



CHAPTER 2

LITERATURE REVIEWS

2.1 Fluorene, fluoranthene, pyrene and other polycyclic aromatic hydrocarbons (PAHs)

Fluorene, fluoranthene and pyrene are members of polycyclic aromatic hydrocarbons (PAHs), which generally arrange with two or more fused aromatic rings in linear, angular or clustered shape. PAHs are hydrophobic substances and their persistence in the environment is chiefly because of their low water solubility (Cerniglia, 1992, and Juhasz and Naidu, 2000).

2.1.1 Chemical structure

Fluorene, fluoranthene and pyrene consists of three or more fused benzene rings as shown Figure 2.1.

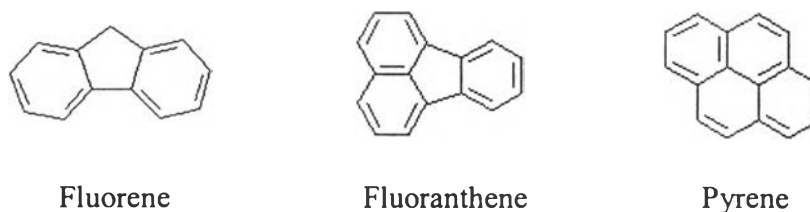


Figure 2.1 Chemical structures of fluorene, fluoranthene and pyrene

16 PAH including naphthalene, acenaphthylene, acenaphthene, phenanthrene, anthracene, fluorene, fluoranthene, chrysene, pyrene, benzo(a)anthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, indeno(1,2,3,cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene compounds were classified by the U.S. Environmental Protection Agency (U.S. EPA) as the priority pollutants to be removed from environments. The structures of those PAHs were shown in Figure 2.2.

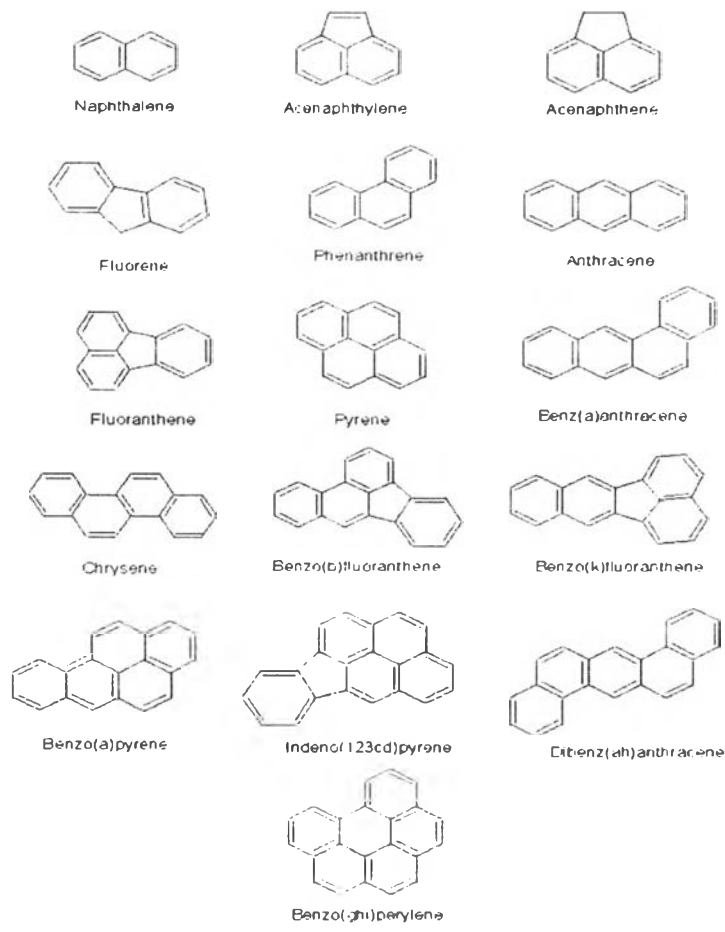


Figure 2.2 Chemical structures of 16 PAHs of U.S. EPA priority pollutants (Fleurat-Lessard *et al.*, 1999)

2.1.2 Source of PAHs

PAHs, including fluorene, fluoranthene and pyrene can be formed naturally, for instance, during thermal geologic reactions associated with fossil fuel and mineral production, and/or during burning of forest (Blumer, 1976, Wilson and Jones, 1993). However, human activities, which lead to generate and distribute significant quantities of PAH-containing materials, are summarized in Table 2.1.

