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Biodegradation of Aromatic Compounds

Mehdi Hassanshahian, Moslem Abarian and
Simone Cappello

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Abstract

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous persistent environmental contaminants generated by natural combustion processes and human activities. PAHs are considered hazardous because of cytotoxic, mutagenic, and carcinogenic effects. Sixteen individual PAH compounds have been identified as priority pollutants by the United States Environmental Protection Agency (U.S. EPA). All substances originated in to the environment by either biogenic or anthropogenic sources. Anthropogenic compounds describe synthetic compounds, and compound classes as well as elements and naturally occurring chemical entities which are mobilized by man's activities. In the marine environment, the fate of pollutants is largely determined by biogeochemical process. Some of these chemical changes enhance the toxicity of the pollutants. Other chemical changes cause the degradation or immobilization of pollutants and, as a result, act to purify the waters. Possible fates for PAHs, released into the environment, include volatilization, photo-oxidation, chemical oxidation, bioaccumulation and adsorption on soil particles, leaching, and microbial degradation. Elevated concentrations of polycyclic aromatic hydrocarbons (PAHs) have been found in mangrove sediments due to anthropogenic compounds.

Pollution and microbial degradation have been suggested as the best way to remove PAHs from contaminated sediments. A significant positive relationship was found between bacterial growth and percentages of aromatic hydrocarbons degradation. The PAH biodegradation ability of the enriched mixed bacterial culture was not related to the degree of PAH contamination in surface sediments. The growth and biodegradation percentages of the enriched mixed culture were not higher that of the individual isolate especially at low salinity (0 and 10 ppt). The principal processes for their successful removal are currently believed to be microbial and bacteria transfor-

mation and degradation. A large variety of bacteria are known, which can utilize PAHs as a sole source of carbon and energy under aerobic and anaerobic conditions. Biodegradation in marine environment was also described attractively. In comparison to their chemically synthesized equivalents, they have many advantages: they are environmentally friendly, biodegradable, less toxic, and nonhazardous. They have better foaming properties and higher selectivity.

Keywords: Bacteria, degradation, polycyclic aromatic hydrocarbons (PAHs), marine

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are the major group of marine contaminants that are made of two or more benzene rings. Polycyclic aromatic hydrocarbons (PAHs) are one of the major categories of pollutants entering the marine environment and finally accumulating in the sediments. Their occurrence raises major concerns for human health, especially during coastal activities (bathing waters, aquaculture, etc.), having combined adverse effects still largely [1]. The solubility of these compounds in the seawater is low, and they tend to be bounded to suspended organic matter in the water column and finally accumulate in the marine sediment [2, 3]. Many researchers have experimentally worked on the biodegradation of PAHs, which involves the use of specific microorganisms, specific reducing culture, metabolic pathways, bioavailability and disappearance, growth of the microbes under aerated and unaerated conditions, pH variation, and kinetics of biodegradation [4]. The bacteria were capable of growing on various hydrocarbons like diesel, petrol, lubricating oil, toluene, naphthalene, and kerosene. The tests were conducted to detect the biodegradation of diesel, by amino-oxygenase biodegradation pathway, and it was observed that diesel was degraded within 12 hours [5].

2. Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are a group of approximately 10, 000 compounds that are atmospheric, water, and soil pollutants. They are organic contaminants that are formed from the incomplete combustion of a variety of organic compounds. The structure of a PAH consists of molecules containing two or more fused six-carbon atom aromatic rings; only hydrogen and carbon are present in the molecules. There are 18 PAHs considered by the Agency for Toxic Substances and Disease Registry that have adverse health effects: acenaphthene, acenaphthylene, anthracene, benzo[a]anthracene, benzo[a]pyrene, benzo[e]-pyrene, benzo[b] fluoranthene, benzo[*ghi*]perylene, benzo[*j*]fluoranthene, benzo[*k*]fluoranthene, chrysene, coronene, dibenz(a, h)anthracene, fluoranthene, fluorene, indeno(1, 2, 3-cd)pyrene, phenanthrene, and pyrene[6, 7]. Figure [1] shows some PAHs.
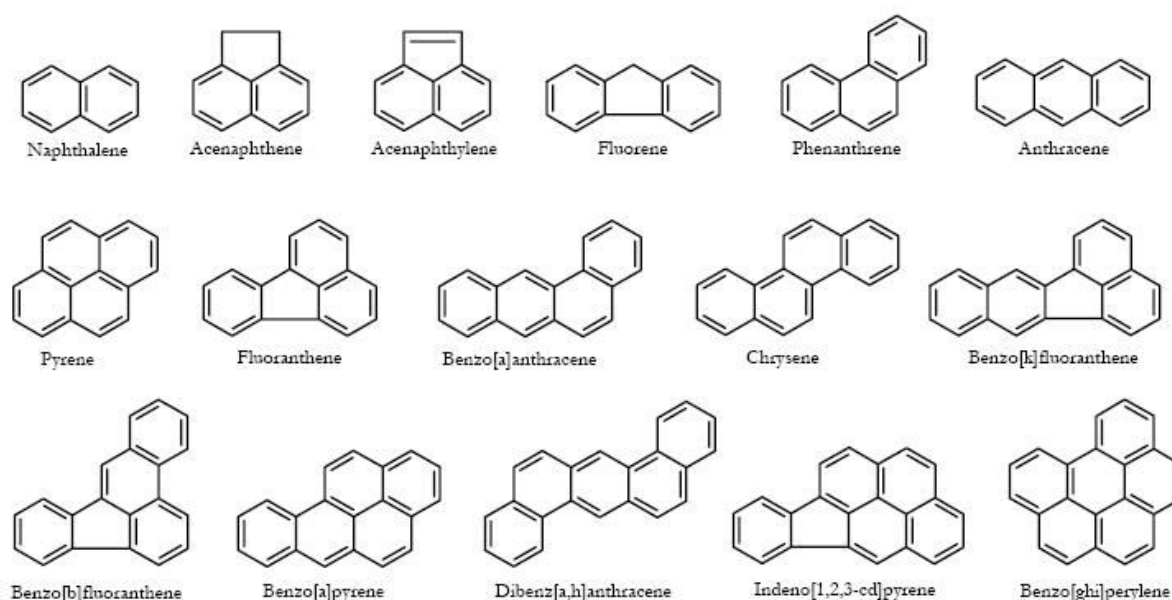
Figure 1. Structures of U.S. EPA's 16 priority pollutant PAH [1].

## 2.1. Phenanthrene (Phe), pyrene (Pyr), and benzo[e]pyrene (BeP)

The most PAHs in oil products have two and three fused benzene rings with one to four carbon atom alkyl group substitutions. Naphthalene, the two-benzene-ring PAH, often present in significant amounts in petroleum, is relatively volatile, soluble, and degradable. Therefore, naphthalene in weathered oil from an offshore spill may not be available when the contaminated water reaches coastal environments; on the other hand, higher molecular weight species such as phenanthrene (three-benzene-ring PAH), pyrene (four-benzene-ring PAH), and benzo[e]pyrene (five-benzene-ring PAH) can be found in weathered crude oils reaching coastal wetlands. The higher the number of benzene-rings the PAH has, the higher the molecular weight, but the lower the solubility in water [17, 29].

3. Sources of PAHs

The sources of PAHs in a marine environment could include volcanoes, natural fires, fossil fuel fired power generation, combustion engines, municipal discharges, oil pipeline spills, oil fields, offshore drilling platforms, natural oil seeps, and shipping accidents [6]. The sources of PAH are illustrated in Figure 2.

