

Role of microbes in the bioremediation of hydrocarbons, pesticides and municipal wastes

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Abstract

With the rapid explosion of industrialization and human population, the levels of pollutants in the environment have become alarmingly high. Many of these persist in the biosphere for a long period of time and cannot be degraded by physico-chemical methods. Microbes with their nutritional diversity can metabolize a vast range of substrates; thus, microbial degradation has been explored for the purpose of reducing the levels of these persistent pollutants. In general, microbial degradation is preferred because it uses the inherent ability of the microbes to metabolize toxic compounds and hence is cost-effective as well as eco-friendly. The microbes involved are mostly bacteria and fungi from marine and soil environment. It is important to understand the physiology and biochemistry of the microbes as well as microbial consortia involved in order to optimize the process of bioremediation. In this review, we discuss the three major categories of pollutants, viz., hydrocarbons from petrochemical industries, pesticides and municipal wastes including solid wastes and wastewater. For each of these areas of microbial degradation, we discuss the different kinds of wastes generated, limitation of physico-chemical methods, the microbial species or consortium involved in the degradation, insight into the mode of action or mechanisms of enzymatic breakdown and the factors affecting the efficiency of the microbes.

Keywords: Microorganisms; Municipal wastes; Hydrocarbons; Pesticides; Pollutants; Degradation

1. Introduction

The result of industrialization is not just the alarming increase in wastes and pollutants, but also the generation of more complex non-biodegradable pollutants like hydrocarbons, highly potent pesticides and xenobiotics. Combined with the boom in human population, there is also the rapid increase in the generation of municipal wastes, leading to a dire need to scale processes of pollutant removal and break-down into non-toxic or less toxic compounds. If left untreated, these pollutants persist in the environment and threaten the health of all living organisms. So far, the widely accepted solution to curtail this global environmental issue in an eco-friendly and sustainable manner is bioremediation (Roychoudhury and Pradhan 2011). By this process, the metabolic capacity and enzymatic action of a variety of microorganisms are used to detoxify the environmental contaminants. It assures a cheap, simple and eco-friendly clean up method. Microorganisms are nutritionally diverse and have shorter generation times, allowing them greater adaptability, enabling degradation of virtually all kinds of organic matter. They utilize intracellular and extracellular enzymes to convert complex pollutants into carbon and energy source. They also have greater genomic plasticity which enables them to develop new enzymatic pathways for the degradation of xenobiotics.

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The present review aims to analyze and understand the microbial consortia, their mode of action, the biochemistry and the influences of different factors on the process of microbial degradation of hydrocarbons, pesticides and municipal wastes. Such a discussion is necessary as it will provide a road map regarding the processes that can be optimized in future to make bioremediation an effective technique for environmental monitoring and clean up.

2. Role of microbes in hydrocarbon decomposition

Hydrocarbons are compounds consisting of hydrogen and carbon in their structure. One of the major sources of energy for industries and daily life is petroleum-based products. On the other hand, petroleum hydrocarbons are one of the most common and major agents for causing environmental pollution. The processes of extracting, transporting and refining petroleum altogether highly pollute the environment and the leakage of crude oil in these processes primarily cause soil and marine pollution. The annual natural crude oil seepage was estimated to be 600,000 metric tons with a 200,000 metric ton uncertainty (Kvenvolden and Cooper, 2003). If the problem of oil spills is not addressed promptly, it can negatively impact the daily life of people in the neighboring areas and also exert a harmful effect on the soil and water resources in the area. Certain hydrocarbons have been shown to be neurotoxic and carcinogenic to many life forms. The increased pollution endangers the marine ecosystem and disturbs the balance between the habitat and the organisms that reside there. Thus, these species (both plants and animals) show altered patterns of growth and reproduction, anatomical distortions and heightened sensitivity to hypothermia. Additionally, such plants and animals may cause more negative impacts through biomagnification when they enter the food chain. Most of the oil pollution is caused by oil spills (large scale or small scale) that are documented every year. A list of the major oil spills over the years is represented in Table 1.

Table 1 Major oil spills in history

Name of the Oil Spill	Date of Occurrence	Location	Amount of oil spilled (Million gallons)
Gulf war Oil spill	January 19, 1991	Persian Gulf, Kuwait	382-520
BP Deepwater Horizon Oil spill	April 22, 2010	Gulf of Mexico	206
Ixtoc I Oil spill	June 3, 1979	Bay of Campeche, Mexico	140
Atlantic Emoress Oil spill	June 19, 1979	Coast of Trinidad and Tobago	90
Kolva River Oil spill	August 6, 1983	Kolva River, Russia	84

Although there are several chemical and physical methods to treat petroleum hydrocarbons, these methods are expensive and fail to remove all contaminants. An ever-evolving technique to remove and degrade various environmental pollutants, including the ones produced by the petroleum industry is thus, the cost-effective process of bioremediation. It employs microorganisms to detoxify or remove contaminants by exploiting their metabolic pathways. It leads to complete or partial mineralization of organic pollutants into carbon dioxide, water and inorganic compounds. The pollutant molecules are used as an energy source and help in synthesizing the cellular components of the microorganisms.

3. Enzymes involved in hydrocarbon degradation

Scientists have identified the enzymes and mechanisms involved in the microbial breakdown of hydrocarbons over time. The heme-thiolate monooxygenases called cytochrome P450 alkane hydroxylases is a ubiquitous enzyme, participating in the microbial breakdown of oil, chlorinated hydrocarbons and fuel additives. The enzyme systems are important to provide oxygen to the substrate to start biodegradation of petroleum hydrocarbons. Higher eukaryotes often have a variety of P450 families, each of which contains a huge number of distinct P450 forms that form an ensemble of isoforms and contribute to the metabolic conversion of the given substrate. In microorganisms, the chances of finding such P450 multiplicity are low. Numerous microsomal cytochrome P450 help several yeast species to utilize n-alkanes and other aliphatic hydrocarbons as their chief carbon and energy sources. The yeast species *Candida maltosa*, *Candida tropicalis* and *Candida apicola* were used in the isolation of the enzyme cytochrome P450 (Van Beilen and Funhoff, 2007). Tables 2 and 3 represent, respectively, the petroleum-degrading microbes and enzymes involved in hydrocarbon degradation.

Table 2 Petroleum degrading microbes and their preferred substrates

Petroleum hydrocarbon components	Bacterial species	Main degrading compound	Reference
Aromatic hydrocarbons	<i>Neptunomonas naphthovorana</i>	Polyaromatics	Hedlund <i>et al.</i> , 1999
	<i>Cycloclasticus</i>		Kasai <i>et al.</i> , 2002
	<i>Pseudomonas aeruginosa</i>	Monoaromatics	Mukherjee <i>et al.</i> , 2017
	<i>Mycobacterium cosmeticum</i>	Monoaromatics	Zhang <i>et al.</i> , 2013
	<i>Aeribacillus pallidus</i>	Mono-/poly-aromatics	Mnif <i>et al.</i> , 2014
	<i>Achromobacter xylosoxidans</i>	Mono-/poly-aromatics	Ma <i>et al.</i> , 2015
	<i>Bacillus licheniformis</i> <i>Bacillus mojavenensis</i>	Poly-aromatics	Eskandari <i>et al.</i> , 2017
Saturated hydrocarbons	<i>Pseudomonas</i> sp.	n-alkanes (C14–C30)	Sugiura <i>et al.</i> , 1997
	<i>Alcanivorax</i> sp.	n-alkanes and branched alkanes	Hara <i>et al.</i> , 2003
	<i>Oleispira antarctica</i>	n-alkanes (C10–C18)	Yakimov <i>et al.</i> , 2003
	<i>Rhodococcus ruber</i>	n-alkanes (C13–C17)	Zhukov <i>et al.</i> , 2007
	<i>Rhodococcus</i> sp.	Cyclohexane	Lee and Cho, 2008
	<i>Dietzia</i> sp.	n-alkanes (C6–C40)	Wang <i>et al.</i> , 2011
	<i>Geobacillus thermodenitrificans</i>	n-alkanes (C15–C36)	Abbasian <i>et al.</i> , 2015
	<i>Gordonia sihwensis</i>	Branched and normal alkanes	Brown <i>et al.</i> , 2016
Resins	<i>Pseudomonas</i> spp.	Resins	Venkateswaran <i>et al.</i> , 1995
Asphaltenes	<i>Pseudomonas</i> spp., <i>Bacillus</i> sp.	Asphaltenes	Tavassoli <i>et al.</i> , 2012
	<i>Citrobacter</i> sp., <i>Enterobacter</i> sp., <i>Staphylococcus</i> sp., <i>Lysinibacillus</i> sp., <i>Bacillus</i> sp., <i>Pseudomonas</i> sp.	Asphaltenes	Jahromi <i>et al.</i> , 2014

Table 3 Enzymes involved in hydrocarbon degradation

Enzymes	Substrate	Microorganism	Reference
Alkane hydroxylase, lipase and esterase	Hexane, hexadecane and motor oil	<i>Alcanivorax borkumensis</i>	Kadri <i>et al.</i> , 2018
Eukaryotic P450	C10–C16 alkanes, fatty acids	<i>Candida maltose</i> , <i>Candida tropicalis</i> , <i>Yarrowia lipolytica</i>	Iida <i>et al.</i> , 2000
<i>AlkB</i> -related alkane hydroxylases	C5–C16 alkanes, fatty acids, alkylbenzenes, cycloalkanes, etc.	<i>Acinetobacter</i> , <i>Alcanivorax</i> , <i>Burkholderia</i> , <i>Mycobacterium</i> , <i>Pseudomonas</i> , <i>Rhodococcus</i> , etc.	Van Beilen and Funhoff, 2007

Alkane oxygenase systems in eukaryotes and prokaryotes, including cytochrome P450 enzyme, soluble di-iron methane monooxygenases, integral membrane di-iron alkane hydroxylases (such as *alkB*), membrane-bound copper-containing

methane monooxygenases, lipase and esterase are actively involved in the degeneration of alkanes under aerobic conditions (Van Beilen and Funhoff, 2005; Das and Chandran, 2011; Kadri *et al.*, 2018).

