DEGRADATION OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS BY PHYLLOSPHERE BACTERIA AND ITS ENHANCEMENT BY SURFACTANT ADDITION

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for the Degree of Doctor of Philosophy Program in Environmental Management
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การย่อยสลายสารพอลิไซคลิกอะโรมาติกไฮโดรคาร์บอนมวลโมเลกุลสูง โดยแบคทีเรียบนใบพืช และการส่งเสริมโดยเติมสารลดแรงตึงผิว

นายวีรยุทธ สิริรัตน์เรืองสุข

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Thesis Title	DEGRADATION OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS BY PHYLLOSPHERE BACTERIA AND ITS ENHANCEMENT BY SURFACTANT ADDITION
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วีรยุทธ สิริรัตน์เรืองสุข : การย่อยสลายสารพอลิไซคลิกอะโรมาติกไฮโดรคาร์บอนมวลโมเลกุลสูง โดยแบคทีเรียบนใบพืช และการส่งเสริมโดยเติมสารลดแรงตึงผิว (DEGRADATION OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS BY PHYLLOSPHERE BACTERIA AND ITS ENHANCEMENT BY SURFACTANT ADDITION) อ.ที่ปรึกษาวิทยานิพนธ์หลัก: รศ. ดร. เอกวัล ลือพร้อมชัย, อ.ที่ปรึกษาวิทยานิพนธ์ร่วม: ศ. ดร. Masami Furuuchi, ผศ. ดร. ทรรศนีย์ พฤกษาสิทธิ์, 180 หน้า.

พอลิไซคลิกอะโรมาติกไฮโดรคาร์บอน (พีเอเอช) เป็นมลพิษหลักในเขตเมือง ไพรีนเป็นสารพีเอเอชมวลโมเลกุลสูง ที่สามารถตรวจพบได้มากในอากาศและฝุ่นตามท้องถนน จึงถูกเลือกเป็นตัวแทนในการศึกษานี้ พีเอเอชที่มีมวลโมเลกุลสูงสามารถรวมตัวกับอนุภาคในอากาศและตกสะสมบนใบไม้ ซึ่งกระบวนการย่อยสลายทางชีวภาพโดยแบคทีเรียที่อาศัยอยู่บนใบไม้ สามารถลดการหมุนเวียนสารพีเอเอชกลับคืนสู่บรรยากาศ งานวิจัยนี้มีวัตถุประสงค์เพื่อตรวจติดตามศักยภาพการย่อยสลายทางชีวภาพของสารพีเอเอชมวลโมลกุลสูงบนใบของไม้ประดับ และเพื่อเติมสารลดแรงตึงผิวสำหรับส่งเสริมการดูดซึมสารพีเอเอชมวลโมเลกุลสูงทางชีวภาพ ในการศึกษานี้ได้ติดตามจำนวนของแบคทีเรียในอากาศและบนใบไม้ รวมถึงความเข้มข้นของสารพีเอเอชในอากาศรอบแนวไม้ประดับบริเวณถนนประชาชื่น กรุงเทพมหานคร พบแบคทีเรียที่ย่อยสลายไพรีนในช่วง 22-152 โคโลนี ต่อ ลบ.ม. อากาศ และมีจำนวนแบคทีเรียย่อยสลายไพรีนบริเวณใกล้เคียงแนวไม้ประดับมากกว่าตามริมถนน ในทางตรงกันข้ามพบความเข้มข้นของฝุ่นละอองที่มีสารพีเอเอชเกาะอยู่บริเวณริมถนนมากกว่าแนวไม้ประดับ พืชที่ทดสอบทุกสปีชีส์พบแบคทีเรียที่สามารถย่อยสลายไพรีนจำนวนเฉลี่ย $5x10^4$ โคโลนี ต่อ กรัมใบไม้ โดยมีสัดส่วนประมาณ 7 เปอร์เซนต์ ของจำนวนแบคทีเรียทั้งหมด ต่อมาได้คัดแยกแบคทีเรียที่สามารถย่อยสลายไพรีน 4 สายพันธุ์ จากต้นเข็มเล็ก (*Ixora coccinea*) เพื่อใช้เป็นต้นแบบของแบคทีเรียบนใบไม้ เพื่อเพิ่มการดูดซึมทางชีวภาพของไพรีนได้เติมสารลดแรงตึงผิวชีวภาพชนิดลิโพเพพไทด์ที่ผลิตจาก *Bacillus* sp. GY19 พบว่าแบคทีเรีย *Kocuria* sp. IC3 ย่อยสลายไพรีนได้สูงสุดในอาหารที่เติมสารลดแรงตึงผิวทางชีวภาพ การศึกษาประสิทธิภาพของพืชตัวอย่างร่วมกับกลุ่มแบคทีเรียบนใบไม้ และสารลดแรงตึงผิวทางชีวภาพ ใช้ขวดโหลขนาด 2.5 ลิตร บรรจุกิ่งของต้นเข็มเล็ก ที่มีความเข้มข้นไพรีนสะสมเริ่มต้นที่ 30 ไมโครกรัม ต่อ กรัมใบไม้ พบว่าการเติม *Kocuria* sp. IC3 ร่วมกับสารลดแรงตึงผิวทางชีวภาพที่ความเข้มข้น 0.1 เท่าของ CMC สามารถกำจัดไพรีนได้ 100 เปอร์เซ็นต์ หลังจากการทดลอง 14 วัน และระบบมีจำนวนแบคทีเรียสูงที่สุด โดยระบบที่ใช้สารลดแรงตึงผิวทางชีวภาพเพียงอย่างเดียว ช่วยส่งเสริมแบคทีเรียดั้งเดิมบนใบไม้ในการกำจัดไพรีนถึง 94% ดังนั้นการเสริมแบคทีเรียบนใบไม้จึงไม่มีความจำเป็น การติดตามกิจกรรมของแบคทีเรียบนใบไม้ต่อการลดลงของพีเอเอชชนิดอื่นๆ ในอากาศ ทำในระบบขยายขนาดโดยบรรจุต้นเข็มเล็กทั้งต้นที่พ่นสารลดแรงตึงผิวแล้วในตู้ทดสอบขนาด 96 ลิตร ที่มีไอระเหยของสารพีเอเอชผสม ต้นเข็มเล็กสามารถลดปริมาณความเข้มข้นของสารพีเอเอชในอากาศและที่สะสมบนใบ โดยสามารถลดฟีแนนทรีน ไพรีน และฟลูออแรนทีน ได้ 64 - 86% หลังจาก 14 วัน ในขณะที่ตู้ทดสอบชุดควบคุมลดสารพีเอเอชเหล่านี้ลงเพียง 20 % ดังนั้นการกำจัดไพรีนและสารพีเอเอชอื่น สามารถทำได้โดยการพ่นสารลดแรงตึงผิวทางชีวภาพลงบนใบไม้พุ่มประดับ ความรู้ที่ได้รับสามารถนำไปพัฒนาวิธีฟื้นฟูทางชีวภาพสำหรับปรับปรุงคุณภาพอากาศในเขตเมืองต่อไป

สาขาวิชา การจัดการสิ่งแวดล้อม ลายมือชื่อนิสิต .....

ปีการศึกษา 2559 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

ลายมือชื่อ อ.ที่ปรึกษาร่วม .....

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KEYWORDS: AIR POLLUTANTS / POLYCYCLIC AROMATIC HYDROCARBONS / PHYLLOSPHERE BACTERIA / BIOSURFACTANT / BIOREMEDIATION

WEERAYUTH SIRIRATRUENGSUK: DEGRADATION OF HIGH MOLECULAR WEIGHT POLYCYCLIC AROMATIC HYDROCARBONS BY PHYLLOSPHERE BACTERIA AND ITS ENHANCEMENT BY SURFACTANT ADDITION. ADVISOR: ASSOC. PROF. EKAWAN LUEPROMCHAI, Ph.D., CO-ADVISOR: PROF. MASAMI FURUUCHI, Ph.D., ASST. PROF. TASSANEE PRUEKSASIT, Ph.D., 180 pp.

Polycyclic aromatic hydrocarbons (PAHs) are dominant air pollutants in urban environment. Pyrene is a major high molecular weight (HMW) PAHs in roadside air and street dust, thus it was chosen as a representative of HMW-PAHs in this study. The HMW-PAHs can combine with airborne particulates and accumulate on plant leaves. The biodegradation of accumulated PAHs by phyllosphere bacteria would reduce their recirculation back into the atmosphere. The objectives of this research were to investigate the biodegradation potential of HMW-PAHs on ornamental plants and to use surfactant for increasing bioavailability of HMW-PAHs. This study monitored the abundance of airborne and phyllosphere bacteria as well as the concentrations of airborne PAHs around ornamental plant swaths on Prachachuen Road, Bangkok. The number of airborne pyrene-degrading bacteria ranged from 22-152 CFU m^{-3} air, and more bacteria were found in the proximity of the ornamental plant swath than along the roadside. On the other hand, the concentrations of particle-bound PAHs were much higher at roadside than those around plant swath. Pyrene-degrading bacteria averaged $5x10^4$ CFU g^{-1} on the leaves of all tested plant species and accounted for approximately 7% of the total population. Four pyrene-degrading bacteria were isolated from *I. coccinea* to use as model phyllosphere bacteria. To increase the bioavailability of pyrene, a lipopeptide biosurfactant from *Bacillus* sp. GY19 was applied. *Kocuria* sp. IC3 showed the highest pyrene degradation in the medium containing biosurfactant. A small 2.5 L glass chamber containing *I. coccinea* twigs was then used for examining the efficiency of plant, phyllosphere bacteria and biosurfactant on removal of deposited pyrene at 30 μg g^{-1} leaf. After 14 days, leaves containing both *Kocuria* sp. IC3 and 0.1X CMC biosurfactant showed 100% pyrene removal with the most abundant bacteria. The system with biosurfactant alone also enhanced the activities of indigenous phyllosphere bacteria with 94% pyrene removal. Consequently, the bioaugmentation of leaves was not necessary. To investigate the activity of phyllosphere bacteria on reducing other PAHs found in the ambient air, the whole plant of *I. coccinea* was sprayed with biosurfactant and placed in a scale-up 96-L gas tight chamber containing vapor of mixed PAHs. The plant was able to reduce 64 – 86% of both airborne and deposited phenanthrene, pyrene and fluoranthene after 14 days, while only 20% of these PAHs was decreased in the control chamber. Consequently, the removal of pyrene and other PAHs could be achieved by spraying biosurfactant on ornamental shrubs. The acquired knowledge could be used to develop a bioremediation approach for improving air quality in urban areas.

Field of Study: Environmental Management
Academic Year: 2016

Student's Signature .....
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Co-Advisor's Signature .....

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CHAPTER I
INTRODUCTION

1.1 Statement of problems

High concentrations of polycyclic aromatic hydrocarbons (PAHs) in the atmosphere have been reported from many cities such as Tennessee, USA (Hussar, Richards, Lin, Dixon, & Johnson, 2012), Shanghai, China (Liang, Fang, Wu, Zhang, & Wang, 2016; Q. Wang et al., 2016) , Nagoya, Japanese (Ohura, Kamiya, & Ikemori, 2016) and Riyadh, Saudi Arabia (Bian, Alharbi, Collett, Kreidenweis, & Pasha, 2016). In Thailand, the amount of atmospheric PAHs in Bangkok roadsides is approximately 41-189 ng m^{-3}, whereas only 1-56 ng m^{-3} PAHs is found in rural areas (Boonyatumanond, Murakami, Wattayakorn, Togo, & Takada, 2007; Ruchirawat, Settachan, Navasumrit, Tuntawiroon, & Autrup, 2007). In addition to vehicle combustion, the presence of high molecular weight PAHs (HMW-PAHs) with four or more rings can be due to the long-range emissions of particulate matters from biomass burnings, as seen in Chiang Mai, Thailand (Wiriya, Prapamontol, & Chantara, 2013). The environmental fate of particle-associated PAHs are influenced by vegetation, which can capture particles from the atmosphere, retain them, encapsulate them into their cuticles and release them into soil and/or lower biomasses (Terzaghi et al., 2013). Tree, shrub, herb and woodland settings have been found to trap more PAHs than those of the grassland (Peng, Ouyang, Wang, Chen, & Jiao, 2012). The biodegradation of accumulated PAHs can reduce their recirculation back into the atmosphere. Some bacteria on leaves of ornamental shrubs are able to degrade both deposited and airborne PAHs. However, previous studies have focused only on low molecular weight PAHs (LMW-PAHs) such as acenaphthylene, acenaphthene, fluorene and phenanthrene (Waight, Pinyakong, & Luepromchai, 2007; Yutthammo, Thongthammachat, Pinphanichakarn, & Luepromchai, 2010). Therefore,

the aim of this study was to investigate the biodegradation potential of HMW-PAHs on ornamental plants that usually grown in urban areas.

Phyllosphere consists of the aerial parts of plants, and phyllosphere microbiomes are related to various processes at the interface among plants, microorganisms and the atmosphere (Bringel & Couee, 2015). Phyllosphere bacteria isolated from diverse plant species have been found to utilize hydrocarbons as carbon sources, such as alkanes (Al-Awadhi et al., 2012; Ali, Sorkhoh, Salamah, Eliyas, & Radwan, 2012), (monoaromatic hydrocarbons), and LMW-PAHs (Al-Awadhi et al., 2012; Waight et al., 2007; Yutthammo et al., 2010) . The variability of bacterial species is low in certain plant species because plant genotypes with specific leaf surface properties and plant hormones are important factors that influence the bacterial composition in the phyllosphere (Vorholt, 2012). This study therefore selected six common ornamental plant species as models for monitoring and isolating HMW-PAH-degrading bacteria. In addition to phyllosphere bacteria, airborne bacteria released from the soil and plant surfaces may be applied for the bioremediation of air pollutants (Smets, Moretti, Denys, & Lebeer, 2016). The correlation between phyllosphere and airborne bacteria on the degradation of PAHs has never been reported. Thus, the amounts of airborne and phyllosphere bacteria were monitored along a roadside in the summer, rainy and winter seasons of 2013 and 2015 to determine their potential role in HMW-PAH removal. Plant leaves and air samples were collected from a roadside with plant swaths on Prachachuen Road, Bang Sue District, Bangkok. The majority of PAHs here come from incomplete combustion of engines in traffic sector. Bang Sue District contains the terminus of sub-railway, junction of railway station and a shuttle bus station. Therefore, the area was considered as a representative as an urban city in Thailand.

A major limiting factor for HMW-PAH biodegradation is their low bioavailability to the degrading microorganisms. Surfactants have been used to increase the solubility of PAHs, thereby enhancing their bioavailability (Aryal & Liakopoulou-Kyriakides, 2013; Rodriguez-Escales, Sayara, Vicent, & Folch, 2011). The effect of surfactants on HMW-PAHs biodegradation depends on the type of surfactant and their applied concentrations, the type of PAHs and the identity of the microorganisms present (Rodrigues, Nogueira, Melo, & Brito, 2013). Some surfactants have negative effects on PAH biodegradation because of their direct toxicity or their ability to solubilize high concentrations of PAHs (Chen et al., 2013). In addition, the preferential degradation of biosurfactants over PAHs has been reported. For example, pyrene degradation by *Pseudomonas aeruginosa* is decreased after the application of rhamnolipid JBR515 (Ghosh & Mukherji, 2016). Consequently, the types and concentrations of surfactants that are used to enhance PAH biodegradation should be optimized for application on ornamental plants. To avoid the potential toxicity of surfactant on plants and phyllosphere bacteria, this study investigated several synthetic surfactants and a biosurfactant from *Bacillus* sp. GY19. The biosurfactant is classified as lipopeptides and has been reported for the ability to solubilize crude oil and various hydrocarbons (Khondee et al., 2015; Rongsayamanont et al., 2017).

