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APPENDICES

APPENDIX A

The reagents of PAHs degrading bacteria isolation and PAH concentration analysis

1) PAHs in Dimethyl sulfoxide solution

PAH powder 0.1 g

Dimethyl sulfoxide 10 ml

Sterilized by filtering through a 0.20 μm pore size filter.

2) 0.85% NaCl

NaCl 0.85 g

Distilled water 100 ml

Sterilized by autoclave at 15 psi for 20 minutes.

3) Methanol 80% (v/v)

Methanol 80 ml

Deionized distilled water (DDW) 20 ml

Filtered methanol through a 0.5 μm FH filter and added filtered DDW

Sonicated the mixture until the bubble left.

4) 2% PAH diethyl ether

PAH powder 0.2 g

Diethyl ether 10 ml

Mixed to completely dissolve and filtered the mixture through a 0.22 μm pore size filter.

Kept in dark bottle (should freshly prepare for every usage).

APPENDIX B

The reagents of genomic DNA extraction

1) 10% Sodium dodecyl sulphate (10% SDS)

SDS	10 g
Distilled water	70 ml

Dissolved at 70 °C.

Brought up with distilled water to 100 ml.

2) Hexadecyl trimethyl ammoniumbromide/sodium chloride solution (CTAB/NaCl solution)

NaCl	4.1 g
Distilled water	80 ml
CTAB	10 g

Slowly added with stirring, while heating at 65°C.

Brought up with distilled water to 100 ml.

3) 5 M NaCl

NaCl	14.61 g
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Distilled water to 50 ml

4) Phenol/chloroform/isoamyl alcohol solution

Phenol	25 ml
Chloroform	25 ml
Isoamyl alcohol	1 ml

Equilibrated by extraction several times with 0.1 M Tris-HCl (pH 7.6).

Stored the equilibrated mixture under equal volume of 0.01 M Tris-HCl (pH 7.6) in dark glass bottle.

5) Phenochloroform/isoamyl alcohol solution

Chloroform	24 ml
Isoamyl alcohol	1 ml

Mixed and stored the mixture at 4°C.

6) 50xTris-acetate/EDTA buffer (50xTAE buffer, pH 8)

Tris-base	242 g
Glacial acetic acid	57.2 ml
0.5 M EDTA	100 ml

Distilled water to 1 liter.

7) TE buffer

1 M Tris-HCl, pH 8	1 ml
0.5 M EDTA, pH 8	0.2 ml

Distilled water to 100 ml.

Sterilized by autoclaving at 15 psi for 20 minutes.

8) Loading Dye

A) Bromophenol blue	0.05 ml
Absolute ethanol	1 ml
B) Sucrose	12 g
Distilled water	17 ml
C) 1 M EDTA, pH 8	2 ml

Autoclaved parted A and B at 15 psi for 20 min.

Mixed part A, B and C after autoclaving and kept at -20 °C

9) Ethidiumbromide solution

Dissolved ethidiumbromide powder in TAE buffer at final concentration of 10 µg/ml and kept in dark.