Table 2.1 Human activities associated with the PAH productions
(Cerniglia, 1992 and Wilson and Jones, 1993)

Anthropogenic activities
Asphalt production
Carbon-black production
Coal-tar/coal-tar-pitch production
Coke production
Fuel/oil storage, transportation, processing
Gasification/liquefaction of fossil fuels
Heat and power generation by using fossil fuels
Incineration
Landfill/waste dumps
Open burning (tires/refuse/coal etc.)
Refining/distillation of crude oil and crude oil derived products
Wood-preservative (e.g. creosote) production
Wood-treatment processes

2.1.3 Occurrence of PAHs

PAHs can be found widely in the diverse environments such as air (Lim *et al.*, 1999), soil (Bogan *et al.*, 1999), and water (Kanaly and Harayama, 2000). PAHs have been detected in the aquatic organisms (Deshpande *et al.*, 2002, and White and Triplett, 2002), and vegetables and fruits (Wagrowski and Hitrs, 1997 and Camargo and Toledo, 2003). PAHs were contaminated into aquatic environments through industrial discharges, petroleum spills, automobile exhausts, urban runoff and atmospheric fall out (Neff, 1979, Hoffman *et al.*, 1984, Latimer, *et al.*, 1990 and Simck, *et al.*, 1996). Various PAHs can be detected in lake and river sediments from many countries as shown in Table 2.2.

Table 2.2 PAH concentrations (ng/g, dry weight) from surface sediments from various international locations

Locations	Acenaphthylene	Acenaphthene	Fluorene	Phenanthrene	Fluoranthene	Pyrene	Benzo(k) fluoranthene	Benzo(a) pyrene	Benzo(g,h,i) perylene	References
Australia Brisbane river				110 -1490	340 -2340	320-2260	80-350	170 -1060	170-680	Kayal and Connell, 1989.
Europe Tamar Estuary (UK)				10-710	40-2260	60-2050	60-2330	60-1230	30-820	Readman <i>et al.</i> , 1986.
Jamesanvessi Lake (Finland)	<5 -1740	<5 -16780	190-33780	2610-188840	2320-29530	< 5	<5 -490	<5 -20000		Hyötyläinen and Oikari, 1998.
Irish sea			2 -28	4 -123	4 -156	3 -117		3-113		Charlesworth <i>et al.</i> , 2002.
USA San Joaquin River (California)	0.1-0.2	0.1-0.5	0.2-1.2	0.5-29	0.4-100	0.3-91	0.1-36	0.4-76	0.4-61	Pereira <i>et al.</i> , 1996.

In Thailand, several researches also revealed that PAHs can be found contaminated in various environments as described in Table 2.3

Table 2.3 PAHs in various environments of Thailand

PAHs	Air ^a (dusts, µg/g)	Soil ^b (µg/kg)	Sediments ^c (mg/kg)
Naphthalene		16.5	
Acenaphthylene		1.0	
Acenaphthene		1.0	
Fluorene		1.2	
Phenanthrene	2.3	10.3	
Anthracene		1.2	
Fluoranthene	131.2	10.3	
Chrysene		8.5	
Pyrene	61.3	10.6	
Benzo(a)pyrene	24.9	5.5	20-89.6
Benzo(e)pyrene		8.2	
Benzo(a)anthracene		5.0	
Perylene		14.3	
Dibenz(a,h)anthracene		1.3	
Benzo(k)fluoranthene			15-66.1
Benzo(b,j,k)fluoranthene		14.2	
Benzo(g,h,i)perylene		11.8	100-282.5
Indeno		8.4	

