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Review Article

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Bacterial Biodegradation Pathways of Low and High Molecular Weight Polycyclic Aromatic Hydrocarbons (PAHs)

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Abstract

Polycyclic Aromatic Hydrocarbons (PAHs) are widespread pollutants in various ecosystems. These pollutants are of great concern due to their potential toxicity, mutagenicity and carcinogenicity as well as recalcitrance in the environment due to their hydrophobicity. United States Environment Protection Agency (USEPA) has enlisted 16 of PAHs as priority pollutants that must be disposed. Physicochemical properties of PAHs and their classification according to carcinogenicity as determined by specific agencies have been recorded. Treatment of PAHs by physicochemical methods are expensive and having limited efficiency. So, obligatory development of alternative technology for *in situ* application must be created. Microbial degradation of PAHs represent the major mechanism responsible for cleaning up of the environment and recovery of PAHs contaminated sites. The main goal of this review is to provide an outline of bacterial degradation pathways of PAHs catabolism. A number of bacterial genera that metabolize PAHs have been isolated (*Alcaligenes*spp. *Bordetella*spp. *Bacillus*spp. *Rhodococcus*spp. *Pseudomonas* spp. and *Mycobacterium*spp.). This review includes the catabolic pathway of the Low Molecular Weight-Polycyclic Aromatic Hydrocarbons (LMW-PAHs) and High Molecular Weight-Polycyclic Aromatic Hydrocarbons (HMW-PAHs) by different bacterial isolates and strains. Also the catabolic enzymes (Monooxygenases and diooxygenases) involved in bacterial catabolic pathways has received a considerable attention for better understanding of the catabolic pathways. Application of bacterial strains in treatments of Refinery Waste Water of Petroleum (RWP) have been taken in consideration to facilitate the development of new treatment methods to enhance PAHs bioremediation as a sole compound or in a mixtures in polluted ecosystems.

Keywords: Bacteria; Biodegradation; High molecular weight- PAHs pathways;Low molecular weight PAHs pathways; Physicochemical structures; Polycyclic Aromatic Hydrocarbons (PAHs) ; RefineryWaste Water of Petroleum (RWP)

Introduction

In recent years, there has been increasing concern over public health threated presented by introduction of petroleum hydrocarbon pollutants in environment due to anthropogenic activities to a greater extent and natural process to less extent [1].

The rapid economic growth achieved in last decade has been paralled by an increase in global petroleum oil consumption [2] in which different petroleum oil industries such as fuel are fast growing, synthetic polymers and petrochemicals**.** Polycyclic Aromatic Hydrocarbons (PAHs) are category of over 100 various, compounds released from incomplete combustion source[3].These sources are either natural i.e. petroleum industry activities as well as accidental spills, bush fire, forest and volcanoes eruptions or manmade combustion i.e. care emission, cigarette smoke, wood burning and combustion of dung and crop residues [4-6].

PAHs are a group of hydrophobic hydro carbonic compounds consisting of two or more combined benzene rings in linear, angular or cluster arrangement[7-9].Most of PAHs persist in the ecosystem for many years owing to their hydrophobicity and their absorption to solid particles[10,11].

Physical and chemical properties of PAHs

PAHs are organic substance made up of carbon and hydrogen they can be divided into two categories: Low Molecular Weight (LMW-PAHs) compounds consisting of fewer than four rings and High Molecular Weight (HMW-PAHs) compounds of four or more rings. Pure PAHs are usually colored crystalline solids at ambient temperature [12].Chemical structures of some commonly PAHs are indicated in Figure 1.

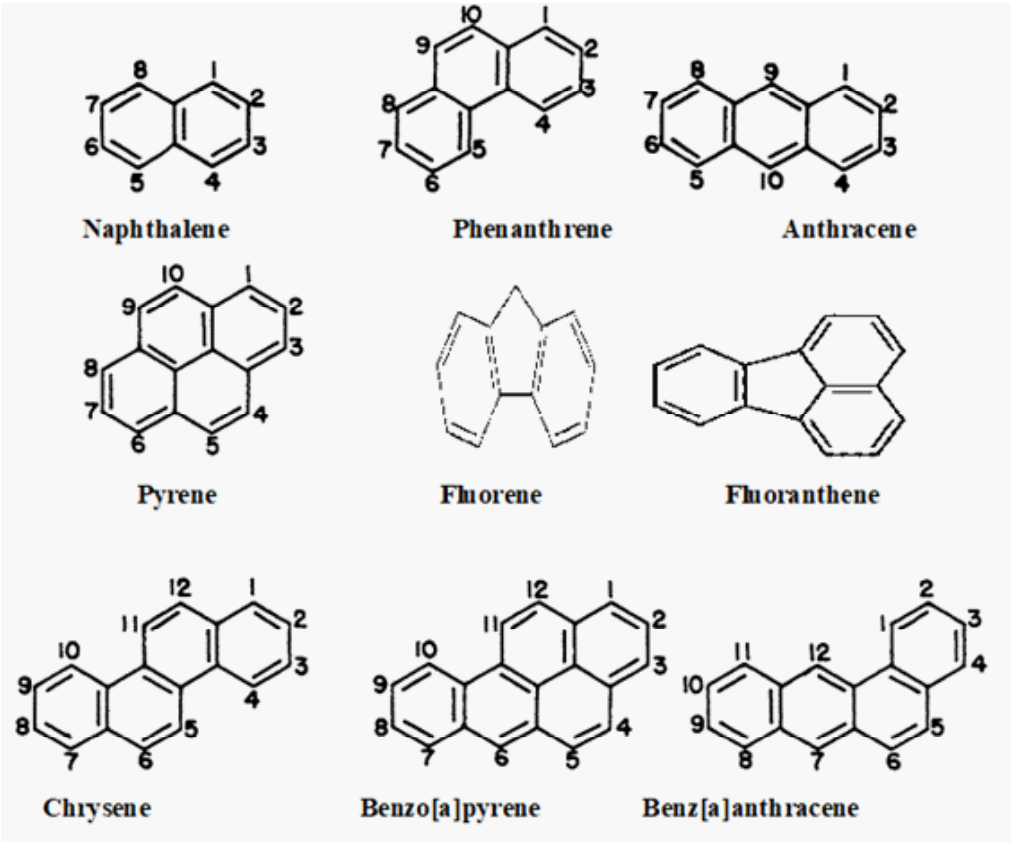
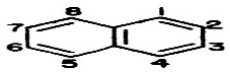
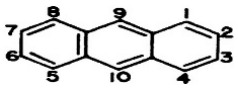
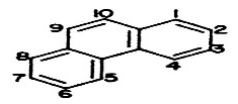


Figure 1: Chemical structures of some PAH compounds.

Physical and chemical characteristics of some priority PAHs listed by the USEPA are shown in Table 1.

Compound	M.F.	Configuration	M.wt.	M.P.°C	B.P.°C	R.N.	D.C.	Class
Naphthalene	$C_{10}H_8$		**128.1**	**80.2**	**218**	**2**	**0**	**LMW**
Anthracene	$C_{14}H_{10}$		**178.1**	**216.4**	**342**	**3**	**0**	**LMW**
Phenanthrene	$C_{14}H_{10}$		**178.1**	**100.5**	**340**	**3**	**0**	**LMW**

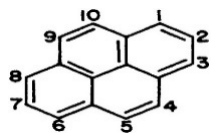
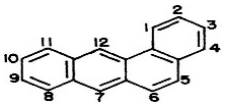
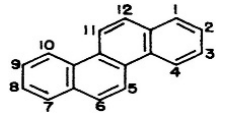
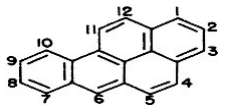
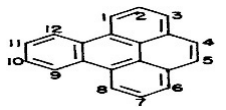
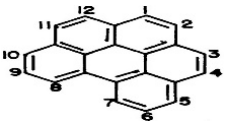
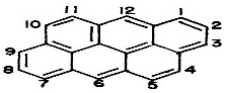
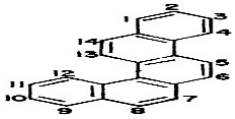
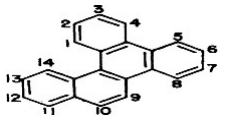
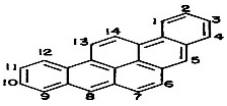
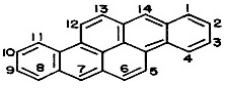
Pyrene	$C_{16}H_{10}$		**202.0**	**393**	**150**	**4**	**-**	**HMW**
Benz[a]anthracene	$C_{18}H_{12}$		**228.1**	**158**	**438**	**4**	**+**	**HMW**
Chrysene	$C_{18}H_{12}$		**228.1**	**254**	**448**	**4**	**+**	**HMW**
Benzo[a]pyrene	$C_{20}H_{12}$		**252.1**	**179**	**495**	**5**	**++**	**HMW**
Benzo[e]pyrene	$C_{20}H_{12}$		**252.1**	**178[13]**	**492**	**5**	**+**	**HMW**
Benzo[ghi]perylene	$C_{22}H_{12}$		**276.1**	**277**	**500**	**6**	**+**	**HMW**
Dibenzo[def,mno] Chrysene (Anthanthrene)	$C_{24}H_{14}$		**276.1**	**264°C [14]**	**497**	**6**	**-**	**HMW**
Benzo[c]chrysene	$C_{22}H_{14}$		**278.1**		**524**	**5**	**+**	**HMW**
Benzo[g]chrysene	$C_{22}H_{14}$		**278.1**	**112-114 °C[15]**	**524**	**5**	**+**	**HMW**
Benzo[rst]pentaphene (Dibenzo[a,i]pyrene)	$C_{24}H_{14}$		**302.1**	**281.5 °C [16]**	**552**	**6**	**++**	**HMW**
Dibenzo[a,h]pyrene	$C_{24}H_{14}$		**302.1**	**315°C [16]**	**552**	**6**	**++**	**HMW**
M.F.; Molecular formula,Mwt; Molecular weight, B.P.°C; Boiling Point Celsius degree, M.P. °C; Melting point Celsius degree, D.C.; Degree of Carcinogenicity, (-); Not available, (0); limit Effect, (+); Moderate effect, (++); High effect, LMW; Low Molecular weight and HMW; High Molecular weight.								

Table 1: Physical and chemical characters of some PAHs [17,18].

PAHs possess very characteristic UV absorbance spectra. As each ring structure has unique UV. Spectrum, each isomer exhibits a unique absorbance spectrum also. This is especially useful in identification of PAHs [19].

Regarding to the mutagenic and carcinogenic effects from chronic exposure to PAHs and their metabolites classifications as enlisted by US. Department of Health and Human Services (HHS), International Agency for Research on Cancer (IARC, 2009)[20]and US. Environmental protection Agency (USEPA, 2007)[21] are indicated in Table 2**.**

Agency	PAH Compound(s)	Carcinogenic Classification
U.S. Department of Health and Human Services (HHS)	• **benz(a)anthracene,** • **benzo(b)fluoranthene,** • **benzo(a)pyrene,** • **dibenz(a,h)anthracene, and indeno(1,2,3c,d) pyrene.**	**Known animal carcinogens**
International Agency for Research on Cancer (IARC)	• **benz(a)anthracene andBenzo (a)pyrene.**	**Probably carcinogenic to humans**
	• **benzo(a)fluoranthene,** • **benzo(k)fluoranthene, andideno(1,2,3-c,d) pyrene.**	**Possibly carcinogenic to humans**
	• **anthracene,** • **benzo(g,h,i)perylene,** • **benzo(e)pyrene,** • **chrysene,** • **fluoranthene,** • **fluorene,** • **Phenanthrene, and pyrene.**	**Not classifiable as to their carcinogenicity to humans**
U.S. Environmental Protection Agency (EPA)	• **benz(a)anthracene,** • **benzo(a)pyrene,** • **benzo(b)fluoranthene,** • **benzo(k)fluoranthene,** • **chrysene,** • **dibenz(a,h)anthracene, andindeno(1,2,3-c,d) pyrene.**	**Probable human carcinogens**
	• **acenaphthylene,** • **anthracene,** • **benzo(g,h,i)perylene,** • **fluoranthene,** • **fluorene, phenanthrene, and pyrene.**	**Not classifiable as to human carcinogenicity**

Table 2: Carcinogenic classification of selected PAHs by specific Agencies.

Pad impact of PAHs on human

PAHs pose high risks on human populations[22,23].United State Environmental protection Agency (USEPA) has enlisted 16 of PAHs as priority pollutants[21].PAHs have a potential to induce malignant tumors that primarily affect skin and other epithelial tissue as they have a great affinity for nucleophilic center of macromolecules like RNA, protein and DNA[24].

PAHs induce Geno toxicity, mutagenicity and carcinogenicity as shown in different living organisms or cell lines [24,25-27]. PAHs are environmental carcinogens[28], associated with skin, lung, pharynx, oral and other cancers [29].

Galicia, eye redness, headache, sore throat, trauma, nausea, dizziness, breathing difficulty and abdominal pain have been reported[30]. Lung cancer is expected to cause 10 million deaths per year worldwide by near 2030[31],also PAH-DNA adducts have been detected in blood from newborns whose mothers were living in polluted sites[32].PAHs from stable and depurating DNA adducts

in mouse skin to induce paraneoplastic mutations. Depurating adducts play a major role in forming tumorigenic mutations[33].A number of PAHs found in cigarette smoke of US and European brands, such as Benz[a]-anthracene and Benz [a]-Pyrene have been classified as carcinogens by the International Agency for Research on cancer[34], causing lung cancer mortality[35-37].

Epidemiological studies have shown evidence that cancer, birth defects, genetic damage[20], immunodeficiency[21], respiratory [38] and nervous system disorders [21] can be linked to exposure to occupational levels of PAHs.

PAHs are rapidly distributed in wide variety of tissues with a marked tendency for localization in body fat. Metabolism of PAHs occurs via cytochrome P460-mediated mixed function oxidase systems with oxidation or hydroxylation as first step[22]. Due to lipophilic characteristics of PAHs they tend to accumulate in food chain[39].PAHs are able to cross placental barrier and are also found in breast milk[40].High prenatal exposure to PAHs is associated with low IQ at age three, increased behavioral problems at age six to eight and childhood asthma[41,42].Furthermore associated with reduced birth weight, length and head circumference, lower scores on childhood tests of neurodevelopment and with symptoms of anxious / depressed and attention problems[43].

Once PAHs enter the human body, PAHs are metabolized in a number of organs and excreted in bile and urine also excreted in breast milk and stored in adipose tissue[44].Pyrene is commonly found in PAH mixtures, and its urinary metabolite, 1-hydroxypyrene, has been used as an indicator of exposure to PAH chemicals [45-49].

Physicochemical degradation of PAHs

Many conventional engineering based physicochemical decontamination methods are expensive due to the cost of excavation and transportation of large quantities of contaminated materials for *ex-situ*treatment viz soil washing, chemical inactivation (use potassium permanganate and/ or hydrogen peroxide as a chemical oxidant to mineralize non-aqueous contaminants such as petroleum) and incineration[50-52].Among physicochemical methods used for PAHs treatment, are dispersion dilution, sorption, volatilization and abiotic transformation[53,54].