APPENDIX C

16S rDNA nucleotide sequence

1) 16S rDNA nucleotide sequence of *Mycobacterium* sp. strain PY1

5' - TTAACACATGCAACGTCGAACGGAAAGGCCCTTCGGGGTACTCGAGTGGCGAACGGGTGA 60
 GTAACACGTGG-TGATCTGCCCTGCACTTTGGGATAAGCCTGGGAAACTGGGTCTAATAC 119
 CGAATAGGACCGCATGCTTCATGGTGTGTGGTGGAAAGCTTTTGCGGTGTGGGATGGGCC 179
 CGCGCCTATCAGCTTGTGGTGAGGTAATGGCTTACCAAGGCACGACGGGTAGCCGGC 239
 CTGAGAGGGTGACCGGCCACACTGGGACTGAGATACGGCCAGACTCCTACGGGAGGCAG 299
 CAGTGGGGAATATTGCACAATGGGCGCAAGCCTGATGCAGCGACGCCGCGTGAGGGATGA 359
 CGGCCTTCGGGTGTAAACCTCTTTGCGCAGGGACGAAGCGCAAGTGACGGTACCTGGAG 419
 AAGAAGGACCGCCAACACTACGTGCCAGCAGCCGCGTAATACGTAGGGTCCGAGCGTTGT 479
 CCGGAATTACTGGGCGTAAAGAGCTCGTAGGTGGTTTGTGCGGTTGTTTCGTGAAAACTCA 539
 CAGCTTAACTGTGGGCGTGGGGCGATACGGGCAGACTTGAGTACTGCAGGGGAGACTGG 599
 AATTCCTGGTGTAGCGGTGGAATGCGCAGATATCAGGAGGAACACCGGTGGCGAAGGCCG 659
 GTCTCTGGGCAGTAACTGACGCTGAGGAGCGAAAGCGTGGGGAGCGAACAGGATTAGATA 719
 CCCTGGTAGTCCACGCCGTAAACGGTGGGTAGGTGTGGGTTTCCTTCCTTGGGATCCGTG 776
 CCGTAGCTAACGCATTAAGTACCCCGCTGGGGAGTACGGCCGCAAGGCTAAAACTCAA 836
 GCAATTGACCGGGGCCCCGACAAAGCGGCGGAGCATGTGGATTAATTCGATGCAACGCGA 896
 AGAACCTTACCTGGGTTTGACATGCACAGGACGCCGGCAGAGATGTCGGTTCCTTGTGG 956
 CCTGTGTGCAGGTGGTGCATGGCTGTCGTGAGCTCGTGTGAGATGTTGGGTTAAGTC 1016
 CCGCAACGAGCGCAACCCCTTGTCTCATGTTGCCAGCACGTTATGGTGGGACTCGTGAGA 1076
 GACTGCCGGGGTCAACTCGGAGGAAGGTGGGGATGACGTCAAGTCATCATGCCCCCTTATG 1136
 TCCCAGGGCTTACACATGCTACAATGGCCGGTACAAAGGGCTGCGATGCCGTGAGGTGG 1196
 AGCGAATCCTTTCAAAGCCGGTCTCAGTTCGGATCGGGTCTGCAACTCGACCCCGTGAA 1256
 GTCGGAGTCGCTAGTAATCGCAGATCAGCAACGCTGCGGTGAATACGTTCCCGGGCCTTG 1316
 TACACACCGCCCGTCACGTCATGAAAGTCGGTAACACCCGAAGCCGGTGGCCTAACCCCT 1376
 TGTGGGAGGGAGCCGTCGAAGGTGGGATCGGCCA - 3'