4. Mechanism of petroleum hydrocarbon degradation

Most organic pollutants degrade quickly and completely in an aerobic environment. The initial oxidative intracellular attack of organic pollutants is the primary enzymatic reaction that is catalyzed by oxygenases and peroxidases. Both the activation and incorporation of oxygen occur during this step. Using the tricarboxylic acid (TCA) cycle as an example, Das and Chandran (2011) described how the pathways of peripheral degradation change the organic pollutants progressively in the intermediates of the central intermediary metabolism. The metabolites of the primary precursors like acetyl-coA, pyruvate and succinate are used in the production of cell biomass. Through gluconeogenesis, the saccharides required for many types of biosynthesis and development are created. According to Rahman *et al.* (2003), there are three potential ways to degrade petroleum hydrocarbons: using a particular enzyme system, microbial cell adhesion to substrates and producing biosurfactants.

According to Varjani and Upasani (2016), a single strain of microbe or a microbial consortium of strains, belonging to the different genera can preferentially metabolize petroleum hydrocarbons. According to Al-Hawash *et al.* (2018), the consortium demonstrates that it is capable of decomposing or metabolizing petroleum hydrocarbons more effectively than individual cultures. The degradation of diesel by HDMP2 has most recently been separated into three stages: surface adsorption, cell absorption and biodegradation. The early stage of the process revealed that the diesel components are immediately absorbed on the surface of HDMP2 (Dias *et al.*, 2012). The majority of the components of diesel were thereafter continually collected, with the exception of a little amount that was put into a cell, and they were ultimately broken down into C_nH_n , CO_2 and H_2O (Yang *et al.*, 2020).

There are four major stages in the microbial breakdown of petroleum hydrocarbons. First, the microorganisms secrete petroleum pollutant-emulsifying surfactants. Next, the emulsified product is adsorbed to the microorganism surface. The petroleum hydrocarbon adsorbed on the cell membrane thereafter enters the cell membrane via active or passive transport and endocytosis. In order to accomplish the goal of decomposing the pollutant, the petroleum hydrocarbon entering the cell undergoes an enzymatic reaction with the appropriate enzyme (Lawniczak *et al.*, 2020).

One of the most important aspects of remediation of pollution is the capacity of microorganisms to degrade petroleum hydrocarbons. Alkanes can be degraded through single-end oxidation, double-end oxidation or secondary-end oxidation. Alcohol, aldehyde, acid and CoA are produced when these three oxidation modes are applied at various points along the carbon chain. Following that, the oxidation is finished, producing energy for microbial life (Li *et al.*, 2019).

The degradation of cycloalkane is slightly different than that of paraffins. The hydroxylase first causes the cycloalkane to oxidize to create a cycloalkanol. To synthesize naphthenic ketone, the cycloalkanol removes the hydrogen from the mixture. The naphthenic ketone is transformed into caprolactone by the ketoxygenase. The caprolactone ring is then broken by the introduction of a H_2O molecule to the ring. This then results in the formation of 6-hydroxycaproic acid that is further oxidized to 6-oxohexanoic acid by the action of 6-hydroxycaproate dehydrogenase (Waikhom *et al.*, 2020). Aldehyde dehydrogenase easily catalyzes the oxidation of aldehydes to produce dicarboxylic acid. To maintain the life activity of the microorganism, the dicarboxylic acid is ultimately oxidized via the TCA cycle. Although it is claimed that co-metabolism and symbiosis play a role in the degradation of naphthenes, this often only applies to the degradation of terpenes, which are more difficult to degrade than alicyclic hydrocarbons.

Olefins can be degraded via a variety of techniques. The double bond undergoes oxidation to a ketone. The microbe then converts the ketone to an ester through esterification. The main alcohol and fatty acid are the two byproducts of the subsequent cleavage reactions of the ester bond. The epoxides created when olefins are added to aldehydes or ketones are directly oxidized by microbes, and they are then gradually oxidized so that they can be utilized by the microorganisms without producing intermediate alcohols. In order to produce energy through oxidative breakdown, some linear paraffins are also directly dehydrogenated by the microbe to produce olefins, which are then further oxidized into alcohols, aldehydes and acids by the catalytic action of the enzyme (Waikhom *et al.*, 2020). Compared to other hydrocarbons, aromatic hydrocarbons have a more difficult degradation mechanism. Due to their many sites of degradation, the same aromatic hydrocarbons will have various degradation sites. For example, there are many pathways for phenanthrene breakdown in *Arthrobacter* and *Myobacterium*. They open at various points relative to the phenanthrene ring and can result in the formation of compounds like 2-hydroxy-1-naphthoic acid and 2,2-biphenyldioic acid. Different polycyclic aromatic hydrocarbons (PAHs) degrade in much the same ways. First, a ring of PAHs is oxidized with the help of a microenzyme in a microbe to form a trans-dihydro-dihydroxy compound which is dehydrogenated to form a thiol epoxide. Salicylic acid, catechol and other compounds are produced as a result of the epoxidative cleavage

of diol into an alcohol with less than one ring. After further oxidation, the end product like gentisic acid passes through alkane oxidation pathway to produce a carboxylic acid, which then combines with CoA to produce acetyl-CoA, which supplies energy for self-metabolism. As the number of benzene rings rises, it becomes increasingly difficult for microorganisms to destroy PAHs with more than four rings. Co-metabolism is the only process that can oxidatively degrade these compounds. Complex PAHs are also synthesized in several ways. Existing biotechnological approaches can only be used to degrade low concentrations of PAHs, since intermediate products like trans-thiols, phenols, naphthalenes and epoxides are poisonous to microorganisms.

5. Microorganisms degrading the hydrocarbons

In the environment, bacteria and fungi are principally responsible for biodegrading the hydrocarbons. Although common in both terrestrial and aquatic ecosystems, the proportion of the total heterotrophic community made up of hydrocarbon-using bacteria and fungi varies greatly, with the reported frequencies for soil fungi, ranging between 6% and 82% and for soil bacteria between 0.13% and 100%. As a result of the limited range of hydrocarbon substrates that can be metabolized by the individual organisms, complex mixtures of hydrocarbons such as crude oil must be broken down in soil, freshwater and marine environment by assemblages of mixed populations with generally broad enzymatic capacities. According to the published reports, *Achromobacter*, *Acinetobacter*, *Alcaligenes*, *Arthrobacter*, *Bacillus*, *Flavobacterium*, *Nocardia*, *Pseudomonas spp.* and the coryneforms are the most significant hydrocarbon-degrading bacteria in both soil and marine environment; the importance of hydrocarbon-degrading strains of the salt-requiring *Vibrio spp.* is restricted to the marine environment. The majority of the isolates (95%) were discovered to be *Pseudomonas*, *Micrococcus* and *Nocardia spp.*, members of the family Enterobacteriaceae, actinomycetes and coryneforms. According to Austin et al. (1977), the most common soil isolates of the fungi are *Trichoderma* and *Mortierella spp.*, while the most common marine isolates are *Aureobasidium*, *Candida*, *Rhodotorula*, *Sporobolomyces spp.*, *Aspergillus* and *Penicillium spp.* that break down the hydrocarbons. The marine, beach-adapted genera, viz., *Corollospora*, *Dendryphiella*, *Lulworthia* and *Varicosporina* are included in the list, based on the research of Kirk and Gordon (1988). The ecosystem and regional environmental factors appear to have an impact on how much bacteria, yeasts and filamentous fungi contribute to the biodegradation of hydrocarbons. Bacteria are typically thought to comprise the main hydrocarbon-degrading component of the microbial population in the marine environment. According to Floodgate (1984), fungi make up a small portion of the marine microflora, but they are becoming more prevalent in salt marshes and mangrove areas, nearshore regions and intertidal zones. According to the existing reports, fungi are also significant occupants of specialized habitats including submerged wood, surface film of water, decomposing algae and surface of tar balls. The level of hydrocarbon breakdown, carried out by bacteria and fungi in the marine environment, has seldom been directly compared. At 0 and 5^o C, all petroleum utilization was accounted for by Chesapeake Bay bacteria. At 10^o C, yeasts, but not filamentous fungi, utilizes petroleum.