^a Karlsson and Frejd, 2003

^b Wilcke, *et al.*, 1999

^c Patarasiriwong and Boonyoy, 2002

Owing to their low solubility and high octanol/water partition coefficient (K_{ow}), contaminated PAHs tend to associate with particular material. They therefore accumulated in bottom sediments, which can be a long-term reservoir of hydrophobic pollutants (Prahl and Carpenter, 1979, Lee, 1980 and Bixian *et al.*, 2001). These accumulated pollutants could be transferred into the food chain through the bioaccumulation and biomagnification effects (Adamo *et al.*, 1997, Brunson *et al.*, 1998)

2.1.4 Exposure routes

Toxical Profile Information Sheet (Agency for Toxic Substance and Disease Registry [ATSDR], 1995) reported that human can be exposed to PAHs with various ways as following; breathing contaminated air containing smoke from fireplaces, wood stoves, furnaces burning coal or oil and from food preparation, eating smoked or charbroiled meat and fish, smoking tobacco products, inhaling vehicle exhaust, inhaling fumes from working with coal tar and asphalt, and working near charbroiling and high temperature frying equipment. Although the direct exposure of PAHs contaminated sediment has less opportunity, it can alternately come to contact with human via the diet in food chain as previously described.

2.1.5 Toxicological properties

The contamination of PAHs is a great concern due to the fact that many low molecular weight compounds; one-, two-, and three-ring, are acutely toxic (Sims and Overcash, 1983), while higher molecular weight PAHs as well as fluoranthene and pyrene are considered to be potential carcinogen (Mersch-Sundermann *et al.*, 1992 and Nylund *et al.*, 1992). Therefore, U.S. EPA has studied and reported the toxicological characteristic of PAHs, particularly fluorene and fluoranthene and pyrene as following (U.S. Environmental Protection Agency's Integrated Risk Information System [IRIS], 2000)

Fluorene is associated with dose-related decreased erythrocyte, hematocrit, and hemoglobin values; increased liver and spleen weights and increased kidney weights; dose-related increased total cholesterol and total bilirubin and decreased blood urea nitrogen level; decreased in spermatozoa based on mice study. Furthermore, fluorene acts as a tumor initiator in dermal application studies with mice.

Fluoranthene is reported that irritate to the dermal and respiration with cough and bronchitis. It is a potent co-carcinogen, which enhances the chemical's carcinogenicity with another carcinogenic chemical presence. A metabolite of fluoranthene, trans-2,3-dihydrodihydroxy fluoranthene was an ultimate mutagen for human lymphoblasts (Irwin *et al.*, 1997).

Pyrene is cause of irritation to exposed skin and eyes, excitement and contraction, kidney weight decrease in mice when inhalation or digestion. However, pyrene is reported as an experimental mutagen on mice.

2.1.6 Laws and regulations

2.1.6.1 United State (Wilson and Jones, 1993)

United States has established the national standards associated with PAHs, which regulates the PAH concentrations in drinking water, effluents for point sources and the brown fields in order to protect the quality of environment and human health. There are several regulations that mention to PAHs. Clean water Act has limited fluoranthene concentration at 42 µg/l for the protection of human health through ingestion water and contaminated aquatic organisms. Under Comprehensive Environmental Response Compensation and Liability Act (CERCLA) U.S. EPA was granted authority to clean up the contaminated sites. The Superfund Amendments and Reauthorisation Act (SARA), section 121, Clean Up Standards, provides rules and specific approaches for remediation.

CERCLA has identified the fluorene, fluoranthene, pyrene and other PAHs containing materials as hazardous waste, which required to notify the National Response Center (NRC) immediately, when there is a release of this designated hazardous substance. Resource Conservation Recovery Act (RCRA) has also listed the fluorene, fluoranthene, pyrene and other PAHs as the hazardous waste when they are discarded commercial chemical products, off-specification species, container residues, and spill residues. Occupational Safety and Health Administration (OSHA) regulates the benzene soluble fraction of coal tar pitch volatiles and mineral oil mist, which contain several of the PAH compounds.

Furthermore, many states have established their own uniform standards or guidance level, for instance, clean up standard and groundwater quality guideline, namely, Arizona, Kansas, Maine, Minesota, New Jersey, New Hampshire, New Maxico and New York.

