CHAPTER 2

LITERATURE REVIEWS

2.1 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic Aromatic Hydrocarbons (PAH) are ubiquitous environmental contaminants that are formed during incomplete combustion of organic materials and from geochemical formation of fossil fuel. Primary natural sources are forest fires and volcanic activity. However, most of the PAH released into the environment arise from anthropogenic sources such as combustion of coal coking, petroleum refining, refuse burning, industrial processes, and motor vehicle exhausts (Pothuluri & Cerniglia, 1998).

The term PAH generally refers to hydrocarbons containing two or more fused benzene rings, which are fused with two neighboring rings sharing two adjacent carbon atoms (ToxProbe Inc., 2002). PAHs are hydrophobic compounds and their persistence in the environment is chiefly due to their low water solubility (Cerniglia, 1992). Generally, PAH solubility decreases and hydrophobicity increases with an increase in number of fused benzene rings as presented in Figure 2.1 (Wilson and Jones, 1993).

PAH are a group of approximately 10,000 compounds, but the most toxic members of this family known to date are PAH molecules that have four to seven rings (ToxProbe Inc., 2002). The U.S. Environmental Protection Agency has identified 16 substituted PAH as priority pollutants including naphthalene, acenaphthylene, acenapthene, phenanthrene, anthracene, fluorene, benzo(a)anthracene, benzo(b) fluoranthene, chrysene, fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo (a,h)anthracene, indeno(1,2,3,-d)pyrene, dibenzo(a,h)anthracene, and benzo(ghi) perylene (US.EPA, 1984), respectively.

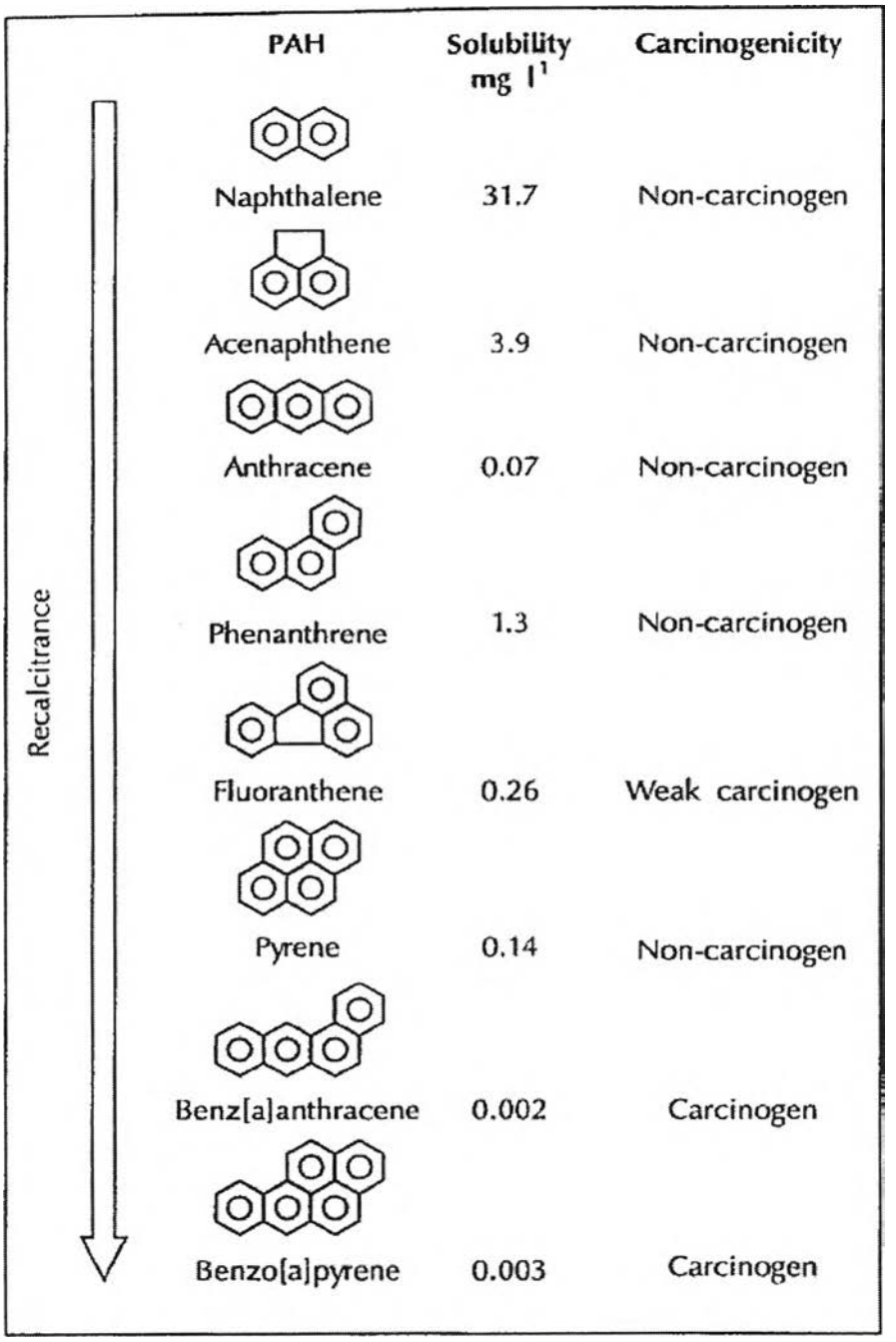


Figure 2.1 Structures, solubility and carcinogenicities of some polycyclic aromatic hydrocarbons (PAHs)

