# Microbial Degradation of Hydrocarbons

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## Abstract

Hydrocarbon contamination in the environment has been a notable problem since years. Particularly the major oil spills in last few decades, and resulting loss to biodiversity has brought public attention to this problem. Bioremediation is a promising approach for recovery of environmental sites contaminated with crude oil and other hydrocarbons. Many microorganisms have been identified to possess hydrocarbon degradation potential. This review covers an overview of common hydrocarbon pollutants, microbes known as hydrocarbon degraders, major pathways and enzymes involved therein, factors affecting hydrocarbon degradation, and various approaches employed to exploit degrading capacity of microbes for remedial purpose. In addition to making use of inherent catabolic ability of degrader populations, metabolic engineering can be of considerable value in dealing with the problem of hydrocarbon contamination.

**Keywords:** Bioremediation, Oxygenase, Biomagnification, Cometabolism, Consortium, Biosurfactant.

## Introduction

Hydrocarbon (HC) group of compounds consist of hydrogen and carbon in their structure. As petrochemical industries are flourishing worldwide, HC contamination has become one of the major environmental problems faced globally. Environment is particularly being contaminated with accidental releases of petroleum products. Some of the HC compounds can prove carcinogenic and neurotoxic to different life forms. Bioremediation is a promising approach for the treatment of HC contaminated locations as it is cost effective and can lead to complete mineralization. Bioremediation strategy exploits the metabolic pathways of living organisms (mainly microorganisms) for biodegradation of organic pollutants, leading to their partial or complete mineralization into carbon dioxide, water, and inorganic compounds. Degrading organisms may use the pollutant molecules as an energy source and for deriving building blocks for synthesis of their cellular components. In the process they transform the complex organic contaminants to simpler (may be less toxic) forms, which can further be utilized by other organisms. HC degradation often requires the presence of oxygen as the initial degradation occurs by the action of oxygenase enzymes (Atlas, 1991), however in subsequent steps nitrate or sulphate may serve as a terminal electron acceptor (Bartha, 1986).Various industries and different daily life processes use petroleum based products as

the major source of energy. Leaks and accidental spills (Table 1) occur frequently during transportation, production, exploration, refining, and storage of petroleum and its derivatives. The amount of natural crude oil seepage was estimated to be 600,000 metric tons per year with an uncertainty of 200,000 metric tons per year (Kvenvolden and Cooper, 2003). Release of HC into marine environment can lead to major water contamination causing extensive damage to biodiversity. The accumulation of these pollutants in marine animal and plant tissues may cause death or mutation. Further when these animals and plants become a part of food chain they may lead to more harmful effects through biomagnification.

**Table 1. Major oil spills in history**

(http://www.mnn.com/earth-matters/wilderness-resources/stories/the-13-largest-oil-spills-in-history; last accessed on 20.3.2013)

| **Oil Spill** | **Date** | **Site** | **Amount of oil spilled** (million gallons) |
|---|---|---|---|
| Arabian gulf/Kuwait | January 19, 1991 | Persian gulf, Kuwait | 382-520 |
| Gulf oil spill | April 22, 2010 | Gulf of Mexico | 206 |
| Ixtoc 1 oil spill | June 3, 1979 | Bay of Campeche, Mexico | 140 |
| Atlantic empress oil spill | June 19, 1979 | Coast of Trinidad & Tobago | 90 |
| Kolva river oil spill | August 6, 1983 | Kolva river, Russia | 84 |
| Nowruz oil field spill | February 10,1983 | Persian gulf of Iran | 80 |
| Castillo de bellven oil spill | August 6, 1983 | Saldanha bay, South Africa | 79 |
| Amoco cadiz oil spill | March 16, 1978 | Portsall, France | 69 |
| ABT summer oil spill | May 28, 1991 | Coast of Angola | 51-81 |
| M/T haven tauker oil spill | April 11, 1991 | Italy | 45 |
| Odyssey oil spill | November 10, 1988 | Coast of Nova scotia, Canada | 40.7 |
| The sea star oil spill | December 19, 1972 | Gulf of Oman | 35.3 |
| The torrey canyon oil spill | March 18, 1967 | Scilly Isles, UK | 25-36 |

