space and the soil can become anaerobic. This can retard the rate of biodegradation and cause major changes in microbial metabolic activity to occur. Conversely, soil moisture content should be between 25–85% of the water holding capacity, and a range of 50–80% is optimal for biodegradation. The water holding capacity of soil is sometimes expressed as the “field capacity” which is the percentage of water remaining in a soil after it has been saturated and gravitational drainage has ceased. “The amount of water in the pore spaces of soil also affects the exchange of oxygen. Under these conditions, oxygen can be consumed faster than it is replenished”.

## ***pH***

Soil pH is a measure of the acidity or alkalinity of water. Biodegradation can occur under a wide-range of pH; however, a pH of 6.5 to 8.5 is generally optimal for biodegradation in most aquatic and terrestrial systems and values ranging from 5 to 9 are considered acceptable. Soil pH may affect the availability of nutrients. For example, the solubility of phosphorus, an important nutrient in biological systems, is maximal at a pH value of 6.5 and decreases at pHs that are either higher or lower than this value.

## **Environmental Factors**

### ***Geologic and hydrogeologic factors***

*Adsorption and absorption:* The binding of an organic compound to the surface of a solid is called adsorption. The extent of adsorption that occurs is determined by the relative affinity of the compound for a solid matrix, the surface area of the matrix and the volatility or solubility of the compound in water. In general, hydrophobic chemicals will tend to adsorb to insoluble organic matter and other hydrophobic materials. Similarly, hydrophilic chemicals may bind to minerals.

Adsorbed contaminants are in equilibrium with the dissolved or volatilized contaminants. So as the concentration in the aqueous or gaseous phases is reduced, the amount of contaminants adsorbed onto a surface will decrease. Constituents that adsorb tightly to solid matrices will be less subject to transport in aqueous and gaseous phases and thus their mobility in a system is retarded. In contrast, constituents that are not tightly adsorbed can be transported through soils, aquatic systems, and the atmosphere. The potential for adsorption is closely associated with soil type and soil organic matter content.

The fraction of organic carbon in surficial soils typically ranges from 1 to 3.5 percent. The organic matter content in subsurface soils is typically an order of magnitude lower because most organic residues are incorporated or deposited on the surface soils and do not readily migrate to subsurface soils. In some cases, surfactants or cosolvents can be added to the aqueous phase of a system to increase the relative solubility or mobility of the contaminant and decrease adsorption.

Absorption is an analogous process wherein a contaminant penetrates into the bulk mass of the soil matrix. Both adsorption and absorption reduce the availability of the contaminant to most microorganisms and the rate at which the chemical is metabolized is proportionately reduced.

“Both adsorption and absorption reduce the availability of the contaminant to most microorganisms and the rate at which the chemical is metabolized is proportionately reduced.”

*Contaminant migration in groundwater:* Contaminant migration within an aquifer is controlled by many chemical and physical properties of the contaminants, and of the hydrogeologic setting in which they are found. Hydraulic conductivity is one of the primary aquifer characteristics that must be understood to effectively predict contaminant migration and evaluate the possibilities for adding materials to enhance bioremediation. It may vary horizontally and vertically and is measured in units of length per time, such as feet per day or centimetres per second, reflecting the rate at which water will flow through rock or unconsolidated materials. Particle size and rock type affect hydraulic conductivity, with smaller particle size, or increasing density of rock, resulting in lower rates of hydraulic conductivity. Typically, hydraulic conductivities for soils in the range of  $10^{-5} - 10^{-3}$  cm/sec will be convenient to biodegradation process, although values below  $10^{-5}$  cm/sec may be acceptable if other site specific conditions are favorable.

Variations in porosity of the unsaturated and saturated zones of the aquifer matrix may influence the movement of other fluids, in addition to water. The ability of the matrix to transmit gases, such as oxygen, methane and carbon dioxide, is reduced in fine grained sediments and also when soils become more saturated with water. This can affect the rate and type of biodegradation taking place. Conversely, fine grained clays and silts tend to retard the movement of contaminants making widespread contamination less likely.

Other factors to consider are dispersion and diffusion of contaminants. Dispersion is the mechanical mixing and spreading of the contaminants that occurs within the aquifer and includes diffusion, the movement of contaminants along a concentration gradient due to their kinetic energy. These are primarily physical processes that reduce contaminant concentrations within the plume and are of particular importance for sites where natural attenuation is the proposed remedy.

### **Bioavailability**

The fraction of contaminant actually available to microorganisms is said to be bioavailable. The concentration of bioavailable contaminant is often less than the total concentration measured (following solvent extraction, for example). It is important to note that the rate and extent of contaminant degradation is proportional to the concentration of bioavailable contaminant and not the total concentration.

### **Soil Matric Potential**

The soil matric potential is a measure of the energy required to overcome capillary and adsorptive forces and thus reflects the “work” that microbial cells must do to extract water from the soil. The matric potential of soil is

strongly dependent on soil type and is correlated with soil moisture content. Soil moisture contents of 50–80% roughly correspond to matric potentials in the range of  $-0.01$  to  $-0.015$  Mpa ( $-0.1$  to  $-0.15$  bar) in sands and  $-0.03$  to  $-0.05$  Mpa ( $-0.3$  to  $-0.5$  bar) in medium to fine textured soils. In general, microbial activity is low in soils with a matric potential of about  $-0.1$  Mpa ( $-1$  bar) and ceases at about  $-8$  Mpa ( $-80$  bars). Matric potentials that range from  $-0.5$  Mpa ( $-5$  bars) to  $-1.5$  Mpa ( $-15$  bars) are generally considered adequate. “If the matric potential is too low then contaminant degradation rates will also be low”.