Source: Cerniglia C.E., 1993

2.2 Benzo(a)pyrene

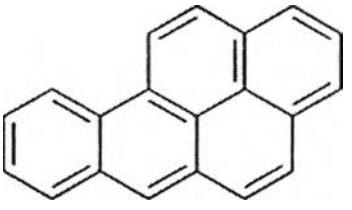


Figure 2.2 Chemical structure of benzo(a)pyrene

Benzo(a)pyrene, shortly named as BaP, is a high molecular weight five-ring polycyclic aromatic hydrocarbon that is mutagenic and highly carcinogenic. Its formula is $C_{20}H_{12}$. The chemical structure of BaP is illustrated in Figure 2.2. It is a crystalline yellow solid. As known as the others PAHs, benzo(a) pyrene is a product of incomplete combustion at temperatures between 300 and 600°C. It is found in coal tar, in automobile exhaust fumes (especially from diesel engines), tobacco smoke, and in charbroiled food. BaP has been classified by the US.EPA as one of the priority pollutants and as one of the most potent carcinogenic PAHs and thus it is the most studied compound of the PAHs class (Juharz&Naidu, 2000).

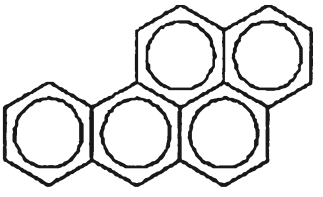
2.2.1 Physicochemical properties of benzo(a)pyrene

The important physicochemical properties including aqueous solubility, vapor pressure, octanol-water partition coefficient, molecular weight are given in Table 2.1. BaP is only slightly soluble in water and is poor volatile.

2.2.2 Benzo(a)pyrene in the environment

Most PAHs either moderately or highly persist in the environment (water, atmosphere, and soil) due to their hydrophobicities. Accordingly, BaP is generally detected in several environmental compartments. For instance, BaP was found in the range of 2.5-667 ug/g organic carbon of the sediment (Ward et al., 1998; Cnyper et al., 1998; Baumard et al., 1998; Viguri et al., 2002; Morse, 2004), while higher contaminated range of 0.69-4.0 mg/kg was found in the municipal sewage sludge (Steven et al., 2003). Furthermore, BaP was reported in fly ash of the concentration of 42.0-48.1 ng/g (Liu et al., 2002). For a long accumulation, BaP was certainly found in soil in many cities of the world, for example, in Helsinki at 236 ug/g soil, Chicaco and

Table 2.1 The relevant physico-chemical properties of Benzo(a)pyrene (adapted from Mackay et al., 2000)

Benzo(a)pyrene	
	
Chemical name	Benzo(a)pyrene
Abbreviation name	BaP
Chemical formula	$C_{20}H_{12}$
Molecular mass	252.31 g/mol
CAS number	[50-32-8]
Density	1.24 g/cm3
Melting point (760 mm Hg)	179 °C
Boiling point (760 mm Hg)	496 °C
Aqueous solubility (mg/l at 25°C)	0.0038
Vapor pressure (Pa at 25°C)	7.0 x10 -7
Log K_{ow} at 25 °C	6.04
Use	Used extensively as a positive control in a variety of laboratory mutagenicity & carcinogenicity short-term tests.
Appearance color	Pale Yellow Monoclinic Needles from Benzene & Methanol; Crystals may be Monoclinic or Orthorhombic.
Odor	Faint aromatic odor

London cities at 1,634 and 1,652 ug/g soil, respectively (Saltiene et al, 2002). Also, in Spain, BaP was found in the industrial area, residential area, and unpolluted area with the amount of 100 ng/g, 56 ng/g, and 21 ng/g dry weight, respectively (Nadal et al., 2004). The maximum allowable concentration of BaP in soil for difference purpose, as well as the classification criteria of polluted or unpolluted area was expressed in Appendix A.

Atmospheric concentration of BaP was detected in many urban area of the world, both of indoor ambient air and outdoor atmosphere (Fang et al., 2004; Fomme et al., 2004). In Japan, BaP was found in the range of 0.27-0.31 ng/m^3 of ambient air, while that of outdoor atmosphere was 0.74 ng/m^3 (Ohura et al., 2004). The city of London and Manchester were of 0.1-1.0 ng/m^3 (Prevedouros et al., 2004). In Brazil, the atmospheric of Amazon forest was detected and found to have the amount of BaP in the range of 0.001-0.660 ng/m^3 (Krauss et al., 2005).

Critically, benzo(a)pyrene was found in fish muscle caught from Red Sea at the mean concentration of 0.5 ng BaP per gram dry weight (DouAbul et al., 1997), while the amount of 0.3-74 μg BaP per kilogram dry weight of smoked fish was revealed (Stolyhwo& Sikorski, 2005). BaP in vegetable oil of about 18 ppb was also reported (Bouman et al., 2000).

In Thailand, BaP was detected in the ambient air of Bangkok in the higher range of 35-190 ng/m^3. The concentration level of BaP in urban areas is significantly 10-time higher than that in rural areas. Also, in semi-aquatic plant, such as water spinach, BaP was found at 19-88 ug/kg wet weight (Soderstrom et al., 2003). In soil, BaP was detected as one of PAHs mixture accumulated in soil of Chiang Mai city which arised from atmospheric deposition (Amagai et al, 1999).