## Hydrocarbon Overview

HCs in crude petroleum can be classified as alkanes, cycloalkanes, aromatics, polycyclic aromatics, asphaltines, and resins. Among the petroleum HCs, n-alkanes are the most amenable to biodegradation. Normally alkanes in the range of $C_5$ to $C_{10}$ are inhibitory to majority of the HC degraders at higher concentration as they disrupt lipid membrane when present as solvent. Alkanes in range of $C_{20}$ to $C_{40,}$ also referred to as waxes, are less biodegradable as they being hydrophobic solids have low solubility in water. During degradation the alkanes are converted to alcohol by the action of oxygenase enzymes that attack the terminal methyl group. The alcohol is further oxidized to aldehyde and then to fatty acids. Further utilization of fatty acid occurs by β-oxidation of aliphatic chain. Higher the methyl branching lower is the extent of β-oxidation.

The cycloalkanes or alicyclic HCs are less degradable than alkanes. Here the biodegradability decreases with increase in number of ring structures. Alkyl substituted cycloalkanes can be degraded more easily as compared to non-substituted HCs. Cycloalkanes are degraded to cyclic alcohol by the action of oxidases which further is dehydrogenated to

ketone. The primary products of metabolism of cycloalkanes are cycloketones and cycloalkane-carboxylic acids.

Aromatic HCs molecules have benzene-based structure. As compared to most other cyclic compounds, aromatic compounds are more stable because of sharing of delocalized electrons by pi bonds. BTEX (benzene, toluene, ethylbenzene, xylene) compounds are comparatively more mobile and water- miscible. There are two major steps involved in biodegradation of an aromatic molecule (1) activation of the ring, (2) ring cleavage (Fig 1). Activation is achieved by the incorporation of molecular oxygen into the aromatic ring leading to dihydroxylation of aromatic nucleus, and the enzymes responsible for this are oxygenases. Monooxygenases, characteristic of fungi and other eukaryotes, catalyze the incorporation of a single atom of oxygen to form an epoxide which can then undergo hydration to yield transdihydrodiols (Rochkind et al., 1986). Dioxygenases catalyze simultaneous incorporation of two atoms of oxygen to form a dihydrodiol. These dioxygenase reactions have been shown to occur for benzene, halogenated benzenes, toluene, para-chlorotoluene, xylenes, biphenyls, napthelene, anthracene, etc (Gibson, 1988). These dihydrodiols are further oxidized to catechols which are precursors to ring cleavage. Catechol can be oxidized either via ortho-cleavage pathway which involves cleavage of the bond between carbon atoms of the two hydroxyl groups to yield muconic acid, or via the meta-cleavage pathway which involves cleavage of the bond between a carbon atom with a hydroxyl group and the adjacent carbon atom to yield 2-hydroxymuconic semi aldehyde (Cerniglia, 1984). These compounds are further degraded to form organic acids which are then utilized by microorganisms for their cell synthesis and energy generation.

Polycyclic aromatic HC (PAH) or polynuclear aromatic HCs (PNA) are produced during high temperature industrial operations such as petroleum refining, coke production, and wood preservation (Park et al., 1990). This group of compounds, which consist of two or more benzene rings, includes 16 priority pollutants (Dzomback et al., 1984; McEldowney et al., 1993). Some of these compounds are suspected to be carcinogens. The increase in molecular weight and number of ring structures of PAHs decreases their solubility and volatility, while increasing adsorption capacity. PAHs are degraded by mechanism similar to that for monoaromatic compounds. Fungal degradation of PAHs is environmentally important because some of the products have been implicated as toxic forms in higher life forms (Cerniglia, 1984).

Asphaltines and resins are high molecular weight compounds containing nitrogen, sulphur, and oxygen in their structure. These compounds are recalcitrant to biodegradation as they are highly insoluble, and consist of functional groups that are shielded from microbial attack by extensive aromatic ring structure.

**Need for Biodegradation**

Dissolved aromatic components of petroleum even at a low ppb concentration, disrupts the chemoreception of some marine organisms. As feeding and mating responses in marine life forms largely depend on chemoreception, such disruption can lead to elimination of many species from the polluted area, even when the pollutant concentration is far below the lethal level as defined in the conventional sense. Another disturbing possibility is that some condensed polynuclear components of petroleum that are carcinogenic and relatively resistant

to biodegradation may move up marine food chains and taint fishes or shellfishes, which further can be used as food or feed.