6. Factors influencing degradation of petroleum hydrocarbon

The biodegradation of petroleum hydrocarbons has been observed to be limited by a number of reasons. The quality and quantity of the hydrocarbon mixture and its constituent features directly influence the environmental factors, our ecology and enzymes having effect on pollutants arising from petroleum. The factors are listed below:

6.1. Temperature

Temperature has an impact on the physical state of hydrocarbons as well as the bacteria that dwell there. Additionally, it impacts the rate of microbial development, gas solubility, soil matrix, metabolism in microorganisms and the physical and chemical condition of the pollutants. According to research by Aislabie et al., (2006), a rise in temperature increases the solubility of the hydrophobic contaminants, reduces viscosity, improves diffusion and moves the long-chain n-alkanes from the solid phase to the liquid phase. The start of biodegradation at low temperature is delayed by an increase in oil viscosity, a decrease in hazardous short-chain alkane volatilization and a decrease in their water solubility. According to Cui et al., (2020), petroleum hydrocarbon degradation rates gradually increased with temperature increase until they reached their optimum levels.

6.2. Nutrients

The type and concentration of carbon and nitrogen sources utilized in the culture medium are crucial for microbial growth. In order for microbes to incorporate these nutrients into biomass, they must be present in the same environment as hydrocarbons. Nitrogen demand is crucial for biodegradation of hydrocarbons, much like the biological oxygen demand. Varjani (2014) examined the fact that some nutrients such as nitrogen and phosphorus, needed for microbial growth, are not present in petroleum hydrocarbons in considerable amounts. However, the ratios of carbon,

nitrogen, phosphorus and potassium (C-N-P-K) can be altered using urea, phosphate, N-P-K fertilizers, ammonium and phosphate salts. C:N:P is typically 100:10:1 to encourage microbial development. The addition of nitrogenous fertilizers results in faster rates of biodegradation of petroleum hydrocarbons. In contrast, the addition of a nitrogen source has no discernible impact on the biodegradation of petroleum hydrocarbons (Atlas 1981, 1984, 1995, 1998); Atlas and Bartha (1992). According to Toda and Itoh (2012), nitrate is the best source of nitrogen for microorganism growth and formation of biosurfactants. According to Walworth et al. (2005), applying nitrogen to a petroleum-polluted site speeds up cell proliferation and hydrocarbon breakdown by reducing the lag phase of microbial growth and keeping microbial populations active. However, it has been demonstrated that too much nitrogen in soil inhibits microbial growth. For the best biodegradation of hydrocarbon pollutants, nitrogen level should be kept below 1800 mg/kg H₂O. Excessive nutrient levels, particularly high NPK levels, prevent hydrocarbon pollutants from degrading naturally. Furthermore, a high concentration of these pollutants in polluted areas disturbs the C:N:P ratio, which impairs oxygen availability (Walworth et al., 2007).

6.3. Oxygen

Oxygen is the most important element for the biodegradation of hydrocarbon. The initial stage of petroleum degradation begins when oxygen is present, and oxygen is necessary for the entire degradation process. Because soil bacteria need molecular oxygen for respiration during the whole degradation pathway and aerobic conditions use a substantial amount of oxygen, oxygen is sufficient for the decomposition of hydrocarbons. 1 mL of hydrocarbon is primarily broken down into water and carbon dioxide, using 3-4 mL of oxygen. Due to the high levels of carbon and hydrogen, but low levels of carbon dioxide in petroleum, the biodegradation process uses a significant amount of oxygen. Due to interaction of air, water, wave action and wind, oxygen concentration in lakes and oceans is high on the surface, but decreases as depth increases. In deep water, low oxygen concentration causes anaerobic breakdown process. If petroleum is dispersed widely and deeply underground, it will take longer to degrade. Due to many mechanical processes used to remove oil and create a boundary for oxygen penetration, the presence of oil pollutants on the water surface prevents the reclamation of oxygen needed for the degradation process. The ability of the microbe to degrade, the kind of soil, the concentration of moisture and the presence of oxygen all affect oil transportation. For the biodegradation of petroleum hydrocarbons, each of these requirements is crucial. However, depending on the microbes used in the degradation, the oxygen concentration will be high. 3.1 mg/mL of oxygen is used by aerobic bacteria to break down 1 mg/mL of hydrocarbon without the requirement of any additional organisms (Curtis and Lammey, 1998).

6.4. pH

It has been stated that the ideal pH for mineralization of oil sediment ranges from 5.0 to 7.8. The majority of the heterotrophic fungi work in acidic conditions. The pH of many hydrocarbon-contaminated areas, such as grass work sites with notable amounts of bricks and concrete, is not optimal for bioremediation. Due to the oxidation and release of sulphides, coal leaching and oxidation, an acidic condition is generated. Microbes cannot breakdown PAHs in an environment that is highly alkaline or acidic.

6.5. Salinity and pressure

Salinity inhibits the growth of microbes as well as formation of their byproducts. According to Atlas (1998), salinity and pressure are the distinctive characteristics of common ecosystems like salty lakes or deep seas, which represent a specific environment, contaminated by the petroleum hydrocarbons. According to Minai-Tehrani et al. (2006), 41% of the crude oil is degraded over the course of four months in soil samples without the addition of NaCl, whereas 12% of crude oil degraded over the same period of time in the same soil samples with the addition of 50 g/L NaCl. At high pressures of 495 atm or 500 atm, experiments for the biodegradation of hexadecane, tetradecane and mixed hydrocarbon substrate, employing a mixed culture of deep-sea sediment bacteria, were carried out. It was observed that hydrocarbon pollutants may contaminate deep benthic zones of the sea when they enter the deep ocean environment. According to Varjani and Upasani (2016), the refractory component of petroleum oil that might remain for a long time (which could be years or decades) causes these contaminants in benthic zones of sea to biodegrade very slowly.

6.6. Substrates and properties

The concentration of petroleum hydrocarbon affects its mineralization (Leahy and Colwell, 1990). The maximum degrading rates and cumulative extents of mineralization rise with higher petroleum oil content, while the lag phase decreases (Towell et al., 2011). The degradability of petroleum hydrocarbons is also influenced by their composition. Linear alkanes are more biodegradable than branched alkanes, low-molecular weight alkyl aromatics, monoaromatics, cyclic alkanes, polyaromatics and asphaltenes in that order. Extremely high full oil hydrocarbon fixations have been

found to be toxic to microbes, which limits the possibility for biodegradation (Admon et al., 2001). Extremely low total petroleum hydrocarbon concentrations can impede biodegradation because they may not provide enough carbon to allow microbial development. Usually, the molecular weight and chemical complexity of a hydrocarbon enhance the biodegradation rate. The same compound(s) in different petroleum crude is degraded to different extents by the same or other organisms/consortium. This can be an effect of bioavailability of the compound(s). An effective bioremediation process depends on the physicochemical characteristics of crude oil and polluted areas (Varjani et al. 2014a, b). The kind, quantity and metabolic activities of every ecosystem microflora are directly influenced by these variables (Admon et al. 2001, Ghazali et al. 2004). Less complex molecules are preferred by hydrocarbon degraders. The makeup of pollutants has an inherent impact on biodegradability. Crude oil that has saturates and/or aromatics as its main constituents are biodegradable; nonetheless, under ideal conditions for microbe growth, only around 11% of heavy asphaltic-naphthalene crude oil may biodegrade in an acceptable amount of time.

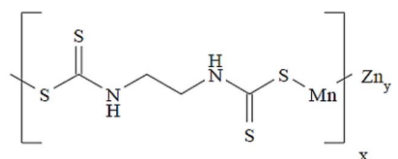
7. Role of microbes in pesticide degradation

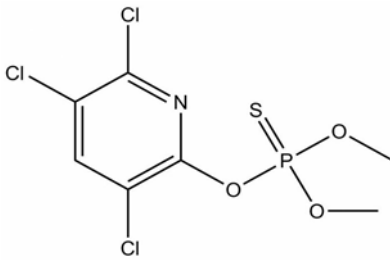
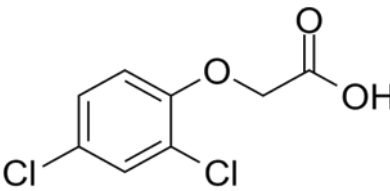
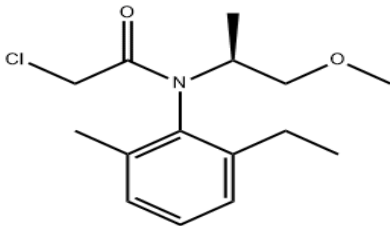
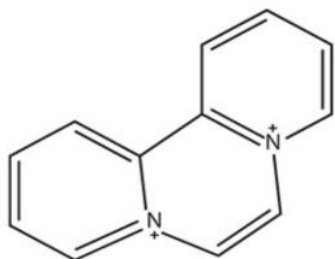
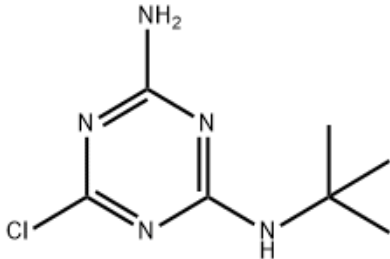
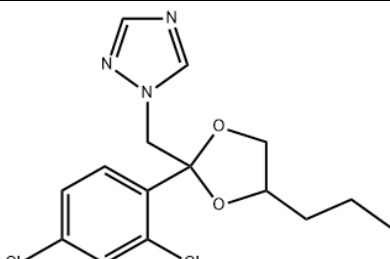
Pesticides are widely used to protect crops from diseases and pest attacks. It has been seen in agricultural studies that natural growth and storage methods of crops can lead to significant loss in yield. For example, China loses about 8.8% of its entire grain output (which is about 40 million tons), to different pest attacks every year. In another instance, India's total grain output stands at about 250 million tons, but it loses 11-15% of it or 27.5-37.5 million tons every year to pests. Thus, to avoid such losses, the amount of pesticides used has steadily increased over the decades. According to Food and Agriculture Organization (FAO), between 1990 and 2018, the amount of pesticides used globally has increased from 1.7 to 2.7 million tonnes (Arbeli and Fuentes 2010; Hugo et al. 2016).