There are another chemical methods e.g, chemical oxidation and photocatalysis remediation[55,56].Due to the increasing cost and limited efficiency of these conventional physicochemical treatments obligatory development of alternative technology for *insitu*application must be created, particularly based on microbial remendation capabilities of microorganisms [51,57].

Microbial degradation of PAHs

Microbial degradation is green technology for cleanup of pollutants by biological means include bioremediation, biodegradation, bio-augmentation, biostimulation and phytoremediation[58-62].

Microorganisms play crucial role in maintaining ecosystem and biosphere to develop sustainable environmental cleaning up [52]. They also used to mitigate adverse effects of pollutants [54,63,64].Bacteria, fungi and alge are reported to be hydrocarbon pollutants degraders [53,65-69].

Resistance of hydrocarbon pollutants to microbial degradation in either soil or water tends to increase with the type as well as molecular weight and number of rings. Naphthalene is ready biodegraded in most situations, however PAHs with four, five or six rings tend to be degraded much more slowly. Generally aerobic biodegradation occurs much more rapidly than anaerobic biodegradation[70].

Polycyclic aromatic hydrocarbons (PAHs) degrading bacteria

A large number of bacteria that metabolize PAHs have been isolated (*Alcaligenes dentrificans, Rhodococcus* sp., *Pseudomonas* sp., *Mycobacterium* sp.) [71].A variety of bacteria can degrade certain PAHs completely to $CO_2$and metabolic intermediates or H_2O[72].*Mycobacterium* spp. *Sphingomonas* spp., *Rhodococcus* spp. and *Nocardia* spp. populations were selectively stimulated in soil contaminated with PAHs or hexadecane[73].

Low Molecular weight PAHs (LM W-PAHs) degrading bacteria

A large number of naphthalene-degrading bacteria including *Pseudomonaspanipatensis ; Pseudomonas putida; P. vesicularis; P. paucimobilis; Bacillus cereus; Mycobacterium* sp.*; Alcaligenes dentrificans; Rhodococcus* sp.; *Corynebacterium venale; Cyclotrophicus* sp.;*Streptomyces* sp.; *Vibro* sp. and *Bordetella avium.* Have been isolated[68,74].In case of naphthalene-degrading bacteria, a different bacteria including *Arthrobacter polychromogenes; Aeromonas* sp.; *Beijerincka* sp.;*Micrococcus* sp. *Alcaligenes faecalis; Mycobacterium* sp.*;Nocardia* sp.; *Bordetella* sp.*; Flavobacterium* sp.*Bacillus* sp.*; Vibro* sp. and*Rhodococcus* sp.[69].The degrading bacterial strains that have been characterized are taxonomically diverse and mainly belong to the genera *Mycobacterium,Pseudomonas, Bacillus, Sphingomonas*, and *Alcaligenes.*[67,75-78].*Bacillus subtilis* showed the highest catechol, 1, 2 dioxygenase activity in MSM supplemented with anthracene with 99% degradation after five days incubation [79].

High Molecular weight PAHs (HM W-PAHs) degrading bacteria

Sphingobium KK22 isolated from soil of Texas, USA. This strain able to grow on phenanthrene and metabolize Benzo[a] anthracene[80].*Mycobacterium* RJG II-135 is capable to degrade phenanthrene, anthracene and pyrene at 10 to 20 fold greater than

Benzo[a]anthracene and Benzo[a]pyrene [81].*Mycobacterium vanbaalenii* PYR-1 is able also to degrade wide range of low and high molecular weight of PAHs [82].*Bacillus subtilis* isolated from contaminated soil with PAHs.*Bacillus subtilis* is able to transform pyrene and Benzo[a]pyrene, but degradation rate of Benzo[a] pyrene is greater than Pyrene [83].

Two microorganisms *Bacillus* SPO2 and *Mucur* SFO6 which are capable to degrade PAHs, were immobilized on vermiculite and investigate their ability to degrade Benzo[a]pyrene. Removal rate in case of immobilized bacterial-fungal mixed consortium was higher than that of freely mobile mixed consortium [84]. In another research, *Bacillus subtilis*DM-04 and *Pseudomonas aeruginosa* mucoid (M) and Non-Mucoid (NM) strains isolated from petroleum contaminated soil samples of North East India were used to degrade pyrene.*Bacillus subtilis* showed higher utilization of pyrene than *Pseudomonas.*

Bacillus subtilis and *Pseudomonas* were able to secreting biosurfactants in the medium which enhanced the solubility of pyrene in aqueous media leading to higher utilization of pyrene [85].Bacterial consortium CON-3, isolated from crude oil contaminated soil of Punjab, India cometabolized 50 µg/ml pyrene in the presence of glucose (0.5% w/v) at 30 ^{o}C.*Bacillus* PK-12,*Bacillus* PK-13 and *Bacillus* PK-14 from CON-3 were able cometabolize pyrene in order PK-12> PK-13> PK-14 after 35 days of incubation [86].Also in India, a bacterial strain*Bacillus thuringiensis* NA2 was isolated from polluted site with petroleum oil.*Bacillus thuringiensis* was able to degrade fluoranthene and pyrene. By optimizing the different factors (PH, Temperature, glucose addition etc…) which increased the biodegradation [87]. Syakti *et.al.,* (2013)[88] isolated 6 viable and cultural bacterial strains from contaminated mangroves. The bacterial strains were identified by 16S RNA as *Bacillus aquimaris, Bacillus megterium* and *Bacillus pumilus* while the other 3 strains were related to *Flexibacteraceae bacterium, Halobacilus trueperi* and *Rhodobacteraceae*bacterium. These strains were able to grow on PAHs (Phenothiazine, fluorine, fluornthene, dibenzothiophene, phenantherene and pyrene). Combination of two bacteria, *Bacillus* PY-1 and *Sphingomonas* PY-2 and a fungus *Fusarium* Py-3 isolated from contaminated soils were able to degrade pyrene and volatize arsenic independently and in combination. Removal of pyrene in high rate was recorded after 9 days in liquid medium and 63 days in soil [89]. Abo-State *et.al*., (2013,2014) [90,91] isolated five bacterial strains from soil and water contaminated with petroleum oil, Cairo, Egypt. The most potent strains (two strains) were identified by16S rRNA as *Bacillus amyloliquifaciens* MAM-62 with accession number 038054 and the other bacterial strain was *Achromobacter xylosoxidans* MAM-29 with accession number 038055.Both of the two bacterial strains were able to degrade pyrene efficiently as a sole carbon and energy source.

Bacillus amyloliquifaciens MAM-62 degrade 94.1%; 90.8%; 90.6; 72.9% and 51.4% of 100,200, 300, 400, and 500 µg/l pyrene after 21 days respectively[91].

However *A. xylosoxidans* MAM-29 degraded 95.0%; 90.5%;90.30%; 71.1% and 50.7% of the Benzo[a]anthracene as same pyrene concentrations respectively[92]. However, [67] isolated eight bacterial strains from soil contaminated with crude petroleum oil from Egypt. The most potant bacterial strain, isolateMAM-P8 was identified by 16Sr RNA as *Bacillus altitudinis* which was able todegrade 91%,, 33% and 97% of PAHs mixture (500 µM pyrene, 500 µM Benzo [a] anthracene and 50 µM Benzo [a] pyrene) respectively.

*Pseudomonas aeruginosa*SP4 isolated from contaminated soil produce surfactant, by enhancing biosurfactant production for more efficient pyrene degradation[93].

Pseudomonas isolate PAHs As^{-1}removed all 60 mgl^{-1}phenanthrene and half of 20 mgl^{-1}pyrene within 60 h respectively[94].*Brevibacillus brevis* adsorbed pyrene initially on their cells and then pyrene was transported and intracellularly degraded. The removal of pyrene (mgl^{-1}) was 0.75 mgl^{-1}after 168 hours. PAHs-utilizing bacteria (26) were isolated from soil of 7 sites of Mathura refinery, India. The most potant strains were 15 strains (*Bacillus, Acinetobacter, Stenotraphomonas, Alcaligenes, Lysinibacillus, Brevibacterium, Serratia* and *Streptomyces* were adapted to utilize mixture of 4 PAHs (anthracene, fluorine, phenanthrene and pyrene). A cosortium of 4 most potant isolates were able to degrade PAHs more efficiently within 7 days[95].

In case of *Stenotrophomonas maltophilia* BR12 which was isolated from oil-contaminated soil in India, it was able to grow best at 50 µgml^{-1}pyrene and degrade nearly 100% of pyrene after 20 days and produce high amount of surfactant[96].

A batch culture of *Proteus vulgaris* CPY1and *Pseudomonas aeruginosa* LPY1 on 100 mgl^{-1}pyrene degrade nearly complete degradation[97].Pyrene and anthracene utilizing bacteria isolated from water used engine oil contaminated soil from Malaysia. Thirteen different bacterial species were isolated including *Bacillusthuringiensis* and *Bacillus megaterium, Salmonella enterica* and *Bacillus toyonesis*. All isolates degraded within 7 days almost all PAHs [98].

Degradation of LMW-PAHs by the marine halotolerant *Achromobacter xylosoxidans* have been determined. Glucose in combination with a triton x-100 and b-cyclodextrine resulted in 2.8 and 1.4 fold increasing in degradation of LMW-PAHs and 7.59 and 2.23 fold increase in degradation of HMW-PAHs respectively[99]. *Mycobacterium gilvum* strain PYR-GCK isolated from an estuary polluted with PAHs and was able to degrade pyrene efficiently [100].

Consortium Y-12 isolated from soil sample in Haikou city, China was able to degrade a mixture of PAHs including phenanthrene, Anthracene, fluoranthene, pyrene and Benz[a] pyrene. A bacterial strain was isolated from consortium Y-12 and identified by 16S rRNA as *Sphingobium* sp.FB3 [101].

Staphylococcus was isolated from diesel contaminated soil sample and identified by 16S rRNA as *Staphylococcus nepalensis* which was able to degrade pyrene at PH8 and 30˚C within 5 days incubation. The best bacterial growth and efficient pyrene degradation have been recorded with Co-substrate (glucose 4% and sucrose 2%) were added [102].

It was shown that mono culture of *Pseudomonas monteilii* P26 and *Pseudomonas* sp. number 3 could degrade efficiently LMW-PAHs but did not show interesting HMW-PAHs removal capabilities whereas, the *Actinobacteria rodococcus* p18, *Gardonia* H 19 and *Rhodococcus* F27 were able to degrade efficiently HMW-PAHs, but they did not remove LMW-PAHs from culture medium. The combination of four of these five strains (called C15 mixed culture) removed naphthalene and phenanthrene completely, and showed the highest pyrene biodegradation activity with removal values close to half , almost 6 times higher than those values recorded with strains in pure culture[103].

The degradation rates of consortium to pyrene and fluoranthene increased compare to pure culture of PY97M [104]. Main while Pyrene was used as sole carbon energy source by isolated strain of *Pseudomonas* JPYR-1 and the maximum pyrene degradation rate was 3.07 $mgml^{-1}h^{-1}$ in 48 h. incubation with initial pyrene concentration of 200 $\mu g/ml^{-1}$[105].But in case of pyrene-degrading endophytic bacterium, *Staphylococcus* BJ06, this strain was capable to degrade pyrene 50 $\mu g/ml^{-1}$[106].

Pyrene can be degraded by functional strain F14 which was constructed through protoplast fusion between *Sphingomonas* GY2B and *Pseudomonas* GP3A.The degradation of Pyrene by F14 was increased as concentration of pyrene decreased from 100$\mu g/ml^{-1}$to 15 $\mu g/ml^{-1}$within 10 days. Pyrene when it was in binary mixture with naphthalene or phenanthracene, Pyrene degradation was enhanced but more efficient naphthalene have been recorded than that of phenanthracene**.** The enzymatic activity of binding efficiency of *Actinobacter radioresistens* deoxygenase with chrysene is lesser binding energy than benzo[a]Pyrene while in case of *Rhodococcus opacus*benzo[a]Pyrene binds with lesser binding energy as compared to chrysene [107].

Four strains that could degrade both LMW-PAHs and HMW-PAHs were isolated from long-term manufacture gas plant site soil. These isolates included *Stenotrophomonas*(MTS-2), *Citrobacter* (MTS-3) and the most efficient isolate was *Pseudomonas*(MTS-1) in degradation of HMW-PAHs[108].

The bacterial strains *Burkholderia fungorum* T3A13001 and *Caulobacter*T2A12002 were pyrene degraders.*Caulobacter* sp, degraded 21% and 24% of Pyrene at 9.0 pH and 5.0 respectively, while *B. fungorum* was active in a wide range of pH values[109]. Main while a new halophilic bacterium capable of degrading HMW-PAHs were isolated from costal soil of the yellow sea, China. This bacterium was identified by 16S rRNA as *Thalassospira* TSL5-1. The Pyrene degradation occurred at salinity ranging from 0.5% to 19.5% with optimal value between 3.5% and 5% and degradation of Pyrene influenced greatly by pH values [110].Twenty one isolates from human skin having abilities to degrade benzo[a] Pyrene have been isolated and characterized. Benzo[a]Pyrene was completely degraded by at least 4 isolates. These isolates included Gram positive and Gram negative with micrococci being predominant[111].

A novel strain of *Bacillus* BMT4i capable of utilize Benzo[a]Pyrene as a sole carbon and energy source via enducible chromosomally encoded pathway was isolated. This strain was improved by inducing random mutations through treating by physical mutagen (UV) or chemical mutagen (ethyle methyl sulphonate [EMS], 5-bromouracil [5BU] and Acridine Orange [AO]). It was found that a UV-mutant (BMT4imuv2) exhibited higher Benzo[a]Pyrene degradation when compared with the wild type[112,113].Also, nine bacterial strains capable of degrading Benzo[a]Pyrene were isolated from Tokyo Bay and Tama River in Japan. The isolates belonged to the phyla Proteobacteria, Actinobacteria, Bacteroidetes and firmicutes. Isolate IT B II was identified by 16SrRNA as *Mesoflavibacter zeaxanthinifaciens*. This strain utilize Benzo[a]Pyrene as a sole carbon and energy source[114].

Over 33 days pyrene sorbed on hydrophobic filters more than half of pyrene than the five ring Benzo[a]pyrene and Benzo[a] fluoranthene by microbes having the ability to specialize in adhesion. Most bacteria enriched by HMW-PAHs were *Bacillus, Mycobacterium* and *Pseudomonas* [115].

Enzymes and genes involved in PAHs-degradation

Enzymes play an important role in microbial degradation of PAHs, oil, fuel activities and many other compounds[116].

Oxidoreductase are enzymes that clear chemical bonds and transfer the electrons from the reduced organic substrate (donner) to another chemical compound (acceptor). During these oxidation reduction reaction, contaminants are oxidized to harmless compounds. Oxygenases classified under the oxidoreductase group of enzymes [117].Oxidation reaction is the major enzymatic reaction of aerobic biodegradation is catalyzed by oxygenases.