Direct of indirect exposure of living organisms to hydrocarbon compounds can have varying ecological and/or economic impacts, as described below:

- Direct exposure to oil spill: A variety of ill effects may develop when the oil spill occurs, mainly to those who live and work in such areas. They may come in contact with gaseous oil compounds or oil compounds adsorbed on particulate matter (dispersed in air) through breathing. Another exposure pathway may relate to activities carried out, in contaminated ground (soil) or directly through skin adsorption on touching the spilled contaminated material.
- Indirect exposure: This happens through consumption of contaminated food or water especially relevant in the case of consumption of fish that was from an oil spill polluted environment. As some oil components can accumulate in living organisms, if a fish lives in a polluted environment, it will keep accumulating in its body some oil components (without excretion) which may reach concentrations several orders of magnitude higher than those of the surrounding waters. Through consumption of such polluted fish meat, humans may become seriously exposed to concentration of oil components higher than in the surrounding environment or as compared to ingestion of the polluted water or bathing in the polluted water.
- Economic impact: Oil spills have hampered many industries such as fishing, tourism, oil industries, etc. Estimated economic toll during the British Petroleum gulf coast spill indicated a huge loss of 17,000 jobs at year's end. The National Wildlife Federation reported that already more than 150 threatened or endangered sea turtles are dead. And 316 sea birds, mostly brown pelicans and northern gannets, have been found dead along the gulf coast as a result of the spreading oil (http://www.care2.com/causes/10-most-horrifying-facts-about-the-gulf-oill-spill.html; last accessed on 14.2.2013).

## Approaches to Biodegradation of HCs

There have been several approaches to treat soil and water contaminated with HCs:

(a) Phytoremediation: This strategy makes use of plants for bioremediation, but is outside the scope of this review.

(b) Use of microbial consortium: This involves use of multiple microbial species together rather than relying on catabolic capacity of any single species. Consortium can be in form of microbial biofilms, where production of certain biosurfactants can enhance oil degradation by increasing its bioavailability. Biofilms may be single-species or multi-species. Some organisms apply behavioral strategies such as adhesion and biofilm formation to acquire carbon and energy from hydrophobic organic compounds (HOCs) contained in marine aggregates. HOCs are weakly soluble in water providing for low bioavailability. *Marinobacter hydrocarbanoclasticus SP17* form biofilms at HOC-water interface enhancing bioavailability of HOCs (Grimaud, et al., 2012). Physiological and proteomic studies revealed that biofilm formation is an efficient strategy to colonize hydrophobic interfaces (Ballihaut, et al., 2004; Vaysse , et al., 2009; Vaysse , et al., 2011). SP17 *M. hydrocarbanoclasticus* is observed to form biofilm at the interface of aqueous phase and HOC substrates like n alkanes, fatty alcohols, or polar lipids, but not on non-metabolizable compounds such as *n*-$C_{32}$

alkanes, pristine, and heptamethylnonane. Since bilfilm is a nutritive interface, it is not observed on non metabolizable compounds.

When more than one type of organisms are present at the contaminated site, some of them can practice cometabolism (fortuitous metabolism), wherein a microorganism transforms the given compound without being able to grow on it or derive energy from it; this transformed compound can be used as a growth substrate by another microorganism present in the same environment or in a mixed culture. Cometabolic transformations may gradually lead to recycling of recalcitrant compounds on which no individual microbial culture can grow. For example, *Mycobacterium vaccae* was reported to cometabolize cyclohexane while growing on propane; the cyclohexane is oxidized to cyclohexanol, which further can be utilized by other bacteria (Beam and Perry, 1974).

(c) Stimulation of anaerobic degradation using alternative electron acceptor: Anaerobic degradation can be used as an alternative where aerobic conditions can not be maintained. Several alternative electron acceptors have been proposed for use in anaerobic degradation, including nitrate, sulphate, iron ($Fe^{3+}$), and carbon dioxide (Maier, 2000).