2.2.3 Environmental fate of benzo(a)pyrene

Benzo(a)pyrene is generally released into air and water since it is a ubiquitous product of incomplete combustion. Consequently, it is largely associated with particulate matters. Although environmental concentrations are greatest near sources, the presence of BaP in distant places indicates that it is reasonably stable in the

atmosphere and capable of long distant transport (Fang et al., 2004; Fomme et al., 2004). Atmospheric losses are caused by gravitational settling and rain out. A half life of 1.4 years has been reported for removal of BaP from the gas phase by rain out (Prevedouros et al., 2004).

Benzo(a)pyrene gets into surface water from dust and precipitation in addition to runoff and effluents. Consequently, it would be expected to bio-concentration in aquatic organism (McCarthy et al., 2003). In water and sediments, it has been shown to be stable towards biodegradation. Nonetheless, BaP is expected to undergo significant photo-degradation near the surface of water. Adsorption to sediment and particulate may significantly retard biodegradation, photo-degradation and evaporation (Pothuluri & Cerniglia, 1998). On land, it is strongly adsorbed to soil and remains in the upper soil layers and should not leach into groundwater (Spectrum laboratory chemical fact sheet).

2.2.4 Toxicity of Benzo(a)pyrene

Among the PAHs, benzo(a)pyrene is widely used as an indicator of human health risks and pollution level, because of its strong and direct carcinogenicity (Ohura et al., 2004). The toxicity of benzo(a)pyrene is explicited by that of benzo(a)pyrene interacts with DNA.

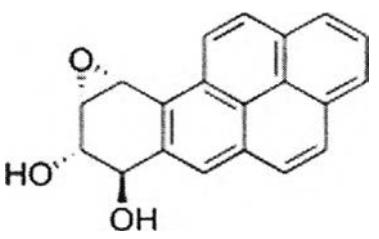


Figure 2.3 Chemical structure of the carcinogen benzo(a)pyrene diol epoxide

Benzo(a)pyrene diol epoxide ((+)-7R,8S-dihydroxy-9S,10R-epoxy-7,8,9,10-tetrahydro benzo (a)pyrene) is the carcinogenic product of three enzymatic reactions. Benzo(a)pyrene is first activated by cytochrome P4501A1 to form benzo(a)pyrene

7,8-epoxide among other products. Next, benzo[a]pyrene 7,8-epoxide is metabolized by epoxide hydrolase to yield benzo(a)pyrene 7,8-dihydrodiol. Benzo(a)pyrene 7,8-dihydrodiol forms the ultimate carcinogen after reacting with cytochrome P4501A1 to yield benzo(a)pyrene diol epoxide ((+)-7R,8S-dihydroxy-9S,10R-epoxy-7,8,9,10-tetrahydro benzo (a)pyrene). The two carbons of the epoxide are electrophillic, due to an unequal sharing of electrons with the oxygen. As a result, this molecule intercalates in DNA, covalently bonding to the nucleophillic guanine nucleobases at the N2 position. A recent X-ray crystallography study shows that this intercalation distorts the DNA. Consquently, this can disrupt the normal process of copying DNA, and may lead to cancer. This mechanism of action is similar to that of aflatoxin which binds to the N7 position of guanine (Wikipedia encyclopedia, Huberman et al., 1976; Xue & Warshawsky, 2005).

2.3 Remediation process of PAHs

According to the persistence of PAHs in the environment and their genotoxicity, much research effort has been aimed at the remediation of PAH contaminated wastes (Potin et al., 2004a,b). Although the possible fates of PAHs are volatilization, photo-oxidation, chemical oxidation, bioaccumulation, and adsorption, PAHs are removed from contaminated sites principally by microbial degradation. The overall removal processes are summarized in Figure 2.4

The use of bioremediation to degrade hazardous chemicals is a practical alternative to traditional remediation treatment technologies. The effectiveness of bioremediation in the detoxification of potentially hazardous chemicals is directly related to the chemical structure of the PAHs. The polycyclic Aromatic Hydrocarbons (PAHs) with four and five fused benzene rings are more resistant to biodegradation than PAHs with two or three rings (Atlas, 1997).

Bioremediation is an option that offers the possibility to destroy or remove various contaminants using natural biological activity. Therefore, this technique requires relatively low cost (see appendix B), non-complicated technology, which generally have a high public acceptance and can often be carried out on site (Vidali, 2001).