Oxygenases metabolize organic compounds, they increased their reactivity, water solubility and cleave the organic ring[118].On the bases of the number of oxygen atoms used for oxidation, oxygenases can be further divided into two groups:I) Monooxygenases and II) Dioxgenases.

Monooxygenases transfer one atom of molecular oxygen to the organic compound and they possess highly region selectivity and steroselectivity on a wide range of substrates[118].The members of the genus *Pseudomonas*are known to have diverse metabolic pathways and grow using different substrates as a source of carbon example *Pseudomonas aeruginosa* N7B1 [119].*Pseudomonas stutzeri* produce catechol 2, 3 dioxygenase responsible for Meta cleavage of catechol[120].

PAH-induced proteins of *Mycobacterium vanbaalenii*PYR-1 grown on pyrene are catalase-peroxidase, putative monooxygenase, dioxygenase small subunit, and small subunit of naphthalene induced dioxygenase and aldehyde dehydrogenase. Main while carbohydrate metabolism related proteins are enolase, 6-phosphogluconate dehydrogenase, indol-3-glycerol phosphate synthase and fumarase[121].Several *Mycobacterium* spp. having multiple dioxygenase [122-124].The genes designated *mid* A3B3 encoding the subunits of terminal dioxygenase detected enzyme of*Mycobacterium vanbaalenii* PYR-1 showed a close similarities to PAH-ring hydroxylating dioxydenases from *Mycobacterium* and *Rhodococcus* spp. but has a highest similarity to α-subunit of *Nocardioides*KP7 fumarase [82].

The *Nah*Ac gene was detected in 13 Gram-negative isolates and sequence of *Nah* Ac-like genes were obtained from *Pseudomonas brenneri, Enterobacter, Pseudomonas entomophila, Pseudomonas koreensis* and *Stenotrophomonas* strains[125].

Four aromatic ring cleavage dioxygenase genes: Phd F, Phd I, Pea G and Pca H critical to pyrene biodegradation were detected in *Mycobacterium gilvum* PYR-GCK[100].

Microbacterium BPW,*Novosphingobium* PCY,*Ralstonia* BPH,*Alcaligenes* SSKIB and *Achromobacter* SSK4 were isolated from mangrove sediment.These strains degrade more than 50% of 100 $u$gml^{-1}of phenanthracene within 2 weeks. Strains PCY and BPW degrade 100% pyrene. The presence of α- subunit of pyrene dioxygenase gene (*nid*A) in Ph/pyrene degrading ability[126].

In Korea, sediment of U involved San Bay, a marine bacterium *Novosphingobium pentaromativorans* sp. US6-1was able to degrade PAHs. Various enzymes including PAH ring hydroxylating dioxygenase α- subunit (RHD α), 4-hydoxybenzoate3-monooxygenase and salicylaldehyde dehydrogenase were associated with PAHs degradation.

Strain US6-1 degrade PAHs via a metabolic route initiated by RHDα and that degradation occurred via salicylate pathway or phatholate pathway. Both of them inter TCA cycle and were mineralized to CO_2 and H_2O[127-129].

A strategy based on selection of *Mycobacterium vanbaalenii* PYR-1 mutant (6GII) that degrades HMW-PAHs but not LMW-PAHs. This mutant was defective inPdoA2 gene encoding an aromatic Ring Hydroxylating Oxygenase [RHO] enzyme. Mutant (6GII) had lower rate of fluorine, anthracene and pyrene degradation[130].Hydrocarbon catabolic genes from 9 different locations around Syowa station, Antarctica have been determined. PAH-ring-hydroxylating dioxygenase coding genes from Gram +ve and Gm –ve bacteria were detected.

Benzo[a]pyrene metabolism involved two transcripts that encode a putative Dsz A/ NtaA like monooxygenase and NifH-like reductase respectively [111].

RHD genes in clone libraries of Gram +ve were releated to I) *nid* A3 of *Mycobacterium* Py146, II) *Pdo* A of *Terrabacterium* HH4, III) *Mid* A of *Diaphorobacter* KOTLB and IV) *Pdo* A2 of *Mycobacterium* CH-2. While that of Gram –Ve, RHD geneswere related to I) Naphthalene dioxygenase of *Burkholderia glathei* II) *Phn* Ac of *Burkholderia satisoli* and III) RHD α- subunit of uncultured bacterium [131].

From costal environment a close to*Burkholderia fungorum* and *Mycobacterium gilvum* had *mid* A, *mid* A3, *Pdo* A2 and *PCaH* genes [132].

The ring-hydroxylating dioxygenase RHDase coding for RHDases and 1-hydroxy 2- naphthoate dioxygenase 1H2Dase genes coding for 1H2Dase enzymes play importantroles in decomposing the intermediates of PAHs which can be separated from *Arthrobacter* sp. SAO_2 and have the capacity of degrading phenanthrene [133].Liang et al., (2019 a, b) [134,135] used for first time gene - targeted metagenomics to investigate the diversity of PAH- degrading bacterial communities in oil field soils and mangrove sediments. A PAH hydratase - aldolase - encoding gene *Pah* E was a superior biomarker for PAH - degrading bacteria instead of *Pah* Ac which encoded the alfa - subunit of PAH ring -hydroxylating dioxygenase as functional marker gene.

Bacterial degradation pathways of PAHs

Biodegradation of pollutant involves series of steps using different enzymes [65]. Hydrocarbons can selectively be metabolized by individual strain of microorganism or consortium of microbial strains belong to either the same or different genera [64,90]However, consortium have been proved to be more efficient than individual cultures for metabolizing or biodegrading pollutants [136-139]**.**

Initial oxidative attack followed by ring cleavage of benzene ring is the key step in degradation of aromatics and polycyclic aromatic hydrocarbons (PAHs) which normally involves diol formation followed by ring cleavage and formation of dicarboxylic acid[140].

First step in the microbial degradation of PAHs is oxidation catalyzed by monooxygenase or dioxygenase[141], which

introduces atom of oxygen at two carbon atoms of benzene ring resulted in the formation of cis-dihydrodiol[142,143]. Aerobic catabolic pathway involves a wide variety of peripheral degradation pathways whichtransform PAHs into small number of intermediates that enter the Tricarboxylic Acid (TCA) cycle[144].

Synthesis of cell biomass formed from central precursor metabolites(Succinate,AcetylcoA,Pyruvate,andGluconeogenesis) which resulted in synthesis of sugars and growth [116].

The most common way of initial oxidation is formation a diol, followed by ring cleavage and formation of dicarboxylic acid [143]and formation of Cis-dihydrodiols by incorporation of both oxygen atoms of an oxygen molecule and then formation of catecols. Ortho- or meta-cleavage pathway lead to formation of central intermediates (e.g.: Protocatechuates and caticols with further steps converted to TCA cycle intermediates[65].Anaerobic degradation is more recent as compared to aerobic degradation [145].This is due to less information is available about the genes and enzymes involved in these pathways[146].

Naphthalene degradation pathways:-

Naphthalene has low water solubility and high solid-liquid distribution ratio [147].Salicylic acid is an intermediate compound formed in microbial pathway of naphthalene degradation as shown in Figure 2[148]by *Pseudomonas putida.*

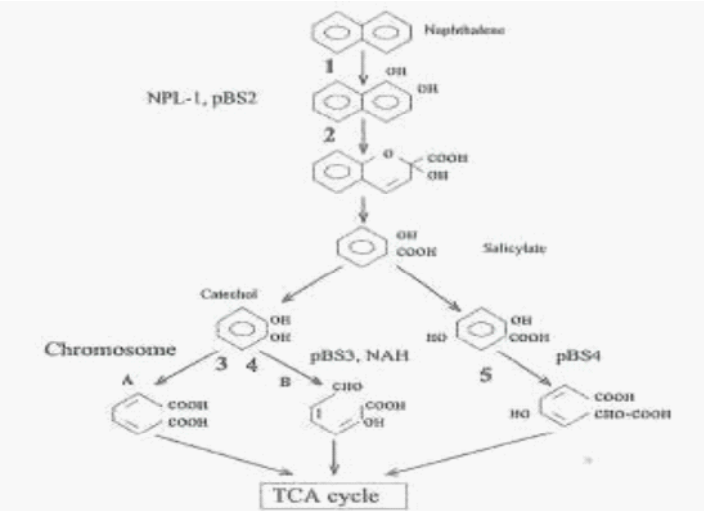


Figure 2: Proposed pathway for the degradation of naphthalene by *Pseudomonas putida* [148].

Streptomyces griseus catalyze the biotransformation of naphthalene to 4-hydroxy-1tetralone in good yield, 2-methyl-1, 4-naphoquinone and 2-methyl-4-hydroxy-1 tetralone as indicated in Figure 3 [149].

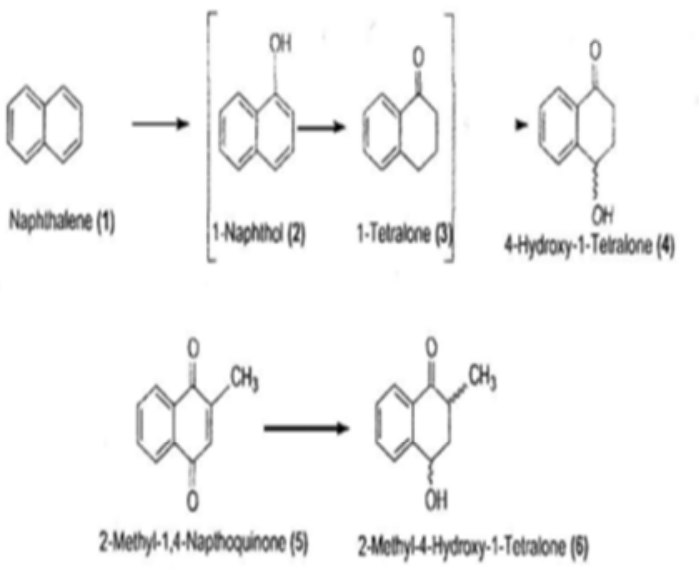


Figure 3: Proposed pathway for the degradation of naphthalene by *Streptomyces griseus* [149].

Degradation of naphthalene starts through the multi-component enzyme naphthalene dioxygenase, which converts naphthalene to Cis-naphthalene dihydrodiol. This diol is transformed to 1, 2-dihydroxynaphthalene via the enzyme Cis-dihydrodiol dehydrogenase. At this point two pathways are possible Figure 4.The ring fission of 1, 2-dihydroxynaphthalene leads to the formation of O-phthalic pathway) which is subsequently converted to intermediates enter the Krebs Cycle (TCA) or the formation of salicylates (Salicylic pathway) and also enter TCA cycle [150,151].In the second pathway, 1, 2-dihydroxy naphthalene is converted to salicylate which is either transformed to caticol or gentisate (salicylic pathway). The plasmid possess degradative genes have been detected in several bacterial species including the plasmid NAH7 of *Pseudomonas putida* strain G [152]and that of strain Ak5 and plasmid of *Gordonia* sp. strain CC-NAPH129-6[153].Hydroxy-phthalic acid is an intermediate arising after O-phthalic have been identified in *Pseudomonas aeruginosa* but not found in *M. radiotolerans* O-phthalic pathway have been proven in many bacteria including *Pseudomonas* sp.[154],*Bacillus fusiformis* [155].*Bacillus thermoleovorans* [156] and *Geobacillus* sp.[157].The phthalic pathway also reported for *Pseudomonas* sp. [154]and *Arthrobacter*sp.[158].Therefore information of bacterial degradation of naphthalene has been used to understand and predict pathways in the degradation of three or more ring PAHs [159,160].The proposed pathway of degradation Naphthelene by *Pseudomonas*sp. CZ2 and CZ5 can be shown in Figure 5[161]**.** GC/MS analysisby Abo-State *et.al*.,(2018)[69] revealed that *Bordetella avium*MAM-P22 degraded Naphthalene

to six intermediate compounds , these compounds were 1,2- benzene dicarboxylic acid, Butyl - 2,4- dimethyl -2 - nitro - 4- Pentenoate, 1- Nonen- 3 - ol, Eicosane Nonacosane as indicated in Figure 6 [69].

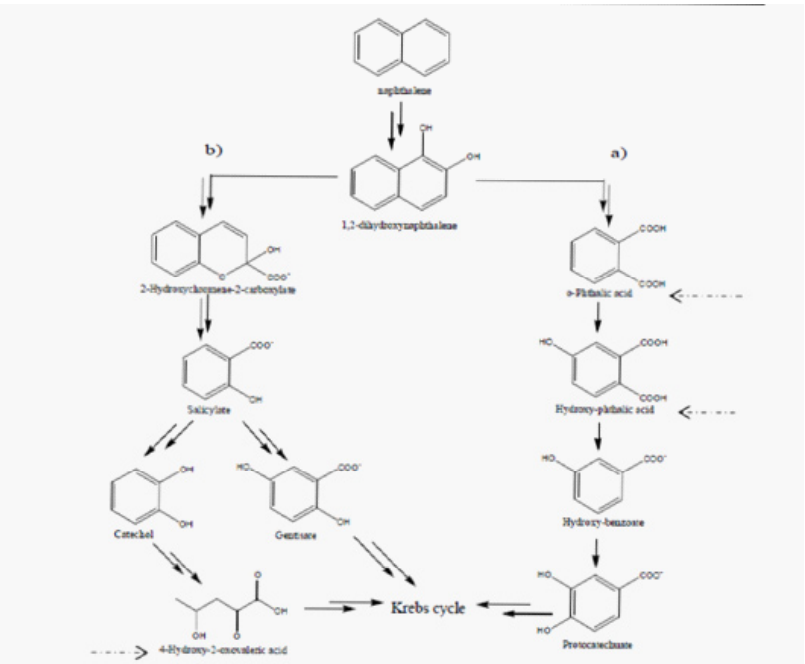


Figure 4: Naphthalene biochemical biodegradation pathways. (a) Phthalic pathway (b) Salicylate pathway[150,151]. Discontinuous arrows show molecules identified by Gas Chromatography (GC) analysis.