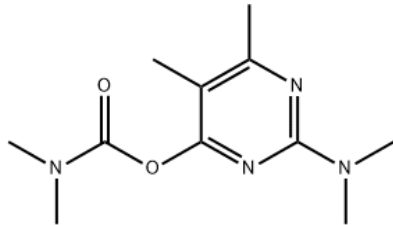
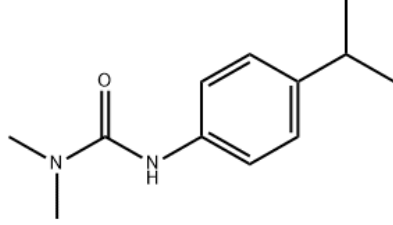
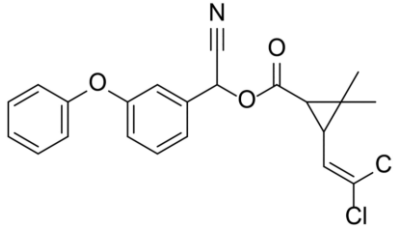
However, while the loss of crops has been reduced, pesticides are a major threat to the environment and cause massive pollution. They pollute the soil and crops, disturbing the natural microbiome, posing hazards to humans, aquatic biota and polluting drinking water. It is seen that pesticides are ubiquitously present in concentration, ranging from ng/liter to µg/liter in groundwater and surface water and even get washed off to marine bodies. Therefore, it becomes imperative to control environmental residues of pesticides and explore modes of its degradation.

Pesticides can undergo three major degradative processes, viz., microbial degradation, chemical hydrolysis and photolysis. While degradation by microbes is a biotic transformation process, chemical hydrolysis and photolysis are abiotic processes. The degradation pathway that a pesticide undergoes depends on its structure, which renders its affinity towards some processes, and also the environmental conditions it is subjected to by virtue of its distribution and transport behavior (Pal et al. 2006). For instance, photolysis or photochemical transformation of pesticides only occurs in areas where sunlight can penetrate like the topmost meters of water bodies, plant surfaces or submillimeter layers of the soil. However, a major drawback of the chemical processes is that it would cause secondary pollution. For example, cypermethrin and other pyrethroid pesticides, degraded under natural conditions, would lead to the production of an intermediate 3-phenoxy benzoic acid (3-PBA). 3-PBA would accumulate in the environment and appear hazardous to the ecological environment and human health than its parent pesticide (Singh 2008).

Table 4 Main environmental degradation routes for top 10 pesticide classes (adopted from Fenner et al., 2013)

Pesticide category	class	Major representative active substance	Major use	Percentage of global pesticide use	Main environmental degradation route
Dithiocarbamates		 Mancozeb	Fungicide	7.1	Acid-catalyzed hydrolysis; formation of potential N-Nitrosodimethylamine (NDMA) precursors

Organophosphates	 <p>Chlorpyrifos</p>	Insecticide	6.7	Microbial transformation (oxidation and hydrolysis)
Phenoxy alkanolic acids	 <p>2,4-D</p>	Herbicide	4.7	Microbial transformation (oxidative dealkylation and aromatic ring cleavage)
Amides	 <p>S-metolachlor</p>	Herbicide	4.2	Microbial transformation (hydrolysis and glutathione coupling)
Bipyridyls	 <p>Diquat</p>	Herbicide	3.2	Slowly biotransformed due to strong sorption to soil matrix
Triazines	 <p>Terbutylazine</p>	Herbicide	2.3	Microbial transformation (oxidative dealkylation and hydrolysis)
Triazoles, diazoles		Fungicide	2.0	Slow microbial transformation (oxidation); phototransformation of specific representatives

	Propiconazole			
Carbamates	 Pirimicarb	Insecticide/herbicide	2.0	Microbial or base-catalyzed transformation (hydrolysis of ester bond); phototransformation of specific representatives
Urea derivatives	 Isoproturon	Herbicide	1.7	Microbial transformation (oxidative dealkylation and hydrolysis)
Pyrethroids	 Cypermethrin	Insecticide	1.3	Microbial transformation (hydrolysis, oxidation); phototransformation (direct and indirect)

The role of microbes in the degradation of pesticides was first observed in the 1940s. It was seen that repeated applications of 2,4-dinitrophenol (2, 4-DNP) in soil environment led to lower efficacy due to metabolic adaptations of the microbes which eventually led to its degradation (Audus, 1949). One of the consequences of these studies was the emphasis on developing the ways of degrading pesticide residues. As a result, recalcitrant pesticides (mainly chlorinated compounds) were replaced by degradable pesticides (mainly organophosphorus and carbamate compounds) (Padhye et al. 2013). For instance, organochlorine pesticides were found to be stable and easily taken up by the organisms and humans by means of food. These included hexachlorocyclohexane (666), 1,1,1-trichloro-2,2-bis(p-chlorophenyl) ethane (DDT) and other organochlorine pesticides. The common pesticides degraded through microbial transformation is represented in Table 4.

8. Microbial species or consortium involved in pesticide degradation

Over the years, a host of microbial flora have been studied for their ability to degrade pesticides, including bacteria, fungi, actinomycetes and algae among others. Kafilzadeh et al. (2015) studied bacteria for the purpose of endosulfan degradation. Bacteria were screened and isolated from natural sediments and water samples from agricultural areas. Five bacterial genera were found that could degrade endosulfan efficiently, viz., *Klebsiella*, *Acinetobacter*, *Alcaligenes*, *Flavobacterium* and *Bacillus*. Jayabarath et al. (2010) tested 319 strains of *Actinomycetes* for their tolerance to the pesticide carbofuran and concluded that only seven of those strains could grow in presence of and degrade the pesticides effectively. The seven strains included *Streptomyces alanosinicus*, *Streptoverticillium album*, *Nocardia farcinia*, *Streptomyces atratus*, *Nocardia vaccini*, *Nocardia amarae* and *Micromonospora chalcea*. Elgueta et al. (2016) studied the action of white-rot fungi in the degradation of atrazine and found that the half-life of atrazine reduced drastically to six days. Kabra et al. (2014) also studied atrazine degradation by green microalga *Chlamydomonas mexicana*. It was observed that the microalga could accumulate atrazine in its cells, followed by degradation at a rate of 14-36%.

Table 5 Common pesticide-degrading microorganisms (Huang et al., 2018)

Types of microorganism	Species	Pesticides degraded
Bacteria	<i>Pseudomonas</i>	Aldrin, chlorpyrifos, coumaphos, DDT, diazinon, endosulfan, endrin, hexachlorocyclohexane, methyl parathion, monocrotophos, parathion
	<i>Bacillus</i>	Chlorpyrifos, coumaphos, DDT, diazinon, dieldrin, endosulfan, endrin, glyphosate, methyl parathion, monocrotophos, parathion, PAHs
	<i>Alcaligenes</i>	Chlorpyrifos, endosulfan
	<i>Flavobacterium</i>	Diazinon, glyphosate, methyl parathion, parathion
Actinomycetes	<i>Micromonospora, Actinomyces, Nocardia, Streptomyces</i>	Aldrin, carbofuran, chlorpyrifos, diazinon, diuron
Fungus	<i>White rot fungi, Rhizopus, Cladosporium, Aspergillus fumigatus, Penicillium, Aspergillus, Fusarium, Mucor, Trichoderma spp, Mortierella sp.</i>	Alachlor, aldicarb, atrazine carbofuran, chlordane, chlorpyrifos, DDT, diuron, endosulfan, esfenvalerate, fenitrothion, fenitrooxon, fipronil, heptachlor epoxide, lindane, malathion, metalaxyl, pentachlorophenol, terbuthylazine, 2,4-D
Algae	<i>Small green algae</i>	Phorate, parathion
	<i>Chlamydomonas</i>	Atrazine, fenvalerate
	Diatoms	DDT, patoran

Microorganisms commonly involved in pesticide degradation are listed in Table 5. It is noteworthy that, of the microbes used commonly to degrade pesticides, the majority are bacteria. All of these microbes succeed in pesticide degradation due to their ability to utilize organophosphate pesticides as sources of carbon and phosphorus due to the presence of hydrolyzing enzymes that can break down phosphotriesters. Bacteria are more likely to harbor these enzymes due to their strong adaptability and ability to induce mutations. Bacteria have short generation times and high plasticity that leads them to express genes, responsible for degradation at a higher rate. In addition, within the same microbial population, horizontal transfer of genes can occur, leading to the spread of newly evolved biodegradation pathways. By virtue of this, bacteria can degrade nearly all known organic materials (Johnson and Spain, 2003) and are thus an excellent choice for biodegradation of pesticides.