2) 16S rDNA nucleotide sequence of *Sphingomonas* sp. strain FT1

5' - ATGCCTAATACATGCAAGTCGAACGAATCTTCGGATCTAGTGGCGCACGGGTGCGTAAACG 71
 CGTGGGAATCTGCCCTTGGGTTCGGAATAACTTCTGGAAACGGAAGCTAATACCGGATGA 131
 TGACGTAAGTCCAAAGATTTATCGCCCAAGGATGAGCCCGCTAGGATTAGCTAGTTGGT 191
 GGGGTAAAGGCTCACCAAGGCGACGATCCTTAGCTGGTCTGAGAGGATGATCAGCCACAC 251
 TGGGACTGAGACACGGCCCAGACTCCTACGGGAGGCAGCAGTAGGGAATATTGGACAATG 311
 GCGAAAAGCCTGATCCAGCAATGCCGCGTGAGTGATGAAGGCCTTAGGGTTGTAAAGCTC 371
 TTTACCCGGGATGATAATGACAGTACCGGGAGAATAAGCTCCGGCTAACTCCGTGCCATG 431
 CAGCCGCGGTAATACGGAGGGAGCTAGCGTTGTTTCGGAATTACTGGGCGTAAAGCGCACG 491
 TAGGCGGCTATTCAAGTCAGAGGTGAAAGCCCGGGGCTCAACCCCGGAACTGCCTTTGAA 551
 ACTAGATAGCTTGAATCCAGGAGAGGTGAGTGGAAATCCGAGTGTAGAGGTGAAATTCGT 611
 AGATATTCGGAAGAACACCAGTGGCGAAGGCGGCTCACTGGACTGGTATTGACGCTGAGG 671
 TGCGAAAAGCGTGGGGAGCAAACAGGATTAGATACCCTGGTAGTCCACGCCGTAAACGATG 731
 ATAAGTAGCTGTCAGGGCACATGGTGTGTTTGGTGGCGCAGCTAACGCATTAAGTTATCCG 791
 CCTGGGGAGTACGGTCGCAAGATTAAGAACTCAAAGGAATTGACGGGGGCTGCACAAGCG 851
 GTGGAGCATGTGGTTTAATTGCAAGCAACGCGCAGAACCTTACAACGTTTGACATCCCTA 910
 TCGCGGATCGTGGAGACACTTTCCTTCAGTTCGGCTGGATAGGTGACAGGTGCTGCATGG 970
 CTGTCGTCAGCTCGTGTGCGTGAGATGTTGGGTTAAGTCCCGCAACGAGCGCAACCCTCGC 1030
 CTTTAGTTGCCAGCATTTAGTTGGTACTTAAAGGAACCGCCGGTGATAACCGGAGGAAG 1088
 GTGGGGATGACGTCAAGTCCTCATGGCCCTTACGCGTTGGGCTACACACGTGCTACAATG 1148
 GCGACTACAGTGGGCAGCCACCTCGCGAGAGGGAGCTAATCTCCAAAAGTCGTCTCAGTT 1208
 CGGATCGTTCTCTGCAACTCGAGAGCGTGAAGGCGGAATCGCTAGTAATCGCGGATCAGC 1268
 ATGCCGCGGTGAATACGTTCCCAGGCCTTGACACACCG-CCGTCACATCCATGGGAGTT 1327
 GGATTCAACTTGAAGGCGTTGAGCTAACCGTAAGGAGGACAGGCGACTCACCAAGTGGGTT 1387
 TAGCGACCTGGGGTGAAGTCGTAA - 3'