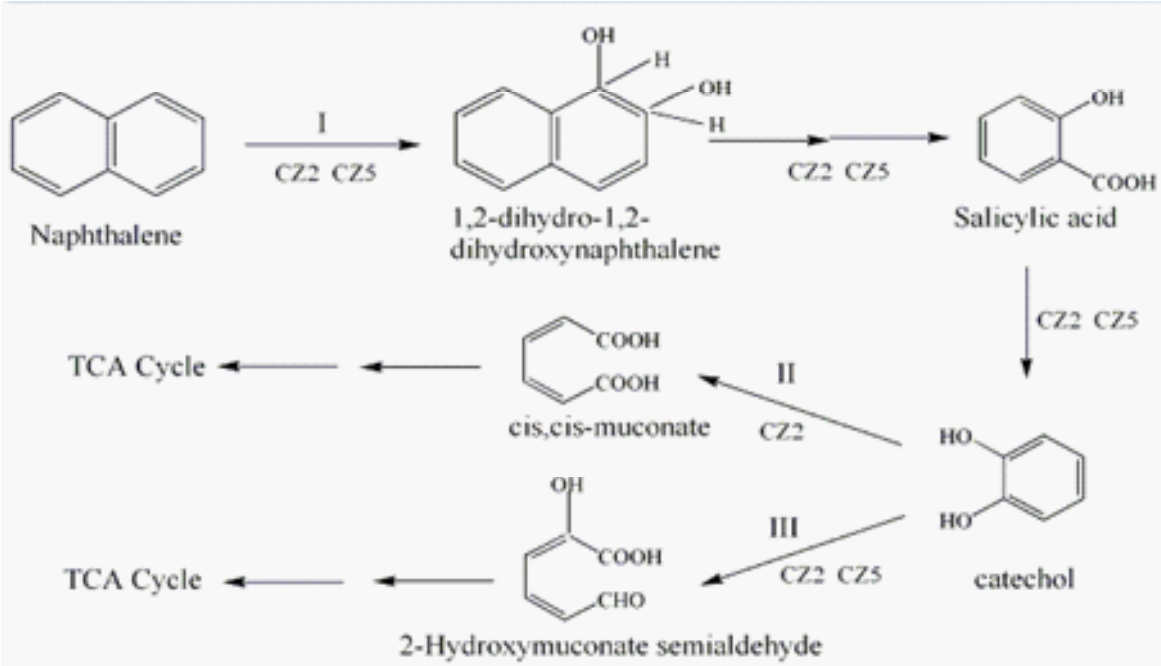


Figure 5: Proposed pathway for the degradation of naphthalene by strains *Pseudomonas* sp. CZ2 and CZ5. I, Naphthalene dioxygenase; II, catechol 1, 2-dioxygenase; III, catechol 2, 3-dioxygenase. CZ2, *Pseudomonas* sp. CZ2; CZ5, *Pseudomonas* sp. CZ5 [161].

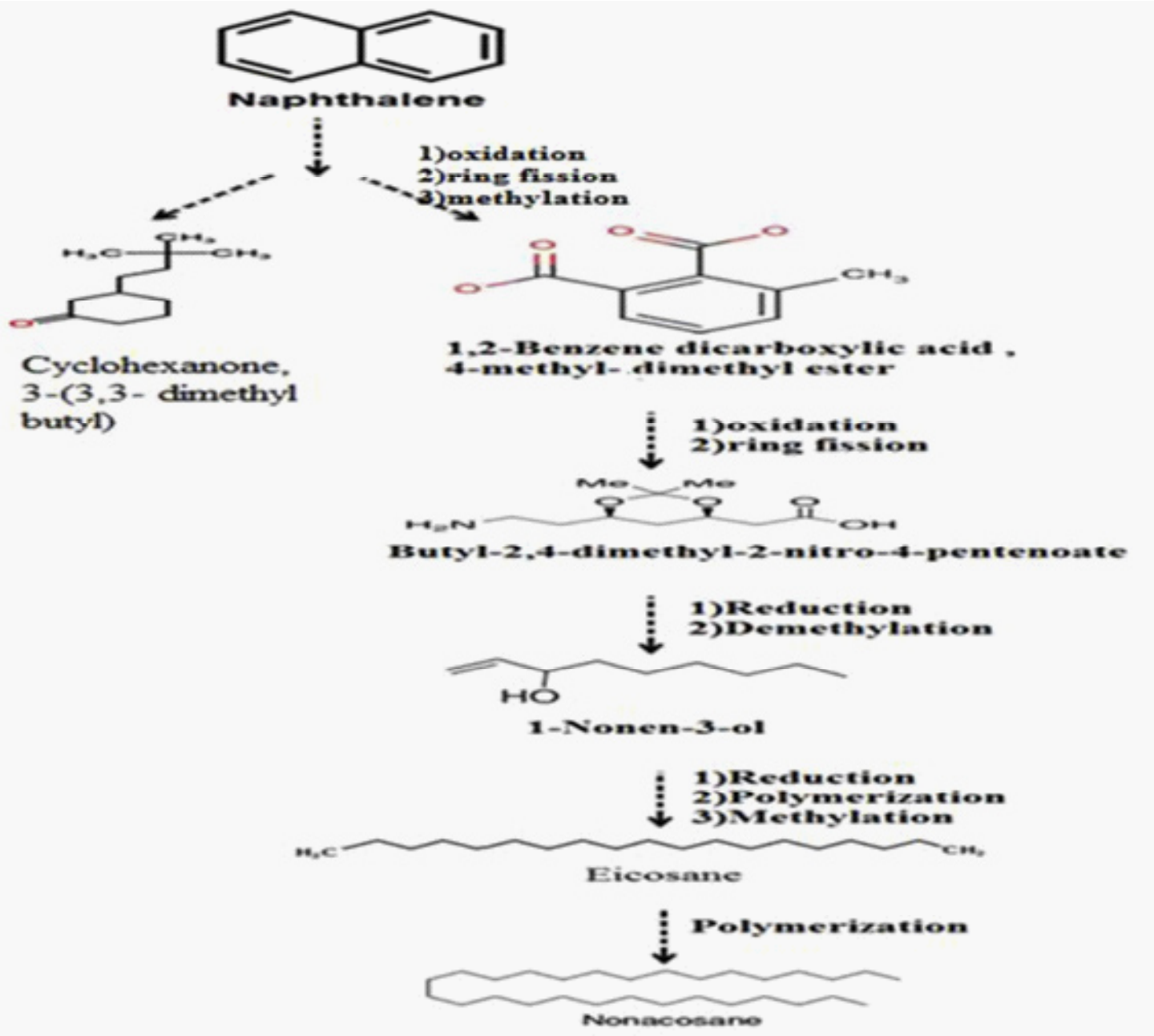


Figure 6: Proposed pathway of Naphthalene by *Bordetella avium* MAM-P22[69].

Phenanthrene degradation pathways

Bacterial degradation of phenanthracene is initiated by 3, 4-dioxygenase to give *cis*-3, 4- dihydroxy 3,4- dihydrophanthrene, which undergoes enzymatic dehydrogenation to 3,4- dihyroxyphenanthrene)[122,158].

The proposed phenanthrene degradation pathway [162]by managrove enriched bacteria consortium was indicated in Figure 7.This pathway followed the phthalic pathway.

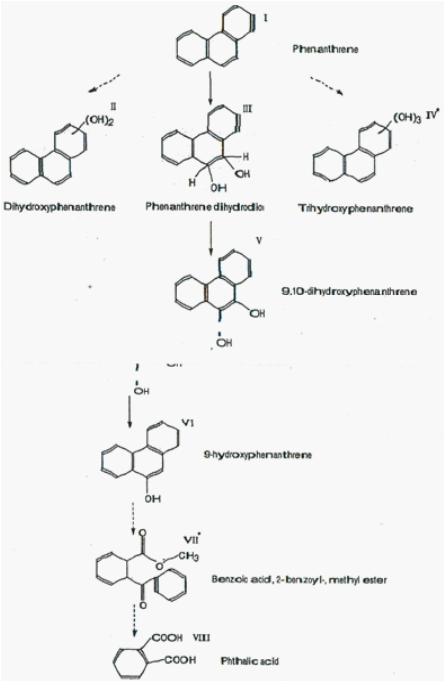


Figure 7: Proposed Phenanthrene degradation pathways by the Managrove enriched bacterial consortium [162].

*Shingomonas*sp. GY2B can degraded Phenanthrrene efficiently as indicated in Figure 8[163],but it is following the salicylate route**.**

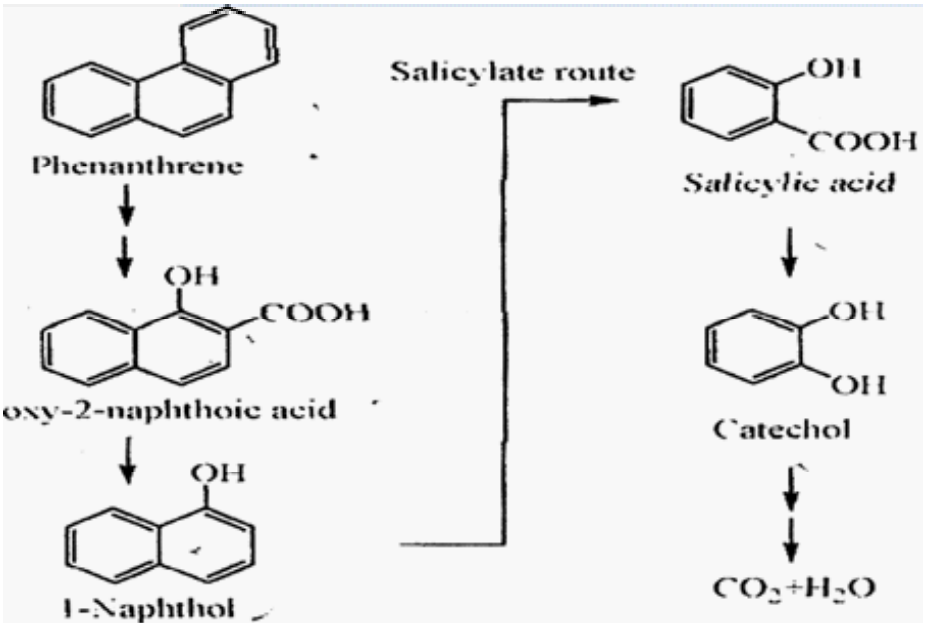


Figure 8: Proposed pathway for the degradation of Phenanthrene by *Sphingomonas* sp.[163].

Bacteria can oxidise Phenanthrene to cis- 1, 2-dihydroxy-1, 2-dihydrophenanthrene which converts to 1,2-dihydrophenanthrene when it undergoes enzymatic dehydrogenation. The compounds can be oxidized further to 1-hydroxy-2-naphthoic acid, 2- carboxy benzaldhyde, O-phthalic acid, and proto-catechuic acid as shown in Figure 9 [164].

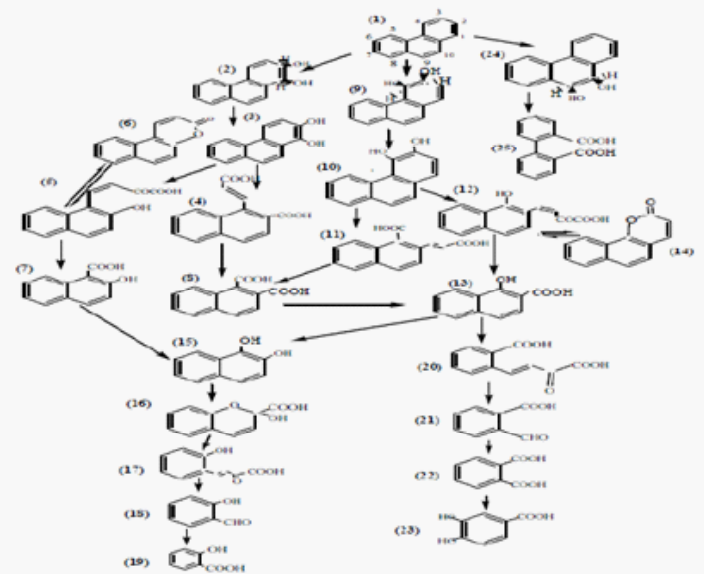


Figure 9: Proposed catabolic pathways of Phenanthrene by aerobic bacteria. The compounds are 1, Phenanthrene; 2, cis -1,2-dihydroxy- 1,2- dihydrophenanthrene; 3, 1,2-dihydroxyphenanthrene; 4, 2-[(E)-2- carboxyvinyl]-1-naphthoic acid; 5, trans-4-(2-hydroxynaph-1-yl)-2- oxobut- 3-enoic acid; 6, 5,6-benzocoumarin; 7, 2-hydroxy-1-naphthoic acid; 8, naphthalene-1,2-dicarboxylic acid; 9, cis-3,4-dihydroxy-3,4- dihydrophenanthrene; 10, 3,4-dihydroxyphenanthrene; 11, 1-[(E)-2- carboxyvinyl]-2-naphthoic acid; 12, trans-4-(1-hydroxynaph-2-yl)-2- oxobut-3-enoic acid; 13, 1-hydroxy-2-naphthoic acid; 14, 7,8- benzocoumarin; 15, 1,2-dihydroxynaphthalene; 16, 2-hydroxy-2H-chromene- 2-carboxylic acid; 17; trans-o-hydroxybenzalpyruvic acid; 18, salicylaldehyde; 19, salyclic acid; 20, trans-2-carboxybenzalpyruvic acid; 21, 2-carboxybenzaldehyde; 22, o-phthalic acid; 23, protocatechuic acid; 24, cis-9,10-dihydroxy-1,2-dihydrophenanthrene; 25, 2,2/-diphenic acid[164].

4-[1-hydroxy (2-naphthyl)-2-oxobut-3enoic acid] which was considered an intermediate product of Phenanthrene biodegradation by *Pseudomonas*sp.BZ-3.strain BZ-3 initiates its attack on Phenanthrene by deoxygenating at C-3 and C-4 position to produce cis-3, 4 dihydrodiol. Which converted to salicylic acid pathway as indicated in Figure 10 [165].

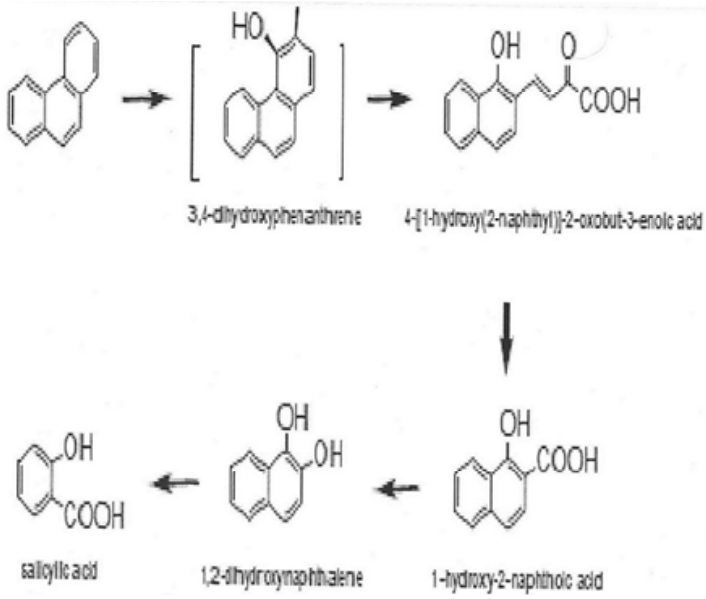


Figure 10: A proposed pathway for the degradation of Phenanthrene by *Pseudomonas* sp. BZ-3[165].