2.1.6.2 Canada (Canada Council of Ministry of the Environment, CCME, 1999)

PAHs are controlled under various laws, regulations and agreements designed to protect the environment and human health under the Canadian Environmental Protection Act (CEPA). The standard values of PAHs in any media have not been set. However, PAHs were judged to be toxic to the environment based, in part, on observed effects on aquatic life at several sites in Canada including industrialized harbors. PAHs are included in ocean dumping provisions of CEPA, which regulates allowable levels of contaminants in materials disposed of at sea. In addition, Environmental Canadian administrative Section 36 of the Fisheries Act, which prohibits depositing harmful substances, such as PAHs, in waters used by fish. Other federal legislation, which deals with the regulation of PAHs, includes the Pest Control Products Act, the Transportation of Dangerous Goods Act and the Food and Drug Act. Finally, CCME has published technical guidelines for the safe design and operation of wood preservation facilities, which use creosote-based pesticides.

2.1.6.3 Thailand (PCD, 1992)

Thailand has not established the standard value or the regulation of PAH in order to conserve any media. Although Thailand does not limit the PAH concentrations, they are regulated as well as hydrocarbon. The Enhancement and Conservation of National Environmental Quality Act B.E.2535 section 78 has been promulgated that all kinds of hydrocarbon shall be prevented and controlled of pollution both in and on land. Furthermore, section 79 involves the proper and technical management, treatment and disposal of the hazardous wastes that generated from production and usage of chemical in the industries.

2.1.7 Decontamination of PAHs

There are various methods to decontaminate PAHs containing materials as following; (Lodolo, 2003)

- Chemical process : the chemical structure of pollutants is changed by means of chemical reaction to produce less toxic substances such as solvent extraction.
- Physical process : the phase transfer of contaminant is induced by the physical mechanism without chemical structure modification for instance solidification or stabilization.
- Physico-chemical process : the physical and/or chemical properties of contaminants or of the contaminated medium are utilized to destroy (i.e., chemically convert), separate or contain the contamination.
- Thermal process : the contaminants are increased on the volatility, burned, decomposed, or destroyed by using the heat, for example thermal desorption or pyrolysis. This treatment needs high budget due to high energy, excavation and transportation the contaminated material to facility.
- Bioremediation : the living organisms, such as bacteria or plants are utilized to clean up contamination. Bacteria generally break down the contamination into less harmful components, such as carbon dioxide and water.

Bioremediation can be used to clean up soil, sediment or water. The contamination can be treated in place (*in situ*) or the material can be excavated and treated above ground in a different location (*ex situ*).

2.2 Bioremediation

Bioremediation is a process in which organisms (i.e., bacteria, fungi or plant) metabolize contaminants. Under favourable conditions, microbes can degrade organic contaminants completely into non-toxic by products such as carbon dioxide and water or organic acids and methane. On fundamental process, microorganisms gain energy and build new cell from the transformation of the contaminants in order to survive. In addition the bioremediation has the following advantages (Rockne and Reddy, 2003):

- It may result in complete degradation of organic compounds to nontoxic by products.
- There are minimum mechanical equipment requirements.
- It can be implemented as *in situ* or *ex situ* process. *In situ* bioremediation is safer since it does not require excavation of contaminated sites. Also, it does not disturb the natural surrounding of the site.
- Low cost compared to other remediation technologies.

2.2.1 Biodegradation by microorganisms

The persistence of PAHs in the environment depends on the physical and chemical characteristics of PAH. PAHs are degraded via photooxidation and chemical oxidation (Shiaris and Jambard-Sweet, 1986). Nevertheless, biological transformation is probably the prevailing route of PAHs loss (Mueller *et al.*, 1990b). The microbial degradation of PAHs may be accomplished by complete mineralization and cometabolic degradation (Mahro *et al.*, 1994). Complete mineralization is the total breakdown of organic compounds to water and carbon dioxide. In terms of cometabolic degradation, a recalcitrant compound is degraded in the presence of an analogous compound (Rathbone *et al.*, 1998). Biodegradation in the presence of air or oxygen is called aerobic

biodegradation, which is typically quicker than under anaerobic degradation (Rockne and Reddy, 2003). Numerous genera of microorganisms capable of degrading low- and high-molecular weight PAHs including bacteria and fungi as shown in Table 2.4 and 2.5, respectively.