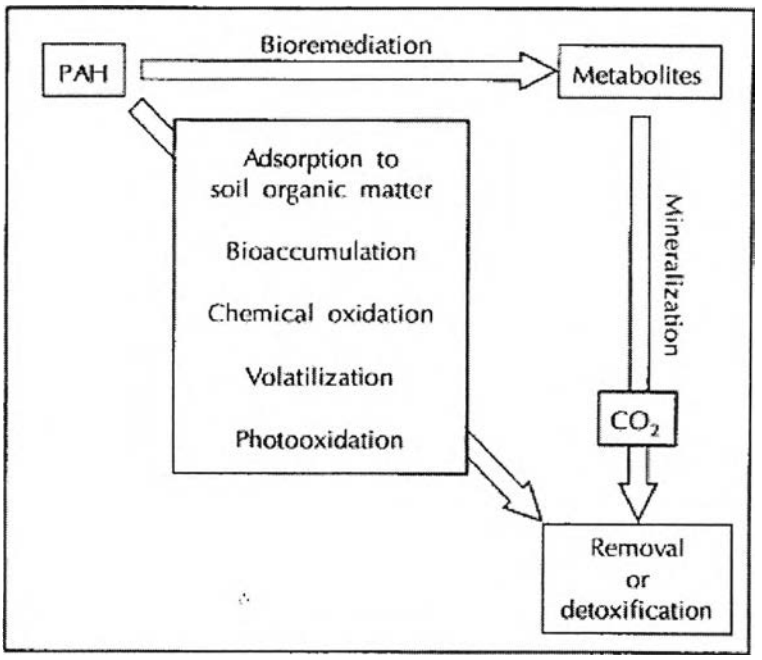


Figure 2.4 Degradation processes of polycyclic aromatic hydrocarbon in the Environmental

Source: Cerniglia C.E., 1993

2.3.1 Bioremediation of Benzo(a)pyrene

The natural presence of PAHs in the environment allows many microorganisms to adapt to exploit these PAHs as the potential growth substrate. Thus, many bacterial, fungal, and algal strains have been shown to utilize a wide variety of PAHs containing from three to five aromatic rings (Gibson, 1993; Pothuluri & Cerniglia, 1998; Bicimi, 2002).

PAHs are degraded slowly by indigenous microorganisms and thus persisted in soil and sediments (Gramss et al., 1993; Potin et al., 2004). The recalcitrant natures of PAHs, attributed in part to the strong absorption of PAHs molecule to soil organic matter and in part to their low solubility, make them less amenable to microbial degradation (Cerniglia, 1992; Roger et al., 2002; Bicimi, 2002). However, compared to other processes whereby these compounds are removed from the environment, the

microbial degradation plays a major role in the remediation of contaminated sites (Cerniglia, 1992; Gibson, 1993; Pothuluri & Cerniglia, 1998; Suillia, 2003).

Over the past 30 years, researches on the microbial degradation have resulted in the isolation of microorganisms able to degrade low molecular weight PAHs (three or less fused rings). The rate, metabolic pathway, enzymatic regulation and genetic regulation have been well documented. However, for the high molecular weight PAHs including BaP, which are generally recalcitrant to microbial attack, only few genera of microorganisms with the ability to degrade them have been observed. Consequently, the reports of the reaction sequence as well as the identification of metabolic product(s) have been limited (Juhasz & Naidu, 2000).

In 1988, Heitkamp and Cerniglia published the first study on the isolation of bacteria capable of extensive degradation of PAHs containing four aromatic rings (Heitkamp & Cerniglia, 1988). In 1989, Muller et al. demonstrated for the first time that the utilization of a PAH containing four or more aromatic rings as a sole source of carbon and energy by bacteria was possible (Muller et al., 1989).

2.3.2 Biodegradation of BaP by fungi

The biodegradation of BaP by fungi has been reported and become more attractive technique than that the use of bacteria, because of the greater degrading potential of fungi by virtue of their aggressive growth, greater biomass production and extensive hyphae reach in soil. The high surface-to-cell ratio of filamentous fungi makes them better degraders the contaminated soils (Vidali, 2001; Sullia, 2003; Robinovich et al., 2004). Moreover, when the fungal mycelia once produced, it can be re-used many times and can be employed without loss of effectiveness in continuous or semi-continuous reactors for several months. Furthermore, the mycelium can be kept in cold storage for several months without loss of activity (Knapp et al, 1999; Kotterman et al., 1999).

Whereas the PAH degradation of bacteria have been well studied, knowledge of similar activities in fungi is limited. As fungi have become as important as bacteria

in the bioremediation of PAH, a diverse group of fungi; ligninolytic and nonligninolytic fungi capable of PAHs oxidation has been reported (Table 2.2)

2.3.2.1 White Rot Fungi and BaP biodegradation

They are several reports on the innovative technique using remarkable potential white rot fungi for biodegradation of PAHs. The ligninolytic fungi, commonly known as white rot fungi, *Phanerochaete chrysosporium* has been used as a model organism to study the potential of white rot fungi to degrade PAHs (Sanglard et al., 1986; Haemmerli et al., 1988; Bumpus et al., 1985, 1989; Barclay et al., 1995; Zheng & Obbard, 2002a). Nevertheless, others white rot fungi, such as *Trametes vericular* (Collin et al., 1996; Majcherzyk et al., 1998; Levin et al., 2003), *Bjerkandera* sp. (Field et al., 1992; Mester et al., 1996; Kotterman et al., 1998), *Pycnoporus cinnabarinus* (Rama et al.,1998), *Pleurotus* sp. (Bazalel et al.,1996; Eggen & Majcherczyk, 1998), and *Strophoria* rugosoannulata (Steffen et al., 2002, 2003) have also shown promises for their abilities to degrade PAHs more rapidly than *P. chrysosporium.*

2.3.2.2 Non white rot fungi and BaP biodegradation

Non-ligninolytic fungi which have the ability to oxidize PAHs were also observed. The fungus *Strophoria coronilla* (Steffen et al., 2003), *Fusarium solani* (Potin et al., and Veignie et al., 2004; Verdin et al., 2004a,b,c), as well as *Penicillium janthinellum* (Launen et al.,1995; Boonchan et al.., 2000), and *Aspergillus* sp.(Captorti et al., 2004) were also reported to oxidize BaP. Furthermore, the same intensive ability to degrade BaP as that of 1000 times of white rot fungi was described from *Cunninghamella elegans* (Cerniglia & Gibson, 1979).