### Redox Potential

The oxidation-reduction potential (redox potential) of a soil provides a measurement of the electron density of the system. Biological energy is obtained from the oxidation of compounds in which electrons are transferred to various more oxidized compounds referred to as electron acceptors. A low electron density (Eh greater than 50 mV) indicates oxidizing, aerobic conditions, whereas high electron density (Eh less than 50 mV) indicates reducing, anaerobic conditions. High positive Eh values ( $+100$  mV to  $+400$  mV) indicate well aerated conditions that are optimal for biodegradation. It should be noted that “Eh may vary considerably over a very small distance within soils and it is generally not practicable to directly measure this parameter for a specific site”.

As many microbial processes, optimizing the environmental conditions in the biodegradation processes is a central goal in order that the microbial physiological and biochemical activities are directed towards biodegrading the target contaminants. Deviation of the environmental factors that reported to influence microbial growth and bioactivity, away from the potential conditions will reduce rates of microbial growth and transformation of target substrate and perhaps cause failure of the biodegradation process. The potential of the process may also be limited by the toxicity of the pollutants to the degrading microorganism<sup>[17]</sup>.

However, as a rule, mineralization of organic compounds is characteristic of growth linked biodegradation, in which the organism converts the substrate to  $\text{CO}_2$ , cell components and products typical of the usual catabolic path ways. It is likely, however, that mineralization in nature occasionally may not be linked to growth but instead results from non-proliferation population. Conversely, some species growing at the expanses of a carbon compound may still cannot mineralize and produce  $\text{CO}_2$  from the substrate; however, if  $\text{O}_2$  is present, the organic product excreted by one species probably will be converted to  $\text{CO}_2$  by another species, so that even if the initial population does not produce  $\text{CO}_2$ , the second species will. The net effect is still one of mineralization.

A compound, such as many environmental pollutants, that represent a novel carbon and energy source for a particular population is still transformed by the metabolic pathways that are characteristic of heterotrophic microorganisms. For the organism to grow on the compound, it must be converted to the intermediate that characterize there metabolic sequences. If a compound cannot be modified enzymatically to yield such intermediate, it will not serve as carbon and energy source because the energy yielding and biosynthetic process cannot function.

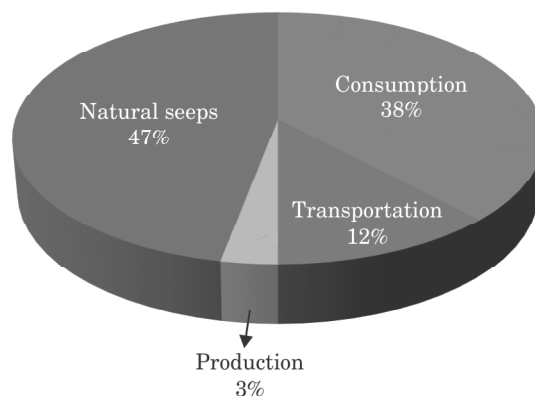
The initial plan of the biodegradation thus involves modification of the substrate to yield a product that is an intermediate or following further metabolism, is converted to an intermediate in there iniquitous metabolic sequences. This need to convert the synthetic molecule to intermediate is characteristics of both aerobes and anaerobes as they drive carbon and energy source from the substrate<sup>[6]</sup>.

Several conditions must be satisfied for biodegradation to take place in an environment. These include the following<sup>[6]</sup>:

1. An organism that has the necessary enzymes to bring about the biodegradation must exist. The mere existence of an organism with the appropriate catabolic potential is necessary but no sufficient for the process to occur.
2. That organism must be present in the environment containing the chemical. Although some microorganism are present in essentially every environment near the earth's surface, particular environments may not contain an organism with the necessary enzymes.
3. The chemical must be accessible to the organism having the requisite enzymes. Many chemicals persist even in environments containing the biodegrading species are not degraded simply because the organism does not have access to the compound that it would otherwise metabolize. Inaccessibility may result from the substrate being in a different environment from the organism, *e.g.*, in a solvent not miscible with water, or sorbed to solid surfaces.
4. If the initial enzyme bringing about the degradation is extracellular, the bond acted upon by the enzyme must be exposed for the catalyst to function. This is not always the case because of sorption of many organic molecules.
5. Should the enzymes catalyzing the initial degradation be intracellular, the molecule must penetrate the surface of the cell to the internal sites where the enzyme acts. Alternatively, the products of an extracellular reaction must penetrate the cell for the transformation to proceed further.
6. Because the population or biomass of bacteria or fungi acting on many synthetic compounds is initially small, condition in the environment must be conducive to allow for proliferation of the potentially active microorganisms.

## OIL BIODEGRADATION

Petroleum-based products are the major source of energy for industry and daily life activities. Petroleum is also the raw material for many chemical products such as plastics, paints, and cosmetics. The transport of petroleum across the world is frequent, and the amounts of petroleum stocks in developed countries are enormous. Petroleum oil contains aromatic compounds that are toxic for most life forms. Episodic and chronic pollution of the environment by oil causes major ecological perturbations. Marine environments are especially vulnerable since oil spills of coastal regions and the open sea are poorly containable and mitigation is difficult. In addition to pollution through human activities, about 250 million litres of petroleum enter the marine environment every year from natural seepages, Fig. 1<sup>[18]</sup>. Despite its toxicity, a considerable fraction of petroleum oil entering marine systems is eliminated by the hydrocarbon-degrading activities of microbial communities, in particular by a remarkable recently discovered group of specialists, the so-called hydrocarbonoclastic bacteria (HCB)<sup>[19]</sup>. *Alcanivorax borkumensis* was the first HCB to have its genome sequenced<sup>[20]</sup>. In addition to hydrocarbons, crude oil often contains various heterocyclic compounds, such as pyridine, which appear to be degraded by similar, though separate mechanisms than hydrocarbons<sup>[21]</sup>.