In this study, pyrene was used because it is the major HMW-PAH found in roadside air and street dusts in the Bangkok area (Boonyatumanond et al., 2007) and because it accounts for 8.5% of the total PAHs on the leaves of evergreen urban shrubs in Italy (Fellet et al., 2016). In addition, to enhance the biodegradation of pyrene on plant surfaces, surfactant and isolated pyrene-degrading bacteria were sprayed on plant leaves before pyrene exposure. The mechanisms involving pyrene removal from ornamental plants were expected to include biodegradation by phyllosphere bacteria, solubilization by surfactant and sorption by plant leaves. Moreover, PAHs profile and concentration in the ambient air at the Prachachuen Road were investigated. These

PAHs information was used to simulate the environment with mixed-PAHs for the remediation in a box-model study.

To our knowledge, this is the first study to combine the activity of plant, bacteria and surfactant for reducing the accumulation of HMW-PAHs in ambient air of the urban environment. The acquired knowledge from this study will be used to develop a bioremediation approach for improving air quality in urban areas.

1.2 Research objectives

The goals of this research was to integrate the knowledges on plant-microbe interactions and surfactant technology for air pollution abatement. To achieve these goals, several objectives are established as follows:

1. To characterize and determine the bacteria capable of degrading HMW-PAHs on ornamental plant leaves and in the ambient air.

2. To investigate the effect of various surfactants on enhancing degradation of HMW-PAHs by isolated phyllosphere bacteria.

3. To evaluate the potential of using ornamental plants, their associated phyllosphere bacteria and selected surfactant for reducing HMW-PAHs in the ambient air.

1.3 Research hypotheses

1. Bacteria capable of degrading HMW-PAHs could be found on ornamental plant leaves with the density relatively higher than those in the ambient air.

2. Surfactant addition could increase degradation of HMW-PAHs by isolated phyllosphere bacteria.

3. Addition of non-toxic surfactant on leaf surface containing phyllosphere bacteria could reduce accumulation of HMW-PAHs in the ambient air.

1.4 Scopes of the study

1. Two groups of ornamental plants consists of 3 plant species of groundcover plant and 3 plant species of shrubby plant were used as representatives of plants commonly grown in urban areas.

2. Bang Sue district (Prachachuen Road) was selected as the representative of urban area to collect the plant leaves and ambient air.

3. Air and plant samplings were carried out in 3 seasons; summer (March - July), rainy (August - October) and winter (November - February) of 2013 and 2015.

4. Pyrene were used as representative of HMW-PAHs.

5. Pyrene-degrading bacteria isolated from selected plant species were used as model phyllosphere bacteria. Their pyrene removal efficiencies were investigated in culture broth with and without surfactant.

6. The toxicity of biosurfactant produced from *Bacillus* sp. GY19 on isolated pyrene-degrading bacteria and ornamental plants were investigated and compared to synthetic surfactants such as SDS, Dehydol LS7TH, Dehydol LS9TH, Triton® X-100, Tween 20, and Dispersants-Slickgone NS.

7. Two scales of gas tight chamber-experiment including 25 L flip-top jar and 96 L acrylic box were performed to investigate pyrene-degradation and mixed HWM-PAHs removal efficiency by the selected plants and their associated bacteria, respectively.

8. All experiments in this research were performed in lab scale.

1.5 Experimental framework

The experimental set-up was divided into 3 phases as in the following diagram (Figure 1.1).

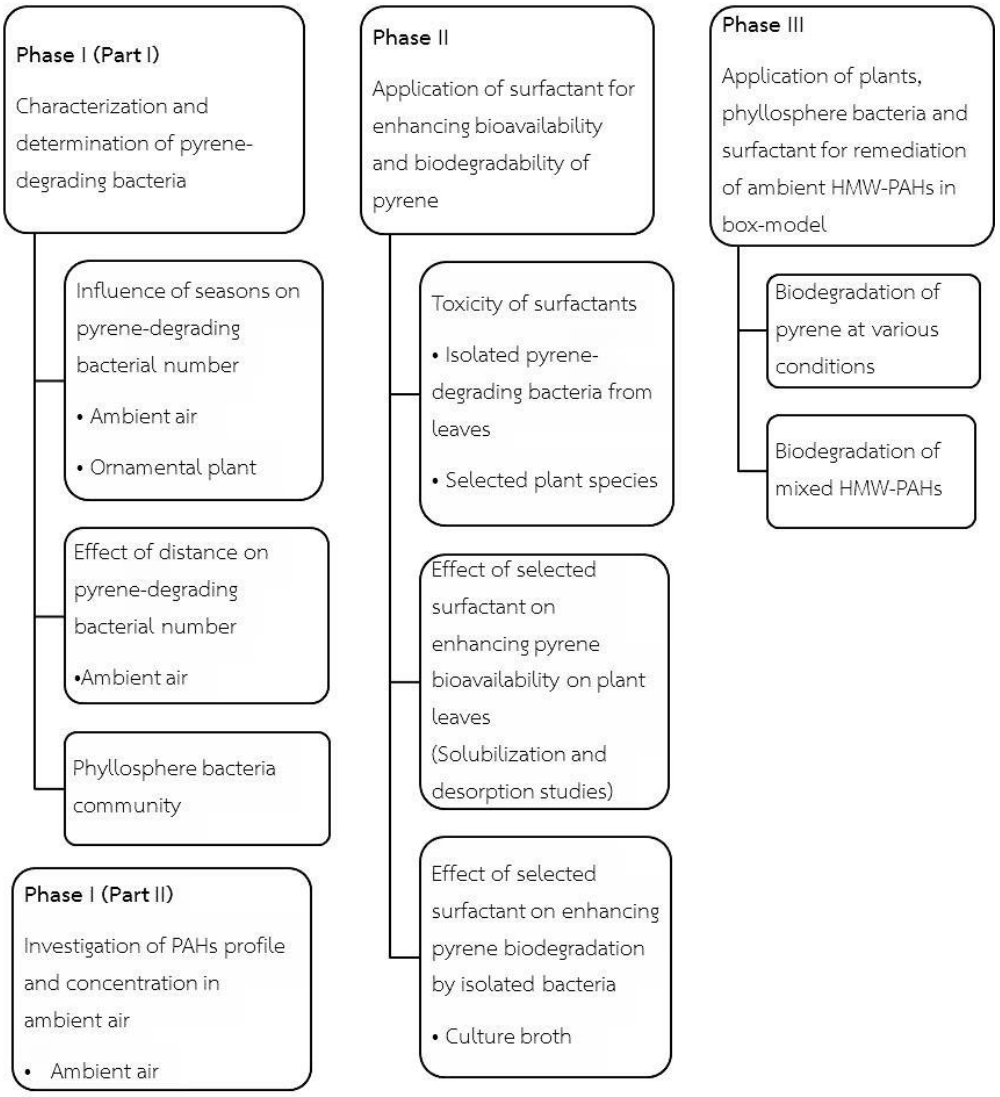


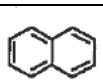
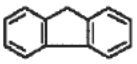
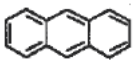
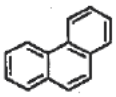
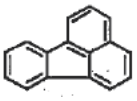
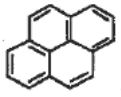
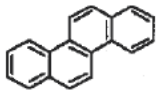
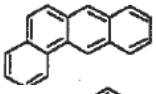
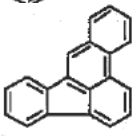
Figure 1.1 Flow chart of the research

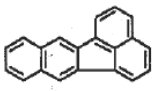
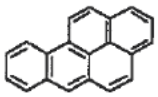
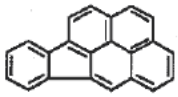
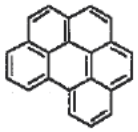
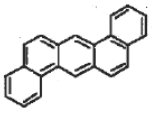
CHAPTER II
LITERATURE REVIEWS

2.1 Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are the world's largest class of carcinogens known to date, not only because of their ability to cause gene mutation and cancer, but due to their persistency in the environment. They are particularly recalcitrant due to their molecular weight, hydrophobic nature and thus, accumulate in various matrices in the environment. PAHs, also known as polyarenes or polynuclear aromatic hydrocarbons, are formed and released into the environment through natural and anthropogenic sources. Natural sources include volcanoes and forest fires while anthropogenic sources include, majorly, incomplete combustion of fossil fuels, wood burning, municipal and industrial waste incineration (Wang et al., 2007). PAHs containing two or three fused benzene rings are classified as low molecular weight (LMW) PAHs and are more water soluble while those with four or more benzene rings are referred to as high molecular weight (HMW) PAHs. They tend to adsorb onto soil and sediment thus, making them recalcitrant in the environment. Sixteen of these organic compounds have been identified as priority pollutants due to their hazardous properties (Table 2.1), with HMW PAHs being considered as potential human carcinogens, by the United State Environmental Protection Agency (USEPA).

Table 2.1 Name, structure, molecular weight, and molecular structure of PAHs by the environmental Protection Agency (EPA) and European Union (EU) (Purcaro, Moret, & Conte, 2013)

Compounds	Abbreviation	Molecular Weight	Molecular structure
Naphthalene	Nap	128	
Acenaphthylene	Acy	152	
Acenaphthene	Ace	154	
Fluorene	Flu	166	
Anthracene	Ant	178	
Phenanthrene	Phe	178	
Fluoranthene	Fluo	202	
Pyrene	Pyr	202	
Chrysene	Chy	228	
Benzo[a]anthracene	BaF	228	
Benzo[b]fluoranthene	BbF	252	

Compounds	Abbreviation	Molecular Weight	Molecular structure
Benzo[k]fluoranthene	BkF	252	
Benzo[a]pyrene	BaP	252	
Indeno(1,2,3-cd)pyrene	Ind	276	
Benzo[g,h,i]perylene	BgP	276	
Dibenzo[a,h]anthracene	DaA	278	

2.1.1 Chemical characteristics of PAHs

The general characteristics of PAHs are high melting and boiling points (therefore they are solid), low vapor pressure, and very low aqueous solubility. The latter two characteristics tend to decrease with increasing molecular weight, on the contrary, resistance to oxidation and reduction increases. Aqueous solubility of PAHs decreases for each additional ring (Masih, Masih, Kulshrestha, Singhvi, & Taneja, 2010). Meanwhile, PAHs are very soluble in organic solvents because they are highly lipophilic. PAHs also manifest various functions such as light sensitivity, heat resistance, conductivity; emit ability, corrosion resistance, and physiological action (Akyuz & Cabuk, 2010).

Table 2.2 Chemical characteristics of PAHs

PAHs	No. of benzene rings	Melting point (°C)	Boiling point (°C)	Water solubility (μg L^{-1})	Vapor Pressure (at 20°C)
Naphthalene	2	80	218	31.5	$4.9x10^{-2}$
Acenaphthylean	3	92	265	3.93	$2.9x10^{-2}$
Acenaphthene	3	96	279	3.47	$2.0x10^{-2}$
Fluorene	3	116	293	1.98	$1.3x10^{-2}$
Phenanthrene	3	101	340	1.29	$6.9x10^{-4}$
Anthracene	3	216	340	0.07	$1.9x10^{-7}$
Fluoranthene	4	111	375	0.26	$6.0x10^{-6}$
Pyrene	4	149	360	0.14	$6.9x10^{-7}$
Benzo[a]anthracene	4	158	400	0.014	$5.0x10^{-9}$
Chrysene	4	255	448	0.002	$6.3x10^{-7}$
Benzo[b]fluoranthene	5	168	481	$1.2x10^{-3}$	$5.0x10^{-7}$
Benzo[k]fluoranthene	5	217	480	$5.5x10^{-4}$	$5.0x10^{-7}$
Benzo[a]pyrene	5	179	496	$3.8x10^{-3}$	$5.0x10^{-7}$
Inden[1,2,3-cd]pyrene	6	163	536	0.062	$1.0x10^{-10}$
Benzo[g,h,i]perylene	6	278	500	$2.6x10^{-4}$	$1.0x10^{-10}$
Dibenzo[a,h]anthracene	5	267	524	$5.0x10^{-4}$	$1.0x10^{-10}$

2.1.2 Fate and transport of PAHs in environment

2.1.2.1 Fate and transport of PAHs in ambient air

PAHs entering the atmosphere derived from the combustion and from volatilization (Figure 2.1). They are present in the ambient air as vapors or adsorbed into airborne particulate matter. Gas-to-particle partition of PAHs depends on the molecular weight of the compounds, temperature, humidity and precipitation. In general, low-volatile PAHs with lower 5-rings, characterized by relatively high temperature of condensation, are adsorbed on the airborne particles. They are classified in the low mobility category of POPs subjected to rapid deposition and retention close to the source. The lower-molecular weight compounds with 2-3 rings, exhibiting low temperatures of condensation, are more abundant in the gas phase. These hydrocarbons (included in the high or moderately high mobility categories, undergo world-wide atmospheric dispersion and preferentially accumulate in polar latitudes. Semi-volatile 4-rings PAHs (like pyrene or phenanthrene) can be found in both phases and their gas-to-particle partition coefficients are most susceptible to the influence of environmental factors. They are deposited and accumulate mainly in mid-latitudes, with high summer temperatures (or in tropical regions), the concentrations of PAHs in the gas phase increase whereas during winters (or in Arctic regions) particulate phase PAHs dominate. Higher PAH concentrations (in general measured in the particulate phase) in the winter months were related also to the higher emission of these pollutants from domestic heating sources. The adsorption of PAHs onto particulate phases may be affected not only by temperature but by humidity as well: it was found that the gas-to-particle PAH ratio decreases with increasing humidity. The range of PAH adsorption on atmospheric sorbents depends also on the quantity of the suspended particulates and their nature (soot, dust, fly-ash, pyrogenic metal oxides,

pollens, etc., of different particle size) and a significant correlation was found between the amount of dust in the air and PAH concentrations in the particulate phase.

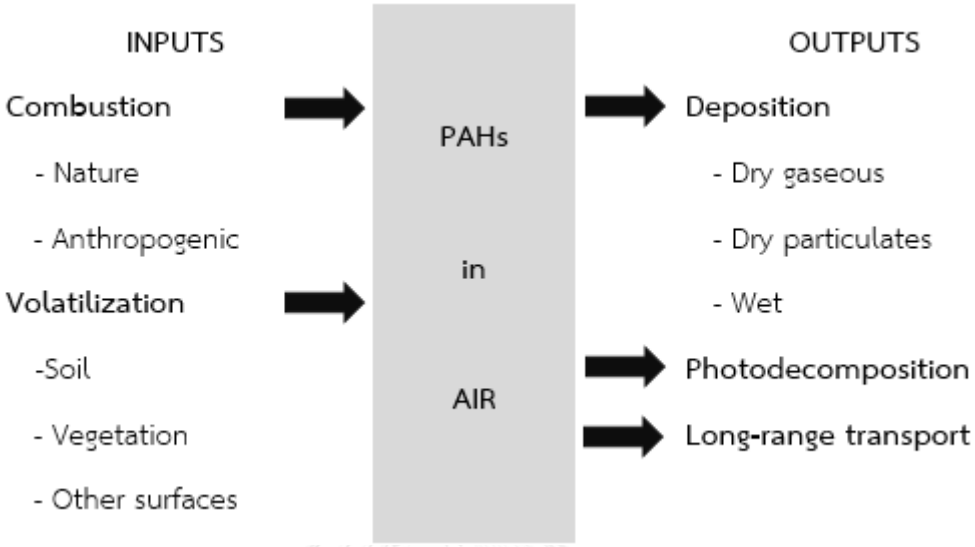


Figure 2.1 Schematic diagram of PAH fate in atmosphere

2.1.2.2 PAHs deposition on plants

PAHs accumulation in plants is a function of: (a) each particular PAHs (e.g. lipophilicity, vapor pressure that controls the vapor-particle partitioning); (b) plant species: surface cuticular waxes, hairs, and numbers of stomata; (c) environmental conditions like temperature, humidity, precipitations, photolysis and atmosphere stability. Therefore, the concentration of PAHs in leaves is affected by a combination of factors such as their atmospheric concentrations and air mass circulation, the kinetics of their uptake in leaves, and their partitioning between air and vegetation. Several papers have previously reported results of PAH analysis in plant matrices like tree leaves, mosses, pine needles, and pasture vegetation. Plant lipid content plays a double role in PAHs determination because high lipid content on leaves favors capture of lipophilic compounds from air. These lipids are comparable to many lipophilic organic pollutants including PAHs in terms of their physicochemical properties, e.g.

solubility or molecular size (Sanz-Landaluze, Bocanegra-Salazar, Ortiz-Perez, & Camara, 2010).