Phenanthrene degradation by *Pseudomonas mendocina*revealed that high level accumulation of the (1H2N) was observed[166].The 2-naphthol (decarboxylated product) of 2H1NA was detected as minor metabolite in the degradation of Phenanthrene by *Staphylococcus* sp. Strain PN/Y[167].Which was further metabolized by unique meta-cleavage dioxygenase, leading to TCA intermediates [168].

Figure 11 indicated that Phenanthrene is initial transformed to *cis*-dihydro- diol by PAH dioxygenase (a multi component of dioxygenase enzyme system); dihydrodiol dehydrogenase converts dihydrodiol to caticol and then caticol is degraded into aldehyde or acids by 2, 3 dioxygenase [150],as shown by aerobic bacteria. Pagnot *et.al.,*(2007)[169] isolated and characterized the gene cluster involved in Phenanthrene degradation by 3, 4 Phenanthrene dioxygenase and meta-cleavage. A high branched metabolic pathways of Phenanthrene biodegradation by *Mycobacterium aromativorans* strain JSI9b1T including deoxygenation on C-1,2 and C3,4 and C-9,10 position and ring opening via both ortho- and meta cleavage [170,171].Dimethylphthalate formation proved that *Psudomonas* sp.USTB-RU degraded phenanthrene via protocatechuate pathway, while *Stenotrophomonas maltophilia* C6 degraded Phenanthrene via protocatechuate and salicylate pathway [172].

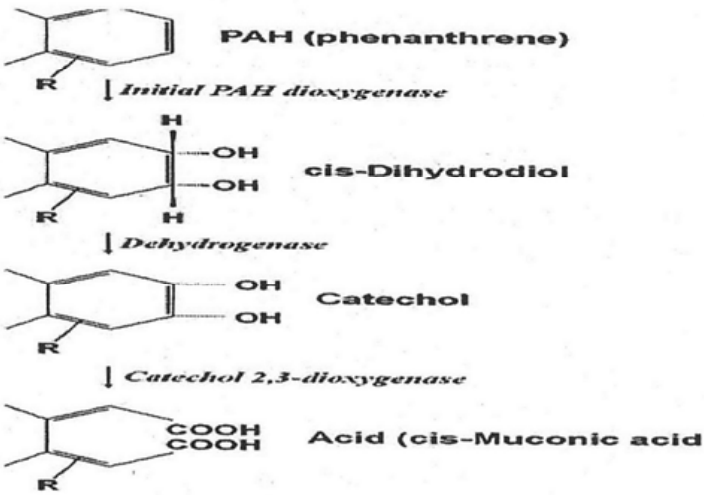


Figure 11: Postulated metabolic pathway of PAH-degradation in aerobic bacteria. Enzymes involved in the degradation of PAHs are oxygenase and dehydrogenase [150].The initial PAH dioxyganase and catechol 2,3- dioxygenase are encoded by *nah*A and *nah*H, respectively.

Benzo[a] anthracene degradation pathways:

Initial enzymatic oxidation of aromatic ring system of B-[a]-anthracene may occur at various locations on the molecule, including 1,2 or 3,4-carbon positions, an angular Kata-type initial deoxygenation, via the 9,10- or 10, 11- carbon positions a linear kata-type initial deoxygenation, or via the K-region at 5,6-carbon position as indicated in Figure12.Metabolites from the biotransformation of Benzo[a]anthracene (B[a]A) by bacteria have identified from only six organisms (i) *Shingobium yanoikuyae* mutant strain B8/36**.** Initial step of B[a] anthracene was oxidation to produce Benzo[a]anthracene 7, 12 dione, in which further oxidation and ring fission transformed to indo-5-aldhyde and benzene ethanol and number of acids, alchols and esters[173].The two proposed pathways for the parent strain MAM-62 and gamma induced mutant strain MAM-62(4) revealed that the parent and mutant are different in some of their metabolites as summarized in Table 3 and Figures13, 14 [92].

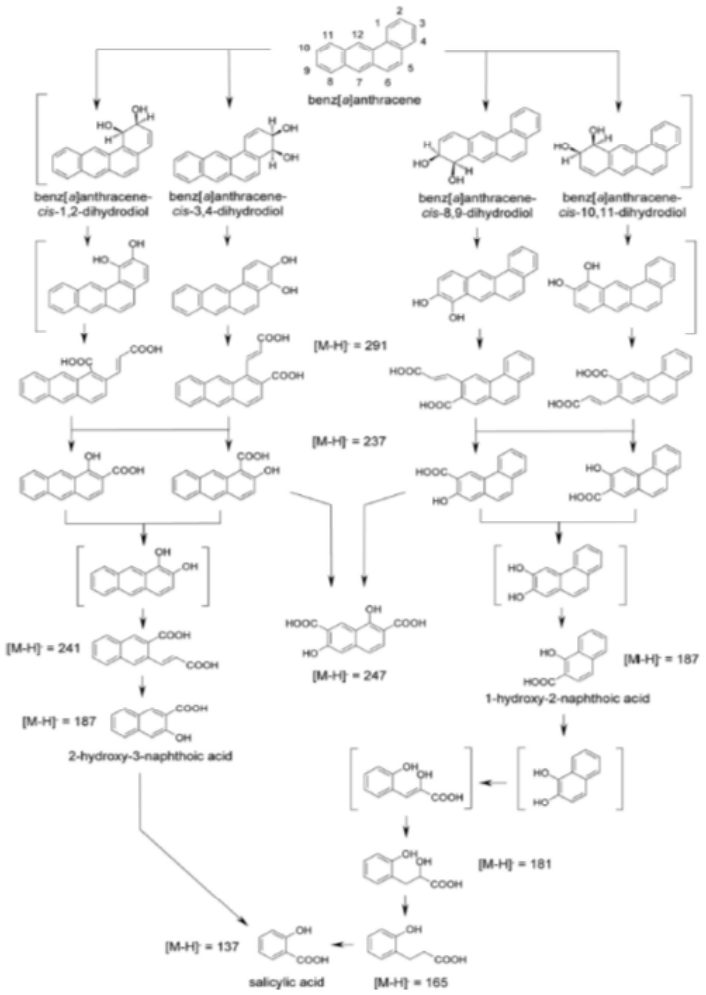


Figure 12: Pathways proposed for the biotransformation of Benz[*a*]anthracene by *Sphingobium*KK22. Metabolites in brackets were not identified in the culture medium [173].

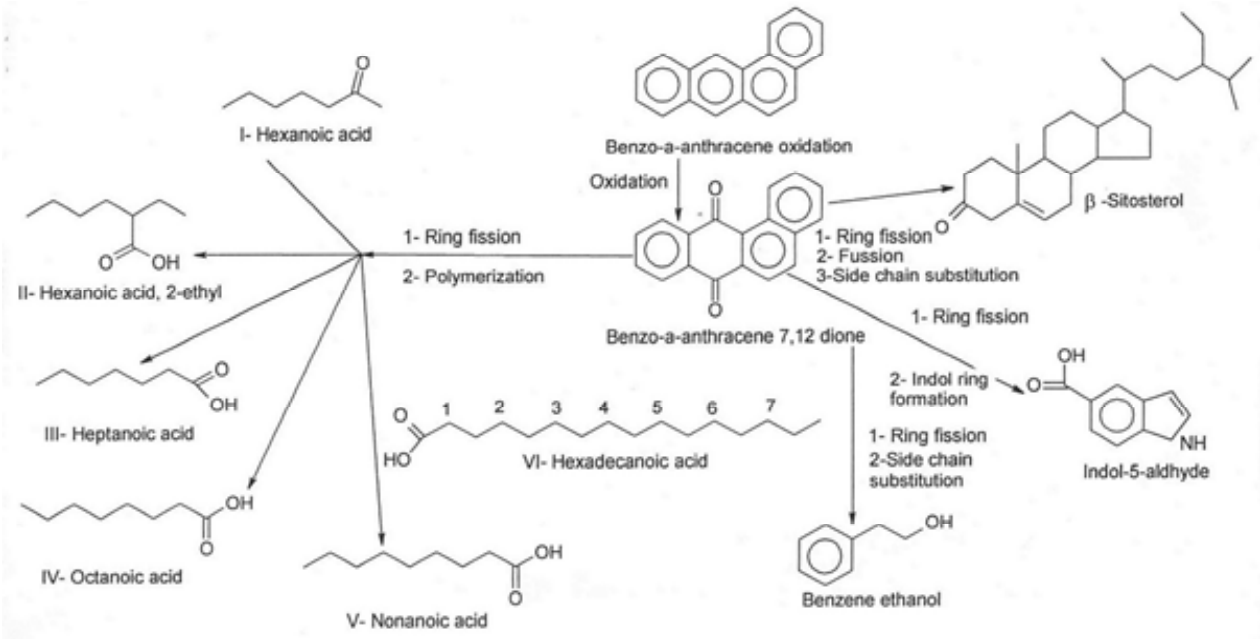


Figure 13: Proposed pathway of benzo-a- anthracene degradation by *B. amyloliquefaciens* MAM-62 [92].

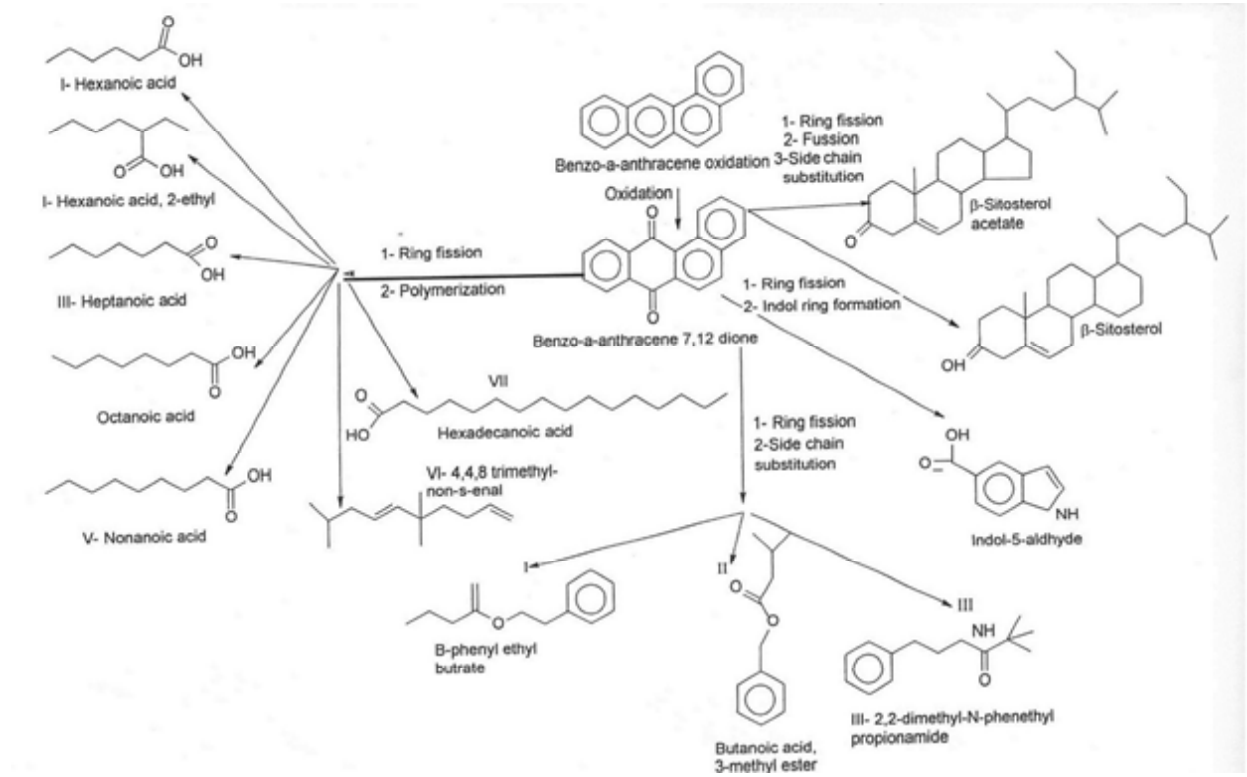


Figure 14: Proposed pathway of of benzo-a- anthracene degradation by *B. amyloliquefaciens* MAM-62(4)[92].

R.T	MAM-62	Formula	MAM-62(4)	Formula
12.272	Hexanoic acid	$C_6H_{12}O_2$	Hexanoic acid	$C_6H_{12}O_2$
15.166	Hepatanoic acid	$C_7H_{14}O_2$	Heptanoic acid	$C_7H_{14}O_2$
16.184	Benzeneethanol	C8H10O	-	-
16.289	Hexanoic acid,2- ethyl	$C_8H_{16}O_2$	Hexanoic acid, 2-ethyl	$C_8H_{16}O_2$
17.553	-	-	N-1-(2-chloro-2- ethylbutylidene)-T- butylamine	$C_{10}H_{20}ClN$
18.276	Octanoic acid	$C_8H_{16}O_2$	Octanoic acid	$C_8H_{16}O_2$
19.574	-	-	Propanamide, N-1(1,1 dimethyl)2,2-dimethyl	$C_9H_{19}NO$
21.084	Nonanoic acid	$C_9H_{18}O_2$	Nonanoic acid	$C_9H_{18}O_2$
29.380	-	-	β-Pheylethyl butyrate	$C_{12}H_{16}O_2$
31.348	-	-	2,2-Dimethyl-N- phenethyl- propionamide	C13H19NO
31.752	-	-	Butanoic acid, 3- methyl,2-phenylethyl ester	$C_{13}H_{18}O_2$
33.841	Indol-5-aldhyde	C_9H_7NO	Indol-5-aldhyde	C_9H_7No
36.666	n-Hexadecanoic acid	$C_{16}H_{32}O_2$	Hexadecanoic acid	$C_{16}H_{32}O_2$
43.383	-	-	4,4,8-trimthylnon-5- enal	$C_{12}H_{22}O$
47.992	Benz(a)anthracene 7,12 dione	$C_{18}H_{10}O_2$	Benz(a)anthracene7,12- dione	$C_{18}H_{10}O$
57.368	-	-	βSitosterol	$C_{29}H_{50}O$
61.832	b-Sitosterol acetate	C29H48	b-Sitosterol acetate	C29H48

Table 3: Intermediates determined by GC-MS analysis of benzo-a- anthracene degradation by *B*. *amyloliquefacien*s MAM-62 and its mutant MAM-62(4) after 24 hours incubation [92].

Pyrene degradation pathways:

Mycobacterium AP1 grew with pyrene as sole carbon and energy source. This strain initiates its attack on pyrene by either monooxygenase or dioxygenase at its C4, C5 positions to give Trans - or cis-4, 5 dihydroxy-4, 5- dihydropyrene. Dehydrogenation of the latter, ortho cleavage of the resulting diol to form phenanthrene 4, 5-dicarboxylic acid and the subsequent decarboxylation to phenanthrene 4-carboxylic acid, the latter with further degradation via phthalate pathway continue to TCA cycle A metabolite (6, 6-dihydroxy-2, 2-biphenyl dicarboxylic acid indicated a new branch in the pathway [174]as indicated in Figure 15.