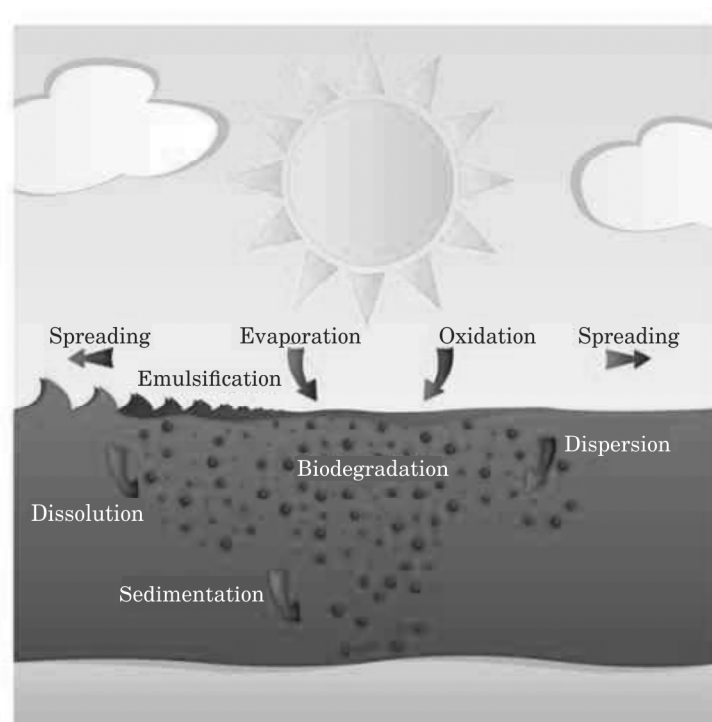
**Fig. 1:** Sources of oil in seas<sup>[22]</sup>

## Fate of Oil Spills

When petroleum is spilled into the sea, it spreads over the surface of the water, Fig. 2. It is subjected to many modifications, and the composition of the petroleum changes with time. This process is called weathering, and is mainly due to evaporation of the low-molecular-weight fractions, dissolution of the water-soluble components, mixing of the oil droplets with seawater, photochemical oxidation, and biodegradation. Those petroleum components with a boiling point below 250°C are subjected to evaporation. Therefore,



the content of n-alkanes, whose chain length is shorter than C14, is reduced by weathering, Fig. 2.



**Fig. 2:** Fate of oil pollution in marine environment Ocean world<sup>[24]</sup>

N-alkanes, a major group in crude oil, are readily biodegraded in the marine environment. They are aerobically biodegraded by several pathways. The degradation of n-alkanes of medium chain length by *Pseudomonas putida* containing the OCT plasmid is initiated by alkanes hydroxylase. Some of these processes, like natural dispersion of the oil into the water, lead to the removal of the oil from the sea surface, and facilitate its natural breakdown in the marine environment. Others, particularly the formation of water-in-oil emulsions, cause the oil to become more persistent, and remain at sea or on the shoreline for prolonged periods of time.

The potential for oil spills is significant, and research on the fate of petroleum in a marine environment is important to evaluate the environmental threat of oil spills, and to develop biotechnology to cope with them. Crude oil is constituted from thousands of components which are separated into saturates, aromatics, resins and asphaltenes. Upon discharge into the sea, crude oil is subjected to weathering, as stated, the process caused by the combined effects of physical, chemical and biological modification. Saturates, especially those of smaller molecular weight, are

readily biodegraded in marine environments. Aromatics with one, two or three aromatic rings are also efficiently biodegraded; however, those with four or more aromatic ring are quite resistant to biodegradation. The asphaltene and resin fractions contain higher molecular weight compounds whose chemical structures have not yet been resolved. The biodegradability of these compounds is not yet known. It is known that the concentrations of available nitrogen and phosphorus in seawater limit the growth and activities of hydrocarbon-degrading microorganisms in a marine environment. In other words, the addition of nitrogen and phosphorus fertilizers to an oil-contaminated marine environment can stimulate the biodegradation of spilled oil. This notion was confirmed in the large-scale operation for bioremediation after the oil spill from the Exxon Valdez in Alaska. Many microorganisms capable of degrading petroleum components have been isolated. However, few of them seem to be important for petroleum biodegradation in natural environments. One group of bacteria belonging to the genus *Alcanivorax* does become predominant in an oil-contaminated marine environment, especially when nitrogen and phosphorus fertilizers are added to stimulate the growth of endogenous microorganisms<sup>[23]</sup>.

The speed and relative importance of the processes depend on factors such as the quantity spilled, the oil's initial physical and chemical characteristics, weather and sea conditions and whether the oil remains at sea or is washed ashore.