2.1.3 Ecotoxicity effects of PAHs

The toxicity of PAHs to aquatic organisms is affected by metabolism and photooxidation. They are commonly more toxic in the presence of ultraviolet light. PAHs have moderate to high acute toxicity to aquatic life and birds. PAHs in soil are unlikely to exert toxic effects on terrestrial invertebrates, except when the soil is highly contaminated. More adverse effects on these organisms include tumors, reproduction, development, and immunity.

Mammals can absorb PAHs by various routes e.g. dermal contact, inhalation, and ingestion (Beyer, Jonsson, Porte, Krahn, & Ariese, 2010; Dong, Chen, & Chen, 2012). Whereas, plants can absorb PAHs from soils through their roots and translocate them to other plant parts. Uptake rates are generally governed by concentration, water solubility, and their physicochemical state as well as soil type. PAHs-induced phytotoxic effects are rare. Full information and the database on this are still limited. Certain plants contain substances that can protect against PAH effects. Other plants can synthesize PAHs that act as growth hormones (Beyer et al., 2010).

PAHs are moderately persistent in the environment, and can be bio-accumulated. The concentrations of PAHs found in fish and shellfish are expected to be much higher than in the environment from which they were taken. Bioaccumulation has been also shown in terrestrial invertebrates. Nevertheless, metabolism of PAHs is sufficient to prevent biomagnifications (Inomata et al., 2012).

2.1.4 Removal of PAHs

PAHs are reduced from the environmental matrixes by several methods, including: biodegradation (Abdel-Shafy & Mansour, 2016) and photochemical

degradation. On the other hand, removal of PAHs from atmosphere was also reported (Zhong et al., 2014; Zhu et al., 2008).

PAH degradation

Degradation of PAHs in the environment includes: biodegradation, photooxidation, and chemical oxidation adsorption to soil particles, leaching, bioaccumulation (Haritash & Kaushik, 2009). Each of these processes affects individual PAHs in a different manner. This is mainly due to the fact that each PAH has a unique structure and a set of physical, chemical, and biological properties. The majority of the articles examined for this literature study discussed biodegradation by either anaerobic or aerobic microorganisms. Few articles studied other modes of PAH degradation, such as photolysis or oxidation.

(1) PAH biodegradation

Biodegradation of PAHs was the most recurrently studied degradation process. The first PAH biodegradation studies focused on aerobic degradation. Nevertheless, anaerobic degradation has been demonstrated, more recently, under denitrifying conditions (Haritash & Kaushik, 2009). In order for bacteria to degrade any given PAH, it must be made available for uptake by the bacteria (Fredslund et al., 2008). PAHs become bioavailable when they are in either the dissolved or the vapor phase. PAHs sorbed onto soil particles cannot be readily degraded by bacteria because the PAHs are separated from the enzymes that are used by bacteria to break them down (Kim, Lindsay, & Pfaender, 2007). However, the bioavailability of a given PAH is complicated. For example, the age of PAHs in the soil has been shown to affect how rapidly they will be desorbed from the soil. PAHs will also desorb at different rates over time.

Such a change in desorption rate is caused mainly by decreases in the concentration gradient as the PAHs desorbed. As the concentrations of the individual PAHs reach the aqueous solubility levels, the rate of desorption will decrease because

the concentration gradients between the sorbet and aqueous phases have decreased. The important factor in the PAHs bioavailability is their solubility character. The aqueous solubility of PAHs is strongly dependent on their molecular weights (Johnsen, Wick, & Harms, 2005). This makes these angular structures more biodegradable than the linear or clustered structures. PAH degradation also can be impacted by competitive inhibition.

On the other hand, the rate of PAH degradation can be reduced if the bacteria involved in degrading PAHs find a chemical that is utilized more easily as a food source. Competitive inhibition occurs when the active sites of enzymes used by bacteria to break down PAHs as a carbon source are non-specific. These non-specific enzymes can attach themselves to a number of different chemicals. If other chemicals are present that are more easily broken down, the enzyme will degrade those chemicals as its carbon source and the PAHs will persist (Wang et al., 2009) It is also evident that anthracene could be completely mineralized by *Sphingomonas, Nocardia*, *Beijerinckia, Paracoccus*, and *Rhodococcus* with dihydriol as the initial oxygenated intermediate (Teng et al., 2010).

(2) Chemical degradation

Chemical oxidation appears to be a minor PAH degradation mechanism under most environmental conditions. In the case of chemical oxidation, either naturally or as part of treatment technologies (Abdel-Shafy & Mansour, 2016), the rates of PAH oxidation depend on several properties of the system. The molecular weight and structure of the compound, its physical state, temperature, and the strength of the oxidizing agent all impact the outcome. Zhang et al. (2008) also reported that the synergistic effect of UV irradiation and TiO_2 or ZnO catalysis was efficient for degradation of PAHs in contaminated soil. Iron oxides and oxalic acid can set up a photo-Fenton-like system without additional H_2O_2 in solid phase to enhance the photodegradation of pyrene under UV irradiation (Wang et al., 2009). On the other

hand, sono-chemical degradation of PAHs using high frequency of ultrasound was also investigated (Manariotis et al., 2011).

2.2 Plant leaves and phyllosphere

The aerial parts of living plants including leaves, stems, buds, flowers and fruits provide a habitat for microorganisms termed of Phyllosphere (Whipps, Hand, Pink, & Bending, 2008). Foliage is always inhabited by a large number of microbes including epiphyte and endophyte. Phyllosphere microflora is mainly composed of bacteria, filamentous fungi, yeasts and a small bit of algae, protozoa and nematodes. Bacteria are the most abundant inhabitants within phyllosphere and also the greatest contributors to phyllosphere ecosystem (Lindow & Brandl, 2003).

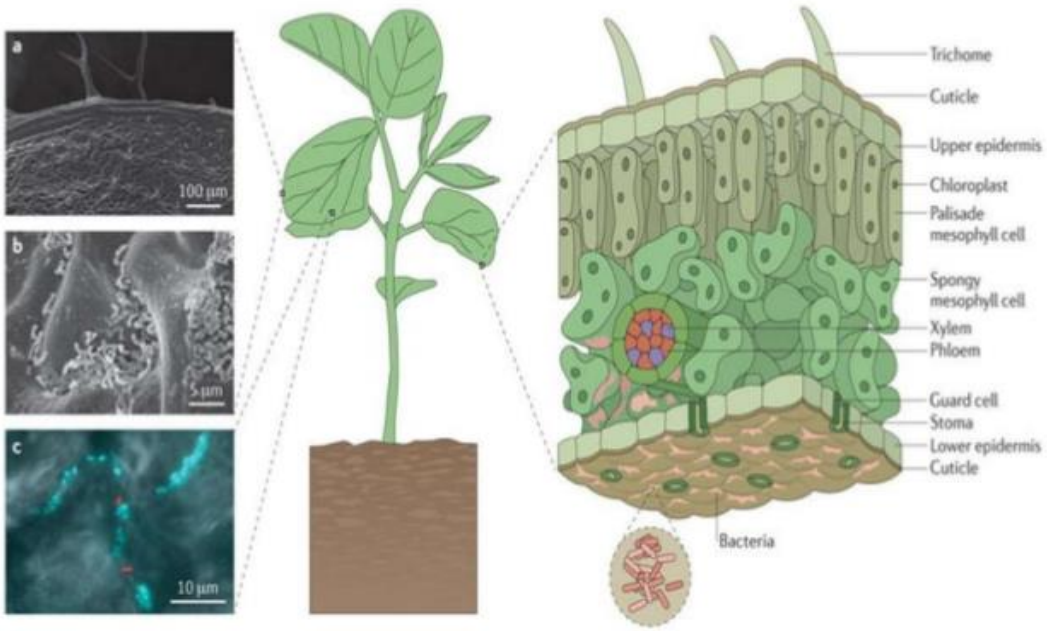


Figure 2.2 Microscopic appearance of phyllosphere bacteria (Lindow and Brandl, 2003)

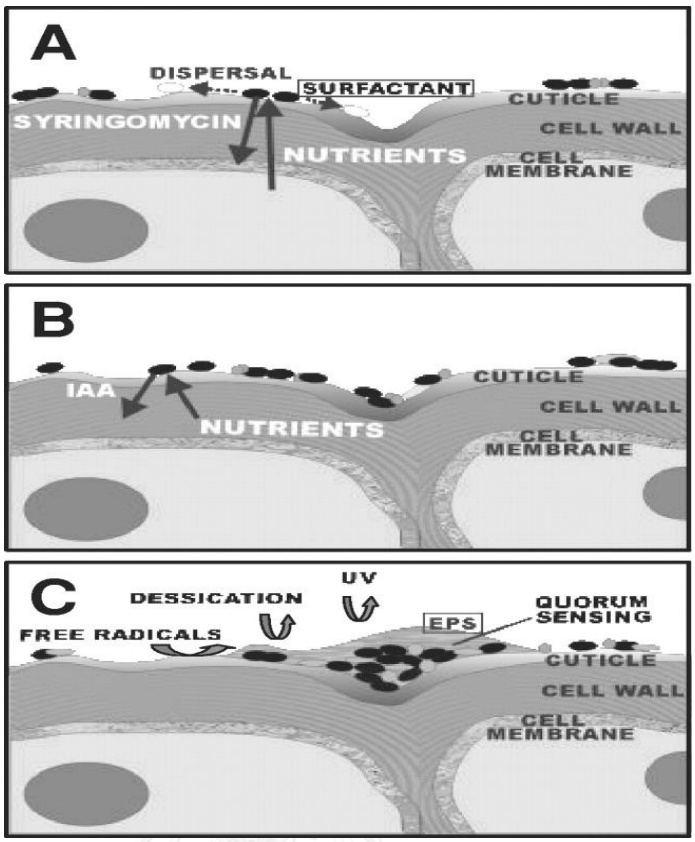


Figure 2.3 Schematic diagram representing various hypothetical bacterial-habitat modifications in the phyllosphere, such as the release of nutrients from plant cells and bacterial cell dispersal effected by the production of syringomycin, which may act both as a phytotoxin and as a surfactant (A); the release of saccharides from the plant cell wall, caused by bacterial secretion of auxin (B); and protection from environmental stresses via production of EPS in bacterial aggregates (C) (Lindow and Brandl, 2003).

Phyllosphere environment is generally considered as a rather hostile habitat for microorganisms due to the tremendous physical fluctuation during day and light, direct UV radiation and oligotrophy. Interestingly, bacteria are often detected in population averaging from 10^6 to 10^7 cells every centimeter (up to10^8 cells g^{-1}) in the hostile habitat (Lindow & Brandl, 2003; Yang, Crowley, Borneman, & Keen, 2001). It is reasonable to believe that, such organism possess special ecological relationships with the hosts. Inconsequential (transient epiphytic saprophytes) and substantial

(commensals, mutualistic symbionts, endophytes or pathogens) could be sum-up between the relationships of plant-microbe and microbe-microbe in phyllosphere ecosystem. Leaf-associated microbes play important roles in their hosts, such as nitrogen fixation, pathogen prevention, and growth stimulation.

The atmospheric microflora can vary in composition and concentration diurnally and seasonally as well as in response to environmental events such as rainfall and high wind, directly influencing the immigration of microorganisms to the phyllosphere. Immigration of microorganisms to leaves from the atmosphere can take place through impaction onto the leaf surface, sedimentation or rain splash as well as from contamination with soil.

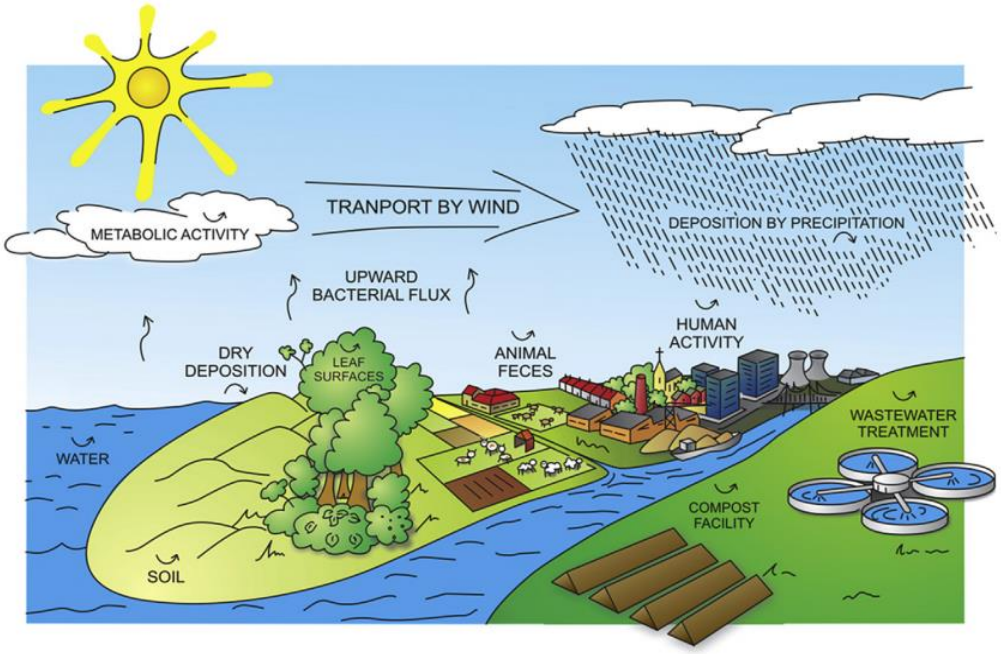


Figure 2.4 Scheme of typical processes that determine the composition of local airborne bacterial communities. Abundant sources of aerosolized bacteria are marked with an upward arrow. Soil and leaf surfaces are often considered the main contributors of airborne bacteria (Smet et al., 2016)

Microorganisms that have arrived in the phyllosphere have to become established and colonize the leaf to become a residual epiphyte. The pattern of distribution of microorganisms on leaves is not even. The most common sites of bacterial colonization are in the epidermal cell wall junctions especially in protected sites in grooves along the veins, at stomata and at the base of trichomes. In general, greater numbers of bacteria are found on lower than upper leaf surfaces possibly because of the lower leaf surface having a greater density of stomata or trichomes, or a thinner cuticular layer. Clearly, not all microorganisms that arrive in the phyllosphere are able to colonize and grow. To some extent this reflects processes of emigration through dispersal mechanisms such as rain splash, wash-off, bounce-off, water movement or removal by insects. Ability to survive and grow are dependent on the environmental, physicochemical and genetic features of the plant and specific

properties exhibited by the phyllosphere microorganisms, which together determine the structure and diversity of the microbial community (Whipps et al., 2008).

2.3 Bioremediation

One of the encouraging technologies to recover hydrocarbon-contaminated sites is bioremediation, using microorganisms beside algae, bacteria, yeast and fungi to transform or decontaminate toxic pollutant to less hazardous chemicals. It is due to high efficiency, low cost, and eco-friendliness (N. Das & Chandran, 2011). Bioremediation has three main approaches (Perelo, 2010; Yu, Ke, Wong, & Tam, 2005)

2.3.1 Natural attenuation utilizes fundamental degradation capacities of the autochthonous microbial population to degrade contaminant. The benefit is the ability to avoid damaging the ecologically sensitive habitat. However, this method often takes a long time to complete because population size of native microorganism is small.

2.3.2 Bioaugmentation, introducing appropriate bacteria (a versatile microorganism or an efficient consortium), is a possible mean to enhance biodegradation of contaminants. Several research indicate that the bioaugmentation is a promising technique to solve toxic substrates and enhance removal efficacy (Wu et al., 2008). Interestingly, it has been reported that it is suitable to treat water and sediment contaminated by chemicals as an alternative method (Semrany, Favier, Djelal, Taha, & Amrane, 2012). For example, the response of the indigenous soil bacteria to the presence of gas-oil in microcosm. The initial concentration of gas-oil was 14,380 μg g^{-1} (dry weight). Indigenous bacteria removed 35% of the total hydrocarbon concentration (THC) while bioaugmentation with *Acinetobacter* sp. strain B2-2 caused a decrease of 65% in 51 days. The results showed that B2-2 strain, bioaugmented bacterium, could improve the biodegradation efficacy representing a valuable alternative tool (Ruberto, Vazquez, & Mac Cormack, 2003).