(d) Nutrient augmentation (Biostimulation): Large amount of contaminants enter the soil most commonly from leaking underground storage tanks, landfills, waste disposal ponds etc. (Eweis et al., 1998). Assimilation of HCs may be done by microorganisms already present in soil, albeit at a slow pace. However relying solely on this small population of naturally present organisms is not sufficient for effective bioremediation in most cases. Natural environments are often deficient in nutrients. Microbial growth rate can be accelerated to enhance soil remediation by providing exogenous supply of nutrient, especially nitrogen and phosphorous to the contaminated soil. Other nutrients present in limiting concentrations can also be added. This approach is known as nutrient augmentation. HC degradation in contaminated soil can be enhanced by applying surfactants (along with other nutrients) to soil, which makes HCs more easily available to microorganisms. After applying nutrients, they are mixed with soil by convenient means such as tilling, disking, rototilling, etc.

(e) Bioaugmentation: When a contaminated site contain insufficient number of endogenous oil-degrading microbes, exogenous microbes with known capacity for efficient HC degradation are added to supplement the existing microbial population, and the approach is known as bioaugmentation.

Bioremediation of contaminated soil can be carried out either *in situ* or *ex situ*. In latter case contaminated soil is excavated and treated at a separate treatment facility, whereas in the former organisms are added directly to the contaminated site. One of the *In situ* methods for treating soil contaminated dominantly with volatile pollutants includes soil venting. Bioventing, which is applicable in case of semi-volatile and non-volatile contaminants, involves addition of oxygen directly to a site of contamination in the unsaturated zone (Maier, 2000). *Ex situ* methods include composting, slurry-phase bioreactors, land treatment (a form of land farming), etc. Both *ex-situ* and *in-situ* approaches may include a combination of biological and non-biological processes.

Bioavailability of a particular compound depends on its solubility in water. A water soluble contaminant can be rapidly degraded, however degradation can be limited if contaminant is sparingly soluble in water resulting in reduced bioavailability. Microorganisms growing on organic compounds with limited water solubility face a problem as they will obtain only a fraction of the substrate from surrounding aqueous

phase. With a low solubility compound the opportunity for contact between degrading organism and organic compound becomes limited. Liquid HCs form separate phase above or below water surface being less or more dense than water. For example, polychlorinated biphenyls (PCBs) are denser than water and form a separate phase below the water surface, whereas benzene and petroleum being less dense form a separate phase above the water surface (Maier, 2000). There are mainly three modes of liquid organic uptake by microorganisms:

1. Utilization of solubilised organic compound
2. Use of organic compound(s) present in direct contact of cells. This can be mediated by cell appendages such as fimbriae (Rosenberg et al., 1982), or by modifying surface hydrophobicity of the cells, which increases attachment of cell to the organic compound. *Pseudomonas aeruginosa* strains capable of degrading octadecane at faster pace were reported to possess high cell hydrophobicities (Zhang and Miller, 1994).
3. Direct contact with fine or submicrometer-size substrate droplets in the aqueous phase (Maier, 2000)

Production of biosurfactants by microbes or emulsifiers can enhance the rate of uptake and biodegradation. Biosurfactants can be effective in two ways. First, they can form micelles or vesicles which can effectively increase the aqueous solubility of the HC. Second, they make the cell more hydrophobic which can facilitate attachment of cells to the HC and thus able to stick to a separate oil phase in a better way.

## Laboratory Methods for Studying Hydrocarbon Degradation

While assessing HC degradation potential of a test microbe in lab, direct addition of some aromatic compounds to growth media can be toxic to the organism. To overcome such problem few methods suggested in literature (Kukor et al., 2007) are noted below:

(a) Vapour phase method: This method can be used for both solid and liquid culture medium. For liquid medium in a flask, a glass bulb containing liquid volatile substrate is suspended in the flask from above which evaporates in the atmosphere of flask and diffuses into the culture medium. For solid medium a small tube containing liquid volatile substrate is placed in the lid of Petridish which evaporates and diffuses in solid medium.

(b) Direct method: This method is suitable for the substrates which have low toxicity levels. For solid medium crystals of these substrates can be directly placed on solid medium.

(c) For less volatile and insoluble aromatic hydrocarbons three different techniques can be used: Top agar, spraying the surface of Petri plates, or deposition by sublimation.