Ultimately, the marine environment usually eliminates spilled oil through the long-term process of biodegradation. Studies on the environmental fate of petroleum have demonstrated the nearly ubiquitous distribution of microorganisms that can metabolize hydrocarbons. The rates of degradation depend upon the concentrations of such microbes and upon the environmental characteristics of an oil-contaminated ecosystem as referred before. Given the appropriate environmental conditions, microorganisms effectively decontaminate, by their biodegradative metabolism, environments that have received petroleum pollutants. Higher-molecular-weight compounds, especially those with multiple condensed ring structures and with highly branched or substituted compounds, are relatively resistant to microbial attack. Despite the fact that a genetically engineered hydrocarbon degrader was the first organism ever patented and that seed cultures are produced by various commercial firms, enhanced biodegradation as a result of seeding generally has not been shown to be effective. Also, even though some anaerobes have now been demonstrated to be capable of hydrocarbon metabolism, hydrocarbons persist indefinitely in anoxic environments. Environmental modification, on the other hand, such as that achieved by aeration or fertilization with nitrogen and phosphorus, has been shown to enhance biodegradative removal of hydrocarbons.

Having considered the various factors that influence the rates of hydrocarbon biodegradation, we are left with the question of what to do

when environmental oil contamination occurs in order to minimize its persistence and thus its long-term effects. Clearly, treatment methods should enhance rather than inhibit the natural rates of oil biodegradation. In some cases, it is possible to modify environmental parameters to enhance rates of hydrocarbon biodegradation, but such methods are rarely undertaken. Specifically designed organisms are needed to degrade toxic aromatic components of refinery waste streams before environmental treatment. Specially designed reactors with specific microbial populations are also needed if oily sludge are to be degraded by biological means, either aerobically or anaerobically, in contained, environmentally safe reactors<sup>[25]</sup>.

### Biodegradation Mechanism of Oil Spill

Sea water contains a wide range of microorganisms that use hydrocarbons as a source of energy and can partially or completely degrade oil to water soluble compounds and eventually to carbon dioxide and water.

Many types of marine microorganism exist and each tends to degrade a particular group of compounds in crude oil. However, some compounds in oil are very resistant to attack and may not readily degrade. The main factors affecting the efficiency of biodegradation, are the levels of nutrients (nitrogen and phosphorus) in the water, the temperature and the level of oxygen present as clarified.

As biodegradation of oil requires oxygen, this process can only take place at the oil-water interface since no oxygen is available within the oil itself. The creation of oil droplets through dispersion, increases the surface area to volume ratio of the oil, and therefore increases the area available for micro-organisms to attach to the surface of the oil and for biodegradation to take place.

It is well known that the composition of petroleum or some of its processing products changes in the environment mostly under the influence of microorganisms. The hydrocarbons of a petroleum pollutant are degraded by microbial cultures which were isolated as dominant microorganisms from a surface water of a wastewater canal of an oil refinery and a nitrogen plant. *Phormidium foveolarum* (filamentous Cyanobacteria-blue-green algae) and *Achnanthes minutissima* (diatoms, algae), microbial cultures isolated as dominant algae from a surface water in a wastewater canal of an oil refinery and a nitrogen plant, have degradable effects dominantly involving petroleum hydrocarbons. Petroleum microbiological degradation is more intensive when inorganic medium (in the light) is applied. Having in mind that the inorganic pollutants have been released into the canal as well, this medium reflects more the natural environmental conditions. Polycyclic alkanes of sterane and triterpane type, in spite of the fact that these compounds could be degraded, have remained unchanged regarding abundance and distribution. Since this is the case even for naphthenic type petroleum (which is depleted

in n-alkanes), it can be concluded that the biodegradation of petroleum type pollutants, under natural conditions, will be restrained to the n-alkanes and isoprenoid degradation<sup>[26]</sup>.

In oil spill investigations, one of the most important steps is a proper choice of approaches that imply an investigation of samples taken from different sedimentary environments, samples of oil contaminants taken in different periods of time and samples taken at different distances from the oil spill. In all these cases, conclusion on the influence of the environment, microorganisms or migration on the oil contaminants' composition can be drawn from the comparison of chemical compositions of the investigated contaminants. However, in case of water contaminants, it is very important to define which part of organic matter has been analyzed. Namely, investigations showed that there were some differences in chemical composition of the same oil contaminant depending on the intensity of its contact with ground water. It was undoubtedly confirmed that there were specific differences in the compositions of the different extracts depending on the intensity of the interaction between the oil contaminant and the surface water<sup>[27]</sup>.

### Study Case

Cyanobacterial mats developing in oil-contaminated sabkhas along the African coasts of the Gulf of Suez and in the pristine Solar Lake, Sinai, were collected for laboratory studies. Samples of both mats showed efficient degradation of crude oil in the light, followed by development of an intense bloom of *Phormidium* spp. and *Oscillatoria* spp. isolated cyanobacterial strains, however, did not degrade crude oil in axenic cultures. Strains of sulfate-reducing bacteria and aerobic heterotrophs were capable of degrading model compounds of aliphatic and aromatic hydrocarbons. Results indicate that degradation of oil was done primarily by aerobic heterotrophic bacteria. The oxygenic photosynthesis of oil-insensitive cyanobacteria supplied the molecular oxygen for the efficient aerobic metabolism of organisms, such as *Marinobacter* spp. The diurnal shifts in environmental conditions at the mat surface, from highly oxidic conditions in the light to anaerobic sulfide-rich habitat in the dark, may allow the combined aerobic and anaerobic degradation of crude oil at the mat surface. Hence, coastal cyanobacterial mats may be used for the degradation of coastline oil spills. Oxygen microelectrodes detected a significant inhibition of photosynthetic activity subsequent to oil addition. This prevailed for a few hours and then rapidly recovered. In addition, shifts in bacterial community structure following exposure to oil were determined by denaturing gradient gel electrophoresis of PCR-amplified fractions of 16S rRNA from eubacteria, cyanobacteria and sulfate-reducing bacteria. Since the mats used for the present study were obtained from oil-contaminated environments, they were believed to be pre equilibrated for petroleum remediation. The mesocosm system at Eilat

provided a unique opportunity to study petroleum degradation by mats formed under different salinities (up to 21%). These mats, dominated by cyanobacteria, can serve as close analogues to the sabkhas contaminated during the Gulf War in Kuwait and Saudi Arabia<sup>[28]</sup>.