3.1. PAHs in water

The main sources of PAHs in water bodies are atmospheric particulate matter deposition, runoff of polluted ground sources, and pollution of river and lakes by industrial effluents, municipal wastewater discharge, and oil spills [14]. Since PAHs have low solubility and tend
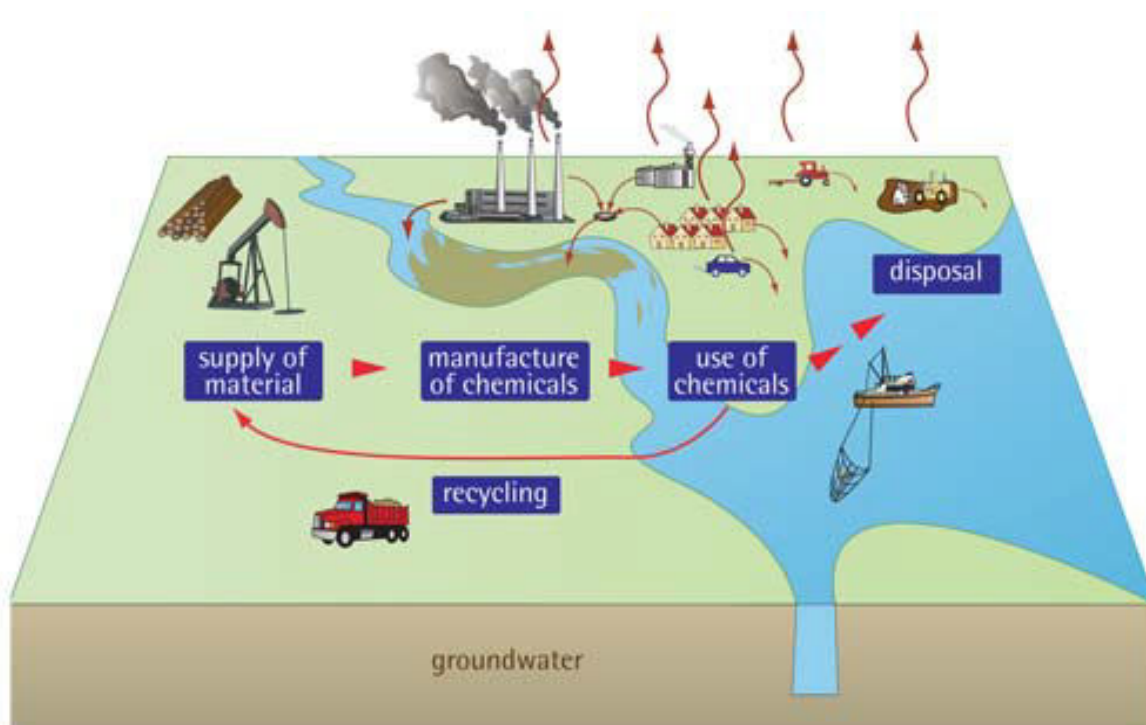
Figure 2. Diagram of the transfer of PAHs in the environment [24].

to adsorb to particulate matter, they are usually found in low concentrations in water bodies. Some PAH concentrations that have been measured in water include marine waters with levels of nondetected to 11 g L^{-1} wastewater in North American and European municipalities with levels of <1 to 625 g L^{-1} and urban runoff in the U.S. with levels of <0.05 to 560 g L^{-1} [14, 15].

3.2. Sources of PAHs in coastal environments and marine

PAHs in coastal environments can come from many sources naturally as well as through anthropogenic activity. They adsorb and tend to stay longer in sediment due to their hydrophobicity (low solubility and high octanol-water partition coefficient). The main sources of PAHs in a marine ecosystem are atmospheric deposition, river runoff, domestic and industrial wastewater, and direct spillage of oil or petroleum products [16]. Figure 3 shows the source of PAH in the marine environment.