2.3.3 Biostimulation, supplying additional nutrients, which affect the growth of indigenous population, is a strategy to promote biodegradation.

2.4 PAHs-degrading bacteria

The first step in the microbial degradation of PAHs is the action of dioxygenase, which incorporates atoms of oxygen at two carbon atoms of a benzene ring of a PAH resulting in the formation of *cis*-dihydrodiol, which undergoes rearomatization by dehydrogenases to form dihydroxylated intermediates. Dihydroxylated intermediates subsequently undergo ring cleavage and form TCA-cycle intermediates (Samanta et al., 2002). A large number of naphthalene-degrading microorganisms (including *Alcaligenes denitrificans*, *Mycobacterium* sp., *Pseudomonas putida*, *P. fluorescens*, *P. paucimobilis*, *P. vesicularis*, *P. cepacia*, *P. testosteroni*, *Rhodococcus* sp., *Corynebacterium venale*, *Bacillus cereus*, *Moraxella* sp., *Streptomyces* sp., *Vibrio* sp. and *Cyclotrophicus* sp.) has been isolated and examined for mineralization (Samanta, Om V. Singh, & Jain, 2002).

Fluoranthene metabolites resulting from degradation by a *Mycobacterium* sp. have been reported and more than 95% fluoranthene can be degraded if efficient organic nutrients are provided in a mineral medium. Several bacteria, such as *Mycobacterium* sp., *Gardona* sp. and *Rhodococcus* sp., were isolated from varying hydrocarbon contaminated soils and each uses fluoranthene, pyrene and chrysene as sole carbon and energy sources (Bastiaens et al., 2000).

In the past time, research into the bacterial biodegradation of PAHs composed of more than three rings has advanced significantly. Of the four-ring PAHs, biodegradation of fluoranthene, pyrene, chrysene and benz[a]anthracene has been investigated to various degrees (Juhasz, Stanley, & Britz, 2000).

A few studies have also reported the bacterial degradation of PAHs with five or more rings in both environmental samples and pure or mixed cultures. However, most research has been targeted towards benzo[a]pyrene (BaP), a five-ring molecule, abundantly present as an active component of coal tar. Although BaP has been detected in a variety of environmental samples, so far, no microorganism has been reported that can use BaP as a sole source of carbon and energy. A slight degradation of BaP in a six-component PAHs mixture has been reported with *Mycobacterium* sp. and *S. paucimobilis* can degrade the five ring PAH dibenz[a,b]anthracene and benzo[b]fluoranthene from 7.5% to 33% (Samanta et al., 2002).

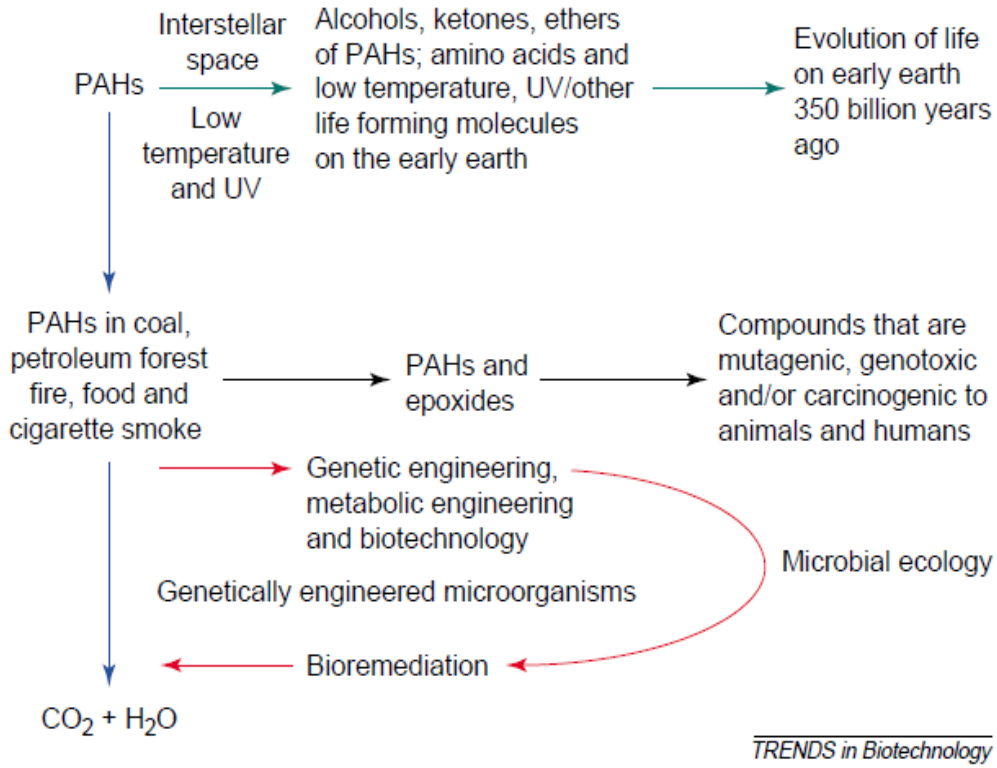


Figure 2.5 Fate, toxicity and remediation of polycyclic aromatic hydrocarbons (PAHs) from the environment. A wide variety of PAHs are abundant in nature owing to incomplete combustion of organic matters. The PAHs from extraterrestrial matter are also oxidized and reduced owing to prevalent astrophysical conditions and resulting in the formation of various organic molecules, which are the basis of early life on primitive earth. The microorganisms (naturally occurring or genetically engineered) can mineralize toxic PAHs into CO_2 and H_2O (Samanta et al., 2002).

2.5 Surface active compounds

Surface active agents (surfactants) are amphiphilic molecules with both hydrophilic and hydrophobic moieties (Figure 2.6), which show a wide range of properties, including the lowering of surface and interfacial tension of liquids, and the ability to form micelles and microemulsions between two different phases. Surface active agent can produced from chemical and microbial (Biosurfactant). The hydrophilic moiety of a surfactant is defined as the “head”, while the hydrophobic one is referred to as the “tail” of the molecule which generally consists of a hydrocarbon chain of varying length. Surfactants are classified as anionic, cationic, non-ionic and zwitterionic, according to the ionic charge of the hydrophilic head of the molecule (Figure 2.7). The most common hydrophobic parts of chemically synthesized surfactants are paraffins, olefins, alkylbenzenes, alkylphenols and alcohols; the hydrophilic part is usually a sulphate, sulphonate or a carboxylate group in anionic surfactants, a quaternary ammonium group in cationic surfactants and polyoxyethylene, sucrose or a polypeptide in nonionic surfactants.

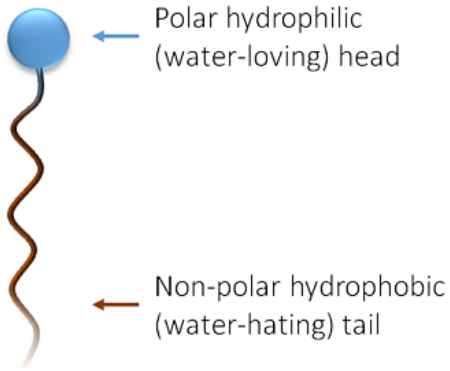


Figure 2.6 General structure of a surfactant molecule.

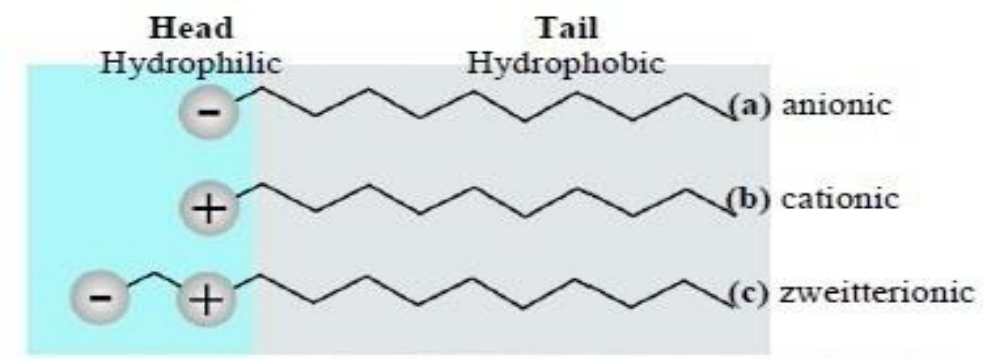


Figure 2.7 Surfactant classification according to the composition of their head: (a) anionic (negatively charged), (b) cationic (positively charged) and (C) zweitterionic (two oppositely charged groups)

Another fundamental property of surfactants is the ability to form micelles which is responsible for the excellent detergency and dispersing properties of these compounds. When dissolved in water in very low concentrations, surfactants are present as monomers. In such conditions, the hydrophobic tail, unable to form hydrogen bonding, disrupt the water structure in its vicinity, thus causing an increase in the free energy of the system. At higher concentrations, when this effect is more pronounced, the free energy can be reduced by the aggregation of the surfactant molecules into micelles, where the hydrophobic tails are located in the inner part of the cluster and the hydrophilic heads are exposed to the bulk water phase. The concentration above which the formation of micelles is thermodynamically favored is called Critical Micelle Concentration CMC). The number of molecules necessary to form a micelle generally varies between 50 and 100; this is defined as the aggregation number. As a general rule, the greater the hydrophobicity of the molecules in the aqueous solution, the greater is the aggregation number. CMC is commonly used to measure the efficiency of a surface active agent. The CMC of surfactants in aqueous solution can vary depending on several factors, such as molecule structure, temperature, presence of electrolytes and organic compounds in solution. At soil

temperatures, the CMC typically varies between 0.1 and 1 mM. The size of the hydrophobic region of the surfactant is particularly important for the determination of the CMC: in fact the CMC decreases with increasing hydrocarbon chain length, i.e. increasing hydrophobicity. However, anionic surfactants have higher CMCs than nonionic surfactants even when they share the same hydrophobic group. At concentrations above the CMC, additional quantities of surfactant in solution will promote the formation of more micelles. The formation of micelles leads to a significant increase in the apparent solubility of hydrophobic organic compounds, even above their water solubility limit, as these compounds can partition into the central core of a micelle. The effect of such a process is the enhancement of mobilization of organic compounds and of their dispersion in solution. This effect is also achieved by the lowering of the interfacial tension between immiscible phases. In fact, this contributes to the creation of additional surfaces, thus improving the contact between different phases. The main surfactant-mediated mechanisms which may potentially enhance hydrophobic organic compound remediation include the reduction of interfacial tension, micellar solubilization and phase transfer (Franzetti, Gandolfi, Bestetti, & Banat, 2010).

Nowadays biosurfactant is become more interesting in many applications. They are a wide group of structurally diverse surface active compounds produced by a variety of microorganisms which are mainly classified by their chemical structure and their microbial origin. They are generally composed of a hydrophilic part, consisting of amino acid or peptide anions or cations, mono- or polysaccharides, and a hydrophobic part consisting of saturated, unsaturated or fatty acids. The term “biosurfactants” should be correctly used to identify low-molecular-weight microbial surfactants. In contrast, high-molecular-weight polymers can be collectively defined as bioemulsifiers also otherwise known as bioemulsifiers. In fact, the former group includes molecules which efficiently lower surface and interfacial tension, while the latter is composed of

amphiphilic and polyphilic polymers which are more effective in stabilizing oil-in-water emulsions but do not lower the surface tension as much. The low-molecular-weight biosurfactants are generally glycolipids, such as rhamnolipids, trehalose lipids, sophorolipids and fructose lipids, or lipopeptides, such as surfactin, gramicidin S and polymixin. The high-molecular-weaight bioemulsifiers are amphiphilic or polyphilic polysaccharides, proteins, lipopolysaccharides and lipoproteins (Smyth, Perfumo, Marchant, & Banat*, 2010).

Table 2.3 Biosurfactant application and their producing bacteria

Type	Application	Producing bacteria	References
Lipopeptide	- Enhance oil recovery - Bioremediation of petroleum hydrocarbon from contaminated site	*Azotobacter chroococcum,* *Alcanivorax dieselolei* B-5	Thavasi et al., 2011; Qiao and Shao, 2010
Rhamnolipid	Bioremediation and petroleum hydrocarbon contaminated site	*Pseudomonas aeruginosa,* *Pseudomonas aeruginosa* J4	Arutchelvi and Doble, 2010; Nguyen et al., 2010
Glycolipid	Bioremediation crude oil and PAHs in contaminated site	*Aeromonas* spp., *Bacillus* sp. NO4, *Pseudomonas putida* IR1	Rizi et al., 2012; Sadouk et al., 2008; Saeki et al., 2009
Surfactin	Enhance oil recovery and biodegradation of hydrocarbon	*Bacillus subtilis* ATCC 21332	Whang et al., 2008 Pacwa-Płociniczak et al., 2016

Biosurfactant generally classified by their chemical structure to low-molecular-weight and high-molecular-weight polymers (Banat et al., 2010). The hydrophobic moiety of biosurfactant is either long chain fatty acid, hydroxy fatty acid, or α -alkyl-β-hydroxy fatty acid and the hydrophilic moiety can be carbohydrate, amino acid, cyclic peptide, phosphate, or carboxylic acid alcohol (Bordoloi and Konwar, 2009).

2.6 Surfactant enhancing PAHs bioavailability and biodegradability

Degradation of PAHs in the environment occurs predominantly by microbial processes (K. Das & Mukherjee, 2007) and in recent years the study of the utilization of bacterial processes for PAHs bioremediation has increased significantly. However, biodegradation of HMW-PAHs is more difficult than low molecular weight (LMW) PAHs due to its low bioavailability to the degrading microbes (Vandermeer & Daugulis, 2007). To overcome this problem, surfactant is introduced into the HMW-PAHs bioremediation. It has been reported that surfactants can enhance the solubilization of pollutants in the medium, thus improving their bioavailability (J.-L. Li & B.-H. Chen, 2009). Moreover, surfactants have been used to increase the solubility of PAHs, thereby enhancing their bioavailability (Aryal and Liakopoulou-kyriakides, 2013, Rodriguez et al., 2013). However, the effect of surfactants on HMW-PAHs biodegradation depends on the type of surfactant and their applied concentrations, the type of PAHs and the identity of the microorganisms present (Rodrigues et al., 2013).

Although some surfactants exhibit negative effects on biodegradation process of PAHs owing to their toxicity to microorganisms (Fernando Bautista, Sanz, Carmen Molina, González, & Sánchez, 2009), some less toxic ionic surfactant or non-ionic surfactants have been chosen to enhance the biodegradation of PAHs (Gonzalez et al., 2011). Surfactant seems to be an effective means to improve the bioavailability of HMW-PAHs in biodegradation. However, the relationship between biodegradation of

PAHs and surfactant in pure culture is still not clear (Doyle, Muckian, Hickey, & Clipson, 2008), which limits its application in bioremediation. As such, the study of the effect of non-ionic surfactant on biodegradation of PAHs is particularly important for understanding the biodegradation processes and also useful for the choice of suitable surfactant for bioremediation of PAHs.

2.7 Related researches

2.7.1 High Molecular Weight (HMW)-PAHs in the environment

High concentrations of polycyclic aromatic hydrocarbons (PAHs) in the atmosphere have been reported from many cities such as Tennessee, USA (Hussar et al., 2012), Shanghai, China (Liang et al., 2016), Nagoya, Japanese (Ohura et al., 2016) and Riyadh, Saudi Arabia (Bian et al., 2016). In Thailand, the amount of atmospheric PAHs in Bangkok roadsides is approximately 41-189 ng m^{-3}, whereas only 1-56 ng m^{-3} PAHs is found in rural areas (Boonyatumanond et al., 2007; Ruchirawat et al., 2007). In addition to vehicle combustion, the presence of HMW-PAHs with four or more rings can be due to the long-range emissions of particulate matters from biomass burnings, as seen in Chiang Mai, Thailand (Wiriya et al., 2013). The environmental fate of particle-associated PAHs are influenced by vegetation, which can capture particles from the atmosphere, retain them, encapsulate them into their cuticles and release them into soil and/or lower biomasses (Terzaghi et al., 2013). Tree, shrub, herb and woodland settings have been found to trap more PAHs than those of the grassland (Peng et al., 2012). The biodegradation of accumulated PAHs can reduce their recirculation back into the atmosphere. Some bacteria on leaves of ornamental shrubs are able to degrade both deposited and airborne PAHs. However, previous studies have focused only on low molecular weight PAHs (LMW-PAHs) such as acenaphthylene, acenaphthene, fluorene and phenanthrene (Waight et al., 2007; Yutthammo et al., 2010).