Although diverse bacteria capable of degrading petroleum hydrocarbons have been isolated and characterized, the vast majority of hydrocarbon-degrading bacteria, including anaerobes, could remain undiscovered, as a large fraction of bacteria inhabiting marine environments are uncultivable. For example, using culture-independent rRNA approaches, changes in the structure of microbial communities have been analyzed in marine environments contaminated by a real oil spill and in micro- or mesocosms that mimic such environments. *Alcanivorax* and *Cycloclasticus* of the gamma-Proteobacteria were identified as two key organisms with major roles in the degradation of petroleum hydrocarbons. *Alcanivorax* is responsible for alkanes biodegradation, whereas *Cycloclasticus* degrades various aromatic hydrocarbons. This information will be useful to develop *in situ* bioremediation strategies for the clean-up of marine oil spills<sup>[29]</sup>.

## BIODEGRADATION OF PLASTICS<sup>[30–32]</sup>

Every year, large quantities of waste polymers are produced from industrial, agricultural and household activities. It has been reported that plastics make up more than 12% of municipal solid waste stream, a dramatic growth from 1960, when plastics were only 1% of the waste stream<sup>[33]</sup>. The widespread applications and non-biodegradability of these polymers create huge amounts of waste and disposal; what results in a serious environmental problem<sup>[34]</sup>.

### Plastics Biodegradation Phases

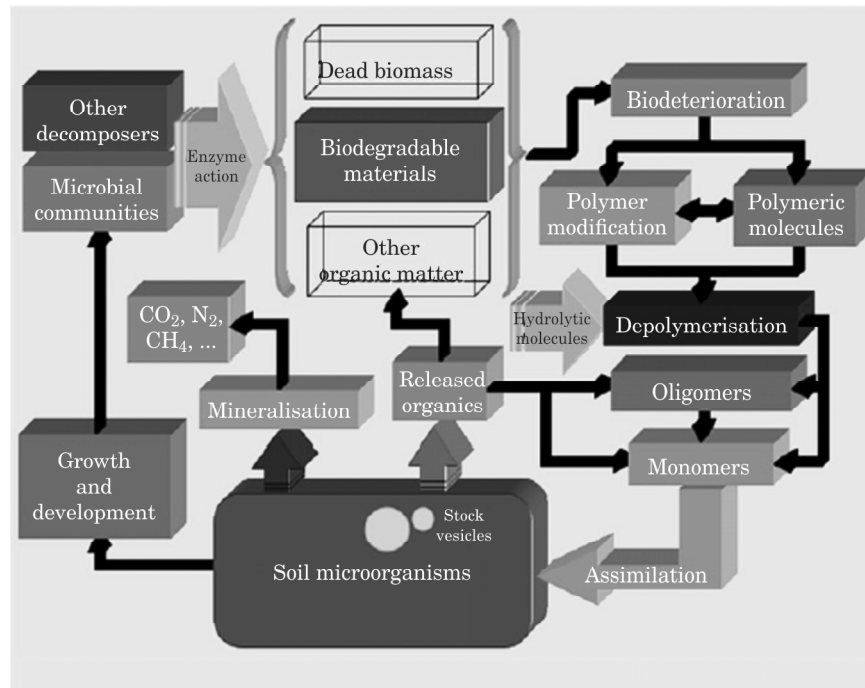
There are four phases for the plastics biodegradation:

1. **Aerobic:** During the first few days, aerobic microbes become establish as the moisture build up in the refuge. Biodegradable plastics start to swell of their molecular structure and weaken of the polymer to create space, where moisture and microbial growth can rapidly begin an aerobic degradation. O<sub>2</sub> replace with CO<sub>2</sub>.
2. **Anaerobic:** At about two weeks to six months later, O<sub>2</sub> concentration decline and an anaerobic process begins. During the initial phase, microbes' colonies eat particulates and reduce large polymers into simple monomers though an enzymatic process. The secreted monomers mix with organic plastic additives causing further swelling, and opening of the polymer chains. Acidogenesis occur, where simple monomers are converted into fatty acids. CO<sub>2</sub> production takes place rapidly at this stage.

3. **Anaerobic: methanogenic, unsteady phase:** About 6–18 months, microbe colonies continue to grow, eating away at the polymer chain and creating increasingly larger molecular spaces. Acetogenesis occurs, converting fatty acids into acetic acid,  $\text{CO}_2$  and  $\text{H}_2$ .  $\text{CO}_2$  rate gradually decline and  $\text{H}_2$  production eventually ceases.
4. **Anaerobic: methanogenic, steady phase:** About 1–5 years, the final stage of decomposition occurs. This final step involves methanogenesis. As colonies of microbes continue to eat away at remaining of the polymer surface, acetates are converted into methane and  $\text{CO}_2$  while  $\text{H}_2$  is consumed. This methane can be turned into energy efficient biotechnology<sup>[6]</sup>.

