CHAPTER 5

CONCLUSIONS AND DISCUSSIONS

The surface of river and canal sediment samples were collected under the water at the depth of 3-4 meters by using the stainless steel grab sampler from eight sampling sites in Bangkok. The samples were analyzed for their PAH concentrations and used for isolation of the bacteria capable of PAHs degradation. For PAH concentration analysis, the sediment samples were extracted by dichloromethane and PAH concentrations were determined by HPLC equipped with UV detector. This study was focused only on 7 PAHs, namely acenaphthylene, acenaphthene, fluorene, dibenzofuran, phenanthrene, fluoranthene and pyrene. Four sampling sites were found of the contamination with PAHs. Only fluoranthene and phenanthrene were found in the range of 6.5 to 8.2 μg/g dry weight and 0.13 to 0.20 μg/g dry weight of sediment, respectively. These results correlated with several studies, which determined PAH concentration in river sediment that fluoranthene and phenanthrene were abundance in sediment (Bixian *et al.*, 2001, Macias-Zamora *et al.*, 2002 and Verrhiest *et al.*, 2002). The disappearance of low molecular weight of PAHs (acenaphthylene, acenaphthene, dibenzofuran and fluorene) was possibly due to volatilization during air-dried step before the solvent extraction. (Stout *et al.*, 2001). As a result of comparison of fluoranthene and phenanthrene in sediments in this study to those in the overseas values (Table 5.1), phenanthrene concentration are comparable to, and higher than those observed in industrialized and domestic areas the Pearl River (Bixian *et al.*, 2001) and Napa River and Petaluma River (San Francisco Estuary Institute, 2000). The fluoranthene concentrations were relatively higher than those found in those river contaminated sediments.

In addition to river sediment, PAHs could also be found in various kinds of sediments. Both fluoranthene and phenanthrene concentrations in the sediment of Lake Jämsänvesi (Finland, Hyötyläinen and Oikari, 1998), Barent sea (Russia, Sovinov *et al.*, 2003) and Bedford harbor (USA, Pruell *et al.*, 1990) were higher than the results in the present study. Since the Lake Jämsänvesi was highly polluted by creosote, which contain

about 85% PAHs, meanwhile both Bedford harbor and Barent sea were associated with industrial and anthropogenic activities. There were several studies found that the industrial and anthropogenic activities including; oil spills of shipping activities, engine exhausts, improper handle of petroleum hydrocarbon, atmospheric deposition and runoff from the streets may be act as the major source for these substances contamination in respective sites (Hyötyläinen and Oikari, 1998 and Zakaria *et al.*, 2002). The concentration of fluoranthene and phenanthrene in the Chao-Phraya River were higher than other rivers sediments (Bixian *et al.*, 2001 and San Francisco Estuary Institute, 2000). Since S_5 located at the Phrachulachomklao Royal Navy Dockyard, it may always contaminated with the engine oil or petroleum hydrocarbon.

Table 5.1 Comparative values of fluoranthene and phenanthrene concentrations in different sources.

Locations	**PAH concentration (µg/g dry weight)**		**References**
	Fluoranthene	**Phenanthrene**	
Pearl River, China	1.32	1.46	Bixian *et al.*, 2001
Napa River, US	0.02	0.02	San Francisco Estuary Institute, 2000
Petaruma River, US	0.12	0.05	
Lake Jämsänvesi, Finland	295.3	188.4	Hyötyläinen and Oikari, 1998
Barent sea, Russia	43-399	28-362	Sovinov *et al.*, 2003
Bedford harbor, US	1.3-21	0.45-17	Pruell *et al.*, 1990
Chao-Phraya River, Saen-Saeb canal and Padungkrungkasem canal, Thailand	6.5-8.2	0.13-0.2	This study

Besides fluoranthene and phenanthrene, benzo(a)pyrene, benzo(k)fluoranthene and benzo(g,h,i)perylene could also be detected in the Chao-Phraya River at range of 20-89.6, 15-66.1 and 100-282.5 mg/kg (Patarasiriwong and Boonyoy, 2002).

However, the other countries have established sediment quality guideline as demonstrated in Table 5.2. U.S. EPA has established the draft sediment quality criteria that fluoranthene and phenanthrene should not more than 620 and 180 μg/g dry weight of sediment, respectively. Meanwhile Canada has set the interim sediment quality guideline, which proposed concentration of phenanthrene and fluoranthene in sediment should not be exceed that 41.9 and 111 μg/g dry weight sediment, respectively. From these guidelines, the concentration of phenanthrene and fluoranthene are acceptable values. Since there are the presence of PAHs in river and canal sediment and the PAHs standard regulation of Thailand has not been established. The PAHs monitoring is necessary in order to observe tend of PAH concentration for protection of the aquatic organisms and the human being.

Table 5.2 The proposed PAH concentrations in the sediment quality guideline.