Some pesticide transformations can occur by both biotic and abiotic processes. Dechlorination of atrazine to hydroxyatrazine was assumed to be carried out by abiotic processes until atrazine-dechlorinating enzymes in bacteria were recognized that could catalyze the same reaction with a second-order rate constant of $10^5 \text{ M}^{-1} \text{ s}^{-1}$. Comparing this to the rate of abiotic transformation revealed that under the natural environment, enzyme-catalyzed microbial degradation was the dominant process (Lindsey, 2018, pp. 44–46).

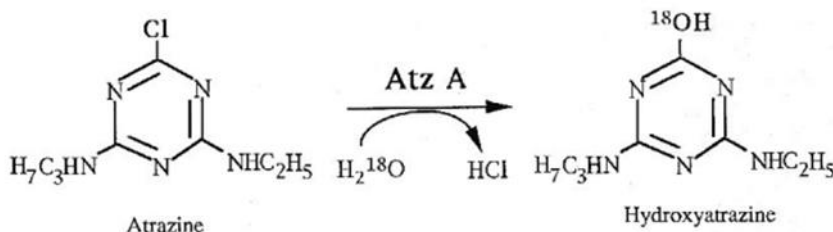
However, even though biotic processes are often dominant and microbes can generally degrade pesticides completely, there might still occur accumulation of biotransformation products in the soil or environment. Taking the same example of atrazine metabolism, hydroxyatrazine is further metabolized to carbon dioxide and ammonia. However, studies have shown, in both whole cell (Devers et al., 2008) and using purified enzymes (Seffernick et al., 2007), that the enzyme producing hydroxyatrazine acts at a faster rate than the downstream enzyme responsible for its removal. As a result, hydroxyatrazine may accumulate to substantial levels. Another reason why pesticides may persist over decades despite degradative microbe being present in abundance is due to threshold concentration. This refers to the phenomenon where below a threshold concentration of pesticides, microbial degradation pathways stall (Egli, 2010).

9. Mechanisms of enzymatic breakdown

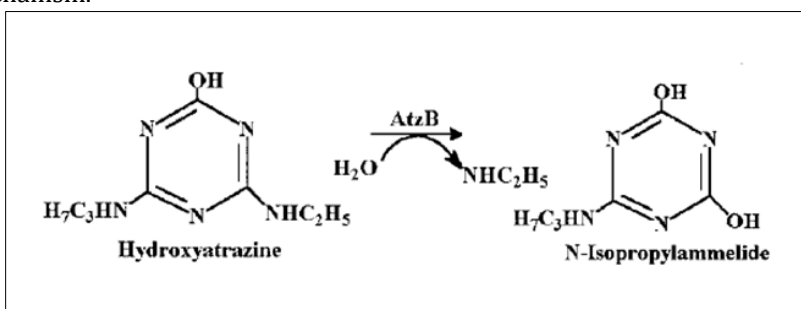
Microbial degradation uses enzymatic action to use the pesticides as sources of nutrients and breaking it down to intermediates. They further breakdown the biotransformation products to small, non-toxic molecules like carbon-

dioxide and water. The reaction proceeds first by internalization of the compound within the body of the microbe and then a series of enzyme-catalyzed physiological and biochemical reactions take place until the pesticide is completely degraded and forms smaller compounds which are either non-toxic or have less toxicity.

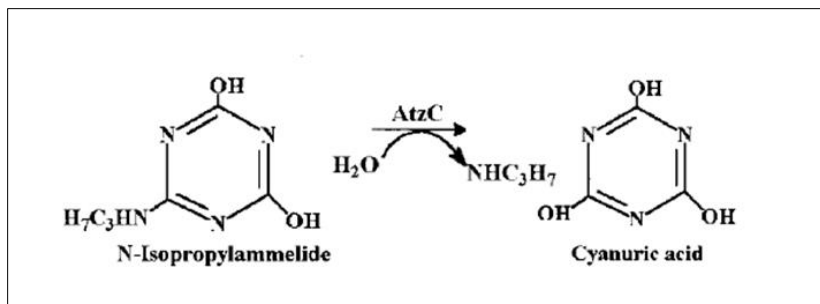
For example, enzymatic degradation of atrazine by *Pseudomonas* sp. strain ADP occurs by means of three enzymes acting upon it successively (deSouza et al. 1996).



- In the first step, the enzyme AtzA catalyzes the dechlorination of atrazine to non-toxic hydroxyatrazine by a hydrolytic mechanism.



- The second enzyme AtzB catalyzes the dehydrochlorination of the hydroxyatrazine to produce N-isopropyl cyanuric amide.



- The third enzyme AtzC forms cyanuric acid which is eventually degraded to carbon dioxide and ammonia (Wackett et al. 2002).

The entire degradation mechanism of a pesticide by a microbe can be divided into three segments. Firstly, the target compound, i.e., the pesticide is adsorbed on to the cell membrane surface. Second, internalization of the compound occurs through the cell membrane into the cell. Finally, specific enzyme-catalyzed reactions are directed to degrade the xenobiotic compound.

Degradation pathways can be chemically of many types including:

- Oxidation - this includes hydroxylation reactions, such as aromatic hydroxylation, aliphatic hydroxylation, N-hydroxylation, epoxidation, N-oxidation, P-oxidation, S-oxidation, oxidative dealkylation, oxidative dehalogenation and oxidative deamination
- Reduction of nitro group, quinone reduction and reductive dehalogenation
- Hydrolysis of some esters such as thiophosphate, thiocarbamate, etc.

- Other mechanisms include dehydrogenation, dehalogenation, decarboxylation, condensation, synthesis and so on.

Further degradation of pesticides occurs either by one of these two main mechanisms, viz., mineralization or co-metabolism. Mineralization broadly describes the conversion of organic compounds to inorganic compounds by the soil microbes. In such cases, the microbe could even uptake the pesticide as a nutrient source and degrade it to simple inorganic compounds like carbon dioxide, water or ammonia. This process of degradation is ideal since the products formed are completely non-toxic and leave no residues. In co-metabolism, the pesticide is a chemical substance that is not available under natural conditions and the microbe cannot degrade it easily. It requires an additional amount of exogenous organic matter to serve as the primary energy source. The degradable products in this case may not be completely mineralized. Thus, in most cases, a synergistic effect of a series of reactions is required to completely degrade a pesticide rather than a single reaction.

10. Factors affecting microbial degradation rates

The rate of biodegradation in soil depends on a large number of variables which can broadly be divided into internal and external factors. While external factors refer to the environmental conditions, internal factors comprise the physiological status and its adaptability which largely originate from the structure of the pesticide. Specifically speaking, the factors affecting the degradative rates of pesticides are physicochemical properties of the pesticide (e.g., structure, concentration, water solubility, sorptive affinity), characteristics of the soil (pH, organic matter content, microbial biomass, redox status, moisture content, salinity level), environmental conditions (temperature, moisture) and management practices (application rate, formulation type). It is also important that there is sufficient microbial biomass and sustainable populations of the microorganisms. For effective rates of bioremediation, it becomes important to maintain niche microhabitats where the microbes can be exploited to the maximum.

11. Microbial species and their metabolic activity and adaptability

It has been observed in studies, that different microbial species or even different strains of the same species have different reactions and capacity to degrade the same organic substrate. The microorganisms also have a strong ability to adapt and thus develop new enzyme systems to degrade a new chemical that it could not metabolize. In a specific mode of adaptation, it was observed that cross-acclimatization of microbes to pesticides also took place. This refers to the ability of microbes to degrade a new kind of pesticide due to exposure to a different pesticide, usually from the same family (Singh et al., 2005). For example, soil with microbes that have acclimatized to carbofuran can degrade other carbamate pesticides such as bendiocarb, carbaryl, cloethocarb, isoprocarb and propoxur at accelerated rates (Read, 1987; Racke and Coats, 1988)

12. Pesticide structure

The structure of a pesticide molecule determines its inherent biodegradability. Its molecular weight, stereochemistry, the number and type of substituents and location of substitutions can all vastly alter the rates of degradation. Sometimes, even minor alterations in structure cause a drastic change in the biodegradation rates of the compound. The differences in the position or nature of substituents in the pesticides of the same class change their rate of degradation. Polar groups such as OH, COOH and NH₂ may act as a site of attack for the microbial enzymes. Halogen or alkyl substituents tend to make the molecule more resistant to biodegradation. As observed in chlorinated hydrocarbons such as DDT, pentachloro and dieldrin, their resistance to degradation is due to the fact that these compounds are insoluble in water, sorb tightly to soil making them less available for biodegradation. Similarly, carbofuran and 2,4-D, which are of different molecular structure, are more susceptible to degradation, and can be broken down in just a few days in field soil.