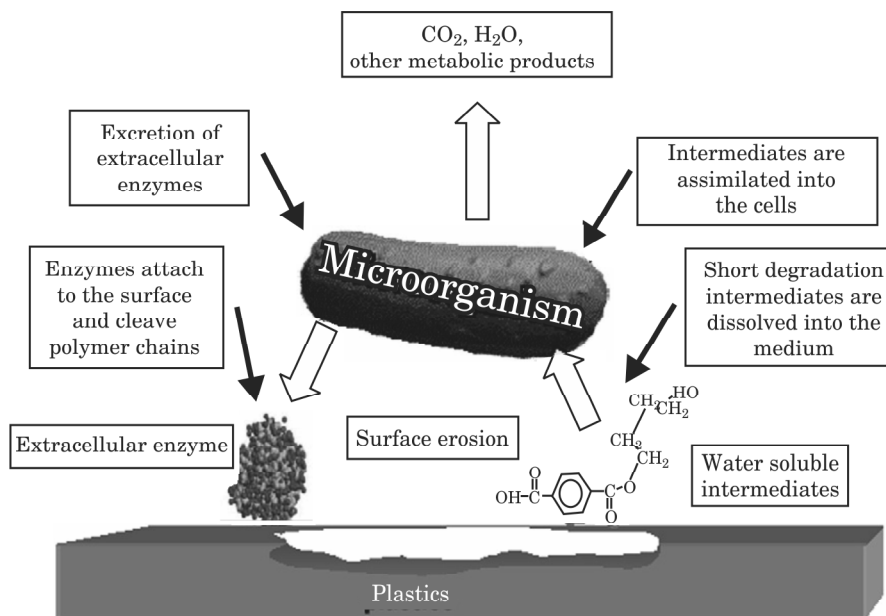
Microorganisms such as bacteria and fungi are involved in the degradation of both natural and synthetic plastics<sup>[35]</sup>. The biodegradation of plastics proceeds actively under different soil conditions according to their properties, because the microorganisms responsible for the degradation differ from each other and they have their own optimal growth conditions in the soil. Polymers especially plastics are potential substrates for heterotrophic microorganisms<sup>[36]</sup>.

The biodegradation of polymeric materials includes several steps and can be summarized in Fig. 3.



**Fig. 3:** Polymer biodegradation scheme<sup>[37]</sup>

Biodegradation is governed by different factors that include polymer characteristics, type of organism, and nature of pretreatment. The polymer characteristics such as its mobility, tacticity, crystallinity, molecular weight, the type of functional groups and substituents present in its structure, and plasticizers or additives added to the polymer all play an important role in its degradation<sup>[38–39]</sup>. During degradation the polymer is first converted to its monomers, and then these monomers are mineralized. Most polymers are too large to pass through cellular membranes, so they must first be depolymerized to smaller monomers before they can be absorbed and biodegraded within microbial cells. The initial breakdown of a polymer can result from a variety of physical and biological forces<sup>[40]</sup>. Physical forces, such as heating/cooling, freezing/thawing, or wetting/drying, can cause mechanical damage such as the cracking of polymeric materials<sup>[41]</sup>. The growth of many fungi can also cause small-scale swelling and bursting, as the fungi penetrate the polymer solids<sup>[42]</sup>. Synthetic polymers, such as poly (caprolactone)<sup>[43]</sup>, are also depolymerized by microbial enzymes, after which the monomers are absorbed into microbial cells and biodegraded<sup>[44]</sup>. Abiotic hydrolysis is the most important reaction for initiating the environmental degradation of synthetic polymers<sup>[45]</sup> like polycarboxylates<sup>[46]</sup>, poly(ethylene terephthalate)<sup>[47]</sup>, polylactic acids and their copolymers<sup>[48–49]</sup>, poly (β–glutamic acids)<sup>[50]</sup>, and polydimethylsiloxanes, or silicones<sup>[51]</sup>. Generally, an increase in molecular weight results in a decline of polymer degradability by microorganisms. In contrast, monomers, dimers, and oligomers of a polymer's repeating units are much easily degraded and mineralized. High molecular weights result in a sharp decrease in solubility making them unfavorable for microbial attack because bacteria require the substrate to be assimilated through the cellular membrane and then further degraded by cellular enzymes. At least two categories of enzymes are actively involved in biological degradation of polymers: extracellular and intracellular depolymerases<sup>[52,39]</sup>. During degradation, exoenzymes from microorganisms break down complex polymers yielding smaller molecules of short chains, *e.g.*, oligomers, dimers, and monomers, that are smaller enough to pass the semi-permeable outer bacterial membranes, and then to be utilized as carbon and energy sources. The process is called depolymerization. When the end products are CO<sub>2</sub>, H<sub>2</sub>O, or CH<sub>4</sub>, the degradation is called mineralization<sup>[53]</sup>. It is important to note that Biodeterioration and degradation of polymer substrate can rarely reach 100% and the reason is that a small portion of the polymer will be incorporated into microbial biomass, humus and other natural products<sup>[54–55]</sup>. Dominant groups of microorganisms and the degradative pathways associated with polymer degradation are often determined by the environmental conditions as stated. When O<sub>2</sub> is available, aerobic microorganisms are mostly responsible for destruction of complex materials, with microbial biomass, CO<sub>2</sub>, and H<sub>2</sub>O as the final products. In contrast, under anoxic conditions, anaerobic combinations of microorganisms are responsible for polymer deterioration. The primary products will be microbial biomass, CO<sub>2</sub>, CH<sub>4</sub> and H<sub>2</sub>O under methanogenic (anaerobic) conditions<sup>[56]</sup> (*e.g.*, landfills/ compost) (Fig. 4).