4. PAHs biodegradation

PAHs are toxic to marine species and to the environment. As they are absorbed into soil/sediment, many hydrophobic PAHs in soil-water interfaces undergo some physical, chemical, and biological degradation, but biodegradation is the most effective process. Microorganisms utilize only dissolved substrates; and the utilization rates of PAH degradation products are related to sorption/desorption rates of PAHs to and from soil. Since biodegradation is assumed
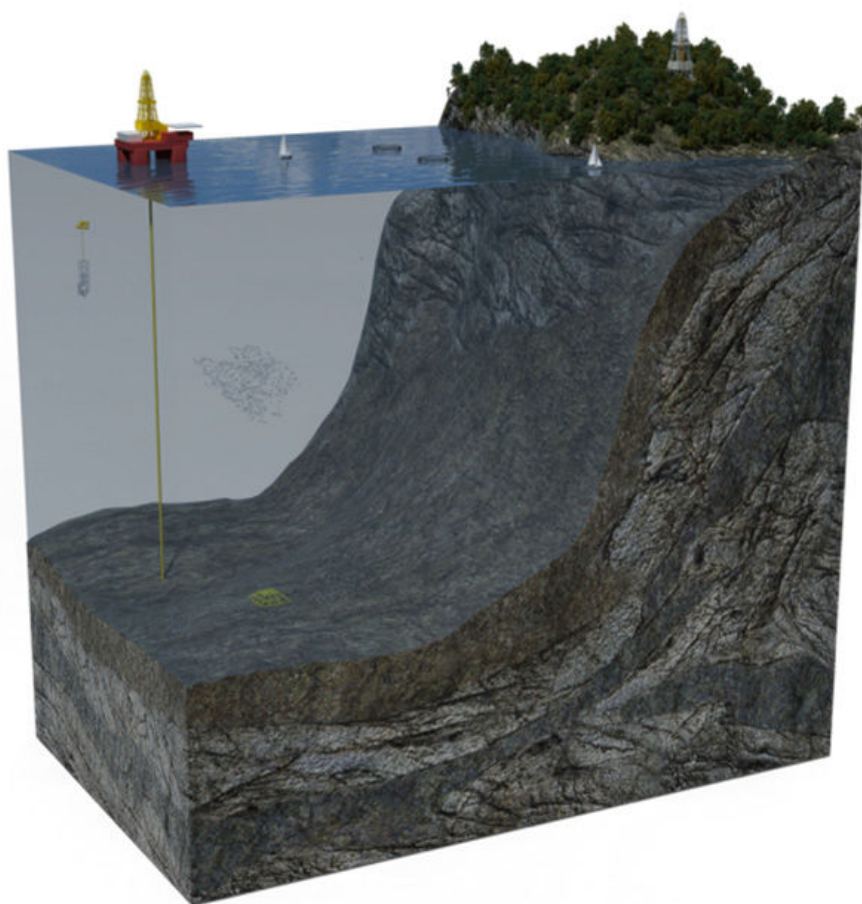
Figure 3. Seeps, oil spills from boats or platforms, and produced water discharge from oil- and gass-producing instal-lations such as the one shown. PAHs in produced water and oil seeps represent a chronic release to the marine enviro-ments [37]

to be the main reaction in PAH decay in soil, microorganisms require some nutrients to survive and to function. The nutrients in shortest supply are usually nitrogen (N), phosphorus (P), or both, while the supply of K, S, Mg, Ca, Fe, and micronutrient elements is nearly always greater than the demand [16]. Some nutrients can be added to wetland soil and water to enhance the degradation of organic material. The capacity of different wetland soil to retain nutrients may be different. Therefore, it is necessary to investigate PAH degradations in the subtidal and the intertidal wetland soil with the addition of nutrients.