PAH compounds	Concentration μg/g dry weight of river sediment	
	U.S. EPA[a]	**CCME**[b]
Acenaphthene	130	-
Phenanthrene	180	41.9
Fluoranthene	62	111
Chrysene	-	57.1
Pyrene	-	153
Benzo(a)anthracene	-	31.7
Benzo(a)pyrene	-	31.9

[a]Draft sediment guideline criteria, U.S. EPA

[b]Interim Canadian sediment quality guideline, Canadian Council of Ministry of the Environment, 1995

Another objective of this study is to isolate the PAHs degrading bacteria from PAHs contaminated sediment. The sediment samples were enriched in CFMM medium supplemented with fluorene, fluoranthene and pyrene as a sole carbon and energy source at concentration of 100 mg/l. From the sampling site S_1, Saen-Saeb Canal, three pyrene degrading bacterial strains could be enriched. After purification, only strain PY1 could degrade pyrene. Although no pyrene was detected in sediment sample, pyrene degrading strain PY1 could be obtained from site S_1 which contaminated by phenanthrene. Many reports suggested that phenanthrene acts as an inducer for pyrene degradation (Molina *et al.*, 1999 and Ho *et al.*, 2000). Meanwhile at the Phrachulachomklao Royal Navy Dockyard (Chao-Phraya River) the fluoranthene enrichment culture that consisted of 2 strains was obtained. Fluoranthene detected sampling site, therefore, could isolated the fluoranthene degrading bacteria. However, the fluorene degrading bacteria could not isolate due to fluorene was not found in sediment samples. It could explain that the degrading ability of the indigenous microbes were representative of the contaminant in natural freshwater sediments (Verrhiest *et al.*, 2002).

Strain PY1 was Gram positive, acid-fast and rod shape. 16S rDNA nucleotide sequences (1417 bp), which was isolated from the Saen-Saeb Canal, showed 99% homology to bacteria in Genus *Mycobacterium*. On basis of morphological and biochemical characteristics and 16S rDNA sequence, the isolated strain PY1 was concluded to be in genus *Mycobacterium*. Although, *Mycobacterium* sp. is an aerobic bacterium, the strain PY1 could be isolated from the sediments, which were 3-4 meters under the surface water level in the Saen-Saeb Canal. The daily shipping activity in this canal may result in the enough soluble oxygen via the spin of propellers. This strain could degrade pyrene for 90.4 % (90.4 mg/l) at the initial concentration of 100 mg/l within 14 days. Several studies reported that bacterium in genus *Mycobacterium* was frequently isolated from PAHs contaminated river sediments as described in Table 5.3. *Mycobacterium* sp. strain PY1 is more effective in pyrene degradation than the other *Mycobacterium* sp. from river sediment as described in Table 5.3. *Mycobacterium* sp. strain MR1 from PAHs contaminated river sediments could mineralize pyrene 63% at the concentration of 6 μg/ml in 18 days (Molina *et al.*, 1999) and was better than

Mycobacterium flavescens, which can degrade pyrene 45 μg/ml in 7 days (Dean-Ross and Cerniglia, 1996). Some *Mycobacterium* strains, such as PYR9-1 (Ho *et al.*, 2000), had better activity in pyrene degradation (0.3 mg/ml within 7 days) than the new isolated strain PY1. Besides pyrene, this new isolated strain, PY1 in this study could utilize other PAHs as a sole carbon and energy source namely, acenaphthylene (98.59%), acenaphthene (99.37%), dibenzofuran (99.64%) and phenanthrene (100%) in 7 days of cultivation. This property has also been reported in many PAHs degrading *Mycobacterium* strain as summarized in Table 5.3.

Table 5.3 List of *Mycobacterium* strains capable of PAHs degradation

Strains	Sources	PAHs degrading ability	References
Mycobacterium sp.	Chronically exposed hydrocarbon sediments	Naphthalene 59.5%, phenanthrene 50.9%, fluoranthene 89.7% and pyrene 63% (0.5μg/ml) in 2 weeks	Heitkamp and Cerniglia, 1988
Mycobacterium sp. strain BB1	Former coal gasification soil	Phenanthrene 90 mg/l, 212 mg/l of pyrene and 160 mg/l of fluoranthene in 144 hrs	Boldrin *et al.*, 1993
Mycobacterium flavescens	Creosote polluted river sediments	Pyrene 45 μg/ml in 7 days and phenanthrene and fluoranthene 3μg/ml in 2 weeks	Dean-Ross and Cerniglia, 1996
Mycobacterium sp. strain CH1	PAHs contaminated freshwater sediments	55% and 60% of pyrene and fluoranthene at the concentration of 25 mg/l in 26 days	Churchill *et al.*, 1999

Strains	Sources	PAHs degrading ability	References
Mycobacterium sp. strain MR1	PAHs contaminated river sediments	Pyrene 63% of 6 μg/ml of initial concentration within 18 days	Molina *et al.*, 1999
Mycobacterium sp. strain KR20	PAHs contaminated soil	60% of fluoranthene at the concentration of 0.5 mg/l in 10 days	Rehmann *et al.*, 2001
Mycobacterium sp. strain PYR9-1	Fuel contaminated river sediments	Pyrene 0.3 mg/ml within 7 days	Ho *et al.*, 2000