The number of benzene rings in PAH is also an important factor. PAHs with two or three cyclic rings (naphthalene, phenanthrene, anthracene, fluorene, etc.) were more easily degraded with the bacteria being able to utilize the compounds, as their sole carbon source. However, PAHs with four or more cyclic rings were more stable, resistant to degradation, with the exception of white rot fungi. Increase in number of rings usually decreases the solubility of the compounds reducing their bioavailability. It can be countered by the use of surfactants that reduce the interfacial tension between soil and water and increases the solubility of the compounds, except surfactants that are toxic to microorganisms (Chen et al. 2011).

13. Environmental Factors

These include temperature, humidity, salinity, pH, nutrition, carbon dioxide, oxygen, substrate concentration, surfactant, etc. Lack of nutrients is a limiting factor and can lead to collapse of the microbial populations, responsible for degradation. A normal ratio of C:N:P in the microenvironment is sufficient to promote degradation of the pesticides.

Temperature and humidity were the most important factors, which affected the growth and reproduction of bacteria. Water acts as a solvent for pesticides, provides a medium for diffusion and is also required for microbial metabolism because of which pesticide degradation is slow in dry conditions. However, heavily-watered environment like those of paddy fields can limit oxygen diffusion causing anaerobic conditions to prevail. The effect of oxygen availability varies from microbial species and pesticide combinations. While phorate persists longer in flooded soil (Walter-Echols and Liechtenstein, 1978), atrazine and trifluralin are degraded sooner under anaerobic conditions.

Temperature is crucial for optimum enzyme activity and also adsorption of the pesticides by altering solubility, thereby impacting its bioavailability to the microbes. Thermodynamically, adsorption is an exothermic process, so that its efficiency reduces with increase in temperature, leading to a corresponding increase in pesticide solubility. Vischetti et al. (1996) found that the half-life of rimsulfuron decreased drastically from 14.8 days at 10°C to 3.5 days at 25°C.

14. Role of microbes in the degradation of municipal wastes

14.1. Solid municipal wastes

The components of solid waste differ according to the population and considerably change over time. The majority of the waste in nations with effective waste recycling systems consists of stubborn wastes like single-use plastic and non-recyclable materials. Coal ash from open fireplaces make up the majority of residential garbage. Food wastes, plastic containers, market wastes, manufactured goods packaging materials, and other varied solid wastes from dwelling, commercial, organizational and industrial sources make up the majority of the waste stream in urbanized countries without significant recycling practices. Manufacturing waste, agricultural waste, medical waste, radioactive waste and sewage sludge are typically excluded from municipal solid waste classifications (Sundberg et al. 2013).

Within a predetermined area, the municipality collects waste. Remaining waste describes materials that have not been separated or recycled (Palaniveloo et al, 2020). There are numerous ways to categorize waste, but the traditional classification includes the biodegradable trash. Green waste, food waste and kitchen waste papers, cardboard, glass, bottles, jars and tin cans are among the recyclable items. Inert wastes include soil, pebbles and debris from building and demolition. Electrical and electronic waste (WEEE) includes devices like watches, televisions, computers, washing machines and other electrical equipment. Composite wastes include used garments and plastic bags, hazardous wastes include majority of paints, chemicals, tires, batteries, light bulbs, electrical equipment, fluorescent lights, aerosol spray cans and fertilizers.

Lignin and lignin-related compounds are being produced in large quantities at present as waste effluent from the paper and pulping industries (De los Santos Ramos et al., 2009). Due to recent developments aiming to replace fossil feedstocks with lignocellulosic biomass for the manufacture of chemicals and fuel, it is anticipated that this number will rise in the near future. Due to the biorefinery method only being applicable to the hemicellulosic portion, lignin components typically stay as a low-value waste stream (Stewart 2008). While being burnt, lignocellulosic biomass produces heat and electricity (Zaldivar et al., 2001; Ragauskas et al., 2006). To increase the variety of products that may be made from lignocellulosic biomass, the lignin components can be utilized as raw materials rather than being burnt for energy and heat. This will allow for the production of valuable compounds like substituted aromatics. Bacterial lignin peroxidases could provide the most efficient alternative. Other enzymes that contribute to the biodegradation of municipal solid wastes include bacterial oxygenases, monooxygenases, deoxygenases, laccases, hydrolytic enzymes, lipases and cellulases (Karigar and Rao, 2011). A list of bacteria, degrading the municipal wastes is represented in Table 6.

Table 6 Municipal wastes and the degradative bacteria involved

Source	Bacteria	References
Municipal solid waste	<i>Bacillus sp.</i> , <i>Pseudomonas sp.</i> , <i>Arthrobacter sp.</i> , <i>Alcaligenes</i>	Stofella and Kahn (2001)
	<i>Staphylococcus sp.</i>	Hassen et al. (2001)
	<i>Bacillus sp.</i>	Abdullah et al. (2014)
	<i>Halomonas sp.</i> , <i>Luteimonas marina</i> , <i>Bacillus megaterium</i> , <i>Bordetella petrii</i>	Siddiqui et al. (2017)
	<i>Zoogloea ramigera</i>	Adebayo and Obiekezie (2018)
	<i>Micromonospora sp.</i> , <i>Streptomyces sp.</i> , <i>Actinomyces sp.</i>	Stofella and Kahn (2001)

14.2. Enzymes involved in solid waste decomposition

Microorganisms like bacteria and fungi secrete enzymes to break down and utilize solid waste products as energy source. A significant portion of the proteinaceous components of the municipal solid waste is broken down by bacterial proteases (Karigar and Rao, 2011). Bacterial protease is one of the top three categories of industrial enzymes. The most utilized proteases now are alkaline serine protease.

15. Composting as a method of solid waste decomposition

Composting is an aerobic, biochemical and microbiological process that involves the hydrolysis of organic material into humus, a stable and sanitized residue. Here, the bacteria break down organic material using oxygen, water, carbon dioxide, heat and compost that improves the soil. The generated compost has a high concentration of humic compounds that are physiologically stable, making it a great component for soil amendment (Jurado et al. 2014; Bialobrzewski et al., 2015). A natural increase in temperature during the process aids in the elimination of the pathogens, making the compost that is produced safer to use. The composting primarily consists of three phases, (i) the initial mesophilic phase, where the mesophilic bacteria and fungi rapidly raise the temperature to degrade simple compounds like sugars, amino acids, etc; (ii) the second thermophilic phase, where thermophilic microbes break down the organic matter (fat, cellulose, hemicellulose and lignin). Due to metabolic activity of the heat-tolerant microbes, the organic carbon concentration of the feedstock decreases during this phase; (iii) last but not least, cooling phase is characterized by lower temperature and a decline in microbial activity. Mesophilic microbes recolonize the compost mass, degrading the remaining sugar, cellulose and hemicellulose to produce humic-like compounds. The rate of organic matter breakdown declines, while the rate of humification and polymerization of the organic compounds rises. The succession of microbes during the composting process is crucial for the efficiency of the process. The quality of the compost produced depends on the presence of specific microbes which also affect the pace of biodegradation and compost maturity (Jurado et al., 2015). Additionally, the microbial inoculants affect the composting process by affecting the breakdown of cellulose, hemicellulose and lignocellulose, which changes the temperature and nitrogen levels during composting. Although composting is thought to be oxygen-demanding (aerobic) process, anaerobic organisms like *Clostridium sp.* have also been mentioned as part of the process (Bhatia et al., 2013).

16. Microbes involved in the degradation of solid wastes

The most prevalent and quickly emerging microorganisms during composting are bacteria and fungi. The substrates used and the microorganisms active during the process have a significant impact on the compost quality (Villar et al., 2016). The microorganisms release several substrate-based hydrolytic enzymes that break down the complex structured molecules and produce water-soluble chemicals (Lee, 2016). Thus, they aid in the organic degradation process during composting. In addition to metabolizing the organics, they also make easily utilizable chemicals that, when applied to soil, improve agricultural potential and maintain the natural environment. Organic waste decomposes spontaneously through moist decomposition. A mixture of dominant and non-dominant microorganisms, with the former taking a more active role, make up an efficient microorganism (EM) culture. Studies by Karnchanawong and Nissaikla (2014), Onwosi et al. (2017) and Manu et al. (2017) showed how EM addition to the treatment mixtures

increased the waste degradation rates. Additionally, these additives can either be created using culture mixtures made of soil, cow dung, straw, etc. or isolated from the microbial communities, based on specific degradative functions. To be more specific, the added inoculum could be a mature compost (Karnchanawong and Nissaikla, 2014; Kinet et al., 2015), a commercial strain mixture (Hou et al., 2017), a single strain of EM or a commercial strain mixture. It is well known that the majority of the organic component of municipal solid waste is mostly made up of plant biomass, upon which the cellulase enzymes work together. However, there are currently only a small number of microbial strains known to release cellulase enzyme and digest municipal solid waste through cellulose hydrolysis (Gautam et al., 2012).