## Microorganisms Known to Degrade HCs

A wide variety of organisms are known to degrade HC compounds under different environmental conditions. These organisms belong to different groups like thermophiles, alkaliphiles, halophiles (Table 2), etc. Many of these organisms are more effective in form of consortium. *Pseudomonas putida* is well known for HC degradation. *P. putida* can efficiently degrade benzene and toluene (Gibson et al., 1970). *P. oleovorans* is reported to degrade tetrahydrofuran (THF). *P. oleovorans* DT4 can also utilize and biotransform THF and BTEX

compounds (Zhou et al. 2011). Many of these HC degraders employ their plasmid encoded machinery for degradation. The TOL plasmid pWWO *xyIN* gene product from *Pseudomonas putida* was reported to be involved in *m*-xylene uptake (Kasai et al., 2001). Alkylbenzoate degradation genes in *P. putida* are located on its TOL plasmid (Kaldalu., 2000). *P. putida* is considered to be more suitable for bioremediation application, as it is not known to be human pathogen, unlike *P. aeruginosa*.

We are currently investigating growth of *Viribacillus salarius* isolated by us from saline soil of Khambhat, India (Solanki and Kothari, 2012). We found this organism to be capable of growth on benzene, toluene, and ethylbenzene, using any of these compounds as sole carbon source. We detected (methyl) catechol 2,3-dioxygenase, and chlorocatechol 1,2-dioxygenase activity in this organism. Hydrocarbon degradation by halophilic / halotolerant organisms is of special interest. In case of marine oil spills any organism which is to be used for bioremediation should be capable of surviving high salinity of sea water, and temp fluctuations typical of such natural environment.

**Table: 2 Halophiles known to degrade HC**

| Microorganism | Target substrate | Reference |
|---|---|---|
| *Marinobacter hydrocarbanoclasticus* | Multiple HCs | Gauthier et al., 1992 |
| *Arhodomonas rozel* | Benzene, toluene | Dalvi et al., 2012 |
| *Marinobacter antarcticus* | - | Liu et al., 2011 |
| *Thalassobacillus devorans* | Various aromatic HCs, phenol | García et al., 2005 |
| *Arthrobacter halodurans* | - | Chen et al., 2009 |
| *Alcanivorax borkumensis* | $C_{14}$-$C_{15}$ n-alkanes | Yakimov et al., 1998 |
| *Acinetobacter venetianus* | n-alkanes | Cello et al., 1997 |
| *Oceanobacter kriegii* | Petroleum | Teramoto et al., 2009 |
| *Marinobacter vinifirmus* | PAH, naphthalene, phenanthrene, pyrene. | Cui et al., 2008 |
| *Stappia aggregate* | | |
| *Pseudoalteromonas ganghwensis* | | |
| *Thalassospira lucentensis* | | |
| *Kaistia adipata* | | |
| *Marinobacter alkaliphilus* | | |

**Enzymes Involved in HC Biodegradation**

Over the years investigators have characterized pathways (Fig 1)of microbial degradation of hydrocarbons and enzymes involved (Table 3). Cytochrome P450 alkane hydroxylases constitute a super family of ubiquitous heme-thiolate monooxygenases which play an important role in the microbial degradation of oil, chlorinated HCs, and fuel additives (Van Beilen and Funhoff, 2007). The diversity of alkane oxygenase systems in prokaryotes and eukaryotes that actively participate in the degradation of alkanes under aerobic conditions like cytochrome P450 enzymes, integral membrane di-iron alkane hydroxylases (e.g. *alkB*), soluble di-iron methane monooxygenases, and membrane-bound copper containing methane monooxygenases has been discussed by Van Beilen and Funhoff (2005).