Additional to strain PY1, another isolated strain FT1 was also isolated by using the same enrichment procedure from the Chao-Phraya River sediments at the area of Phrachulachomklao Royal Navy Dockyard. The newly isolated strain FT1 was Gram negative, rod shape bacteria. 16S rDNA nucleotide sequences (1,446 bp) showed 99% homology to bacteria in genus *Sphingomonas*. Based on the morphological and biochemical characteristics and 16S rDNA sequence, the new fluoranthene degrading strain FT1 was grouped into bacteria genus *Sphingomonas*. The strain FT1 could oxidize fluoranthene only about 39% at the concentration of 100 mg/l. This result could be explained that for the high molecular weight PAHs, such as fluoranthene, degradation would be completely degraded by a bacterial consortium (Trzesicka-Mlynarz and Ward, 1995, and Weissenfels *et al.*, 1991). When mixed bacteria was isolated and purified, the pure strain often yielded lower PAHs degrading activity than the bacterial consortium. In addition, this newly isolated strain exhibited the broad range substrate specificity as carbon and energy source such as acenaphthylene, acenaphthene, dibenzofuran and phenanthrene, which can be degraded for 98.90, 89.85, 67.48 and 99.90%, respectively. This strain could oxidize fluoranthene for only 39%, while it was able to degrade 99.90 % of phenanthrene. Due to the structure of phenanthrene is angular arrangement, which consists of Bay region and K region (Narro *et al.*, 1992). The oxygenase can attache these regions in order to catalyze the degradation process. These regions therefore are

favorable for degradation by microorganisms (Saiphet, 2002). *Sphingomonas* sp. is a bacterium found relative ubiquitously in soil, water and sediment (Kazunga *et al.*, 2001 and Shi *et al.*, 2001) and plays as important role in PAHs degradation. Many *Sphingomonas* sp. strains capable of PAHs degradation were isolated from various environments as summarized in Table 5.4. *Sphingomonas* sp. strain FT1 could degrade fluoranthene less than *Sphingomonas paucimobilis* strain EPA505, which completely utilized fluoranthene at the concentration of 100 mg/l within 2 days as sole carbon and energy source (Mueller *et al.*, 1990a). In addition, in term of other PAHs utilization, the strain FT1 showed the better degrading capability than *Sphingomonas* sp. strain SP2 (Saiphet, 2002). Due to strain FT1 could utilize a broad rang of substrate as well as acenaphthylene, acenaphthene, dibenzofuran and phenanthrene whereas strain SP2 could utilize only acenaphthene.

Table 5.4 List of *Sphingomonas* strains capable of PAHs degradation

Strains	Sources	PAHs degrading ability	References
Sphingomonas paucimobilis strain EPA505	Coal tar creosote contaminated soil	Fluoranthene 100 mg/l within 48 hrs 10 mg/l of pyrene (80%), benzo(a)anthracene (72.9%), chrysene (31.5%), benzo(a)pyrene(33.3%), benzo(b)fluoranthene (12.5%) and dibenzo(g,h) anthracene (7.8%) in 16 hrs	Mueller *et al.*, 1990a and Ye *et al.*, 1996
Sphingomonas sp. strain LH162 and strain LH227	PAHs contaminated sludge	Phenanthrene and dibenzothiophene 20 mg/l in 5 days	Bastiaen *et al.*, 2000
Sphingomonas sp. strain P2	Contaminated soil with lubricant oil	Phenanthrene 100 mg/l within 72 hrs, cometabolism of fluoranthene (86%) and pyrene (36%)100 mg/l with phenanthrene 100 mg/l in 7 days	Supaka *et al.*, 2001
Sphingomonas sp. strain SP2	Wastewater contaminated with gas and oil	900 mg/l of acenaphthene within 6 days	Saiphet, 2002

In conclusion, Both genera *Mycobacterium* sp. strain PY1 and *Sphingomonas* sp. strain FT1 could frequently be isolated from PAHs contaminated sites and exhibited the PAHs degrading ability. These genera specialized in degrading such less-bioavailable compounds. Due to their a particular outer cell wall layer, i.e., glycosphingolipids for *Sphingomonas* (Yabuuchi *et al.,* 1990) and glycolipids such as mycolic acids for *Mycobacterium* (Sayler and Whitt, 1994), which may be important for the interaction with or uptake of hydrophobic compounds (Nohynek *et al.,* 1995).

Mycobacterium sp. strain PY1 and *Sphingomonas* sp. strain FT1 also exhibited the wide variety of substrate utilization including acenaphthylene, acenaphthene, dibenzofuran and phenanthrene. Stringfellow and Aitken (1995) suggested that PAH degrading bacteria utilized common enzymes the degradation of two or more PAHs. They, therefore, are possible for using in bioaugmentation for the removal of the mixture of PAHs contaminated in environments.