Appendix D

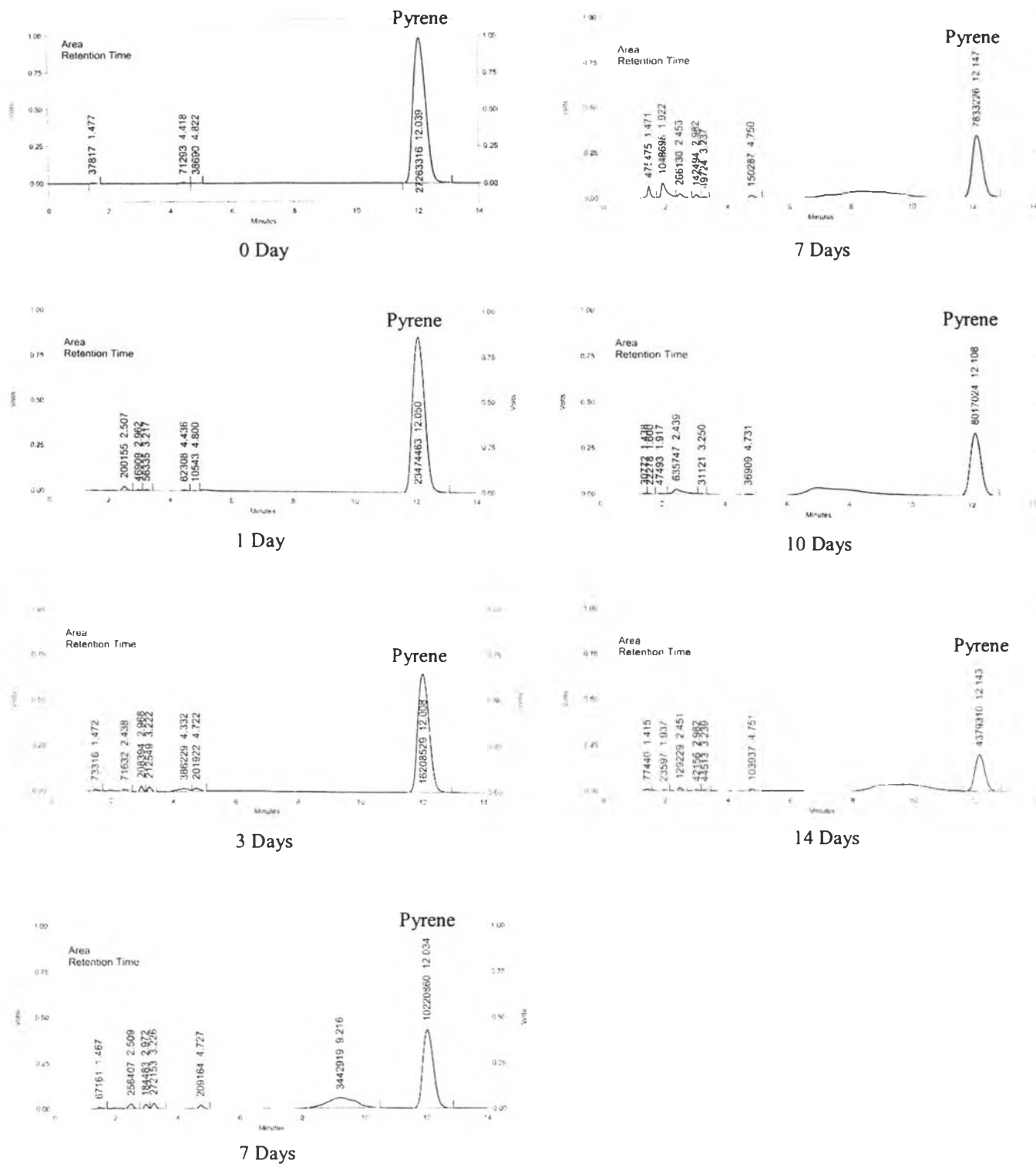


Figure D.1 Reverse phase HPLC profile of pyrene degradation from *Mycobacterium* sp. strain PY1 in 0, 1, 3, 5, 7, 10 and 14 days.