Specific characteristics of coastal wetland ecosystems that make them suitable for PAH biodegradation processes are the large quantity of water, the oxic and anoxic soil for the breakdown of organic matter, and the supporting highly productive, tall emergent vegetation. Some organic compounds degrade favorably under aerobic conditions, such as naphthalene; other compounds degrade favorably under anaerobic conditions, such as DDT; and some others degrade favorably under moderately anaerobic conditions, such as PCBs. Understand-ing of PAHs degradation pathways and kinetics provides guidance for the selection of effective methods and technology to remediate a contaminated site [18]. The schematic biodegradation of PAHs is described in Figure 4.
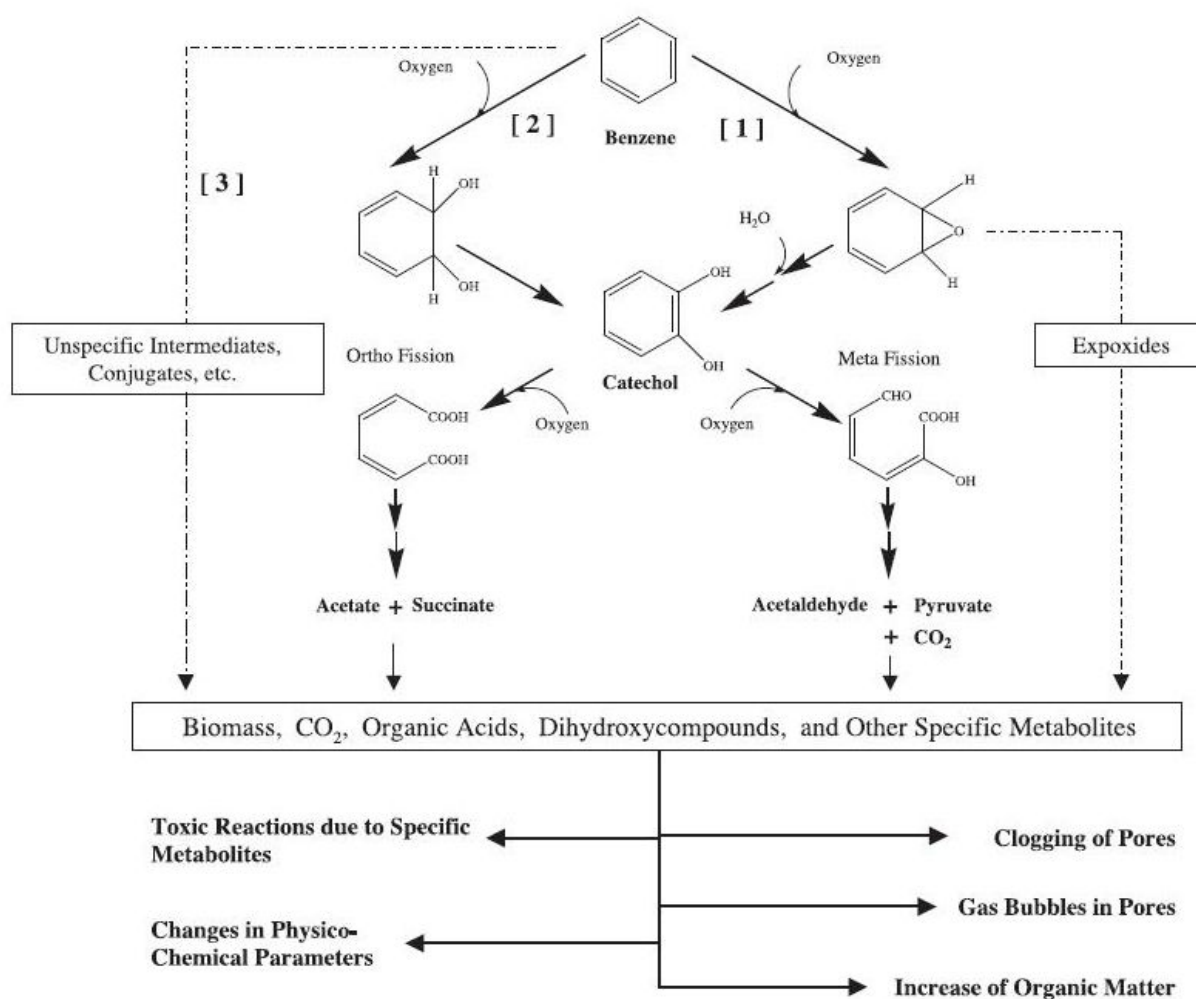
Figure 4. Biodegradation of aromatic hydrocarbons: metabolism begins with the activity of a mono-oxygenase or a di-oxygenase [36].

4.1. Properties PAHs in biodegradation

Pure compounds of PAHs with higher molecular weights are more resistant to biotransformation and pure compounds of PAHs with lower molecular weights transform more rapidly [30]. However, the presence of one PAH can decrease the biodegradation of other PAHs. With the simultaneous presence of phenanthrene, acenaphthene, fluorine, anthracene, pyrene, and benzo[a]pyrene, the biodegradation was decreased for phenanthrene and acenaphtene but was enhanced for fluorine, anthracene, and pyrene (while biodegradation of benzo[a]pyrene did not occur within a 12-day incubation period [31]. There are thousands of PAH compounds in the environment, but in practice, PAH analysis is restricted to the determination of 6 to 16 compounds. Individual PAHs differ substantially in their physical and chemical properties. Generally, the high molecular weight compounds (≥ 4 aromatic rings) are less water soluble, less volatile, and more lipophilic than lower molecular weight ones. The best known model compound from this group is highly

carcinogenic benzo[a]pyrene (BaP). PAHs are listed by the U.S. Environmental Protection Agency and the European Commission as priority pollutants [32].

4.2. PAH-degrading bacteria

PAH degradation has been known for many years to occur in marine sediments. Previous results indicate that culturable hydrocarbon-degrading and PAH-degrading populations are widely distributed and can be enriched from sites of contamination in marine environments [7, 8, 9, 10]. However, other studies indicate that high levels of PAH can be toxic to marine bacteria [11, 12, 13]. Therefore, highly contaminated sites might be inhibitory to PAH-degrading bacteria and other microorganisms.