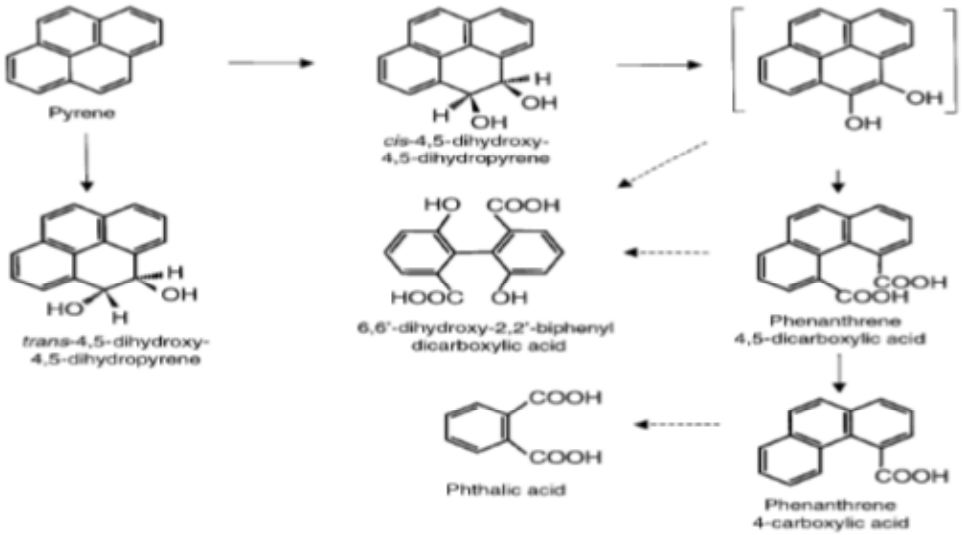


Figure 15: Schemic pathway proposed for the degradation of Pyrene by *Mycobacteruim* AP1. The product in brackets has not been isolated. Dotted arrows indicate two or more successive reactions, [174].

The major pathways for the metabolism of Phenanthrene and pyrene by another *Mycobacterium* sp. strain *vanbaalenii* PYR-1 were initiated by oxidation at the K-regions [174]. Phenanthrene 9, 10 and pyrene-4, 5 di-hydrodiols were metabolized via transient catechol to the ring fission products, 2, 2-diphenic acid and 4, 5-dicarboxyphenanthrene respectively[122].Also another *Mycobacterium* sp. strain KMS can grow on pyrene. Various key metabolites including pyrene-4, 5-dione, cis-4, 5-pyrene-dihydrol, phenanthrene-4, 5- dicarboxylic acid and 4- Phenanthroic acid [123].The same bacterial strain PYR-1 was able to utilize pyrene as sole carbon and energy source and produces 7 metabolites as indicated in Figure 16. These metabolites including four ring metabolities (mono-hydroxy pyrenes and three different di-hydroxy pyrene) and three-ring metabolites (dihydroxyphenanthrene, 4-phenanthrene-carboxylic acid and 4- phenanthrol), of which more 4- ring metabolites accumulated compared with 3-ring metabolites [175]as indicated in Figure16.

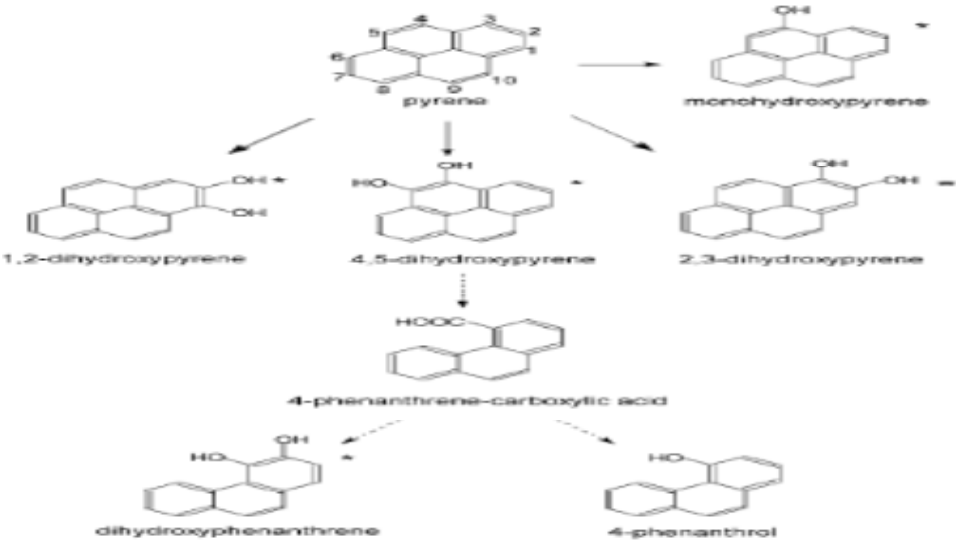


Figure 16: Proposed degradation pathways of Pyrene by *Mycobacteruim*A1-PYR. An asterisk indicates that the position of the substitutes was hypothesized. A solid arrow indicates a single reaction and a broken arrow represents two or more transformation steps. COOH -carboxyl group, OH - hydroxyl group, [175].

As shown in Figures (17-19), the initial step in pyrene degradation pathway was the oxidation of K region by dioxygenase to form cis-4, 5- pyrene-dihydrol. When 3, 4- di-hydroxy Phenanthrene is formed it enters the Phenanthrene degradation pathway [159,176,177]. One of the main metabolites 4-hydroxy-Phenanthrene was transformed into naphthol and 1, 2- dihydroxy- naphthalene which was further degraded through salicylic acid pathway and phatholic acid pathway separately[178].

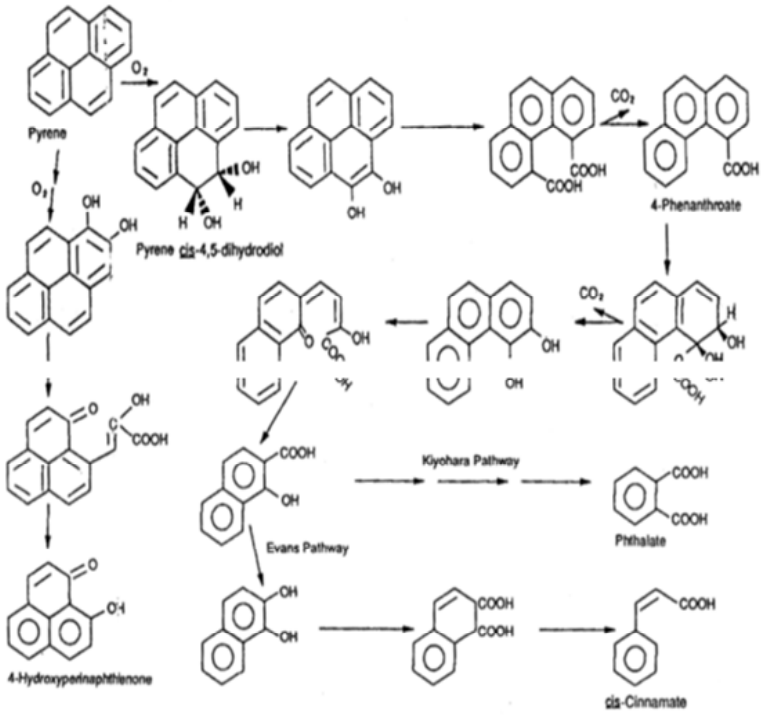


Figure 17: Proposed pathway for the degradation of pyrene by *Mycobacterium* sp. strain PYR-1[159].

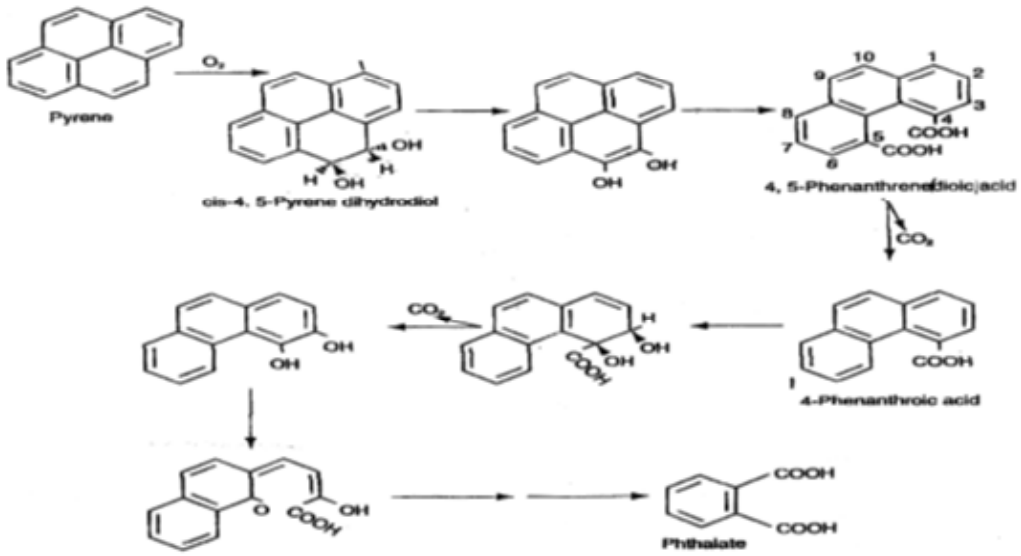


Figure 18: Proposed pathway for the degradation of pyrene by *Mycobacterium flavescens* [176].

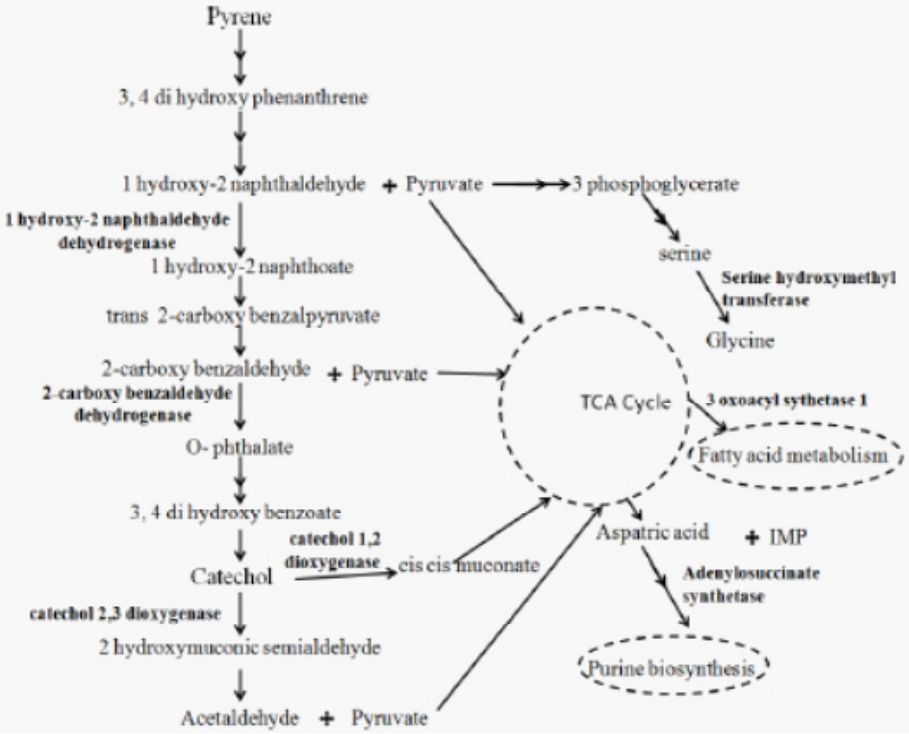


Figure 19: Elucidation of Pyrene degradation pathway in *Pseudomonas*-BP10,[177].

Abo-State *et.al*.,(2014)[91] proposed pathways of pyrene degradation by the parent strain *Bacillus amyloliquefaciens*MAM-62 and its gamma radiation induce[d mutant MAM-62 (4) as summarized in Table 4and Figure 20 revealed that none of the metabolites formed by the mutant strain and also none of the metabolites formed by the mutant have been recorded by the parent *Bacillus*strain. Pyrene by successive oxidation and ring fission produces benzene ethanol and 2, 4, 6 cycloheptatriene-1-one and acids by the parent strain while it produces butonic acid, 3-methyle 2-phenyl ethyl ester and methyl-2, 3-di-O-acetyl-B-D-xylopyranoside by the mutant MAM-62 (4)[91].

R.T	MAM-62	Formula	MAM-62(4)	Formula
16.176	Benzene ethanol	$C_8H_{10}O$	-	-
16.791	Hexanoic acid 3,5,5'-trimethyl	$C_9H_{18}O_2$	-	-
17.336	2,4,6-cycloheptatri- iene-1-one	C_7H_6O	-	-
18.252	-	-	Ethanol,2-(2-but- oxyethoxy)-	$C_8H_{18}O_3$
18.853	-	-	Cyclopropane,2-(1,1- dimethyl-2- pentenyl)1,1-diemthyl	$C_{12}H_{22}$
22.403	-	-	Methyl2,3-di-o-acethyl- B-D-xylopyranoside	$C_{10}H_{16}O_7$

31.713	-	-	Butanoic acid-3-methyl- -2phenyl ethyl ester	$C_{13}H_{18}O_2$
32.468	-	-	Pentachlorophenol	C_6HCl_5O
32.566	Tetradecanoic acid	$C_{14}H_{28}O_2$	-	-

Table 4: Intermediates determined by GC-MS analysis of pyrene degradation by *B. amyloliquefaciens* MAM-62 and its mutant MAM-62(4) after 24 hours incubation [91].