Pyrene, a four-ring PAH with a wide distribution in the environment, represents a major portion of the total PAHs found in contaminated sites (Xu et al., 2007). It is listed in the priority pollutants of the United States Environmental Protection Agency (US.EPA) and has been adopted as an indicator of PAHs contamination in waste monitoring (Dissanayake, Piggott, Baldwin, & Sloman, 2010). Pyrene is shown to be toxic to a wide range of organisms such as microalgae, copepods, mussels and fish (Oliveira, Slezakova, Delerue-Matos, Pereira Mdo, & Morais, 2016) and it has been used as a model compound in metabolism studies of HMW-PAHs biodegradation due to its relatively simple oxidation and structural similarity to the other carcinogenic PAHs (Beach, Quilliam, Rouleau, Croll, & Hellou, 2010). Moreover, pyrene was major constituent of HMW-PAHs in street dust and roadside air that monitor in Bangkok, Thailand (Boonyatumanond et al., 2007).

From above reasons, pyrene were used as the representative of HMW-PAHs in this research. Other HMW-PAHs were simulated as same as the profile and concentration of HMW-PAHs at sampling site.

2.7.2 Plant leaf as PAHs-biomonitor and for reduction of atmospheric PAHs

Plants leaves play an important role in the global cycle of PAHs (Collins, Fryer, & & Grosso, 2006); however, the processes of accumulation, transport and transformation within the plant have not yet been fully established (Lin, Tao, Zuo, & Coveney, 2007). Various groups of plants (e.g., aquatic plants, grasses, vegetables and trees) have been recommended as biomonitors of PAHs because they may accumulate these species in their organs (De Nicola, Claudia, MariaVittoria, Giulia, & Anna, 2011; Lehndorff & Schwark, 2010; Orecchio, Gianguzza, & Culotta, 2008; Rodriguez-Escales et al., 2011). The magnitude of this accumulation and the efficiency of plants as biomonitors depend on their morphological and physiological characteristics (De Nicola et al., 2011; Wild, Dent, Thomas, & & Jones, 2006) in addition to the

physicochemical properties of PAHs and the local meteorological conditions (St-Amand, Mayer, & Blais, 2008). In fact, high amounts of PAHs were reported on plants growing along roadsides, for example the amounts of total PAHs in orange jasmine leaves, *Murraya paniculata* (L.) Jack, collected from Bangkok roadsides ranged from 63.99 to 82.46 mg kg^{-1} leaves (Karnchanasest & Satayavibul, 2005).

At the same time, plant leaves can minimize the atmospheric PAHs by foliar uptake to inner tissue. The study on deciduous forest in Southern Ontario, Canada, confirmed that the amounts of phenanthrene, anthracene, and pyrene were reduced within and above the forest canopy during bud break in early spring (Choi et al., 2008). The major factor that affects the concentration of PAHs in plant tissue is supposed to be plant species, which is possibly due to plant-specific morphological and chemical constitutions (Bakker et al., 2001; Kipopoulou et al., 1999). However, phyllosphere bacteria on plant leaves could be another factor that influences the fate of gaseous and deposited PAHs on leaves.

The biodegradation of leaf-deposited PAHs was first investigated with phenanthrene and *Ixora sp*. leaves by Waight et al (2007). They found that several phyllosphere bacteria (similarity to the genera *Pseudomonas*, *Microbacterium*, *Rhizobium*, and *Deinococcus*) were able to utilize the deposited phenanthrene as carbon source. The experiment studied the leaves removed from *Ixora* sp. to exclude other plant activities. Phenanthrene concentration remained almost constant in control treatment throughout the study. On the other hand, the amount of phenanthrene decreased rapidly in bioaugmentation treatment and there was only 23% of remaining phenanthrene on the leaves after 48 h. In the non-augmented leaves, phenanthrene was gradually decreased to 62% at the end of study. The results indicated that the decrease of deposited phenanthrene was mainly due to bacterial activities, in which the inoculated *Sphingomonas* sp. P2 degraded phenanthrene more rapidly than the indigenous bacteria.

In addition, Yutthammo et al, (2010) found PAHs-degrading bacterial species such as *Acinetobacter*, *Pseudomonas*, *Pseudoxanthomonas*, *Mycobacterium*, and uncultured bacteria from ornamental plant leaves. PAH degradation activity of *Wrightia religiosa* and their bacteria was monitored in gas-tight systems comparing sterilized or unsterilized leaves. The results indicated that phyllosphere bacteria on unsterilized leaves were able to enhance the activity of leaves for phenanthrene removal. In addition, phyllosphere bacteria on *W. religiosa* were able to decrease other PAHs such as acenaphthylene, acenaphthene, and fluorene in both small 60-mL glass vials and in a 14-L glass chamber. When using 60-mL glass vials, the unsterilized leaves had higher PAHs removal efficiencies than dried and sterilized leaves, thus confirming the role of phyllosphere bacteria on the degradation of gaseous PAHs. PAHs removal efficiencies of dried and sterilized leaves were not significantly different. The removal mechanism of PAHs by leaves without bacteria is therefore suggested to be sorption rather than degradation. The efficiency of phyllosphere bacteria in PAHs removal was more distinct when using a 14-L glass chamber containing *W. religiosa* branches with fresh leaves. Due to the low leaf mass to air volume ratio in this system, the maximum sorption capacity of leaves was probably reached rapidly, and the remaining available PAHs were gradually degraded by phyllosphere bacteria. In this study, unsterilized leaves had high efficiency in the removal of all PAHs. This suggested that phyllosphere bacteria could degrade PAHs in both gaseous and deposited forms.

These researches investigated potential ornamental plant to reduce the atmospheric PAHs with their indigenous phyllosphere bacteria. Moreover, the ornamental plant leaves will be used as the sources for isolate the latency strains to degrade the HMW-PAHs.

2.7.3 Biocompatibility of biosurfactant

Surfactants may be useful for the bioremediation of sites polluted with PAHs since they enhance the solubility of hydrophobic compounds (Boonchan, Britz, & & Stanley, 1998). Many studies have been conducted to enhance the biodegradation of PAHs using surfactants to increase their solubility by decreasing the interfacial surface tension between PAHs and the soil/water interphase. When surfactant concentration is above the critical micelle concentration (CMC), micelle aggregates provide an additional hydrophobic area in the central region of micelles enhancing the aqueous solubility of PAHs (J. L. Li & B. H. Chen, 2009). The addition of non-ionic surfactants as additives has a positive effect on PAHs biodegradation (Volkering, Breure, Van Andel, & & Rulkens, 1995). However, other studies have reported negligible or even negative effects of surfactants. Possible reasons for this include the competition for substrate utilization and the toxicity of the surfactants toward PAHs-degrading bacteria. In addition, some surfactants have negative effects on PAH biodegradation because of their direct toxicity or their ability to solubilize high concentrations of PAHs (Chen et al., 2013). Therefore, the preferential degradation of biosurfactants over PAHs has been reported. For example, pyrene degradation by *Pseudomonas aeruginosa* is decreased after the application of rhamnolipid JBR515 (Ghosh and Mukherji, 2016).Therefore, PAHs-degradation processes involving surfactant utilization need to be optimized for each of the factors influencing biodegradation, including surfactants type and concentration, PAH specificity and the microorganisms present in the process (Jin, Jiang, Jing, & Ou, 2007).

Vipulanandan and Ren (2000) have compared the solubilization of the PAH, naphthalene, by a rhamnolipid, sodium dodecyl sulfate (SDS), an anionic surfactant and Triton X-100, a non-ionic surfactant. The biosurfactant increased the solubility of naphthalene by 30 times. However, biodegradation of naphthalene (30 mg/L) took 40 days in the presence of biosurfactant (10 g L^{-1}) compared to 100 h for Triton X-100 (10

g L^{-1}). It appeared that the biosurfactant was used as a carbon source instead of the naphthalene, which did not occur in the case of Triton X-100. Naphthalene in the presence of SDS was not biodegraded.

Deschenes et al. (1994) also showed that the rhamnolipids from the UG2 strain in a bioslurry were more effctive than SDS (up to five times) as they could enhance the solubilization of four-ring PAHs more significantly than three-ring PAHs. There were also indications of higher levels of toxicity by SDS compared to the biosurfactant as the concentration of the surfactants increased above 100 mg kg^{-1}. HMW-PAHs were not biodegraded despite surfactant addition.

Dean et al. (2001) who investigated the bioavailability of phenanthrene in soils to two strains of microorganisms knew to biodegrade this substrate. Either rhamnolipid or a biosurfactant-producing strain of *P. aeruginosa* ATCC 9027 was added. Results were mixed and difficult to interpret. One strain (strain R) showed enhanced biodegradation when the surfactant was added but the other (strain P5-2) did not. Co-addition of the strain–strain enhanced mineralization of phenanthrene only by strain R. There seemed to be some interaction between the two strains, which will need further investigation. Addition of the rhamnolipid enhanced release of the phenanthrene but did not necessarily enhance biodegradation.

Pseudomonas marginalis also indicated that the produced biosurfactants solubilized polycyclic aromatic hydrocarbons (PAHs) such as phenanthrene and enhanced biodegradation (Burd and Ward, 1996). Other research by Garcia-Junco et al. (2001) indicated that addition of rhamnolipids led to attachment to the phenanthrene that enhanced bioavailability and hence degradation of the contaminant by *P. aeruginosa.*

Therefore, HMW-PAHs degradation processes involving surfactant utilization need to be optimized for each of the factors influencing biodegradation, including

surfactants type and concentration, PAH specificity and the microorganisms present in the processed.

CHAPTER III

MATERIALS AND METHODS

3.1 Materials and instruments

3.1.1 Chemicals and solvents

1. Agarose, Research Organics, USA
2. Ammonium nitrate (NH_4NO_3), Merck, Germany
3. Bacto agar, Difco, USA
4. Benzo (a) pyrene, Sigma, USA
5. Calcium chloride ($CaCl_2 \cdot 2H_2O$), Merck, Germany
6. Di-potassium hydrogen phosphate (K_2HPO_4), Merck, Germany
7. Di-sodium hydrogen phosphate ($Na_2HPO_4 \cdot 12H_2O$), Merck, Germany
8. Ethanol, Merck, Germany
9. Ethidium bromide, Promega, USA
10. Ethyl acetate, Merck, USA
11. Ferric chloride ($FeCl_3 \cdot H_2O$), Merck, USA
12. Fluoranthene, Sigma, USA
13. Glycerol ($C_3H_8O_3$), Research Organics, USA
14. GoTaq®qPCR Master Mix, Promega, USA
15. n-Hexadecane, Sigma, USA
16. Isopropanol, Merck, Germany
17. Magnesium chloride ($MgCl_2$), Merck, Germany
18. Magnesium sulfate ($MgSO_4 \cdot 7H_2O$), Carlo Erba, France
19. Manganese chloride ($MnCl_2$), Merck, Germany
20. Methanol, Merck, Germany
21. Phenanthrene, Sigma, USA
22. Phenol, Merck, Germany

23. Potassium di-hydrogen phosphate (KH_2PO_4), Merck, Germany

24. Pyrene, Sigma, USA

25. Sodium chloride (NaCl), Merck, Germany

26. Sodium dodecyl sulfate (SDS), NacalaiTesque, Japan

27. Sodium hydroxide (NaOH), Merck, Germany

28. Sodium sulfate anhydroud (Na_2SO_4), Merck, Germany

29. Urea (CH_4N_2O), Research Organics, USA

3.1.2 Instruments

1. Autoclave, Kakusan, Japan

2. Centrifuge (Model 1920), Kubota, Japan

3. Deep freezer - 20°C (model MDF-U332), Sanyo Electric, Japan

4. Deep freezer - 80°C (model ULT1786), Forma Scientific co.th, USA

5. DNA Thermal Cycle (model UV-160A), Gene Technologies co.th, England

6. Dynamic contact angle meter and tensiometer (Model DCAT21), Future

7. Digital balance, Scientific Corp., USA.

8. Gas chromatography (GC) equipped with 320 µm × 30 m HP5 column coated with 5% of phenyl methyl ciloxane (0.25 µm)

9. Gel documentation system (model Gel Doc 2909TM), Bio-Rad co.th, USA

10. High-Performance Liquid Chromatography (HPLC) equipped with 4.6 × 150 mm Senshu Pak Pegasil ODS column (C18), pump (model LC 10AD*VP*), autosampler (model SIL-10ADVP), and UV-Visible detector (model SPD-10AD*VP*), Shimadzu Corp., Japan

11. Hot air oven (model D06063), Memmert, Germany

12. Incubator 30°C (model BE800), Memmert, Germany

13. Laminar flow (model HT-122.5), International Scientific Supply corp., USA

14. Mini agarose gel electrophoresis system, Cosmo Bio, Japan

15. Oven, Contherm Scientific, New Zealand
16. pH meter (model 240), Corning, USA.
17. Spectrophotometer, Thermo Spectronic, USA.
18. Shaker, GFL 3017
19. Vortex mixerG-560 E, Scientific Industries
20. 20, 100, 200, 1000 and 5000 µL micropipette, Eppendorf®

3.2 Culture medium

Carbon free mineral medium (CFMM) containing pyrene as sole carbon source was used to cultivate pyrene-degrading bacteria (Klankeo, Nopcharoenkul, & Pinyakong, 2009) . The chemicals used to prepare CFMM were purchased from Merck. For the general cultivation of phyllosphere bacteria, a half-strength of nutrient broth (0.5X NB) containing beef extract 1.5 g L^{-1} and peptone 2.5 g L^{-1}, pH 7.0 was prepared by diluting NB medium (Difco). The 0.5X NB was used to simulate the environment with low nutrient concentrations on plant leaves. Synthetic surfactants including Sodium dodecyl sulfate (SDS), Triton® X-100 and Tween-20 were purchased from Sigma-Aldrich, whereas Dehydol LS7TH and Dehydol LS9TH were obtained from Thai Ethoxylate. Slickgone NS, an oil dispersant, was obtained from Thai Oil. The lipopetide biosurfactant was produced by *Bacillus* sp. GY19 and prepared following Khondee et al. (2015).

3.3 Plant leaves

Six ornamental plants were divided into 2 groups and consisted of 1) shrubby plants (i.e., *Ixora coccinea* (Ic), *Wrightia religiosa* (Wr) and *Leucophyllum frutescens* (Lf)) and 2) ground cover plants (i.e., *Epipremnum aureus (Ea), Axonopus compresus* (Ac) and *Nephrolepis exaltata* (Ne)). These plant species are usually grown in Thailand and other tropical regions. The characteristics of leaves from each plant species are listed

in Figure 3.1 and Table 4.1. In this study, these plants were grown as mixed swaths along Prachachuen Road in the Bang Sue District of Bangkok, Thailand (geographic coordinate; 13°48′35′′N, 100°32′15′′E) (Figure 3.2). The size of swath was 1.0 - 1.5 m wide, 200 - 500 m long and 0.8 - 1.5 m high depended on the landscape design.

To determine the number of pyrene-degrading bacteria, leaf samples were collected in the morning from matured plants of the same species. The height of shrubby plants was approximately 1 m. There were 6 sampling times for summer, rainy and winter seasons of 2013 and 2015. At each point, triplicate samples of matured leaves from each plant species were collected. For the pyrene removal experiment, a selected plant species was purchased in one batch from Chatuchak florist shop in Bangkok, Thailand. The age of this plant was approximately 3 months, and it had a height of 30 - 40 cm.