4.3. Community from the Surface Microlayer (SML) in an estuarine

The sea surface microlayer (SML) represents the interface between the atmosphere and the hydrosphere. Organisms within the SML are known as neuston, and the community of bacteria present within this neuston layer is named bacterioneuston. *Acinetobacter* and *Rhizobium* were also found among the PAH-degrading isolates retrieved from the SML. Many environmental strains of *Acinetobacter* with hydrocarbon-degrading capacities have been isolated in terrestrial and marine environments [19]. The subclass gamma-Proteobacteria was relatively more abundant in bacterioneuston (SML) than in bacterioplankton. The gamma-Proteobacteria subclass includes major PAH-degrading genera, such as *Alcanivorax*, *Cycloclasticus*, *Pseudomonas*, *Oleiphilus*, *Oleispira*, and *Thalassolituus* [20, 21].

4.4. PAH-degrading bacteria in coastal sediment

It is well known that bacterial degradation plays an important role in PAHs removal from the marine environment. Many PAH-degrading bacteria have been found in coastal sediments, including bacteria of *Cycloclasticus*, *Marinobacter*, *Pseudoalteromonas*, *Marinomonas*, *Sphingomonas*, *Vibrio*, and *Halomonas.* However, knowledge about deep-sea environments is relatively less. In previous studies on the deep-sea sediments of Atlantic Ocean and Pacific Ocean, *Cycloclasticus* was the most important bacterium, in addition to *Alteromonas* and *Novosphingobium* [22, 23].

5. Toxicity and the effects of PAHs

PAHs reveal their toxicity following biotransformation to toxic metabolites, which can be bond covalently to cellular macromolecules such as DNA, RNA, and protein. The major PAH-metabolizing enzymes are cytochrome P450 mono-oxygenases, epoxide hydrolase, and several conjugating enzymes [25]. Highly carcinogenic 7, 8- and 9, 10-dihydrodiols are the major PAH metabolites produced by fish microsomes. The metabolites of PAH are mainly conjugated with glucuronic acid. Most conjugates are water-soluble organic anions and are rapidly excreted mostly via the gallbladder or in urine PAHs with high molecular weight, which are not acutely

toxic to fish. However, in the presence of solar ultraviolet radiation, many of them are acutely toxic. Exposure to PAHs causes the suppression of the immune system. A decreased number of melano-macrophage centers in the liver and suppression of proliferative responses of T-lymphocytes [26, 27, 28].