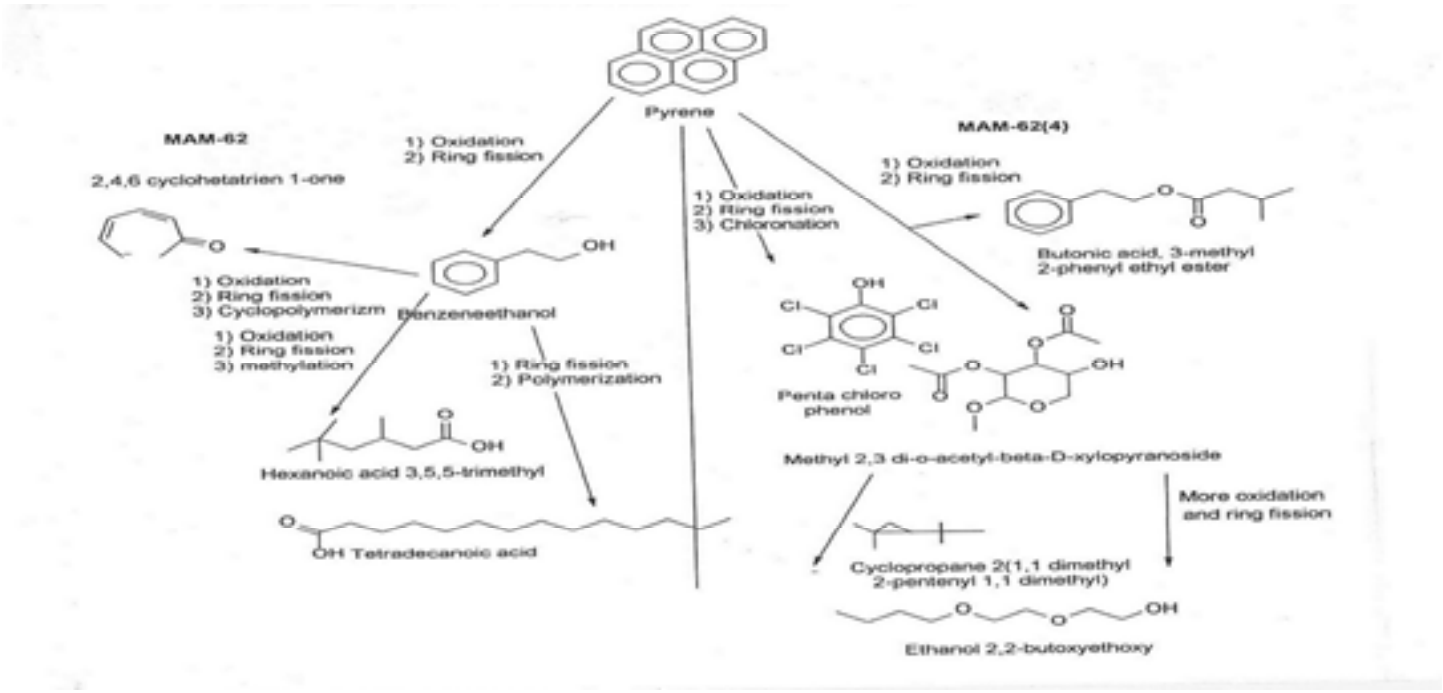


Figure 20: Proposed pathway of of pyrene degradation by *B. amyloliquefaciens*MAM-62 and MAM-62(4)[91].

The role of mononuclear iron in dihydroxylation reaction for pyrene have been indicated in Figure 21 [179].In case of pyrene degradation pathway by *Pseudomonas stutezeri* CECT930, it produces 1-hydroxy-2-naphthoic acid, phthalic acid and cinnamic acid as shown in Figure 22 [180]. Main while, the degradative pyrene proposed pathway by *Bacillus altitudinis* MAM-8 identified by16 S rRNA reveled the formation of the following metabolites 1-[(hexadeulerio)phenyl] naphthalene; trans-4, 4-di methyoxy -beta methyl chalcone, phthalic acid monocyclohexyl ester, phatholic acid monobutyl ester, dimethoxybenzyl-ide neacetone and phathalic anhydride. Abo-State *et.al*., (2017) [67] found that,the previous metabolites indicated that pyrene degradation by *Bacillus altitudinis* MAM-8 followed the phthalic pathway as indicated in Figure 23 [67]. In another study, Abo-State *et.al.,(*2018a)[68]proposed that pathway of pyrene by the isolated strain from petroleum contaminated soil of Suez Canal, Egypt and identified by16S rRNA as *Pseudomonas panipatensis* MAM-P39 with accession number MF150314b produced 14 intermediates. These metabolites including 3-methyl penta-1, 4- diene-3-ol; 3-methyl-2-butenoic acid, 3-methyl-but-2-enyl ester; 3 hexanone; 3-methyl-2- butenoic acid, 2-pentyl ester and benzene, (3,3- dimethyl-4- pentyl- as shown in Figure 24 [68].Not only single bacterial isolates or strains were able to degrade pyrene, but also mangrove enriched bacterial consortium. It is well known that consortium having a number of different bacterial collection owing a battery of degradative enzymes more efficient than single bacterial strain. The proposed pathway was indicated in Figure 25 [162].

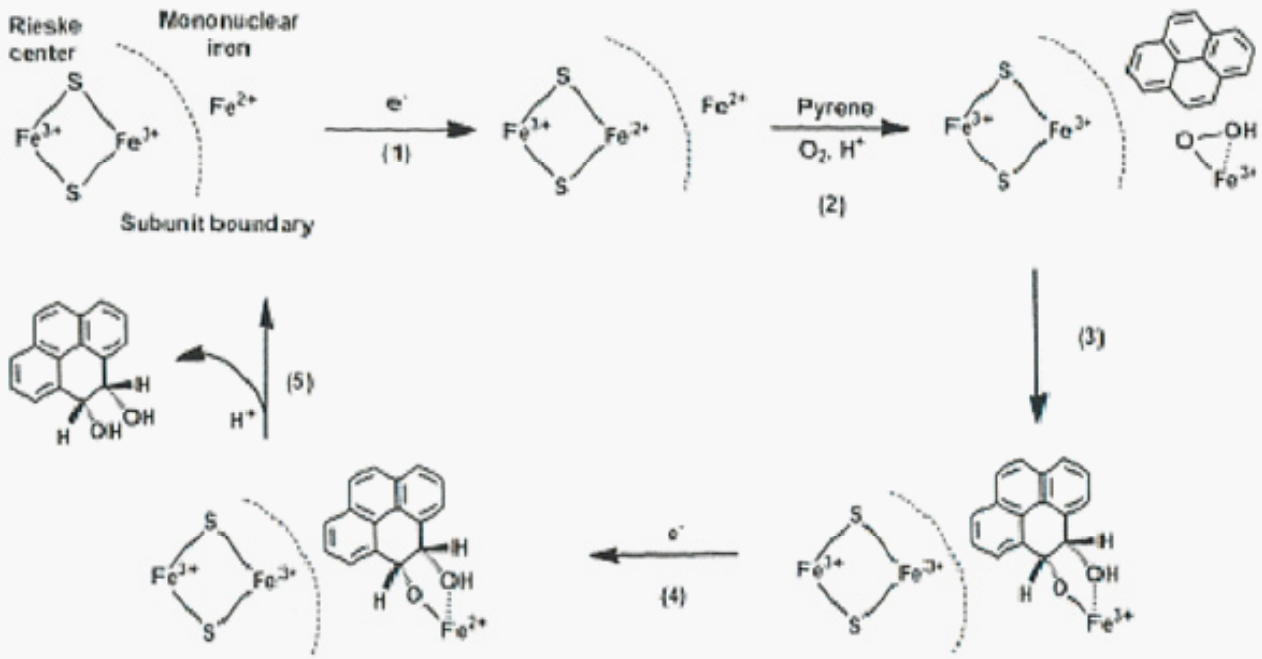


Figure 21: A feasible pathway of dihydroxylation reaction catalyzed by R-NDO in strain ustb-1, the Figure displays that the resting R-NDOhas a mononuclear iron in ferrous status and an oxidized Rieske [2Fe-2S] center in the active site. At first, one ferric ion in Rieske [2Fe-2S] center is reduced by an external electron from NADH to form a fully reduced R-NDO. Following the binding with the substrate, the dioxygen molecule is activated by the two electrons derived from the mononuclear iron and the reduced Rieske [2Fe-2S] center.Subsequently, the binary complex will quickly react with the carbon–carbon double bond of pyrene at C4-C5 positions to form a Fe-O2-pyrene ternary complex which is a promising intermediate in the formation process of the product. Then a second external electron is used to reduce the ferric ion in the ternary complex. Finally, a proton is introduced to the complex, and then the dihydroxylation product was released. Simultaneously, the mononuclear iron and Rieske [2Fe-2S] center recover the initial states and are ready for the next cycle of the reaction [179].

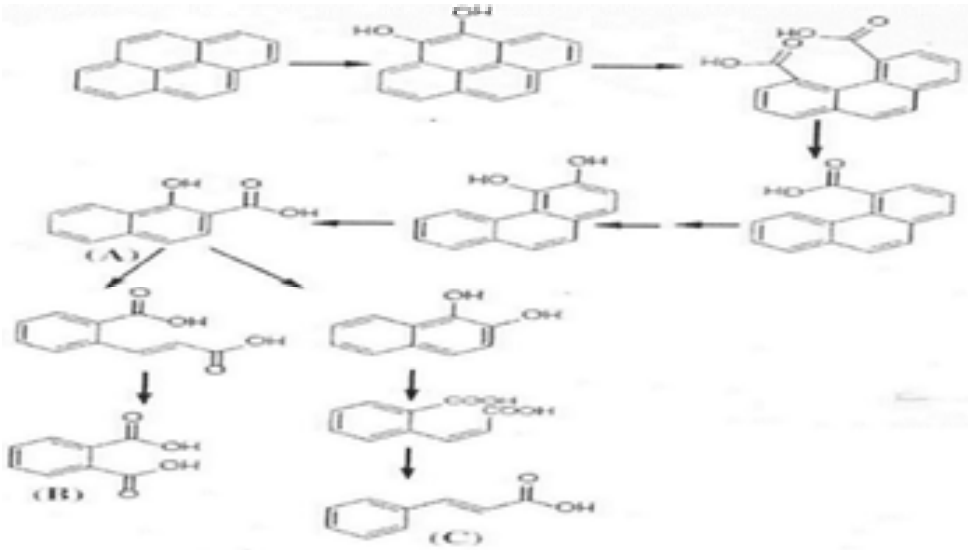


Figure 22: Proposed metabolic pathway of pyrene by *Pseudomonas stutzeri* CECT 930 [180].

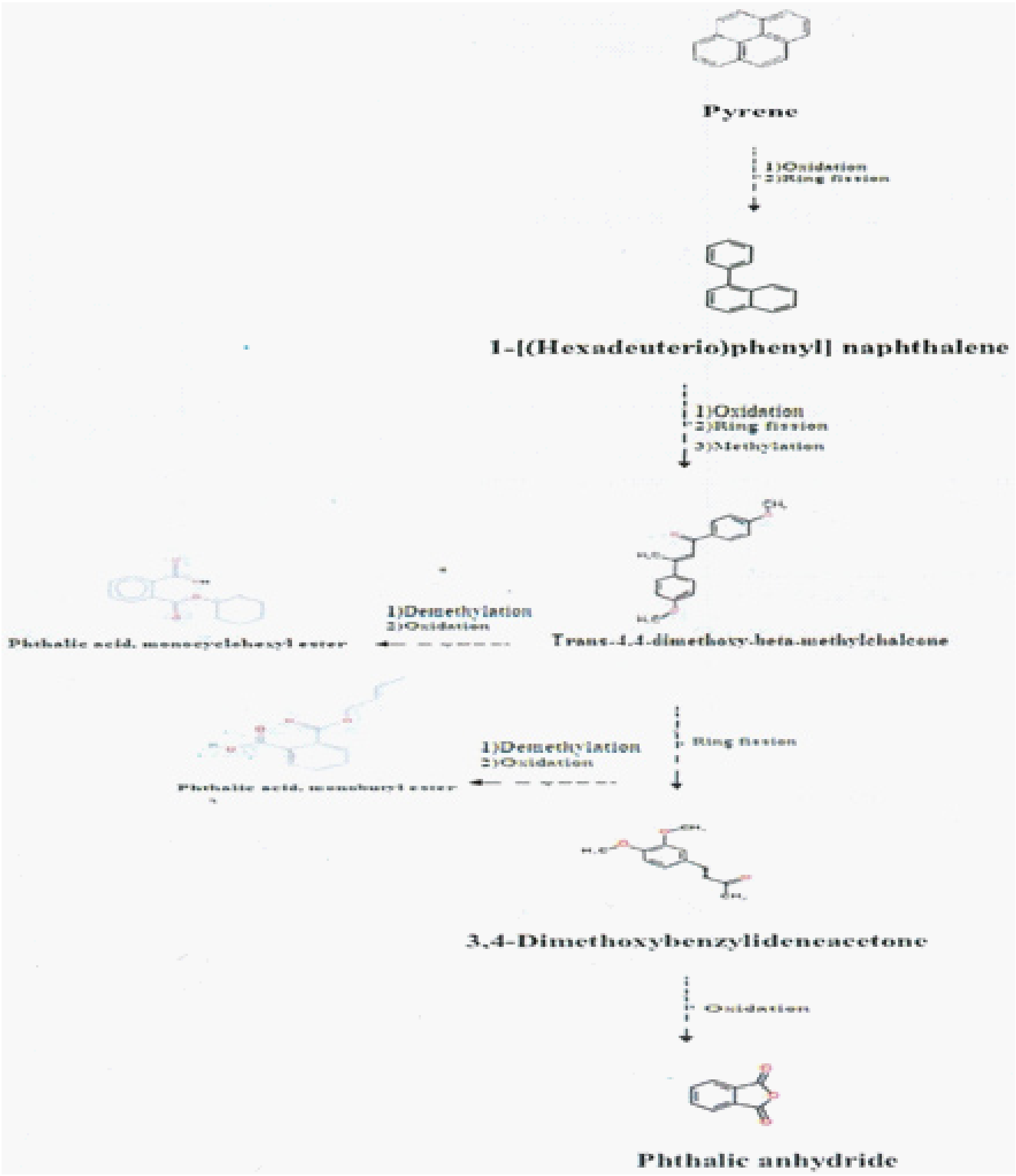


Figure23: Proposed metabolic pathway of pyrene by *Bacillus altitudinis* MAM-P8[67].