Ixora coccinea

(Jungle geranium)

Wrightia religiosa

(Water jasmine)

Leucophyllum frutescens

(Texas barometer bush)

Epipremnum aureus

(Golden pothos)

Axonopus compresus

(Tropical carpet grass)

Nephrolepis exaltata

(Boston fern)

Figure 3.1 Ornamental plant leaves characteristics

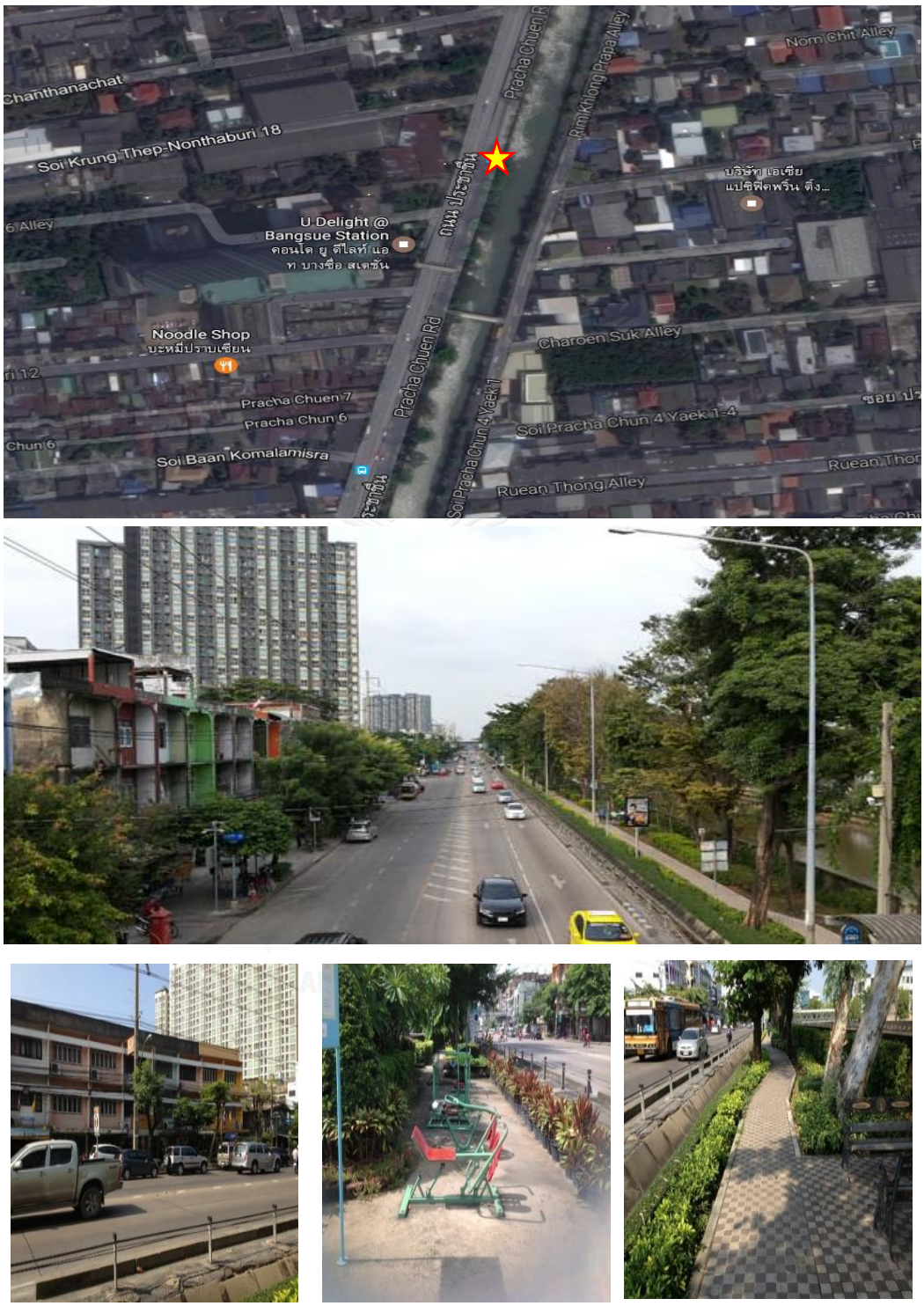


Figure 3.2 Map and locations of ornamental plant swath and roadside area for plant leaves and ambient air collection.

3.4 Research design

3.4.1 Characterization and determination of pyrene-degrading bacteria

This phase aimed to determine the relationship between bacterial species and number of pyrene-degrading bacteria on six ornamental plant leaves and ambient air in each season. Plant leaves and air samples were collected from roadside of streets adjacent to the Bang Sue (Prachachuen Road), Bangkok where the PAHs come from incomplete combustion engine in traffic sector. Moreover, the samples were compared in summer, rainy and winter seasons of 2013 and 2015. Pyrene were considered as representative of HMW-PAHs because it has the highest concentrations than other HMW-PAHs in roadside air and street dust in Bangkok areas (Boonyatumanond et al., 2007).

3.4.1.1 Determination of pyrene-degrading bacterial number in ambient air

To investigate the relationship between airborne pyrene-degrading bacteria and plants, this study compared bacterial numbers from twenty sampling points in proximity to the ornamental plant swaths (i.e., 0.0, 0.5, 1.0 and 2.0 m distant) and five sampling points from the roadside (10.0 m distant opposite the ornamental plant swath) at 0.5 and 1.0 m height from ground level (Figure 3.3). The active air samplings were carried out in the morning on the same day of leaf collection in the summer, rainy and winter seasons of 2013 and 2015. The meteorological conditions of each sampling time are in Table 4.1. Briefly, SAS super 100 (PBI International) microbial air sampler (Figure 3.4) was operated with a flow rate of 100 L min^{-1} and a total suction volume of 200 L. The air flow was directed onto the surface of a CFMM agar plate supplemented with nystatin in dimethyl sulfoxide (5 mg mL^{-1}) to inhibit fungal growth. After air collection, the agar plate was sprayed with 2% pyrene in diethylether and incubated at 30°C for 14 days. All bacterial colonies were counted and multiplied by

five to convert the volume of air to cubic meters. The bacterial number was reported as the colony forming units per cubic meter of air (CFU m^{-3} air). The data were reported as the mean of five replicated samples and standard deviation (SD).

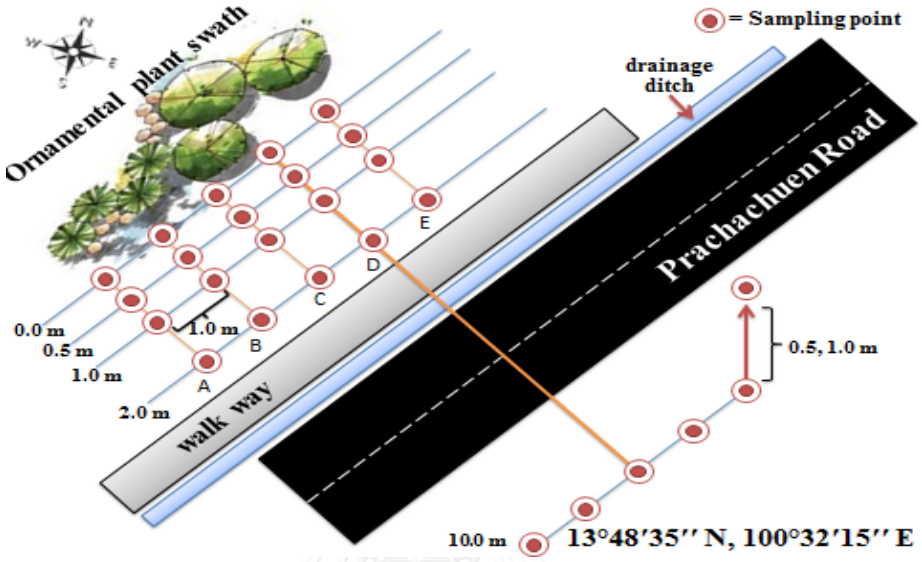


Figure 3.3 The sampling-points for determine the pyrene-degrading bacteria in the ambient air.

Figure 3.4 Microbial air sampler (impactor active sampling) for airborne pyrene-degrading bacteria collection.

3.4.1.2 Determination of bacterial number on leaves of various ornamental plants

The total number of pyrene-degrading and phyllosphere bacteria on leaves was determined using the spread plate method. Phyllosphere bacteria was extracted from the collected leaves by immersing 10 g of freshly picked leaves in 100 mL of 0.1 M potassium phosphate buffer (pH 7.0) and shaken at 200 rpm for 30 minute The samples were later sonicated in an ultrasonic bath for 10 minute to release the adhering microorganisms. The extracted solution was then centrifuged at 8,000 rpm for 20 minute to collect bacterial cells. The cell pellets were diluted in sterilized 0.85% NaCl solution for use as inoculum. The procedure was modified from Yadav et al. (Yadav, Karamanoli, & Vokou, 2005). The media were 0.5X NA supplemented with 2.5% v/v glycerol for total phyllosphere bacteria and CFMM agar spraying with 2% pyrene in diethylether for pyrene-degrading bacteria (Figure 3.5). Bacterial strains with different colony characteristics on pyrene-CFMM agar plates were isolated and purified by repeated streaking. The data were reported as the mean of triplicated samples and standard deviation (SD).

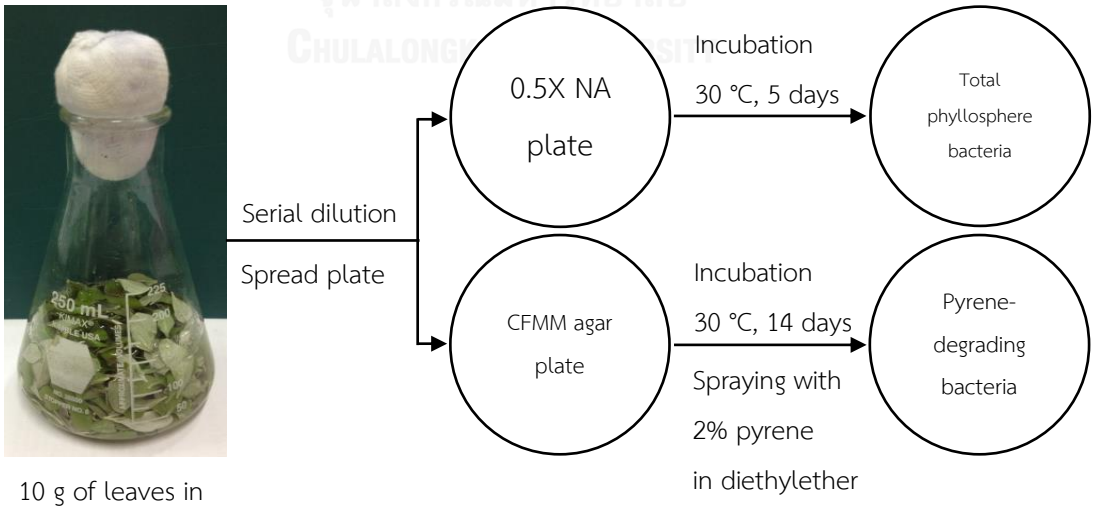


Figure 3.5 Determination the number of total phyllosphere and pyrene-degrading bacteria from plant leaves.

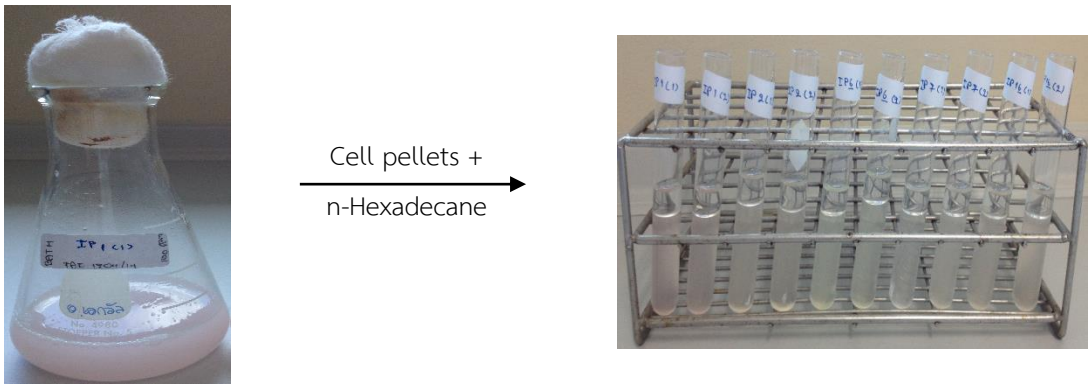
3.4.1.3 Characterization of pyrene-degrading bacteria from plant leaves

The species of isolated pyrene-degrading bacteria were identified from 16S rRNA gene analysis at Macrogen, Inc. The characteristics of bacteria relevant to the enhancement of pyrene biodegradation were studied, including cell surface hydrophobicity, biosurfactant-producing ability and biofilm formation ability.

The isolated pyrene-degrading bacteria were cultivated for 14 days in CFMM with 100 mg L^{-1} of pyrene. To determine cell surface hydrophobicity, bacterial cells were collected by centrifugation before they were used for the bacterial adherence to hydrocarbon (BATH) assay according to Rosenberg et al. (1980) (Figure 3.6). The percentage of hydrophobicity was calculated as follows:

$$\text{Hydrophobicity (\%)} = 1 - \frac{OD_{final}}{OD_{initial}} \times 100 \qquad \text{(Eq. 3.1)}$$

Strains with % hydrophobicity ≥70%, 50-70% and <50% were arbitrarily classified as high, moderate and low hydrophobicity, respectively (Zoueki, Tufenkji, & Ghoshal, 2010).



Isolated pyrene-degrading bacteria

Figure 3.6 Determination of bacterial adherence to hydrocarbon (BATH) assay of isolated pyrene-degrading bacteria from plant model.

The cell-free supernatant from the bacterial culture was submitted to a surface activity measurement by digital tensiometer (Data physics) performed at 25±1°C using the plate method (Figure 3.7).

Figure 3.7 Determination of biosurfactant-producing ability with isolated pyrene-degrading bacteria from plant model.

To quantify the formation of biofilms, the isolated pyrene-degrading bacteria were cultivated in 0.5X NB at room temperature for 24 h for use as an inoculum. Because bacterial growth might be influenced by the cultured medium, the study compared three mediums including 0.5X NB, 0.5X NB supplemented with 2.5% glycerol and CFMM supplemented with 100 mg L^{-1} pyrene. The inoculum was added to each medium at 1% and then dispensed at 150 µL onto flat-bottomed microtiter plates. The plates were incubated aerobically without shaking at room temperature for 24 h. Negative control wells contained culture medium without bacterial inoculum. The biofilm formation was stained with 0.1% crystal violet (Figure 3.8). The interpretation of biofilm formation was done according to the criteria of Stepanovic et al. (2007) as shown in Table 3.1. All of the experiments were carried out in triplicates.

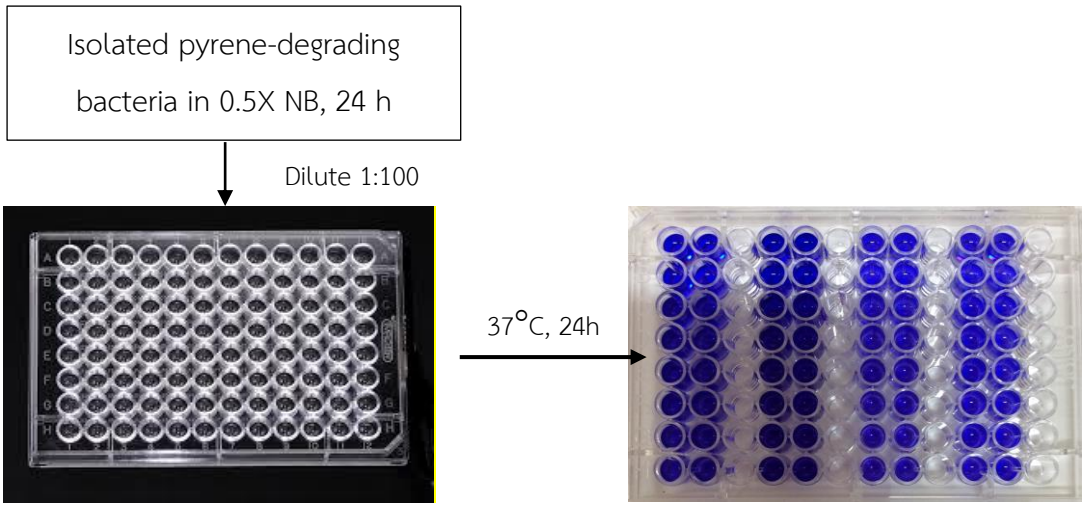


Figure 3.8 Biofilm formation ability of isolated pyrene-degrading bacteria from plant model.