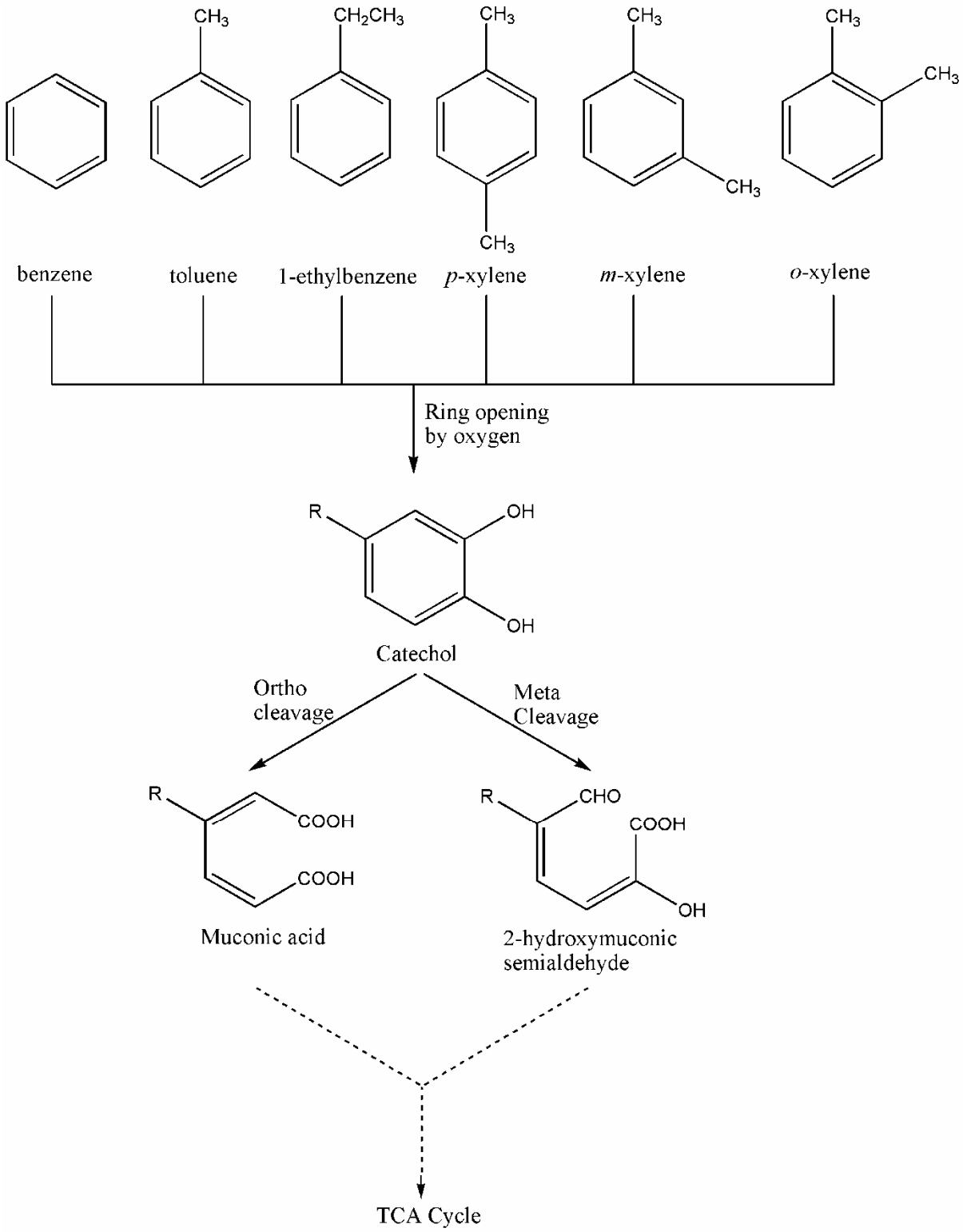


**Fig 1. General pathway for aromatic HC degradation**
(Eweis et al., 1998; Singh and Ward 2004)

**Table 3: Enzymes involved in biodegradation of petroleum HCs**

| Enzyme(s) | Substrate(s) | Degrading microorganisms | Reference |
|---|---|---|---|
| Soluble methane monooxygenases | $C_1$–$C_8$ alkanes alkenes and cycloalkanes | *Methylococcus sp., Methylosinus sp., Methylocystis sp., Methylomonas sp., Methylocella sp.* | McDonald et al., (2006) |
| Particulate methane monooxygenases | $C_1$–$C_5$ (halogenated) alkanes and cycloalkanes | *Methylobacter sp., Methylococcus sp., Methylocystis sp.* | |
| AlkB related alkane hydroxylases | $C_5$–$C_{16}$ alkanes, fatty acids, alkyl benzenes, cycloalkanes | *Pseudomonas sp,. Burkholderia sp,. Rhodococcus sp., Mycobacterium sp.* | Jan et al., (2003) |
| Eukaryotic P450 | $C_{10}$–$C_{16}$ alkanes, fatty acids | *Candida maltose, Candida tropicalis, Yarrowia lipolytica.* | Iida et al., (2000) |
| Bacterial P450 oxygenase system | $C_5$–$C_{16}$ alkanes, cycloalkanes | *Acinetobacter sp., Caulobacter, Mycobacterium* | Van Beilen et al., (2006) |
| Dioxygenases | $C_{10}$–$C_{30}$ alkanes | *Acinetobacter sp.* | Maeng et al., (1996) |