Table 2.4 List of bacteria capable of fluorene, fluoranthene and pyrene degradation

Substances	Microorganisms	References
Fluorene	<i>Mycobacterium</i> sp. <i>Pseudomonas</i> sp. <i>Arthobacter</i> sp.	Boldrin <i>et al.</i> , 1993 Grifoll <i>et al.</i> , 1994 Grifoll <i>et al.</i> , 1992
Fluoranthene	<i>Alcaligenes denitrificans</i> <i>Mycobacterium</i> sp. <i>Sphingomonas paucimobilis</i> <i>Burkholderia cepacia</i> <i>Rhodococcus</i> sp. <i>Pseudomonas</i> sp. <i>Stenotrophomonas maltophilia</i> <i>Acinetobacter alcoaceticus</i> <i>Pseudomonas saccharophila</i>	Weissenfels <i>et al.</i> , 1991 Kelly and Cerniglia, 1991, Kelly <i>et al.</i> , 1993, Sepic <i>et al.</i> , 1998, and Churchill <i>et al.</i> , 1999 Mueller <i>et al.</i> , 1990a, 1990b, Ye <i>et al.</i> , 1996 and Shuttleworth and Cerniglia, 1996 Juhasz <i>et al.</i> , 1997 Walter <i>et al.</i> 1991 Foght and Westlake, 1988 Juhasz, 1998 and Boonchan, 1998 Lal and and Khanna, 1996 Chen and Aitken, 1999
Pyrene	<i>Alcaligenes denitrificans</i> <i>Mycobacterium</i> sp. <i>Rhodococcus</i> sp. <i>Sphingomonas paucimobilis</i> <i>Stenotrophomonas maltophilia</i> <i>Acinetobacter alcoaceticus</i> <i>Burkholderia cepacia</i> <i>Pseudomonas saccharophila</i>	Weissenfels <i>et al.</i> , 1991 Heitkamp <i>et al.</i> , 1988, Jimenez and Bartha, 1996, Schneider <i>et</i> <i>al.</i> , 1996, and Churchill <i>et al.</i> , 1999 Walter <i>et al.</i> , 1991 Ye <i>et al.</i> , 1996 Boonchan, 1998 and Juhasz, 1998, Lal and and Khanna, 1996 Juhasz <i>et al.</i> , 1997 Chen and Aitken, 1999

Table 2.5 List of fungi capable of fluoranthene and pyrene degradation

Substances	Microorganisms	References
Fluoranthene	<i>Cunninghamella elegans</i> <i>Bjerkandera adjusta</i> , <i>Pleurotus ostreatus</i> , <i>Sporormiella australis</i> , <i>Cryptococcus</i> <i>albidus</i> , <i>Cininobolus cesatii</i> , <i>C. blackesleeana</i> , <i>C. echinulata</i> <i>Pestalotia palmarum</i> , <i>Beauveria alba</i> , <i>Aspergillus terreus</i> , <i>Mortierella</i> <i>ramaniana</i> , <i>Rhizopus arrhizus</i> <i>Penicillium</i> sp.	Pothuluri <i>et al.</i> , 1990 and 1992 Salicis <i>et al.</i> , 1999 and Schutzendubel <i>et al.</i> , 1999 Sack and Gunther, 1993
Pyrene	<i>Cunninghamella elegans</i> <i>Phanerochaete chrysosporium</i> , <i>Nematoloma frowardii</i> <i>Kuehneromyces mutabilis</i> , <i>Laetiporus</i> <i>sulphureus</i> , <i>Agrocybe aegerita</i> <i>P. janthinellum</i> <i>Pleurotus ostreatus</i> <i>Syncephalastrum racemosum</i> , <i>Bjerkandera adjusta</i> , <i>Pleurotus ostreatus</i> <i>Pleurotus</i> sp. <i>Mucor racemosus</i> , <i>M. racemosus</i> var <i>sphaerosporus</i> , <i>Gliocladium virens</i> , <i>Penicillium simplicissimum</i> , <i>Phialophora alba</i> , <i>P. hoffmannii</i> , <i>Trichoderma harzianum</i> , <i>Scopulariopsis brumptii</i> , <i>Coniothyrium fuckelii</i>	Cerniglia <i>et al.</i> , 1986 Hammel <i>et al.</i> , 1986 Sack <i>et al.</i> , 1997a Sack <i>et al.</i> , 1997b Boonchan, 1998 and Stanley <i>et al.</i> , 1999 Bezelel <i>et al.</i> , 1996 Launen <i>et al.</i> , 1995 Schutzendubel <i>et al.</i> , 1999 Lang <i>et al.</i> , 1996 Ravelet <i>et al.</i> , 2000