2.4 Fungal biotransformation

Factors effecting BaP biotransformation are:

2.4.1 Carbon source:

In order to biotransform BaP, alternative carbon source may be necessarily provided as a growth substrate in many fungi. Glucose has been used as a versatile carbon source for growth substrate in *Phanerochaete* sp., *Trametes* sp., *Bjerkandera* sp., *Nematoloma forwardii* (Bumpus et al., 1985; Sanglard et al., 1986; Collin et al.,

Table 2.2 List of fungi can degrade of Benzo(a)pyrene (adapted from Juharz & Naidu, 2000)

Fungal species	**References**
Phanerochaete chrysosporium	Sanglard et al., 1986; Haemmerli et al.,1986; Bumpus et al., 1985; Barclay et al., 1995
Aspergillus ochraceus	Ghosh et al., 1983; Datta and Samanta, 1988
Aspergillus Terreus	Capotorti et al., 2004
Cunninghamella elegans	Cerniglia and Gibson, 1980; Cerniglia et al., 1980; Pothuluri et al., 1998
Pleurotus ostreatus	Bezalel et al., 1996
Pleurotus sp. *Florida*	Wolter et al., 1997
Trametes versicolorb	Collins et al.,1996; Majcherczyk et al., 1998
Pycnoporus cinnabarinus	Rama et al., 1998
Bjerkandera sp. Strain BOS55	Field et al.,1992; Kottermann et al.,1998
Penicillium janthinellum	Stanley et al.,1999; Launen et al., 1995
Stropharia coronilla	Steffen et al., 2003
Coniothyrium sp.	Potin et al., 2004a
Cladosporium sphaerospermum	Potin et al., 2004b
Fusarium solani	Veignie et al., 2004
Trichloderma viride	Verdin et al., 2004

1996; Kottermann et al., 1998). While sabourand dextrose broth has been widely applied for *Cunninghamella elegans* (Cerniglia & Gibson, 1980), Malt-Yeast extract-Peptone- Dextrose (MYPD) has good property for *Pennicillium* sp. (Launen et al., 1995). Other growth substrates such as glycerol, malt extract, or even wheat straw have also been reported as a supplemented carbon source (Field et al., 1992; Wolter et al., 1997; Morgan et al., 1993; Barclay et al., 1995), as well as sugarcane bagasses and pine sawdust are the carbon source suitable for P. *chrysosporium.*

2.4.2 Nitrogen source:

Contrarily to carbon source effect, sufficient nitrogen source was mentioned to inhibit the mineralization of BaP, while having a little effect of polar metabolite formation (Kottermann et al., 1996; Boyle et al., 1998). It was shown that sawdust or ground alfafa inhibited formation of polar metabolites, but had little effect on mineralization (Bogan et al., 1996; Boyle et al., 1998).

2.4.3 BaP concentration and biodegradation condition:

Various concentrations of BaP degradation by fungi were reported. The white rot fungi P. *chrysosporium* was showed the ability to maximize degradation of 76 mg/l of BaP within 24 days (Bumpus et al., 1985). As well as the *Cunninghamella elegans* was reported to degrade 53 μmol of BaP within 96hrs (Cerniglia & Gibson, 1980). However, the extent of BaP removal by others ligninolytic fungi were revealed by Launen et al., in 1995 that 100 mg/l of BaP was transformed by *Penicillium janthinella* within 7 days (Launen et al., 1995). The *Aspergillus ochraceus* was discovered to biotranformation of about 80 μmol of BaP within 18 hrs (Ghosh et al., 1983), as well as Datta and Samanta showed the ability of A. *ochraceus* to degrade 0.4 mmol of BaP within 48 hrs (Datta&Samanta, 1988). The degradation of 27.5% of initial concentration of about 25 μg/l of BaP was also degraded by A. *Terreus* within 56 days (Stanley et al., 1999). The *Trichoderma viride* as well as *Fusarium solani*, F. *oxysporum* were also reported to have relative degradation per unit biomass of about 39%, 17% and 8%, respectively, of 100 mg/l of BaP in liquid media after 30 days of incubation (Verdin et al., 2004b).

Several ligninolytic and as well as non-ligninolytic fungi can successively mineralize BaP to CO_2 under different growth and culture conditions as some examples shown below.