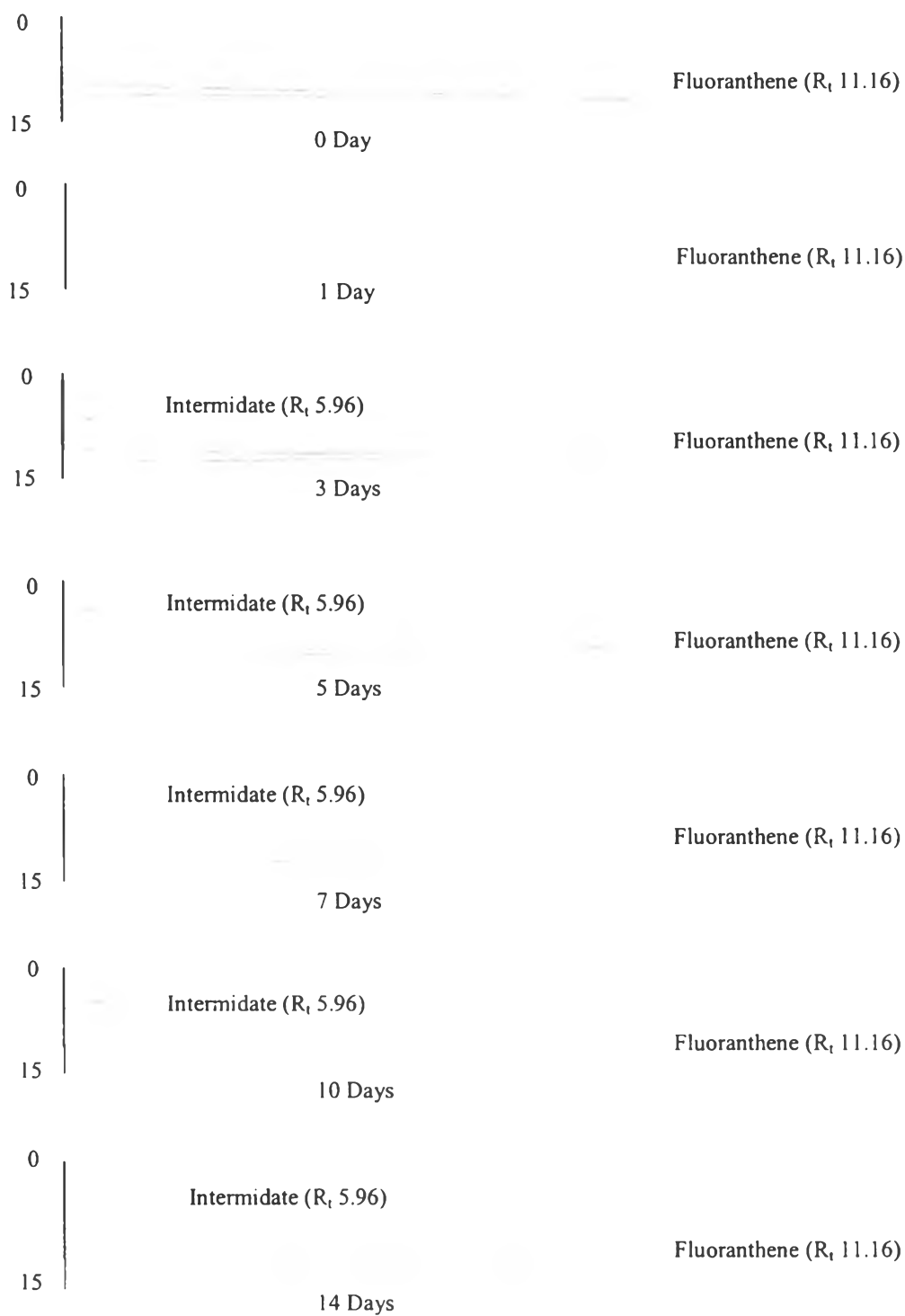


Figure D.2 Reverse phase HPLC profile of fluoranthene degradation by *Sphingomonas* sp. strain FT1 in 0, 1, 3, 5, 7, 10 and 14 days.

Appendix E

Sampling sites in this study



Figure E.1 S₁, Ratchathewi pier (The Saen-Saeb Canal)



Figure E.2 S₂, Pratunam pier (The Saen-Saeb Canal)



Figure E.3 S₃, Panfa-leelard pier (The Saen-Saeb Canal)



Figure E.4 S₄, Wat Sri-boon-reung pier (The Saen-Saeb Canal)



Figure E.5 S₅, Phrachulachomklao Royal Navy Dockyard (The Chao-Phraya River)



Figure E.6 S₆, See-phraya pier (The Chao-Phraya River)



Figure E.7 S₇, Sa-thon pier (The Chao-Phraya River)



Figure E.8 S₈, Padungkrungkasem Canal

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Sampling sites in this study



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Figure E.8 S₈, Padungkrungkasem Canal

BIOGRAPHY

Miss Saranya Prapatsompinyo was born on June 21, 1980 in Chonburi Province, Thailand. She attend Chongunyanukon in Chonburi and graduated in 1998. She received her Bachelor's degree in Science from Department of Microbiology, Faculty of Science, Chulalongkorn University in 2002. She pursued her Master Degree in The International Postgraduate Programs in Environmental Management, Interdepartment of Environmental Management in May 2002.