2.2.2 Biodegradation by the isolated bacteria from the contaminated sediments

In a few decades, several high potential of PAHs degrading bacteria were isolated and characterized from contaminated aquatic environments. The PAH biodegradation of the strains isolated from the hydrocarbon-contaminated environments were higher than those originating from non-contaminated sediments. The certain bacteria could have been acclimatizing and adapting to contaminated environments (Chaineau *et al.*, 1999). These attempts have achieved to gain a great diversity of bacteria capable of PAHs degradation. Heitkamp *et al.* (1988) reported the isolation of pyrene degrading bacterium from petrogenic chemical contaminated sediments by the modified PAH-solution spray method. This bacterium, which was identified as a *Mycobacterium* sp., could mineralize pyrene and other PAHs containing four aromatic rings when grown in a mineral salt medium supplemented with yeast extract, peptone and soluble starch. Furthermore, this strain could also mineralize other PAHs and alkyl- and nitro-substituted PAHs including naphthylene, phenanthrene, fluoranthene, 3-methylcholanthrene, 1-nitrocholanthrene, and 6-nitrochrysene.

Mycobacterium flavescens isolated from the creosote-polluted sediment of Grand Calumet River could utilize pyrene as a sole source of carbon and energy. In addition to pyrene, this strain could mineralize phenanthrene and fluoranthene (Dean-Ross and Cerniglia, 1996).

Mycobacterium sp. strain CH1, which was isolated from PAHs contaminated freshwater sediments by enrichment culture, could mineralize fluoranthene, phenanthrene and pyrene. The mineralization of pyrene showed a lag phase for at least 3 days, however the lag phase diminished when phenanthrene was added in culture. Moreover, strain CH1 was also capable of utilizing a broad range of branched alkanes and n-alkanes as a sole carbon and energy source (Churchill *et al.*, 1999). Another researcher, Molina (1999) reported that *Mycobacterium* sp. strain MR1, which was

isolated from PAHs contaminated river sediments, could utilize pyrene as a sole carbon and energy.

A group of bacteria capable of degrading aromatics in marine environment is the member of the genus *Cycloclasticus*. These bacteria have been isolated from several locations including sediments in the Gulf of Mexico by using a modified most-probable-number technique. The specific substrate utilization experiment presented that these isolates utilized naphthalene, phenanthrene, anthracene, acenaphthene and fluorene, whereas pyrene and fluoranthene could co-metabolize with phenanthrene (Geiselbrecht *et al.*, 1998). Another strain of genus *Cycloclasticus* was isolated from Kamaishi Bay in Japan by enrichment with 2-methylnaphthalene, phenanthrene, or anthracene as a carbon and energy source. This strain could grow on crude oil and degraded PAHs components of crude oil namely unsubstituted and substituted naphthalene, dibenzothiophenes, phenanthrene, and fluorene (Kasai *et al.*, 2002).

Phenanthrene degrading bacteria and yeast were obtained from a stream heavily contaminated with hydrocarbon. *Rhodotorula glutinis* and *Pseudomonas aeruginosa* could utilize phenanthrene at the concentration of 100 mg/l to remain at 0.518 and 0.198 mg/l, respectively, within 31 days of incubation (Romero *et al.*, 1998).