- *Phanerochaete chrysosporium* culture grown on excess glucose (10g/l), at 37 °C showed 14.6% of 150 nmol of initial BaP mineralization after incubation periods of 168 hours (Sanglard et al., 1986).
- *Pleurotus* strain was demonstrated to mineralize 1% BaP in solid state fermentation over a 15-week incubation period in wheat straw. The utilization rate was independent of the initial concentration of BaP at the concentrations tested from 5 to 100 mg/kg (Bezalel et al., 1996; Wolter et al., 1997; Eggen & Majcherzyk, 1998).
- *Fusarium solani* mineralized of 1.2 % of 188 mg/l of BaP to CO_2 at the first stage of the fermentation process within 15 hrs. (Vienie et al., 2002, 2004).
- *Bjerkandera* sp. was shown to have the ability to mineralize 8.4% of 20 mg/l of BaP to CO_2 in the excess of glucose (5g/l) at 30 ° C (Kottermann et al., 1998; Bicimi, 2002).
- *Penicillium janthinella* mineralized 50 mg/l of initial BaP up to 25% within 49 days and at 53% within 100 days (Boonchan et al., 2000).
- Steffen used enzyme which extracted from *Strophoria coronilla* to mineralize 100 mg/l BaP to 12% CO_2 within 6 weeks with the enhancement of Tween 80 (Steffen et al, 2003)
- The greater biotransformation efficiency (90-99%) were also observed with the following fungal activities: such as P. *chrysosporium* could remove of about 99% of 150 nmol. Of BaP (Sanglard et al., 1986). *Pycnoponus cinnabarinus* could remove of 95% of 0.1 mmol. Of BaP (Rama et al., 1998). *Trametes versicolor* was reported to remove of 90% of 25 µmol of initial BaP (Majcherczyk et al., 1998). Also, *Bjerkandera* sp. BOS55 could degrade up to 99% of 50 mg/l of BaP (Kottermann et al., 1998). As well as *Nematoloma frowardii* was reported to degrade 99% of 10 mg/l BaP (Sack et al., 1997). Moreover, the decrease in BaP concentration resulted in the concomitant increase in water soluble BaP products (Kottermann et al., 1998, 1999).

2.5 Fungal metabolism of BaP and its metabolic pathway

The bacterial degradation of PAHs initially proceeds through a dioxygenase which attacks on an aromatic ring to form a cis-dihydrodiol (Figure 2.5). The

dihydroxylated cis-dihydrodiol intermediates formed are oxidized to catechols of which ring fission by dioxygenase enzymes is occurred to give aliphatic intermediates. These intermediates are then be oxidized to provide energy or for biosynthesis of cell constituents.

In contrast to bacteria, fungi do not utilize PAHs as their sole sources of carbon and energy but transform PAHs co-metabolically to detoxify chemical products. Two main enzyme groups involved in the initial attack on PAHs by fungi are the cytochrome P-450 monoxygenases and the lignin peroxidases. Both enzymes are relatively non-specific for the PAHs that they metabolize.

2.5.1 Extra-cellular enzymes

Lignin peroxidases, manganese peroxidases and laccases, extracellular enzymes produced by the white rot fungi, are the best characterized enzymes involving in biotransformation (Rama et al., 1998; Mester&tien, 2000; Steffen et al., 2003; Dodor et al., 2004). The specificity of these enzymes is low; therefore the substrate spectrum is broad. This non-specific mode of enzyme action is advantageous in that it does not require preconditioning to individual pollutants and avoid the uptake of potential toxic substances (Bicimi, 2002; Szewezyk et al., 2003). The mechanism of lignin peroxidases found in P. *chrysosporium*, C. *elegans*, A. *ochraceus*, T. *versicolor* and P. *cinnabarinus* can be described as followed.

The extra-cellular lignin peroxidases initiate a free radical attack on PAHs, by a single electron transfer, to form quinones (Figure 2.5). One of the phenolic metabolites, 6-hydroxybenzo[*a*]pyrene, is further oxidized to the 1,6-, 3,6-, or 6,12-quinones as BaP polar metabolites (Haemmerli et al., 1986; Field et al., 1992; Bogan & Lamar., 1996; Majcherczyk et al., and Rama et al., 1998) show in Figure 2.6. The phenols, quinones, and dihydrodiols can be detoxified by conjugation to glucuronides and sulfate esters, and the quinones can also form glutathione conjugates (Figure 2.5). Moreover, Kottermann showed that the metabolites of BaP obtained from the degradation by *Bjerkandera* sp. had no mutagenic activity and were water soluble products (Kottermann et al., 1998, 1999).

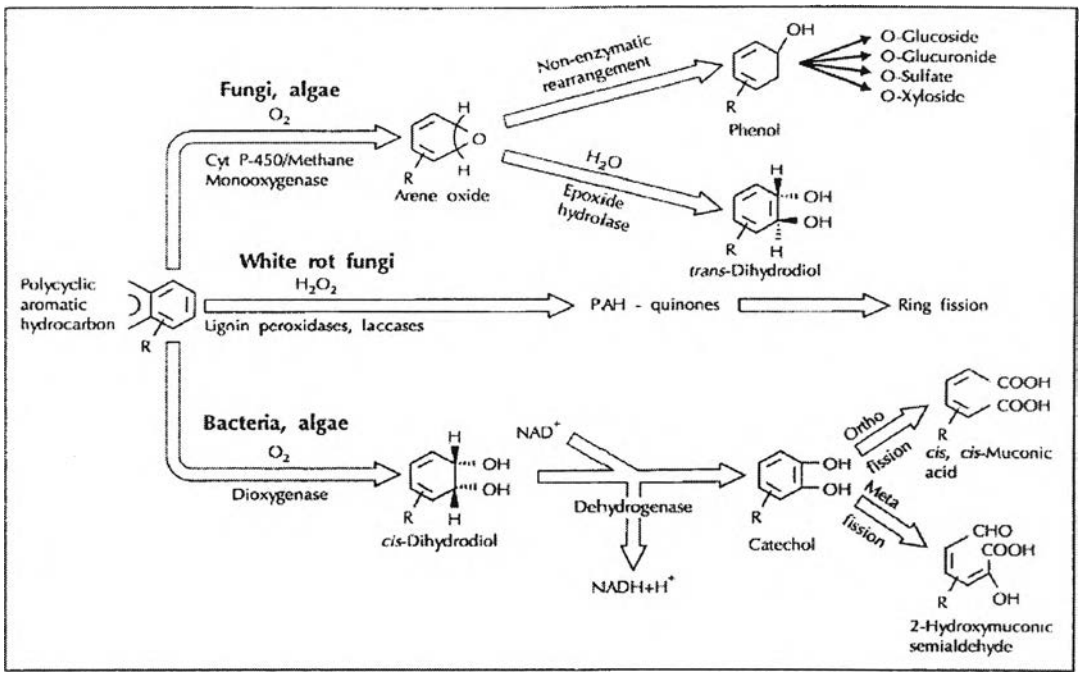


Figure 2.5 Pathways for the microbial catabolism of polycyclic aromatic hydrocarbons