Table 3.4 Interpretation of biofilm production (Stepanovic et al., 2007)

Average OD value	Biofilm formation
$OD \leq OD_c$	non-adherent
$OD_c < OD \leq 2X\ OD_c$	weakly adherent
$2X\ OD_c < OD \leq 4X\ OD_c$	moderately adherent
$4X\ OD_c < OD$	strongly adherent

3.4.1.4 Determination of bacterial community

This study aimed to identify the bacterial species and determine the correlation of pyrene-degrading bacteria species between leaf surfaces and suspended in ambient air. The communities of total phyllosphere bacteria and pyrene-degrading bacteria on leaf surfaces and pyrene-degrading bacteria in ambient air were determined by polymerase chain reaction–denaturing gradient gel electrophoresis (PCR–DGGE) of 16S rRNA genes. Genomic DNA of pyrene-degrading bacteria was extracted from cells after cultivation on CFMM medium supplemented with pyrene at 100 mg L^{-1} for 3

weeks, while total phyllosphere bacteria was directly extracted from bacterial cells that dislodged from leaves. The enrichment process allowed the detection of specific pyrene-degrading populations on plant leaves.

The extraction of bacterial genomic DNA was carried out by NucleoSpin® extraction Kit (MACHEREY-NAGEL, Germany). The extracted DNA was purified by QIAquick Gel Extraction Kit (Qiagen, Germany) to remove impurities that may inhibit PCR reaction. The amplification of V3 regions of 16S rRNA gene was performed with 341F-CG and 534R primers and PCR Master Mix kit (Qiagen, Germany). PCR amplified condition and DGGE were followed Yutthammo et al. (2010). PCR products were analyzed on DCode System (Bio-rad, USA) with 10% acrylamide gel containing 30-70% denaturant (100% denaturing solution contained 40% formamide and 7 M urea). DGGE gel were run at 60 °C, 130 V for 5 h. The number of dominant DNA bands on gel were counted to represent the number of dominant phyllosphere bacterial species on each plant species.

3.4.1.5 Investigation of particulate matter (PM) and particle-bound PAHs profile and concentration at Prachachuen road, Bang Sue district, Bangkok

This study aimed to investigate the PM and particle-bound PAHs concentrations and profiles at the Prachachuen road, Bang Sue district. The information was used to simulate the urban ambient air containing mixed HMW-PAHs in section 3.4.3.2. In addition, this study compared the concentrations of particle-bound PAHs between opposite sampling-points (proximity the ornamental plant swath and roadside area) and hypothesized that the concentration of particle-bound PAHs that proximity to plant might be lower than at the roadside air due to the activities of degrading-bacteria on plant leaves.

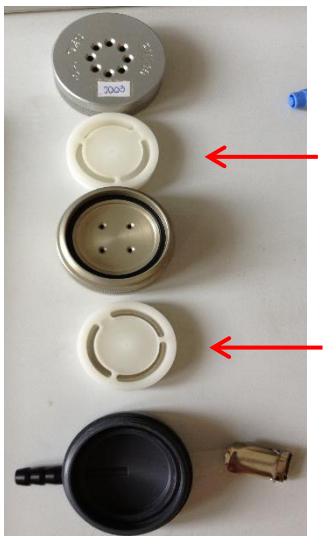
Most of PAHs with low vapor pressure in the ambient air are adsorbed on fine particles. More concentrations of PM presented are also provided more concentration of pPAHs (Srogi, 2007). Thus, PAHs contained in PM might be more

concentrated at the roadside areas than within the proximity to plant swath. To elucidate this phenomenon, the particle-bound PAHs (pPAHs) on filter were extracted and analyzed.

This study investigated the PM and pPAHs at four sampling points divided into 2 sites consists of 2 sampling sites at the roadside (1: the intersection / U-turn point and 2: the avenue) and 2 sampling site proximity the ornamental plant swath (opposite site 1 and 2). Moreover, the concentrations of PM and pPAHs at each sampling point were compared among summer, rainy, and winter seasons, respectively. PM and pPAHs were collected at the same time as the ornamental leaves collection in 2015.

PM2.5 and PM2.5-10 samples were collected on 25 and 37 mm of polytetrafluoroethylene (PTFE) membrane filters, respectively. These filters were soaked with acetone about 15 minute and dry for 15 minute, after that, keep in desiccator at least 24 h. Then, the microbalance (METLER UMX2) with accuracy of 0.001 mg were used to weight the filter before sampling. The samples were obtained using the Personal Modular Impactor (PMI) contains 2 sizes of the filters connected to personal air pump with the flow rate of 3 L $minute^{-1}$ (Figure 3.9). The flow rate was calibrated before and after the sampling in order to calculate the volume of the ambient air.

Personal air pump and PMI



Personal Modular Impactor

Roadside area

Personal air pump connected to PMI setting at sampling site

Plant swath

Figure 3.9 The instruments and sampling location for particulate matter collection.

Gravimetric analysis is the most common way to calculate the mass of fine particulate matters from weighting of the filter before and after sampling by using the ultra-microbalance with a readability of 0.1 μg (Mettler Toledo, UMX2: Greifensee, Switzerland). Standard weight 100 mg and 200 mg were weighted for quality control before and after weighing the filters (Figure 3.10). After that, the filters were weighted for 3 times in order to calculate the average weight and kept the filter in opaque plastic case for the further study of PAHs extraction and analysis. The calculation of particulate matter is shown in equation 3.2 and 3.4.

Total of air volume (m^3)

= Air flow rate (m^3 $minute^{-1}$) × Duration time of sampling (minute) (Eq. 3.2)

Concentration of particulate matter (μg m^{-3})

$$= \frac{\text{Final weight of filter (μg) - Initial weight of filter (μg)}}{\text{Total of air volume } (m^3)} \quad \text{(Eq. 3.3)}$$

Figure 3.10 Digital ultra-microbalance (METLER UMX 2) accuracy of 0.001 mg was used to weight the filters before and after particulate matter sampling.

For the collection of HMW-PAHs in particulate phase were sampling at the same site and during the same period exposure. Two-sampling point's proximity

to the ornamental plant swath were investigated for the profile and concentration of HMW-PAHs and compared with two-sampling points at the roadside area.

After sampling, filters were placed separately in 40 mL vials. In each vial 50 µL of 8000 ng mL^{-1} standard were added and allow to settle for 15 minutes for confirmation the species of PAHs. After that, added 10 mL DCM and extracted by ultrasonic homogenizer in the condition of 80 watts 9 cycles for 30 minutes. PTFE syringe filters were used for filter out extracted solvent to the new 40 mL vials. The extracted filter were rinsed with 10 mL DCM 2 times but the second time added only 5 mL Ultrasonic homogenizer were used for extraction again for 15 minutes. Then, the solvent were transferred to the vials through PTFE Syringe filters again. Solvent were evaporated under N_2 and leveled with 1.5 mL DCM. After that, solvent were transferred to 200 µL insert vials which contained in 2 mL vials and evaporated under N_2 again (Wannavichit, 2005).

The optimum conditions of PAHs analysis were measured by GC/FID (GC2010plus, Shimazdu) with 16-PAHs mixed standard solution (SUPELCO). The oven temperature program were set up as follow: the initial temperature (5°C) were held for 5 minutes, after that, the temperature were raised to 250°C the rate of 15°C per minute. The final step set up the post run at 300°C for 15 minutes in order purge solvents may be left over the column. The total time of samples analysis was about 35 minutes. This analysis were used pure Helium as a carrier gas with the flow rate of 1 µL per minute. Moreover, 1 µL of sample were injected into GC/FID by the auto-sampler to identify retention time of PAHs mix standard.

In order to calculate the concentration of PAHs, PAHs mix standard were spiked. Peak area were found by integration abundance of each species of PAHs. The result of peak integration as indicated in peak area were used for the calculation of PAHs in dimension of ng m^{-3} as shown in below.

$$\text{PAHs concentration (ng m}^{-3}) = (C_x)(10^3) \times \frac{(A)}{(B)} \times \frac{(V_S)(10^{-3})\ \text{mL}}{V_{inj}} \times \frac{1}{(V_A)} \quad \text{(Eq. 3.4)}$$

Where; C_x is initial concentration of PAHs standard (μg mL^{-1})

A is peak area of PAHs target in sample

B is peak area of PAHs target in the standard

V_S is sample volume before analysis on GC/FID (μL)

V_{inj} is injection volume (μL)

V_A is air volume (m^3)

3.4.2 Effect of surfactant on enhancing bioavailability and biodegradability of pyrene

From phase I, the plant with the highest pyrene-degrading bacterial number were selected as a plant model for the following experiments. The isolated pyrene-degrading bacteria from these plants were used to investigate the removal efficiency by various initial pyrene concentrations. Moreover, the various types of synthetic surfactants and a biosurfactant produced from *Bacillus* sp. GY19 were determined for their toxicity to isolated pyrene-degrading bacteria and selected plant species. Therefore, this phase aimed to study the types and initial concentration of surfactant addition to enhancement bioavailability and PAHs degradability.

3.4.2.1 Toxicity of surfactants on isolated bacteria and plants

In this study, the appropriate surfactant for enhancing pyrene removal had low toxicity to phyllosphere bacteria and plant leaves but had high activities on solubilizing PAHs and desorbing PAHs from the leaf surface. There were seven surfactant samples, including a lipopeptide biosurfactant from *Bacillus* sp. GY19 (Khondee et al., 2015), five commercial surfactants (i.e., SDS, Dehydol LS7TH, Dehydol

LS9TH, Triton® X-100, Tween-20) and an oil dispersant, Slickgone NS. The surfactant samples were selected based on their ability to dissolve hydrophobic compounds and availability in Thailand. The lipopeptide biosurfactant was selected due to its more hydrophobicity than other biosurfactants (e.g. rhamnolipids), low toxicity and stable surface activity under wide range temperature (Rongsayamanont et al., 2017).

The toxicity of surfactants on pyrene-degrading bacterial isolates was determined by the minimum bactericidal concentration (MBC) test. The procedure was modified from Andrews (2001). Initially, 50 µL of 0.85% NaCl solution was added to each well of a microtiter plate. To prepare a surfactant dilution series, 100 µL of 100 g L^{-1} surfactant was dispensed to the first well of each row, and a 50 µL solution was subsequently pipetted to the next wells for a 1:1 dilution. Then, 50 µL of isolated pyrene-degrading bacteria (OD_{540} = 0.1) was pipetted to the surfactant solution in each well. After a 72-h incubation, 5 µL of bacteria from each well was cultured on a 0.25X NA plate to determine the bacterial survival. The MBC was the lowest surfactant concentration that could not find bacterial growth.

The non-toxic concentrations of selected surfactants were determined phytotoxicity on selected plant species. Two concentrations of surfactants at below MBC (i.e., 1X and 10X CMC) were sprayed on leaves at 10 mL for each plant. The plant leaves were observed daily for 7 days. Criteria for phytotoxicity was morphology changes relative to the untreated plant leaves, such as the changing of leaf color, number of faded leaves, misshaped or distorted leaves, dead leaf tips or leaf margins, and dead spots or flecks on leaves. All of the experiments were carried out in triplicates. From this study will be found the suitable concentration of selected surfactant to enhance bioavailability on plant leaves that will investigated in section 3.4.3.

3.4.2.2 Effect of surfactant on enhancing pyrene solubilization and desorption

The relatively non-toxic surfactant was selected to investigate pyrene solubilization and desorption. For the solubilization tests, five concentrations of surfactant solution were prepared including 0.1X, 0.5X, 1X, 5X and 10X CMC. An excess amount of pyrene (1 g L^{-1}) was added to a 10 mL surfactant solution in a screw cap vial with Teflon septum and equilibrated for 48 h on a shaker at room temperature. To separate the non-dissolved pyrene, the sample was centrifuged at 5,000 rpm for 30 minute (Wei, Huang, Yu, & An, 2011). The dissolved pyrene was extracted with ethyl acetate twice and dried with anhydrous sodium sulfate. Finally, the ethyl acetate phase was evaporated, and the residual pyrene was dissolved in 1 mL of methanol. The concentration of dissolved pyrene was analyzed by High Performance Liquid Chromatography (HPLC), and UV-Visible detector (model SPD-10AD*VP*) from Shimudzu Corp., Japan following Klankeo et al. (2009). The data were reported as the mean of triplicated samples and standard deviation (SD).

The ability of a selected surfactant for desorption of pyrene from a leaf surface was determined in a batch scale. The tested concentrations of surfactant were 0.1X, 0.2X, 0.5X, 1X, 2X, 5X and 10X CMC. The leaves of a plant model were surface sterilized to avoid the activity phyllosphere bacteria by a modified technique from Sandhu et al. (2007). Briefly, the fresh leaves were immersed in 0.2% Tween 80 for 3 minute, 5% sodium hypochlorite for 3 minute and sterilized distilled water for 1 minute three times (Sandhu, Halverson, & Beattie, 2007). The sterilized leaves were dried in a laminar fume hood for 30 minute and then sprayed with an excess volume of 2% pyrene in diethylether to cover the entire surface area. The pyrene amended leaves were incubated in a black box to avoid photodegradation for 48 h at room temperature. After that, 1 g of randomly selected leaves was immersed in 25 mL of surfactant solution in an Erlenmeyer flask. These samples were equilibrated for 48 h

on a shaker at 200 rpm. The dissolved pyrene in surfactant solution was quantified after solvent extraction and HPLC analysis. The data were reported as mean of five replicated samples and standard deviation (SD).

3.4.2.3 Analysis of pyrene biodegradation by isolated phyllosphere bacteria

The inoculum of isolated pyrene-degrading bacteria from plant model leaves was prepared by culturing in 0.5X NB with shaking at 200 rpm for 48 h. The suspension was centrifuged at 8,000 rpm for 20 minutes to harvest the cells. Then, the cells were washed twice with sterilized 0.85% NaCl solution and resuspended in CFMM broth. The cell concentrations were adjusted to 1.0 at OD_{600}. The cell suspension in CFMM broth was supplemented with 50, 100 or 200 mg L^{-1} of pyrene and incubated at room temperature with shaking at 200 rpm. Moreover, the pyrene removal efficiency was compared between the presence and absence of 1X CMC surfactant, which hypothesized that the presence of a surfactant could enhance the pyrene bioavailability and biodegradability. Controls were CFMM broth supplemented with pyrene in the presence or absence of a biosurfactant. Triplicate samples were collected at 0, 3, 7, 14, 21, 28, 35, 42 and 49 days to determine the remaining pyrene by HPLC. The growth of bacteria was determined by the viable plate count technique on a 0.5X NA plate.

$$\text{Pyrene removal (\%)} = \frac{(C_i - C_f) \times 100}{C_i} \qquad \text{(Eq. 3.5)}$$

Where; C_i is the initial pyrene concentration (mg L^{-1})

C_f is the final pyrene concentration (mg L^{-1})

3.4.3 Potential of selected plant species after spraying with selected surfactant on remediation of HMW-PAHs in box-model

This phase is aimed to study the feasibility of selected plant species from phase I with selected surfactant addition to reduce the HMW-PAHs. This phase were studied the HMW-PAHs removal in vapor condition of different initial concentration and mixture of HMW-PAHs. The removal efficiency of HMW-PAHs were compared among (i) leaves (ii) leaves with surfactant (iii) leaves with surfactant and pyrene-degrading bacteria and (iv) sterilized leaves without phyllosphere bacteria.