6. Anaerobic biodegradation PAHs

The anaerobic degradation of aromatic hydrocarbons is often presumed to be slow and of minor ecological significance. However, anaerobic biotransformation may play a key role in the transformation of aromatic and PAH compounds when oxygen demand exceeds supply in natural environments. As shown in Figure 5, under such conditions, anoxic or anaerobic degradation mediated by denitrifying or sulfate-reducing bacteria can become a key pathway for the cleanup of contaminated sites [33]. The isolation of denitrifying and sulfate-reducing organisms capable of degrading toluene has led to the elucidation of several biodegradation pathways and phylogenetic relationships between bacterial strains. The isolation of denitrifying and sulfate-reducing organisms capable of degrading toluene has led to the elucidation of several biodegradation pathways and phylogenetic relationships between bacterial strains [34, 35].
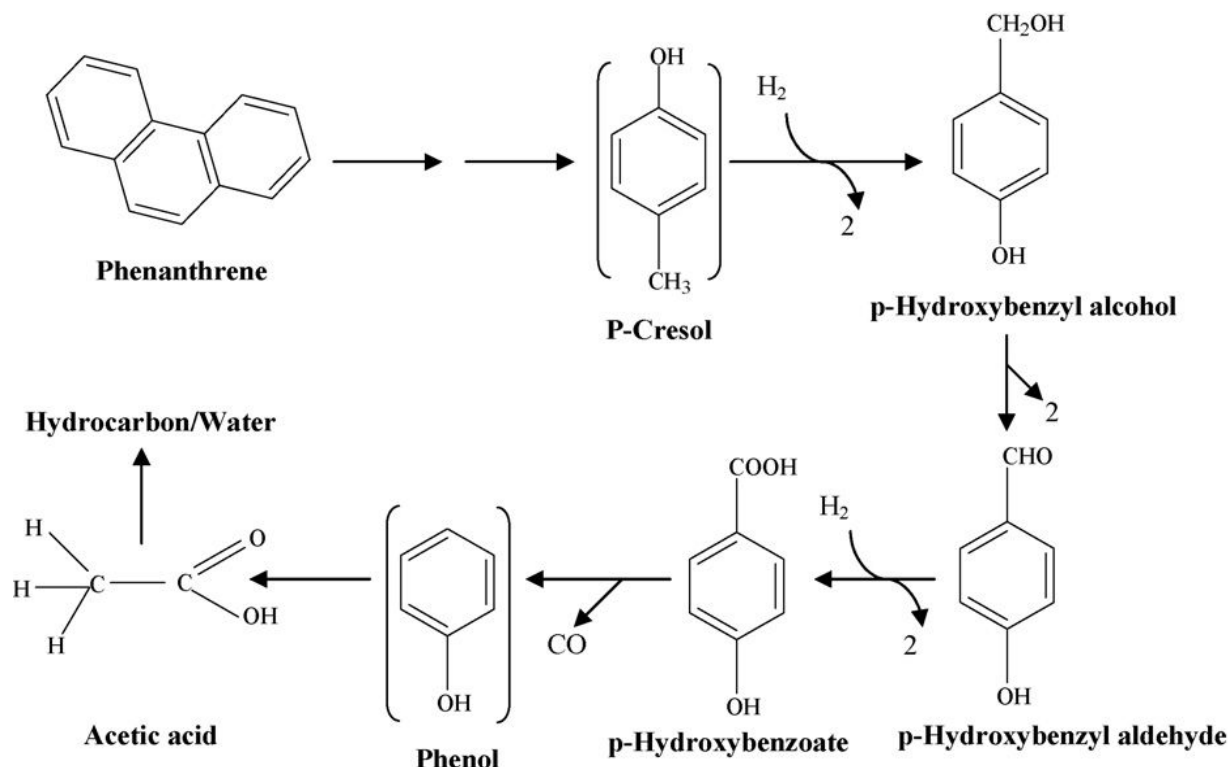

Figure 5. Proposed anaerobic biotransformation pathway of phenanthrene by sulfate-reducing bacteria [38].