**Fig. 4:** General mechanism of plastic biodegradation under aerobic conditions<sup>[57]</sup>

### Pesticide Biodegradation

Approximately  $6 \times 10^6$  chemical compounds have been produced; annually 1,000 new products are synthesized and between 60,000 and 95,000 chemicals are commercially used<sup>[58]</sup>. Among these substances are chemical pesticides, which are used extensively in most areas of crop production in order to minimize pest infestations, to protect the crop yield losses and to avoid reducing the product quality. Different biological systems, as microorganisms, have been used to biotransform pesticides. It has been reported that a fraction of the soil biota can quickly develop the ability to biodegrade certain pesticides, when they are continuously applied to the soil. These chemicals provide adequate carbon source and electron donors for certain soil microorganisms<sup>[59]</sup>, establishing a way for the treatment of pesticide-contaminated sites<sup>[60–61]</sup>. However, the transformation of such compounds depends not only on the presence of microorganisms with appropriate degrading enzymes, but also a wide range of environmental parameters<sup>[62]</sup>. Additionally, some physiological, ecological, biochemical and molecular aspects play an important role in the microbial transformation of pollutants<sup>[63–64]</sup>. Microorganisms have the ability to interact, both chemically and physically, with substances leading to structural changes or complete degradation of the target molecule. Among the microbial communities, bacteria, fungi, and actinomycetes are the main transformers and pesticide degraders<sup>[65]</sup>. Fungi generally biotransform pesticides and other xenobiotics by introducing minor structural changes to the molecule, rendering it



nontoxic. The biotransformed pesticide is released into the environment, where it is susceptible to further degradation by bacteria<sup>[66]</sup>.

## BIODEGRADATION OF RADIOACTIVE WASTES

Radioactive wastes are wastes that contain radioactive material. Radioactive wastes are usually by-products of nuclear power generation and other applications of nuclear fission or nuclear technology, such as research and medicine. Radioactive waste is hazardous to most forms of life and the environment, and is regulated by government agencies in order to protect human health and the environment.

The release of radionuclides from nuclear sites and their subsequent mobility in the environment is a subject of intense public concern and has prompted much recent research on the environmental fate of key radionuclides. The major burden of anthropogenic environmental radioactivity is from the controlled discharge of process effluents produced by industrial activities allied to the generation of nuclear power. Significant quantities of natural and artificial radionuclides were also released as a consequence of nuclear weapons testing in the 1950s and 1960s, through accidental release (*e.g.* from Chernobyl in 1986 and Fukushima, 2011), and from the ongoing storage of nuclear materials amassed over the past 70 years of nuclear activities<sup>[67]</sup>.

Under appropriate conditions stabilization or immobilization of radionuclides and metals is brought by one or more of the following mechanisms:

1. Oxidation–reduction of metals which affect the valance state and stability
2. Changes in pH which affect the ionic state of the metals and solubility
3. Chelation, solubilization, and leaching of elements by microbial metabolism and decomposition products,
4. Immobilization due to formation of stable minerals,
5. Bioaccumulation by cells and exopolymers
6. Biomethylation and production of volatile and /or toxic alkylated metal compounds
7. Biodegradation of organic complexes of radionuclides and metals and
8. Remobilization of biosorbed radionuclides and metals due to mineralization<sup>[68]</sup>.

Treatment of radio-contaminated soils, sediments, sludge and wastes containing:

1. Organic compounds results in the biodegradation of these organic moiety to innocuous products,

2. Toxic metals and radionuclides leads to the dissolution, precipitation, stabilization and recover, and generally
3. Aims at volume reduction and stabilization of the wastes.

Various microbial processes are now exploited in the treatment of radiowastes, contaminated soils, and sediment materials as well. Given the high costs and the technical limitations of current chemical-based approaches, there has been an unprecedented interest in the interactions of microorganisms with key radionuclides in the hope of developing cost-effective bioremediation approaches for decontamination of sediments and waters impacted by nuclear waste. Thus, passive *in situ* biological treatment processes that harness natural biogeochemical cycles for key radionuclides are highly desirable. The inventory of radionuclides generated during the past 60 years of operating fission reactors is long and includes  $^{237}\text{Np}$ ,  $\text{Pu}$  isotopes,  $\text{Am}$ ,  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{85}\text{Kr}$ ,  $^{90}\text{Sr}$ ,  $^{99}\text{Tc}$ ,  $^{129}\text{I}$  and  $^{137}\text{Cs}$  in addition to uranium (*e.g.*,  $^{235}\text{U}$ ) from nuclear fuel. Wastes containing some or all of these radionuclides are produced during the many steps of the nuclear fuel cycle, and vary considerably from low-level, high-volume radioactive effluents produced during uranium mining to the intensely radioactive plant, fuel and liquid wastes produced from reactor operation and fuel reprocessing. Most recent work has focused on the bioreduction of  $\text{U(VI)}$ , addressing the development of more efficient remediation technologies through *in situ* studies and *ex situ* laboratory-based sediment experiments. The mechanisms of enzymatic reduction of soluble  $\text{U(VI)}$  by sulfate-reducing bacteria such as *Desulfovibrio* species remain a topic of interest in several laboratories. Several new organisms have been isolated recently that remove uranium from solution. Heterotrophic bacteria were isolated from an acidic uranium-contaminated sediment at the Midnite mine<sup>[29]</sup>. Five isolates and one reference strain, *D. radiodurans*, were studied for uranium accumulation and resistance to the actinide<sup>[69]</sup>.