Source: Cerniglia C.E., 1993

2.5.2 Intracellular enzymes

Besides the enzymes previously mentioned, many fungi oxidize PAHs via intracellular enzyme such as cytochrome P-450 monooxygenase in which one atom of the oxygen molecule is incorporated into the PAH to form an arene oxide and the other atom into water. Most arene oxides are unstable and can undergo either enzymatic hydration via epoxide hydrolase to form trans-dihydrodiols or non-enzymatic rearrangement to form phenol which can be conjugated with sulfate, glucose, xylose, or glucoronic acid. Further oxidation of BaP dihydrodiols has been demonstrated by Cerniglia and Gibson with *Cunninghamella* elegans (Cerniglia and Gibson, 1980, 1985), *Aspergillus* terreus (Capotorti et al., 2004), ***Penicillium*** sp. (Launen et al., 1995) and *Fusarium* sp. (Suillia, 2003; Verdin et al., 2004 a,b,c).

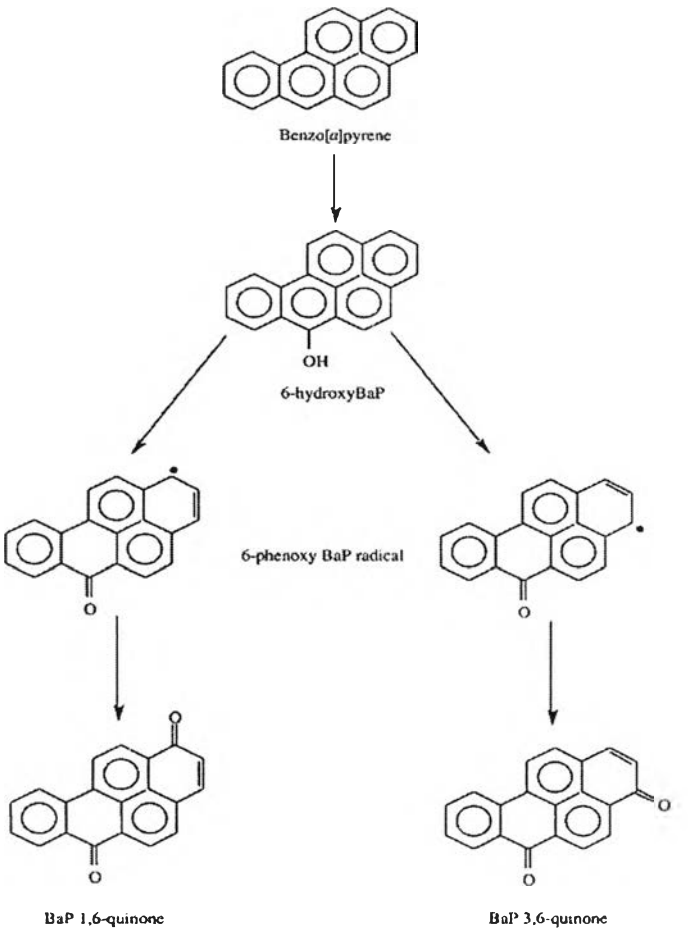


Figure 2.6 Benzo(a)pyrene quinones produced from the fungal oxidation of benzo(a)pyrene (adapted from Cerniglia&Gibson, 1980).

The metabolism of benzo[*a*]pyrene has been extensively studied in the literature; however, only the most important pathways will be presented in this summary. As outlined in IARC (1983), benzo[*a*]pyrene is metabolized initially by the microsomal cytochrome P-450 monooxygenase system to several arene oxides, which may rearrange spontaneously to phenols, undergo hydration to the corresponding trans-dihydrodiols, or react covalently with glutathione, either spontaneously or in a reaction catalyzed by glutathione-S-transferases.

In addition to conjugation, the dihydrodiols undergo further oxidative metabolism. Benzo[a]pyrene is first activated by cytochrome P4501A1 to form (+)-benzo[a]pyrene 7,8-epoxide among other products. Next, (+)-benzo[a]pyrene 7,8-epoxide is metabolized by epoxide hydrolase to yield (-)-benzo[a]pyrene-7,8,dihydrodiol. (-)-benzo[a]pyrene-7,8,dihydrodiol is in part oxidized to 7,8-diol-9,10-epoxide, a compound considered to be the ultimate carcinogenic metabolite of benzo[*a*]pyrene, and further reacting with cytochrome P4501A1 to yield (-7,8,9,10-tetrahydro benzo(a)pyrene) (Figure 2.7).