## Factors Affecting HC Biodegradation

Several environmental factors influence biodegradation of petroleum HC such as temperature, pH, nutrient and oxygen availability, salinity, pressure, and light. Bioavailability of contaminant is also an important aspect of biodegradation. Presence of HC degrading populations of microbes at sufficiently high levels is a prerequisite for an effective bioremediation. Occurrence and abundance of microorganisms in environment depend on availability and diversity of carbon sources. Surface soil has high organic matter but subsurface and deep layers has lower organic content. Because of a low amount of organic matter organisms in these regions are often dormant. Various physicochemical factors affecting HC degradation are described below (Maier, 2000).

- Oxygen availability: HC degradation takes place both in presence and absence of oxygen. However aerobic conditions are more favourable as oxygenases are the primary enzymes needed for degradation to occur. Oxygenases function in presence of oxygen, so degradation rates are higher in aerobic conditions as compared to those under anaerobic conditions.
- Nutrient availability: The process of biodegradation can be enhanced by addition of essential nutrients such as nitrogen and phosphorous. In case of petroleum oil spills where nitrogen shortages can be acute; carbon, nitrogen and phosphorous are added in the ratio of approximately 100: 10: 1 (C: N: P).

- Temperature: HC degradation is known to occur over a wide temperature range (psychrophilic to mesophilic) from close to zero degrees to up to more than 30°C. Bacteria can adapt to temperature fluctuations in order to maintain metabolic activity, however seasonal temperature fluctuations in the natural environment have been shown to affect the rate at which degradation occurs (Palmisano et al, 1991).
- pH: Highest rates of degradation are generally observed at neutral pH. However microorganisms growing on HC have been isolated from historically contaminated sites even at pH 2-3.
- Salinity: Salt concentration has varied effect on HC degradation depending on the type of environment and the type of organisms involved. Higher salt concentrations tend to inhibit degradation. The ability of variation in salinity to affect the rate of HC degradation appears to be dependent on the natural variation in salinity regime of the sample source (Kerr and Capone, 1988).
- Light: Availability of light can have a positive impact on HC degradation by photosynthetic microbes such as algae (Muñoz, 2003). Light can also degrade petroleum compounds by direct photochemical action. Physical properties of petroleum compounds are affected due to the photochemical reactions. Emulsion formation and the solubility of petroleum fractions may get altered by light (Van der Heul, 2009).

**Final Comments**

Quite a lot of research has been carried on hydrocarbon degradation. Due to the major oil spills which took place during last few decades, need for effective strategy to deal with the harmful effect of hydrocarbons on marine life, and also for understanding the risk involved for humans and its adverse effect on whole biosphere, has been felt constantly. The significance of microbial methods for hazardous waste remediation is evident with the changing environmental regulations and social acceptance of bio-based technologies (Zehnder and Wulff 1999; Harayama 2001; Grrommen and Verstraete 2002). It is necessary to assess the impact of hydrocarbons on environment and ecosystem, along with toxicological studies on risk for human health. Aliphatic carbons are volatile compounds, and are not that harmful to organisms due to their structure and characteristics in environment. Non volatile aromatic compounds are easily degraded by bacteria, while cycloalkanes are degraded comparatively at slower pace and are not often used for toxicological studies for assessing risks to humans. The PAHs have carcinogenic effects and are compounds of interest for toxicological studies. However biodegradation is able to degrade nearly all hydrocarbons present in environment (Van der Heul, 2009).

The process of degradation is influenced by multiple physicochemical factors. In future development of effective remediation strategy will focus on: (a) improving activity of indigenous organisms, (b) isolation of novel HC degrading strains, (c) genetic and physiological manipulation and metabolic engineering for strain improvement for creating *superbugs* - organisms that can degrade a multitude of pollutants at rapid rates, (d) forming consortia of suitable organisms, (e) using alternative electron acceptors which stimulates anaerobic degradation, (Maier, 2000). However, a successful implementation of bioremediation technologies will be a multidisciplinary affair, requiring combination of expertise from microbiologists, biochemists, geneticists, and chemical and environmental engineers.

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