The clean-up operation at the Fukushima nuclear disaster site in Japan is being given a helping hand from nature. More than three years after the disaster, caused by the 2011 earthquake and tsunami, researchers have found microalgae and aquatic plants are helping to mop up the radioactive waste contaminating the area. The earthquake caused severe damage to the Fukushima 1 Nuclear Power Plant and since then, scientists in Japan have been trying to determine the impact of radioactive contamination on wild and cultivated plants. Published in the Springer, the experts have now concluded that plants and algae are helping to decrease the radiopollution in the area. Yoshihiro Shiraiwa, of the University of Tsukuba, found that 17 microalgae, aquatic plants and algae in the region are able to efficiently remove radioactive cesium, iodine and strontium from the environment. Researchers believe their findings build on existing clean-up operations at Fukushima to decrease the pollution in the area. In December, Japan's Ministry of Environment announced the \$35bn (£21bn) clean-up operation

would take two to three years longer than originally forecast, missing the March 2014 deadline<sup>[70]</sup>.

Thousands of waste sites around the world contain mixtures of toxic chlorinated solvents, hydrocarbon solvents, and radionuclides. Because of the inherent danger and expense of cleaning up such wastes by physicochemical methods, one alternative is to engineer radiation-resistant microbes that degrade or transform such wastes to less hazardous mixtures. The construction and characterization of recombinant *Deinococcus radiodurans*, the most radiation-resistant organism known, expressing toluene dioxygenase (TDO) was described by Lange *et al.*<sup>[71]</sup>. Cloning of the *tod* genes (which encode the multicomponent TDO) into the chromosome of this bacterium imparted to the strain the ability to oxidize toluene, chlorobenzene, 3,4-dichloro-1-butene, and indole. The recombinant strain was capable of growth and functional synthesis of TDO in the highly irradiating environment (60 Gy/h) of a <sup>137</sup>Cs irradiator, where 5×10<sup>8</sup> cells/ml degraded 125 nmol/ml of chlorobenzene in 150 min. *D. radiodurans* strains were also tolerant to the solvent effects of toluene and trichloroethylene at levels exceeding those of many radioactive waste sites<sup>[71]</sup>.

Bacteria found in anoxic sediments are capable of degrading  $\alpha$  and  $\beta$  forms of isosaccharinic acid (ISA) by utilising a range of terminal electron acceptors at circa neutral pH. Under iron reducing, sulphate reducing and methanogenic conditions the degradation of ISA followed the pathway seen in anoxic environments driven by the degradation of polymeric organic materials; *i.e.*, the fermentation of polymer monomers followed by the degradation of fermentation end products by terminal electron accepting processes. The persistence of bacteria commonly associated with the anaerobic degradation of cellulose (the Clostridia) in these batch fed microcosms suggests that they may play an important role in the metabolism of ISA into common fermentation end products allowing electron and carbon flow within these systems. These findings indicate that the ability to degrade ISA is common in near-surface microbial communities and consequently such communities represent a potential source of ISA degrading consortia<sup>[72]</sup>.

*Pleurotus pulmonarius* has degradative capabilities for some categories of solid low and intermediate level radioactive cellulosic wastes. The medium composition beside the surrounding conditions, not only affect the biodegradation capability of the microorganism, but also resulted in variations in the fungi selectivity<sup>[73]</sup>.

The biodegradation process is an enzymatic dependent and was enhanced by exposing the spawns of mushroom to quantified gamma-irradiation doses. The degradation of the solid cellulosic radioactive wastes is accompanied by bioaccumulation of the radionuclides spiked the substrate<sup>[74]</sup>.

Studies of the mechanisms of biotransformation of uranium and toxic metals under various microbial process conditions has resulted in the development of two treatment processes: (i) stabilization of uranium and toxic metals with reduction in waste volume and (ii) removal and recovery of uranium and toxic metals from wastes and contaminated soils. Stabilization of uranium and toxic metals in wastes is accomplished by exploiting the unique metabolic capabilities of the anaerobic bacterium, *Clostridium* sp. The radionuclides and toxic metals are solubilized by the bacteria directly by enzymatic reductive dissolution, or indirectly due to the production of organic acid metabolites. The radionuclides and toxic metals released into solution are immobilized by enzymatic reductive precipitation, biosorption and redistribution with stable mineral phases in the waste. Non-hazardous bulk components of the waste such as Ca, Fe, K, Mg and Na released into solution are removed, thus reducing the waste volume. In the second process uranium and toxic metals are removed from wastes or contaminated soils by extracting with the complexing agent citric acid. The citric-acid extract is subjected to biodegradation to recover the toxic metals, followed by photochemical degradation of the uranium citrate complex which is recalcitrant to biodegradation. The toxic metals and uranium are recovered in separate fractions for recycling or for disposal. The use of combined chemical and microbiological treatment process is more efficient than present methods and should result in considerable savings in clean-up and disposal costs<sup>[75]</sup>.

## CONCLUSIONS

The ease application of microbiological treatment processes for contaminated materials make them more efficient than many other present conventional methods and result in considerable savings in clean up and disposal costs. The mechanisms described the biodegradation of the organic moiety of the hazardous wastes, beside biotransformation of radionuclides and toxic metals in the contaminated substrates and the wastes, are well developed with the view of using such a knowledge-base for innovating novel biodegradation strategies. Therefore, the potential applications of biotechnology in treating contaminated soils, water, and certain forms of radioactive solid and liquid wastes, looks very promising.

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