Cellulomonas, *Pseudomonas*, *Bacillus* spp., and *Thermoactinomyces* are a few well-known powerful cellulose-producing bacteria. *Aspergillus*, *Trichoderma*, *Sclerotium* and white-rot fungus all produce extracellular enzymes that are responsible for the breakdown of cellulose and lignin during composting (Awasthi et al., 2015). Monitoring the alterations in biological properties (microbial succession), taking place during the process, allows for the evaluation of compost quality. pH, C:N ratio, color, electric conductivity (EC), humic substances (HS), pathogenic activity, germination index (GI), and total NPK contents can all be used to determine the composition of the compost.

17. Factors affecting the decomposition of solid waste

In the broadest sense, there are two groups of factors that have an impact on composting:

- Factors related to the composition of the composting mix like nutrient balance, pH, particle size, porosity and moisture; and
- Process management factors, like oxygen concentration, temperature, water content and compaction (Li et al., 2013).

To gain a clear picture of the ideal process conditions, it may be necessary to regulate variables like pH, bulk density, temperature, porosity, nutrient content, C/N ratio, particle size, moisture and oxygen supply. For energy supplement and developmental activity during composting, bacteria needed C, N, P and K as the key nutrients (degradable organic-carbon) (Iqbal et al., 2015).

17.1. C/N Ratio

To create an effective compost mix, we need a nutritional balance in the form of an ideal C/N ratio. Variations in C/N predicted that as composting progressed over time, the amount of carbon that was converted to CO₂ would determine the rate of organic breakdown. According to Kutsanedzie et al. (2015), the ideal C/N ratio is between 25 and 35, meaning that the microbes need 30 parts of carbon for every one part of nitrogen. However, several authors (Kumar et al., 2010, Yang et al., 2015, Petric et al., 2015) observed good outcomes even with an initial C/N ratio between 20-50.

17.2. pH

Since most raw materials are already sorted within the acceptable pH range, pH is not initially thought to be important (Rich and Bharti, 2015). A reduced pH during composting may be due to the volatilization of ammonia and microbial nitrification, which produce more CO₂ and acids. The increased pH (> 8.0) is explained by protein mineralization (which produces ammonia) and the hindered nitrogen loss through ammonia volatilization (Guo et al., 2012) during later stages of composting. The recommended pH range for composting is typically between 5.5 and 8.0 (Zhang and Sun, 2016); however, Bernal et al. (2009) suggested that a pH value between 6.7 and 9.0 is effective to encourage good microbial action.

17.3. Moisture content

According to Petric et al. (2015), the moisture conditions have a significant impact on the porosity level, temperature, oxygen uptake rate and microbial activity in composting. According to the composition of the raw material, an efficient composting requires a moisture level, ranging between 50 and 60 percent (v/w) (Bernal et al., 2009). The rate of organic matter breakdown was decreased as a result of increased evaporation due to higher temperature. For the waste microbiota to function properly and to maintain adequate moisture levels, treatment piles must be rewetted. On the other hand, excessive moisture levels during composting could result in water logs with widespread anaerobic conditions, which could cause the process to stop (Makan et al., 2013).

17.4. Oxygen supply

Another crucial factor is aeration, which supplies oxygen primarily for microbiological operations, temperature regulation, moisture optimization and elimination of excessive carbon dioxide. According to Latifah et al. (2015), an ideal composting process calls for an oxygen content between 15% and 20%. To ensure that the process is supplied with enough oxygen (Latifah et al., 2015), the oxygen concentration is directly associated with the microbial dynamics (Nakasaki and Hirai, 2017) and temperature (kept below 60–65 °C). Early composting stages with enough aeration shortened the time required for the waste to stabilize, completely converting carbon (C) to carbon dioxide (CO₂) and lowering methane emissions. While excessive aeration inside the matrix may lead to improper composting, it would have a significant impact on the pace of waste decomposition (Palaniveloo et al. 2020). According to a study, a higher aeration rate during waste composting reduced the C/N ratio, NH₃ generation and odor emission, but negatively impacted the compost maturity. The microbial population was impacted by composting at a lower aeration rate, which also caused a slower rate of organic breakdown, less NH₃ production and a considerable decrease in temperature, moisture and heat loss (Guo et al., 2012). A turning regime must be maintained for the compost mass in order to ensure optimal aeration and produce improved stabilization and sanitization of waste.

Furthermore, Getahun et al. (2012) found a significant correlation between turning frequency and a small number of physicochemical factors that might be utilized to determine the compost maturity. For instance, rotating frequency has an impact on the temperature, pH, moisture content, C/N ratio, dry matter and total carbon in the compost pile. According to Mohee et al. (2015), a weekly trash turning schedule is excellent for hastening the decomposition of organic materials. Three days per week was shown to be the most significant turning schedule in a comparative municipal solid waste composting study that involved two turning regimes for bacterial succession. Daily turning did not work well for the bacterial succession. According to Petric et al. (2015), mixing the composting mass for 30 minutes each day improved the co-composting of municipal solid waste and poultry manure. Additionally, forced aeration combined with pile rotating has proven to improve composting as seen by the quality of the finished product (Li et al., 2015; Rasapoor, 2016).

17.5. Temperature

Temperature drives the growth and metabolic activity of the microbial population within the compost material in a manner similar to moisture content and aeration. It may have a direct impact on how quickly organic matter degrades during composting (Waszkielis et al., 2013). Therefore, the requirement for temperature regulation suggests the need to regulate the rate of the process. During municipal solid waste composting, the ambient temperature accelerated the decomposition of the organic substrates and enhanced their rates of biodegradability (Rastogi et al., 2019b).

Temperature between 50 and 55 °C encouraged trash decomposition and guaranteed maximum sanitization during composting. In addition, temperature and processing duration work together to completely eradicate pathogens from the compost pile. Contrarily, prolonged exposure to high heat (i.e., temperature above 70 °C) can deactivate microorganisms (fungi, actinomycetes and bacteria) during the composting process, making temperature control necessary (Varma and Kalamdhad, 2015). By controlling the size and shape of the composting mass by turning operations, the excessive heat may be reduced, which improves cooling and temperature redistribution (Chowdhury et al., 2013). In their study of municipal solid waste composting, Troy et al. (2012) found that the immediate microbial decomposition of carbohydrates, proteins and lipids caused a speedy increase in temperature (which reached 50 °C on day 8) and heat output.

17.6. Particle size

In order to guarantee adequate aeration and control the gas/water exchange, the particle size in the compost mass ensures the porosity level (Zhang and Sun, 2016). The right particle shape and size are crucial for estimating the operational expenses of the process. Ge et al. (2015) mentioned "sieving" as a key technique to establish the ideal distribution of particle size in a compost mass. By chopping and shredding the trash into smaller bits, the particle size can be adjusted. This guarantees that there is a larger surface area accessible for improved microbial activity during composting, leading to rapid breakdown. The feedstock was first compacted because of its small particle size, which increased the likelihood that anaerobic conditions would subsequently be prevalent. While larger particles processed a smaller surface area, making them less accessible for microbial action, they also created significant air pockets that decreased the temperature of the matrix, which led to a slower rate of organic matter decomposition (Verma and Marschner, 2013). When composting tobacco, the best degradation was obtained with waste particles that were 25 mm in size and provided the right physical and chemical conditions for bioactivity.

18. Wastewater treatment by microbial decomposition

Wastewater from municipalities is largely similar to domestic wastewater which includes sewage wastes with fecal matter, wash-water obtained from washing, bathing or cooking and wastewater from small-scale food processing units, offices, institutions or eateries. Sewage wastewater, also termed as black water, includes human urine and feces along with the water used during flushing (Roychoudhury and Das, 2022). Sewage water largely consists of water with only 0.1% of organic or inorganic solids. Organic solids include carbohydrate, lignin, fat, soap, synthetic detergent, protein and their decomposition products.

Wash-water, or more commonly, grey water has high amounts of soap, detergent and antiseptic and is alkaline with a pH reaching up to 8.35 (Bakare et al., 2017). It also contains biodegradable organic materials and nutrients like nitrates, phosphorus and their derivatives, xenobiotic organic compounds (Fatta-Kassinos et al. 2011) and microbes such as fecal coliforms and *Salmonella*. The xenobiotic organic compounds often originate from household and personal care products like cosmetics, aerosols and pigments (Eriksson et al. 2003), while toxic heavy metals such as Pb, Ni, Cd, Cu, Hg and Cr (Aonghusa and Gray 2002; Eriksson et al. 2002), originating from laundry detergents, are also present. Table 7 represents the different components of municipal waste water and their effect on environment.