Two bacterial strains, NAG-2N-113 and NAG-2N-126 were isolated from the creosote contaminated marine sediment based on their ability to utilize vapor phase of naphthalene as a sole carbon and energy source. Each strain degraded 1-methylnaphthalene and 2-methylnaphthalene. In addition, the strain NAG-2N-113 can also degrade 2,6-dimethylnaphthalene and phenanthrene. The co-metabolism of acenaphthylene occurred when both strains were incubated with one of the following PAHs; 1-methylnaphthalene, 2-methylnaphthalene, biphenyl, naphthalene, fluorene, phenanthrene and 2,6-dimethylnaphthalene. 16S rDNA-based phylogenetic analysis was used to identify these strains, most closely related to member of Genus *Oceanospirillum*. Because of some morphologic, physiologic and genotypic differences from *Oceanospirillum*; the isolate were identified as a novel genus and species, *Neptunomonas naphthovarans* (NAG-2N-126) (Hedlund *et al.*, 1999).

Several bacilli capable of degrading fuel-related compounds were isolated from subsurface sediments contaminated with petroleum hydrocarbons. These isolates were obtained using a vapor-plate enrichment technique with the volatile aromatic hydrocarbons including toluene, ethylbenzene, *p*-xylene, naphthalene, and JP-4 jet fuel. Based on 16S rDNA sequence analysis, the isolates were phylogenetically similar to common soil organisms, including members of the genera *Pseudomonas*, *Ralstonia*, *Burkholderia*, *Sphingomonas*, *Flavobacterium* and *Bacillus* (Stapleton *et al.*, 2000).

Pyrene degrader *Mycobacterium* sp. and fluoranthene degrading bacteria *Sphingomonas* sp. were isolated from diesel fuel contaminated sediment of Port Newark, New Jersey, USA by modified spray plate technique. Pyrene degrader produced clear zone on a broad range of substrates include fluorene, phenanthrene, fluoranthene, anthracene, benzo(b)fluorene, and benzo(a)anthracene. Moreover, this bacterial strain could utilize anthracene, benzo(b)fluorene following preexposure to phenanthrene. The fluoranthene degrader could utilize fluorene and benzo(a)anthracene in 6 weeks, and could co-metabolize anthracene, benzo(b)fluorene, benzo(a)anthracene and benzo(a)pyrene when preexposed with pyrene (Ho *et al.*, 2000).

Two phenanthrene degrading bacteria were isolated from burrow wall sediment of marine macro fauna. 16S rDNA sequence analysis indicated that one strain belongs to *Cycloclasticus spirillensus* and the other one represents *Lutibacterium anuloederans*. Both strains could utilize two and three rings PAHs as sole source of carbon and energy for growth. Only *L. anuloederans* presented significant phenanthrene uptake in 35 days of incubation and enhanced phenanthrene degradation in high concentration of contaminants in sediment (Chung and King, 2001).

Bacillus sp. strain MN-003 was isolated as the naphthylene-degrading microorganism by enrichment from oil contaminated tropical marine sediments with naphthylene. In addition, this strain was able to use benzene, toluene, xylene isomers, and diesel oil as sole carbon source, although growth on phenanthrene and anthracene

was not detected. Based on taxonomic and 16S rDNA data, strain MN-003 was designated as *Bacillus naphthovorans* (Zhuang *et al.*, 2001).

PAHs degrading bacteria associated with the rhizosphere of the salt marsh plants were isolated from the contaminated estuarine sediment and the salt marsh rhizosphere by enrichment using of either naphthalene, phenanthrene, or biphenyl as the sole source of carbon and energy. Identification of the isolates based on their fatty acid profiles and partial 16S rRNA gene sequence analysis assigned them to three main bacterial groups: Gram-negative pseudomonads; Gram-positive, non-spore-forming nocardioforms; and the Gram-positive, spore-forming group, *Paenibacillus* (Daane *et al.*, 2001).

Recently, bacterium in genus *Rhodococcus* could also isolated from PAHs contaminated in sediment. *Rhodococcus* sp. was isolated from the contaminated creosote Grand Calumet River sediments. This strain was capable of utilizing anthracene, phenanthrene, pyrene, and fluoranthene as sole source of carbon and energy (Dean-Ross *et al.*, 2002).