7. Aerobic biodegradation

During aerobic degradation by bacteria, PAHs are oxidized to *cis*-dihydrodiols through the incorporation of an oxygen molecule into the PAH. As shown in Figure 6, the *cis*-dihydrodiols are further oxidized to aromatic dihydroxy compounds (catechols), and then PAH rings are cleaved with intracellular dioxygenases. The oxidation of unsubstituted PAHs with very high thermodynamic stability often results in PAH compounds that are less stable than the parent compounds and more susceptible to cleavage [40, 41, 42]. Oxygenase production by bacteria can be increased using biostimulants; for example, salicylic acid is a known inducer of naphthalene dioxygenase. Linoleic acid is a powerful stimulant of pyrene and benzo[a]pyrene degradation by gram-positive bacteria. The addition of humic substances also greatly enhances the microbial degradation of PAHs [43].
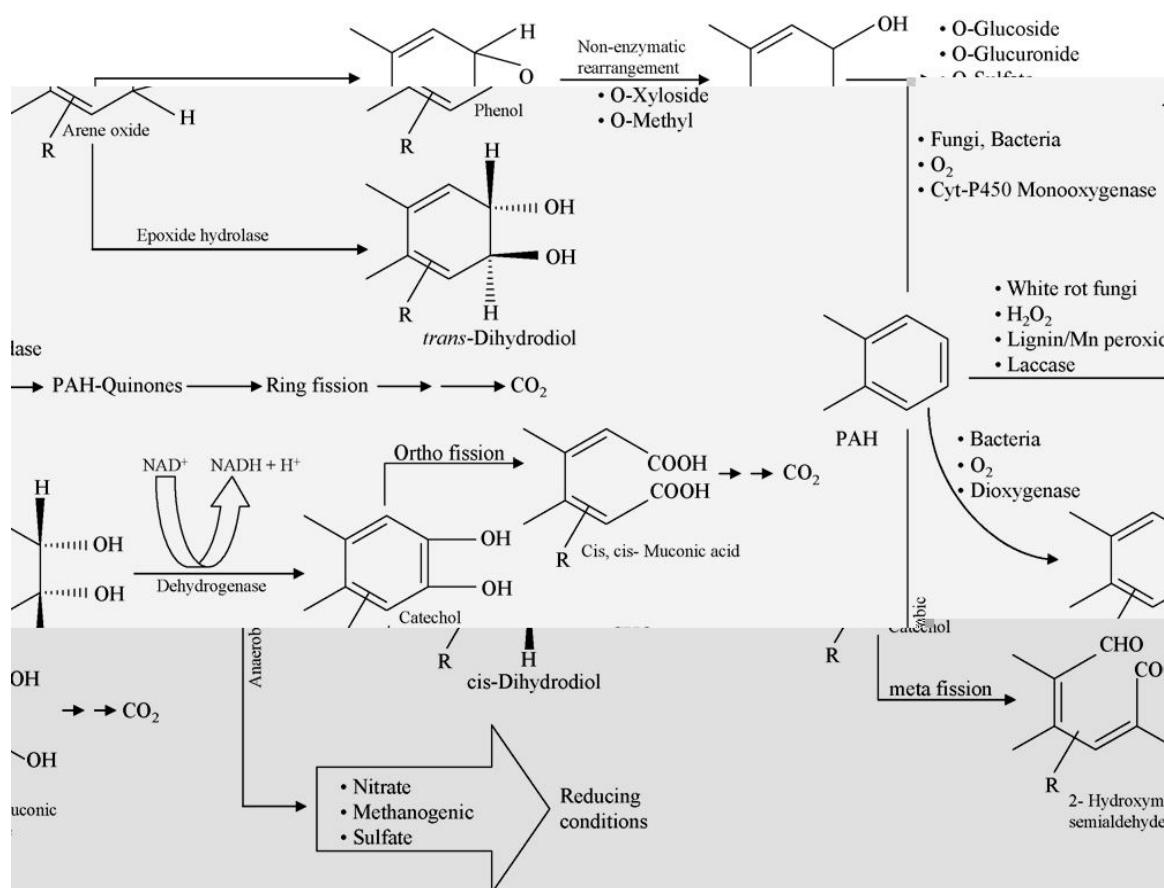
Figure 6. Proposed pathway for microbial catabolism of polycyclic aromatic hydrocarbons [39].

8. Biodegradation of benzo[a]pyrene by marine denitrifying bacteria

Benzo[a]pyrene (BaP) is one of the high molecular weight, 5 ring-PAHs and is listed as one of the priority pollutants classified as a carcinogen by the U.S. Environmental Protection Agency

and the Agency for Toxic Substances and Disease Registry. BaP is ranked number 9 on the priority list of 275 substances. The toxicity of BaP is of high concern because of its ability to accumulate in animal tissues, to cause cancer and hormone disruption, and to affect reproduction. Moreover, BaP was found to depress immune function [44, 45-47]. The principal natural sources of BaP are forest fires, volcanic eruption, pest fires, and burning of crude oil. Anthropogenic sources include the incomplete combustion of coal, oil, gas, wood, rubbish, and other organic substances, incinerator, vehicle exhausts, and cigarette [48]. BaP derived from gasoline automobile accounted for 98% [48, 49]. The natural presence of PAHs in the environment allows many microorganisms to adapt to exploit this naturally occurring as the potential growth substrate. Thus, many bacterial, fungal, and algal strains have been shown to utilize wide varieties of PAHs containing from three to five aromatic rings [50]. The application of microorganisms for bioremediation of PAH-contaminated environment seems to be an attractive technology because they are more specific, effective, economic, and environmental friendly. Biodegradation in marine environment was also described attractively [50, 51]. Denitrification is an important major process; denitrifying bacteria responses to environmental conditions by cycling nitrogen gas back to the atmosphere. These denitrifying bacteria utilize nitrate (NO_3-), nitrite (NO_2-), nitric oxide (NO), and nitrous oxide (N_2O) as terminal electron acceptors in anaerobic respiration. The denitrifying bacteria isolated from marine sediment were *Bacillus subtilis* in majority. *B. subtilis* have the ability to degrade benzo[a]pyrene with a complete degradation efficiency of the initial amount of 200 mg/l supplied over 45 days of incubation at 30°C [52, 53]. *B. subtilis* successfully biodegraded benzo[a]pyrene but failed to increased biomass production [54, 55].

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