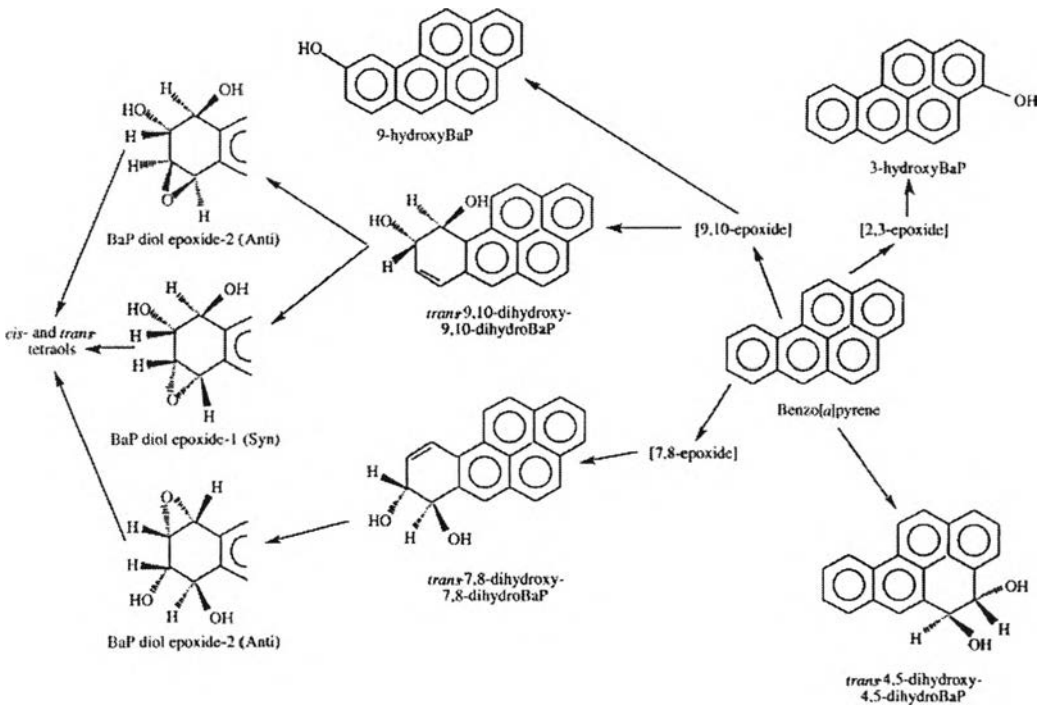


Figure 2.7 Fungal transformation of benzo(a)pyrene. The formation of hydroxylated benzo(a)pyrenes and benzo(a)pyrene dihydrodiols occurs via benzo(a)pyrene epoxides. Dihydrodiols may be transformed to diol epoxides, which may undergo further transformation to form tetraols. (adapted from Cerniglia&Gibson, 1980)

2.6 Application of fungal bioremediation

Some applications of fungal bioremediation were reported. The profound effect on biodegradation of various hydrocarbon compounds by fungi depends on the availability of oxygen molecules (Atlas, 1997). In most cases, field treatment of PAHs contaminated environment involved bio-stimulation (Leahy&Colwell, 1990). The application of fungal bioremediation can be classified into two main types, in *situ* and *ex situ* bioremediation as briefly described below:

In *situ* bioremediation by fungi are mostly reported to treat the long-term PAHs contaminated soil in gas plants, or petroleum refinery, or tar distillation factory sites (Ashok et al., 1995; Pospisil et al., and Sasek & Novotny, 1996; Eggen & Majcherczyk, 1998; Loehr & Webster, 2000; Sasek et al., 2003; Potin et al, 2004 a,b). Some fungi were used to treat the contaminated soil in wood preservation process factory (Hardy & Steward, 1993; Kottermann, 1999). Biodegradation of BaP in soil is reported to have half-lives of 50 days to about 8.2 years (Gramss et al., 1999; ERACL Theme, 2002).

Land farming is a biological treatment technology in which waste materials are applied to soil surfaces as solid sledges or aqueous slurries (Straube et al., 2003).

Composting encourages biological degradation of contaminants. Aeration, leachate and runoff control are built into the system design. Air movement is used to control the concentration of oxygen (Hinchee et al., 1994).

On the other hand, the ex *situ* bioremediation using a bioreactor or on site land farming is commonly approached to treat oily waste (Wilson & Jones, 1993; Atlas, 1997). The study of the treatment of BaP contaminated soil in a batch reactor was previously reported (Wilson & Jones, 1993; Viegnie et al, 2002).

Bioreactors permit the optimization and control of process parameters. Its may use suspended microorganisms or adsorbed bio-film. It may be operated with or without addition of oxygen or other electron accepters and nutrient feed (Atlas, 1995).

In bio-filtration, microorganisms in the form of a moistened bio-film layer attached to compost are used to catalyst chemical reactions (Stone, 1994). Bio-trickling filters are similar to bio-filters, but contain conventional scrubber packing material instead of compost (Levin & Gealt, 1993).

A membrane bioreactor is consisting of liquid-phase bioreactor couple with a membrane clarification unit. The membrane system can be operated in either an aerobic or anaerobic mode (Levin & Gealt, 1993).

A fluidized bed reactor system consists of a column reactor containing media that serves as an attachment surface for the retention of microorganisms. The contaminated liquid flows through the reactor where microorganisms degrade the contaminants present. This is suitable for treating contaminated soils and water (Crawford & Crawford, 1996).