Table 7 Constituents of municipal wastewater and their environmental impact (Henze and Ledin; 2001)

Component	Examples	Environmental effect
Microorganisms	Pathogenic bacteria, virus and worm eggs	Risk when bathing and eating shellfish
Biodegradable organic materials	Oxygen depletion in rivers and lakes	Fish death, stale odors
Other organic materials	Detergent, pesticide, fat, oil, grease, colouring solvent, phenol, cyanide	Toxic effect, aesthetic inconveniences, bio accumulation in food chain
Nutrients	Nitrogen, phosphorus, ammonium	Eutrophication, oxygen depletion, toxic effect
Metals	Hg, Pb, Cd, Cr, Cu, Ni	Toxic effect, bioaccumulation
Other inorganic materials	Acids, hydrogen sulphide, bases	Corrosion, toxic effect
Thermal effects	Hot water	Changing living conditions for flora and fauna
Odour (and taste)	Hydrogen sulphide	Aesthetic inconveniences, toxic effect

Untreated wastewater poses health hazards on multiple counts to humans, plants and animals. Organic chemicals, though very low in concentration, can have detrimental effects on human health over a long period of time. Wastewater can also lead to contamination of crops and groundwater. While there are guidelines for drinking water quality (World Health Organization 1984) to help protect groundwater, bioaccumulation of heavy metals and toxic chemicals in plants and fishes that are later consumed by humans are threats that are difficult to detect and mitigate down the line. Another critical health concern arises from the presence of pathogenic organisms. Raw untreated sewage water, reused to irrigate croplands, led to endemic spread of helminthic diseases, caused by *Ascaris* and *Trichuris*. Thus, it is important that the wastewater generated is treated before its release to the environment.

In sewage treatments, there are three broad steps: primary treatment which is a physical process where the wastewater is filtered for solid wastes; secondary treatment which is the step of microbial action, where under aerobic conditions in trickling filters or activated sludge digesters, 85-95% of BOD is removed; trickling filters use a biofilm of microorganism, whereas activated sludge digesters use "flocs" of microorganisms as the active elements. While purified water is the effluent, these processes also yield a solid sludge which is digested in the anaerobic sludge digesters (Fattal et al. 1985).

The goal of secondary treatment is the biooxidation of the dissolved organic matter to reduce its BOD and chemical oxygen demand (COD). However, the metabolic breakdown of organic matter yields products such as nitrates and phosphates which if released into the environment can cause nutrient richness causing algal blooms and eutrophication in water bodies. Thus, the effluent undergoes a tertiary treatment, or "effluent polishing", which can be chemical or sometimes microbial, to remove excess nitrates and phosphates.

An alternate solution for biological treatment of wastewater is constructed wetlands. These utilize wetland ecosystems for the purpose of purifying wastewater. Compared to septic systems and sewage treatment plants, constructed wetlands have lower costs of construction but are less disruptive of biodiversity than if natural wetland systems were used. Constructed wetlands are also more practical in that they can be designed optimally to reduce BOD, COD and remove nutrients from wastewater. These consist of marshy areas with helophyte plants like *Phragmites australis*, *Typha* spp., *Scirpus* spp. that help filter the water, as well as heterotrophic microorganisms that help in the removal of excess nitrogen and phosphorus. Nitrogen removal occurs by the process of nitrification and denitrification, while phosphorus is removed from water by means of cumulation, sorption and precipitation.

19. Mode of action of microorganisms

Biological wastewater treatment systems rely on the endogenous metabolic action of microorganisms and their ability to degrade organic matter or remove excess nutrients. While removal of organic matter remains the main objective, there are presently additional goals like the conversion of toxins, removal of heavy metals, odors and pathogens. In activated sludge digesters, the microbial community is dominated by Proteobacteria, accompanied by other groups like *Bacteroidetes*, *Chloroflexi*, *Actinobacteria*, *Planctomycetes*, *Firmicutes*, etc (Ferrera and Sánchez, 2016; Zhang et al., 2016).

Recently, microbial fuel cells are being explored as an alternative for wastewater treatment whereupon the removal of the contaminants is accompanied by the generation of electricity. Microbes can transfer electrons to an electrode, using components in their cell wall. The recognized microbial species are *Geobacter* spp., *Shewanella* spp., and phototrophic bacteria like *Rhodospseudomonas* sp. (Xing et al., 2008). The electron transfer efficiency from acetate to electricity was 96.8% with *Geobacter sulfurreducens* cells, whereas that from glucose was reported to be 81% with *Rhodospseudomonas ferrireducens* cells. Mixed cultures are also being explored as they offer simpler operation; other non-electrogenic microorganisms also help microbial fuel cells through syntrophic interactions. The syntrophic propionate-oxidizer *Pelotomaculum thermopropionicum*, co-cultured with *Methanothermobacter thermautotrophicus*, was seen to mediate exocellular electron transfer to an electrode (Ishii et al., 2005).

19.1. Removal of nitrogen

Microbes involved in nitrogen removal generally do so by two key processes of nitrification and denitrification. Beside nitrates, there may be a need to remove ammonia or nitrites, since they are toxic to aquatic life and can lead to eutrophication. Ammonia can be oxidized by aerobic and anaerobic ammonia oxidizing (anammox) bacteria. Proteobacteria carry out aerobic oxidation, in which the main product is nitrite, or anaerobic oxidation of ammonia, yielding dinitrogen, nitrite and nitric oxide, depending on the oxygen concentration. They consist of two classes, the Betaproteobacteria, e.g., *Nitrosomonas* and *Nitrospira*, and the Gammaproteobacteria *Nitrosococcus*, except *Nitrosococcus mobilis* which is a betaproteobacteria.

Anaerobic ammonia oxidizing bacteria, on the other hand, consist of a single group of bacteria within Planctomycetes and includes five genera - *Brocadia*, *Kuenenia*, *Scalindua*, *Anammoxoglobus* and *Jettenia* (Ali et al., 2013). Some archaeobacteria have also been reported to oxidize ammonia; however, its level of contribution is not well ascertained.

Nitrification, which is an oxidative process converting nitrite to nitrate, is carried out by aerobic bacteria from members of the genera *Nitrobacter*, *Nitrococcus* and *Nitrospira*. Of these, *Nitrosomonas* sp. and *Nitrospira* are present in virtually all wastewater treatment systems. Some other less common genera are *Nitrosovibrio*, *Nitrospina* and *Nitrotoga*.

Denitrification is the conversion of nitrate or nitrite to gaseous forms such as dinitrogen or nitrous oxide and takes place under anoxic conditions. It is carried out by different chemoorganotrophic, lithoautotrophic and phototrophic bacteria, as well as some archaeobacteria and fungi. These include the genera *Alcaligenes*, *Pseudomonas*, *Methylobacterium*, *Bacillus*, *Paracoccus*, *Hyphomicrobium*, as well as members of the order *Rhodocyclales*.

19.2. Removal of phosphorus

Phosphorus during sewage treatment is assimilated by the growing biomass and then removed as sludge. The biological process is known as enhanced biological phosphorus removal. Bacteria with a high ability to metabolize phosphorus generally belong to the genus *Acinetobacter*. These bacteria can take up phosphorus in the form of polyphosphates and are also called as polyphosphate accumulating organisms (PAOs). Glycogen-accumulating organisms are also present in this stage and they compete with PAO for carbon. The uptake can occur under both aerobic and anaerobic conditions.

20. Factors affecting the rate of microbial biodegradation for wastewater treatment

The factors affecting the rate of microbial biodegradation in wastewater treatment are broadly classified as environmental and biological factors. These include bacterial to food ratio, concentration of contaminants, temperature, pH and oxygen availability. The concentration of the contaminant and distribution of metabolizing microorganisms greatly influences the rate of degradation. Competition between microbes for nutrients can lead to enzyme inhibition and thus lowered rates of activity. The specificity of the enzyme towards the contaminant as well as high concentration of contaminants can also reduce the efficiency of the process. The degradation pathways are also dependent on temperature and pH changes. Depending on the species of bacteria and whether it is acidophilic, neutrophilic or alkaliphilic bacteria, a suitable media of an appropriate pH range needs to be chosen. Reaction temperature affects both the physical state of the contaminant like solubility, viscosity, etc, and the physiological status of the degradative microbes include growth rate, metabolism, etc.

21. Conclusion

A lot of research is being carried out on the degradation of hydrocarbons from petrochemical industries, pesticides and municipal wastes including solid wastes and wastewater. Due to increasing pollution over the decades caused by these pollutants, a need for finding out an effective strategy to combat the negative effects of these pollutants on environment and life is being felt constantly. Keeping these in mind, bioremediation is the most effective method to deal with pollution. Understanding the method of biodegradation is important for ecological reasons since it depends on intrinsic microbes to alter or mineralize organic pollutants. Microbes utilize their enzymatic properties to break the pollutants down and thus effectively reduce pollution. Although much research has already been done in this regard, further research is necessary to find better and more efficient methods for the removal of the pollutants from the environment with the use of microorganisms. Thus, we can conclude from this review that microbial degradation can be considered as a key component in the cleanup strategy for petroleum hydrocarbons, pesticides and municipal wastes, including solid waste and waste water remediation.

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that there is no conflict of interest in publishing this article.

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