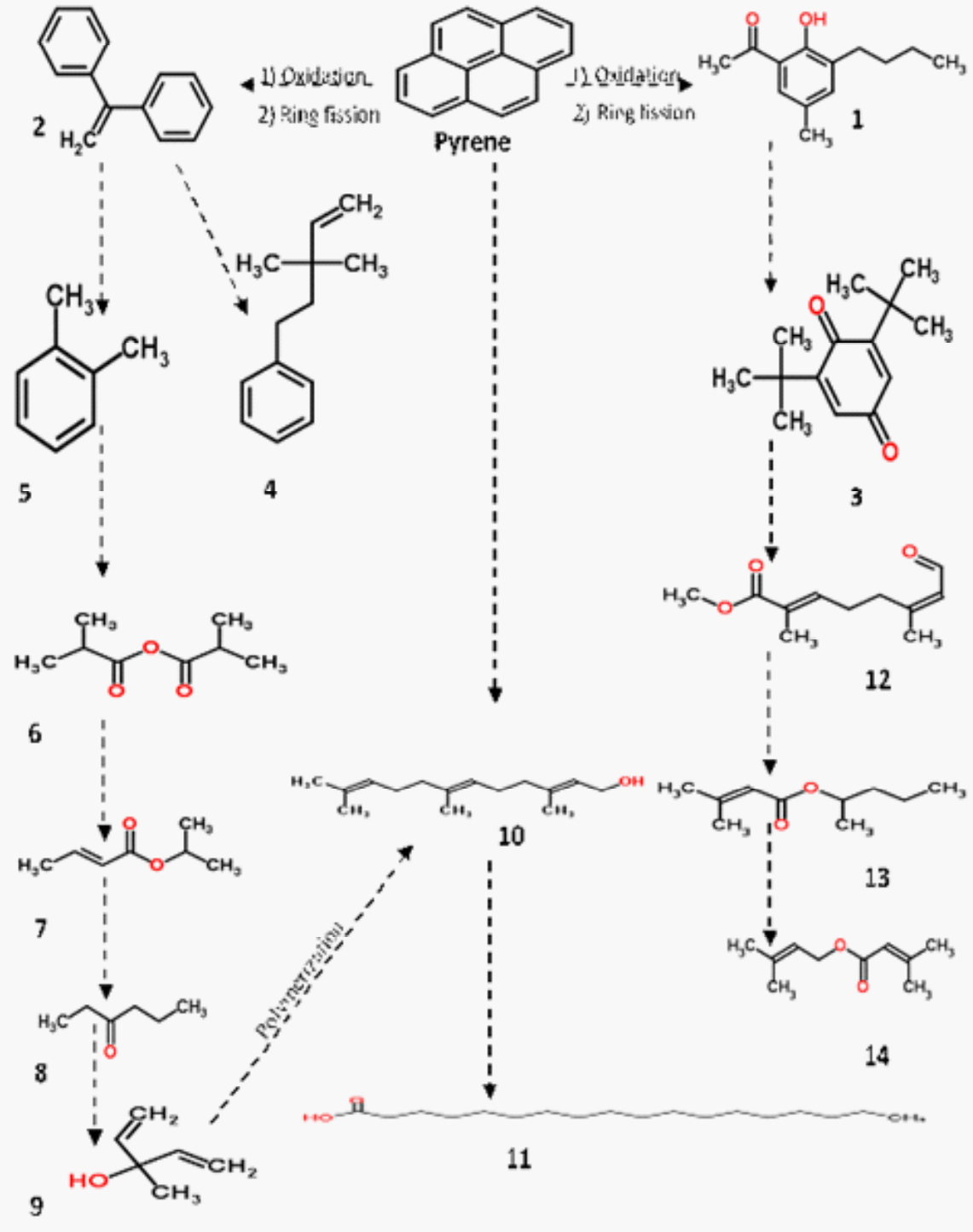


Figure24: Proposed metabolic pathway of pyrene by *Pseudomonas panipatensis* MAM-P39 [68]1 Ethanone,1-(3-buty1-2-hydroxy-5-methylphenyl);2, (1-phenylvinyl) benzene;3, 2,6-di-tert-Butyl-para benzoquinone; 4, Benzene, (3,3-dimethyl-4-pentenyl); 5,2-xylene; 6,isobutyric anhydrid;7 isopropyl (2e)-2-butenoate; 8,3-Hexanone;9, 3- Methylpenta-1,4-diene-3-ol; 10,Farnesol; 11, Octadecanoic acid; 12, 2,6-Dimethyl-8-oxoocta-2,6-dienoic acid, methyl ester; 13, 3-Methyl-2-butenoic acid, 2-pentyl ester; 14, 3-Methyl-2- butenoic acid, 3-methylbut-2-enyl ester.

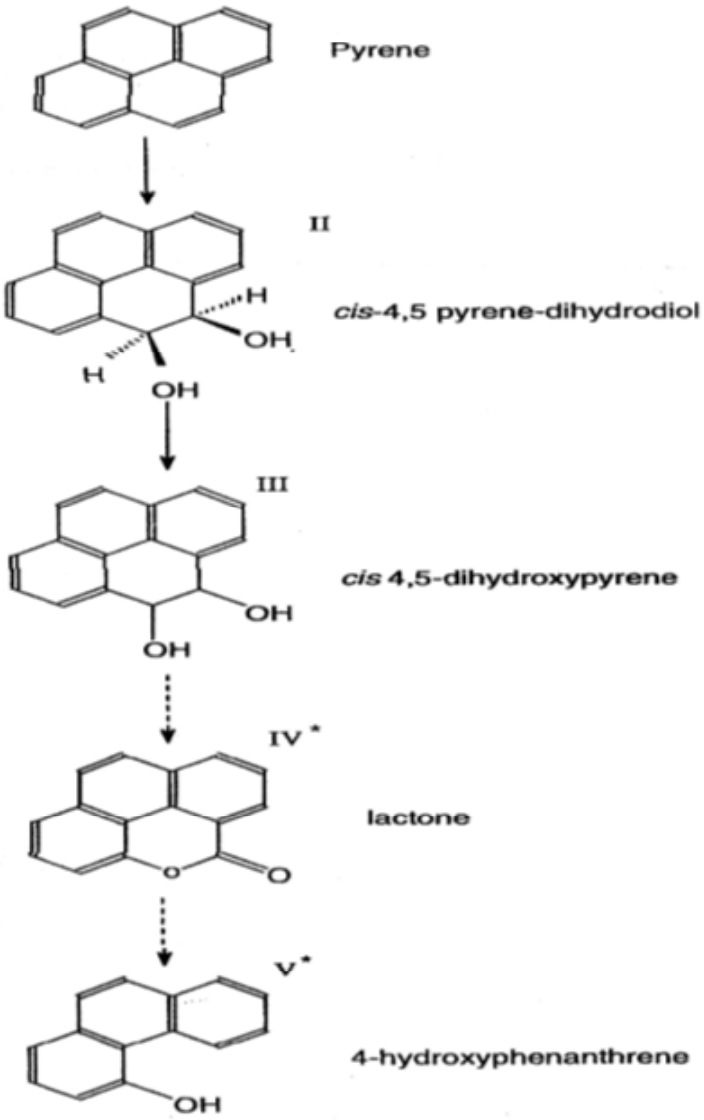


Figure25: Proposed pyrene degradation pathways by the mangrove enriched bacterial consortium [162].

Benzo [a] Pyrene degradation pathways

Few researches have been conducted on HMW-PAHs especially five fussed rings like benzo [a] pyrene. As it is well known that as the number of fussed rings increased, the ability of bacteria to degrade HMW - PAHs decreased. One of the bacterial sp. (*Mycobacterium vanbaalenii* PYR-1) was able to degrade Benzo [a] pyrene as indicated in Figure 26[181].However, O-methylation of benzo [a] pyrene as indicated by Zeng *et al.*, (2013)[182] isthe key of the proposed pathway (Figure 27), the degradation was conducted by two steps.

I) removal of 6 - benzo - [a] pyrenyl acetate to form methoxybenzo - [a] pyrene and

II) Transformation of the tree quinones into dimethoxy benzo [a] pyrene.

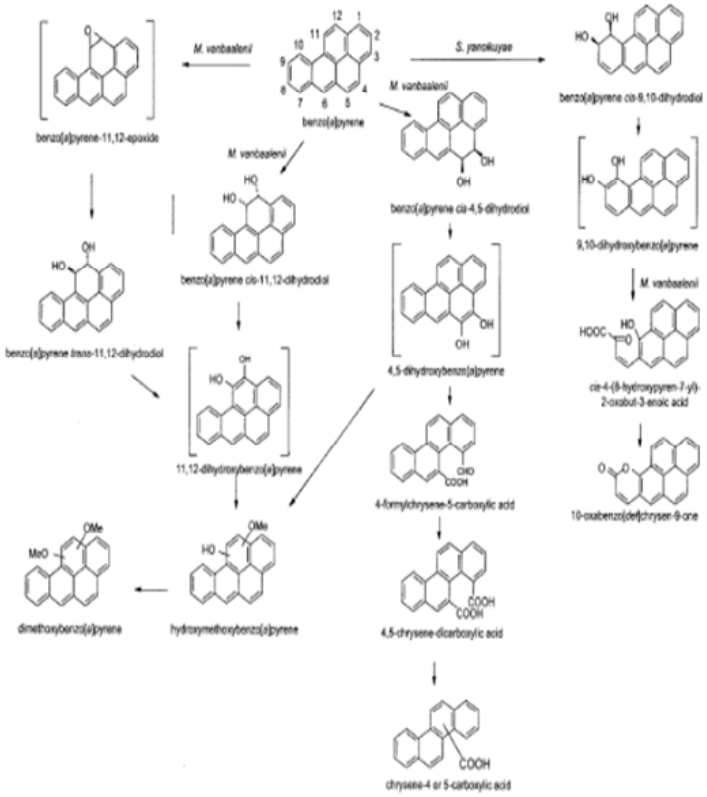


Figure 26: Proposed pathway for the degradation of benzo[a] Pyrene by *M. vanbaalenii* PYR-1. Compounds in brackets are hypothetical intermediates [181].

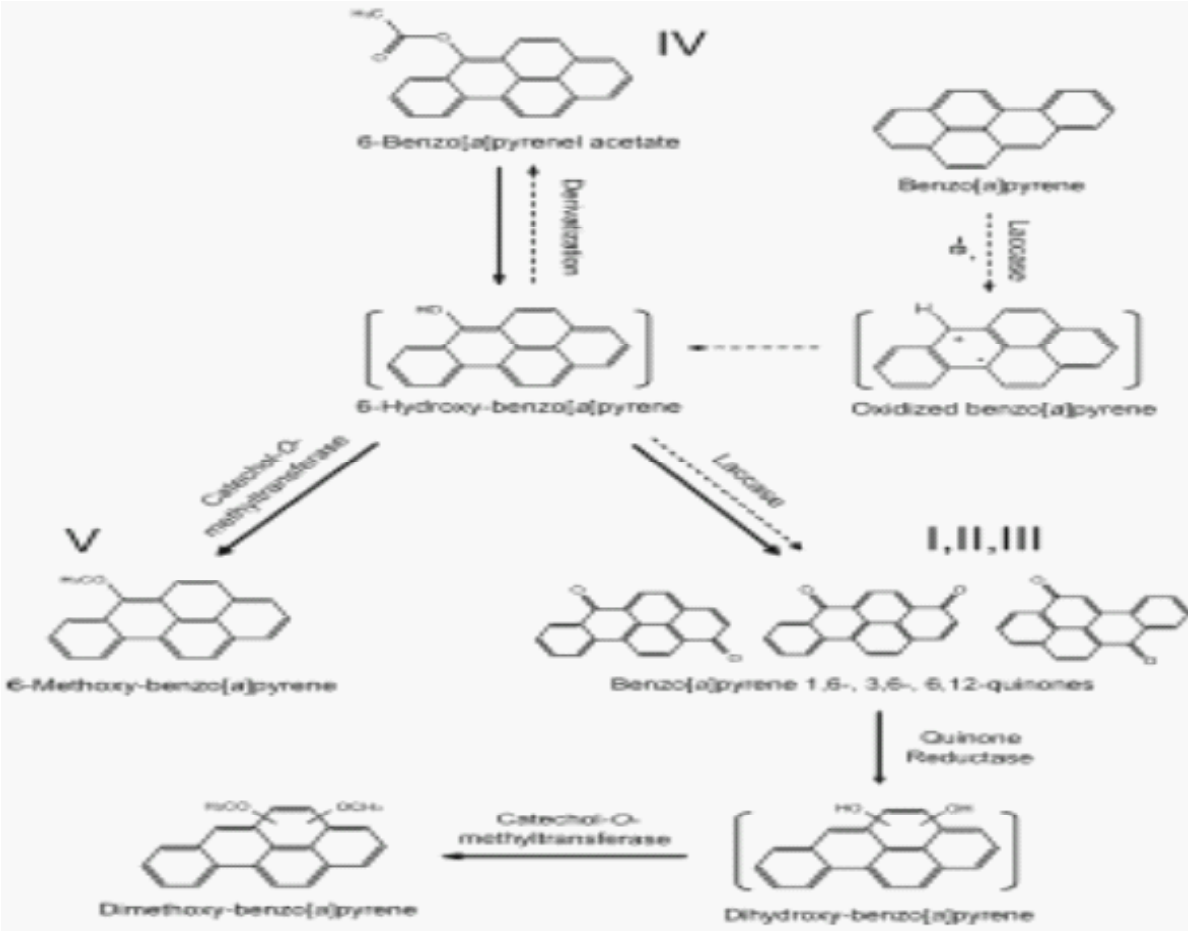


Figure 27: Proposed scheme for the O-methylation mediated pathway involve in successive transformation of benzo[a]Pyrene by laccase and *Mycobacteria*. The compounds in brackets are hypothetical intermediates, short dashed line arrows indicate the steps of B[a]P oxidation by laccase and solid line arrows indicate the steps of successive transformation of the metabolites. The proposed scheme for transformation was composed of these two steps: (1) removal of 6- benzo[a]pyrenyl acetate (IV) to form methoxybenzo[a]Pyrene (V) and the three quinones (I, II, and III), and (2) transformation of the quinones into dimethoxybenzo[a]Pyrene[182].

Treatment of petroleum refinery wastewater (RWP)

Petroleum refinery is an example of an industrial facility which produces a wastewater containing a range of hydrocarbon compounds[183].It also uses a lot of process water [184].This Wastewater released from petroleum refineries is characterized by the presence of large quantity of petroleum products, polycyclic and aromatic hydrocarbons, phenols, metal derivatives, surface active substances, sulfides, naphthylenic acids and other chemicals[185].Wastewaters that containing PAHs must be treated before discharge in water bodies to avoid environmental pollution and comply with environmental protection regulations [186].

Heavy metals together with various pollutants can cause numerous hazards to both human and environment even at low concentration due to gradual accumulation[187].The removal of various toxic substances from wastewater has been a core interest of many researcher [188].

Wastewater may be treated by physiochemical or biological methods, biological treatment is preferred over physicochemical as the former is cost effective, efficient and environmentally friendly [22,189].

Crude oil (C8-C35) was removed by 83.70% by the halotolerant Hydrocarbon Utilizing Bacterial Consortium (HUBC) obtained from on-Shore sites [52].

Consortium of 15 indigenous bacterial isolates removed 94.84% and 93.75% of total Aliphatic and Aromatic Components of Crude Oil (OGDCL, Pakistan) after 24 h respectively [190]. However, the biosurfactant producing *Pseudomonas aeruginosa* UKMP-14T degraded 75.2% of total petroleum hydrocarbon of tap is crude oil after 7 days at 40°C,and 150 rpm[191].

Using *Pseudomonas panipatensis* MAM-P39 for treatment of the petroleum refinery wastewater produced from Suez oil processing company degrade 56.28% of organic compounds as determined by GC/MS. Also, this strain can remove 58.92% of Pb, 64.41% of Cd, 67.87% of as and 99.89 of Hg as verified by ICP analysis [192].

Treatment of petroleum refinery wastewater by physicochemical treatment and that treated with *Bordetella bronchiseptica*MAM-P14 and *Bordetella avium* MAM-P22 revealed that degradation of 9- methylene-fluorene were 69.6%, 42.0% and 76.9% respectively and degradation of 4-chloro-alfa-naphthol were 73.6%, 74.4% and 49.9%. However, treatment by petroleum refinery wastewater by *Bordetella bronchiseptica*MAM-P14 removed 58.5%, 84.8% of vanadium and cadmium respectively. While *Bordetella avium* MAM-P22 removed 71.6% and 82.3% of the same metals [193].

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