Plant species for this phase is plant that highest total phyllosphere bacteria or pyrene-degrading bacteria from phase I. Plant's model were purchased from a florist at Chatuchak market, Bangkok, Thailand.

3.4.3.1 Analysis of pyrene removal by *Leucophyllum frutescens* and *Ixora coccinea* twig, phyllosphere bacteria and biosurfactant in flip-top glass-tight jar

The aim of this experiment is to confirm the ability of phyllosphere bacteria to reduce the accumulation of pyrene with different ornamental plant species. *Leucophyllum frutescens* was selected because it had the highest total phyllosphere bacteria in all seasons; while *Ixora coccinea* was selected due to it had the highest pyrene-degrading bacteria in the previous study.

Pyrene-degrading activities of phyllosphere bacteria on *L. frutescens* and *I. coccinea* plant were determined by comparing pyrene removal efficiency of four treatments: 1) leaves, 2) leaves with biosurfactant, 3) leaves with biosurfactant and pyrene degrading-bacteria and 4) sterilized leaves (without phyllosphere bacteria). The plants were purchased in one batch from a florist in Bangkok, Thailand. To remove phyllosphere bacteria, branch and fresh leaves were partially surface-sterilized by a technique modified from Sandhu et al. (2007). Briefly, the branch and fresh leaves

were immersed in 0.2% Tween 80 for 3 minute, 5% sodium hypochlorite for 3 minute, and in sterilized distilled water three times for at least 1 minute. The sterilized leaves were dried in a laminar fume hood about 30 minute before used. Each experiment was carried out in three replicates.

The batch experiment was made of 2.5 L flip-top glass jar (10×10×25 cm) containing about 3.5 g of branch. To keep the plant alive, the branch was put in a vial containing 60-mL Hoagland's solution as a hydroponic system. Pyrene was dissolved in diethylether to the initial concentration of 100 mg L^{-1}. One-milliliter of pyrene was sprayed onto branches and leaves inside the jar by TLC sprayer, rapidly closed the lid, and sealed with parafilm-tape. To add biosurfactant, one-milliliter of 0.1xCMC biosurfactant was sprayed onto leaves and allowed to dry before spraying pyrene. Bioaugmentation experiment was performed by spraying the leaves with 1.0 mL of 10^8 CFU mL^{-1} pyrene-degrading bacteria (Figure 3.11a, b). The control set consisted of GF/C membrane filter that had similar size to plant surface area. This treatment represented pyrene removal by abiotic mechanisms. All treatments were incubated at room temperature for 3 and 7 days for *L. frutescens* whereas *I. coccinea* were monitored along 14 days. The numbers of phyllosphere bacteria on leaves were examined by spread plate method.

To determine the remaining pyrene, each branch was extracted twice by a mixture of dichloromethane and acetone (5:1) with ultrasonic method (for 10 minute). Moreover, the residual pyrene sticks on inner surface chamber were twice rinsed with same mixture solvent to quantify the remaining pyrene. The remaining pyrene were analyzed by High Performance Liquid Chromatography (HPLC) following the operations of Klankeo et al. (2009).

Figure 3.11 Batch experiment made of 2.5 L flip-top glass jars containing *L. frutescens* (a) and *I. coccinea* (b) leaf branches for evaluating the pyrene-degrading activities of phyllosphere bacteria.

3.4.3.2 Analysis of mixed-PAHs removal by *Ixora coccinea* plant, their associated bacteria, and surfactant in chamber experiment

The aim of this experiment is to ratify the ability of phyllosphere bacteria to reduce the accumulation of mixed-PAHs (Phenanthrene, Fluoranthene, Pyrene and Benzo (a) Pyrene) by ornamental plant leaves. *I. coccinea* was selected because it had the highest number of pyrene-degrading bacteria from the previous experiment. In addition, the phyllosphere bacteria isolated from this plant demonstrated the ability to degrade high pyrene concentration in the carbon free mineral medium (CFMM) culture medium. The activities of phyllosphere bacteria and *I. coccinea* plant were determined for mixed-PAHs removal in both air phase and leaf surface.

The experimental box, a gas-tight chamber 96-L (40×40×60 cm) capacity was made from acrylic plates and silicone sealant. This experiment were performed to simulate the real atmospheric conditions, where the mixture of PAHs is continuously released from the traffic sector and the plants are always exposed to these air

pollutants. Types of HMW-PAHs were applied as same as the dominant PAHs species from 3.4.1.5. Moreover, the study used a whole plant in soil pot to determine the plant's function associated their indigenous phyllosphere bacteria. The whole plant would grow normally and the condition was more natural than that of the plant twig which were cultivated in hydroponic culture.

This chamber consist of sprayed nozzle for PAHs and biosurfactant injection, solid phase microextraction (SPME) –air sampling port (headspace cap with septum), water feeding port, and fan for helping air inside mixing as shown in Figure 3.12. Two-replicates of control set (Fig. 3.12a) consist of PAHs on 47 mm diameter of GF/C membrane filters (≅ 70 cm^2) put on Petri dish within chamber. These chamber without plant leaves which represent the mixed-PAHs removal by abiotic process. Two-replicates of experimental set (Fig. 3.12b) were performed with whole plant (≅ 30 cm height, 90 - 100 g of leaves) of *I. coccinea* that purchased in one batch from a florist in Bangkok, Thailand. To keep the plant alive, the *I. coccinea* pot was watered with 15-mL tap water every week. The experimental set was carried out in two replicates.

Each PAH was dissolved in diethylether to the initial concentration of 1,000 mg L^{-1}, except for benzo (a) pyrene at 100 mg L^{-1}. Five milliliters of mixed-PAHs was sprayed onto branches and leaves inside the chamber by TLC sprayer, rapidly closed the lid, screw on plate-lid, and sealed with masking tape after closing. To add biosurfactant, five milliliters of 0.1X CMC biosurfactant was sprayed onto leaves and allowed to dry before spraying mixed-PAHs. The treatments and control chambers were incubated at room temperature. Mixed-PAHs were analyzed from air samples collected by SPME device that contained a fiber coated with 100 μm poly (dimethylsiloxane) (PDMS; Supleco, USA) every day within 2 weeks. The fiber was exposed to air inside the chamber for 30 minute before transferred to Gas Chromatography (GC) injector. GC analysis was performed with a Hewlett-Packard 6890

(Agilent Technology) equipped with a flame ionized detector (FID) and an HP-5, fused-silica capillary column. The initial column temperature was 80 °C and held at this temperature for 2 minute after that temperature increased at 15 °C $minute^{-1}$ to 185 °C and then increased at 8 °C $minute^{-1}$ to 320 °C and held at this temperature for 10 minute. The injection was splitless. High purity helium was use as the carrier gas at constant flow rate of 1.3 mL $minute^{-1}$. The identification of the individual PAHs was based on retention times of target ion peaks (within ± 0.05 minute of the retention time of the calibration standard). Concentration of PAHs was determined by external standardization using similar experimental setup.

To determine the remaining mixed-PAHs, 10-g of leaves were randomly collected from *I. coccinea* plant. These leaves were extracted twice by a mixture of dichloromethane and acetone (5:1) under rotary shaker after that using ultrasonic method (for 10 minute). The remaining PAHs were analyzed by GC-FID as described before. The residual of mixed-PAHs on leaves and number of phyllosphere bacteria were determined at starting and final day of experimental period. The numbers of PAHs-degrading and total phyllosphere bacteria on *I. coccinea* leaves were examined by spread plate technique using carbon free minimal medium (CFMM) agar spraying with mixed-PAHs (Phenanthrene, Fluoranthene, and Pyrene at 1,000 mg L^{-1}, benzo (a) pyrene at 100 mg L^{-1}) and nutrient agar supplemented with 2.5% (v/v) glycerol, respectively.

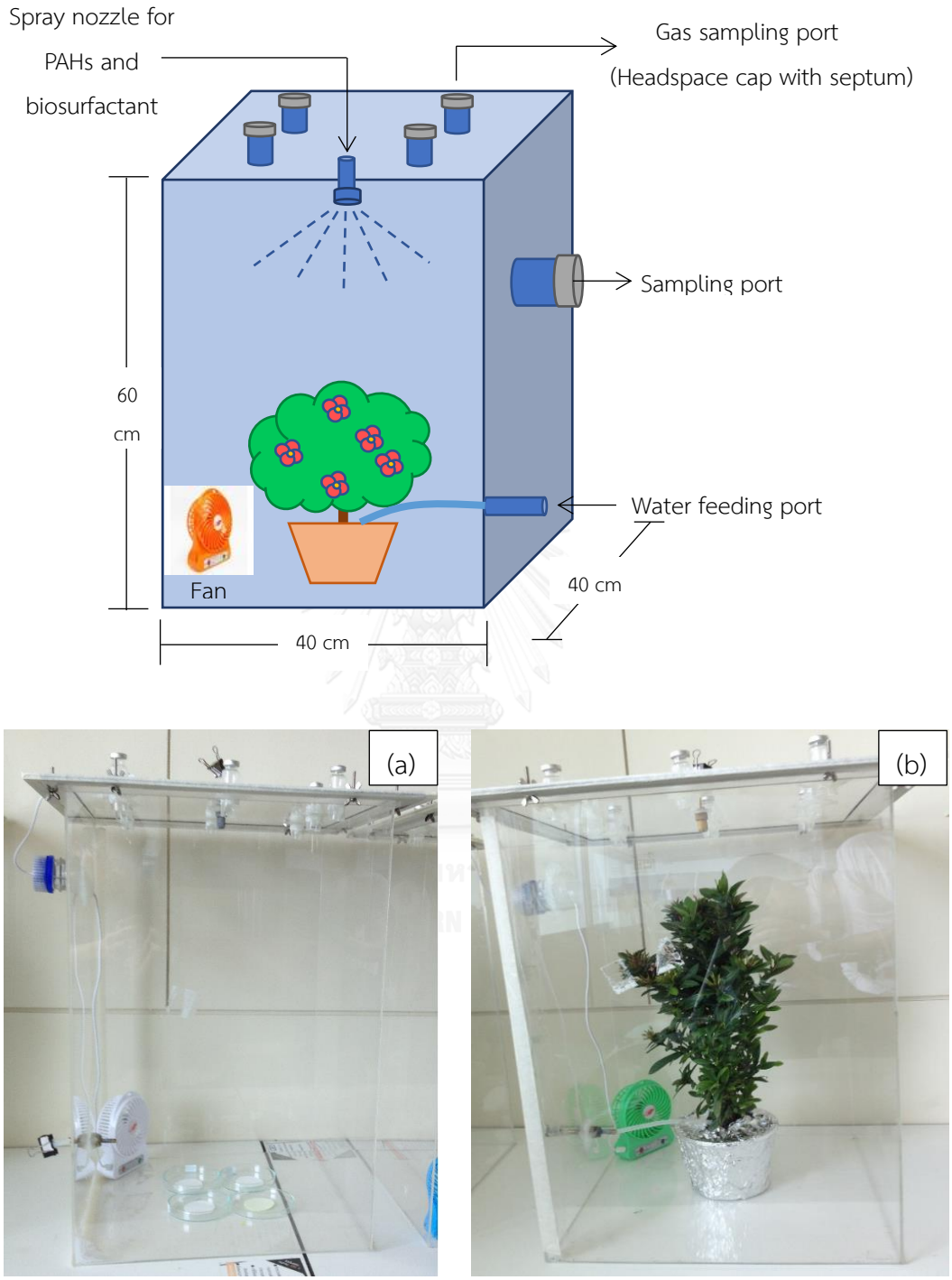


Figure 3.12 A 96-L gas-tight chamber of control set (a) and containing whole plant of I. coccinea (b) for evaluating the mixed-PAHs degrading activities of phyllosphere bacteria with biosurfactant spraying.

3.5 Statistical analysis

The statistical analyses were carried out by SPSS, version 20.0. To assess the statistical significance of bacterial numbers on the distant from plant swath and on the plant species, a one-way analysis of variance (ANOVA) was performed followed by Duncan's multiple range test. Similar procedure was performed to determine the statistical significance of biosurfactant concentrations on pyrene solubilization and desorption as well as of bacterial strains on cell surface hydrophobicity and surface tension. When compared the pyrene degrading activity and bacterial growth in the presence and absence of biosurfactant, an independent sample t-test was used. All results with a $p < 0.05$ were considered statistically significant. The correlation between pyrene-degrading bacteria and total phyllosphere bacteria on leaves were determined by Pearson correlation.

CHAPTER IV
RESULTS AND DISCUSSION

4.1 Characterization and determination of pyrene-degrading bacteria

4.1.1 Presence of pyrene-degrading bacteria in ambient air

The number of airborne pyrene-degrading bacteria in 2013 and 2015 ranged from 69-152 CFU m^{-3} air within the proximity of ornamental plant swaths (i.e., 0.0 - 1.0 m distant from plant swath), while the number of pyrene-degrading bacteria in roadside air (i.e., 10.0 m distant from plant swath) ranged from 23 - 41 CFU m^{-3} air (Figure 4.2). The numbers of pyrene-degrading bacteria from both years at the same sampling locations were not significantly different ($p > 0.05$), thus the study analyzed the data together as replicated samples. The results showed that the numbers of bacteria at 10.0 m distant from the plant swath were significantly lower than those within 1.0 m distant regardless of sampling height (i.e. 0.5 and 1.0 m above ground) and seasons (i.e. summer, rainy and winter) (Figure 4.2). When compared between seasons, the airborne pyrene-degradation in rainy seasons was the lowest with about 18% decrease from summer to winter seasons at all sampling locations of both years (Figure 4.2c, d). The results indicate that a high amount of rainfall led to the wash out of suspended bacteria from the ambient air.

Although plants have been identified as sources of airborne bacteria, this is the first study to show the relationship between airborne pyrene-degrading bacteria and ornamental plants in urban areas. The proportion of airborne bacteria from plants varied from 0.4% to 50.8% and depended on the mass of the plant at a given site (Lymperopoulou, Adams, & Lindow, 2016). In this study, all of the studied plants were grown in traffic congestion area and had dense leaves, thus contributing to the high concentrations of pyrene-degrading bacteria in the air around plant swaths. The abundance of culturable airborne bacteria was varied, such as 101 - 3,800 CFU m^{-3} air

in Dunhuang, China (W. Wang et al., 2010) and 1,018 CFU m^{-3} air in the Mediterranean ecosystem (Vokou et al., 2012). In contrast, the culture-independent technique reported the abundance of airborne bacteria at 3.3×10^{3} to 1.0×10^{6} ribosomal operons m^{-3} air in the urban area of Milan, Italy (Bertolini, 2013). Compared with the results from our 2-years monitoring study, the airborne pyrene-degrading bacteria was considered to form minor populations in the urban area. Consequently, their role in pyrene biodegradation was disregarded in further study.

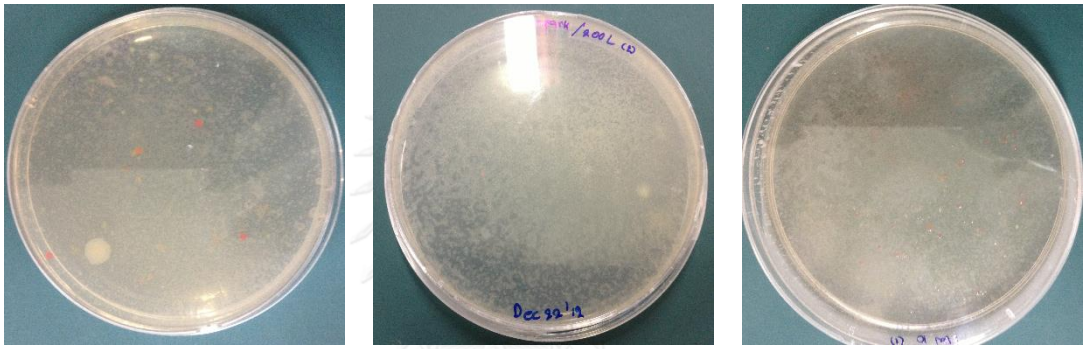


Figure 4.1 Colonies characteristic of airborne pyrene-degrading bacteria at the roadside areas

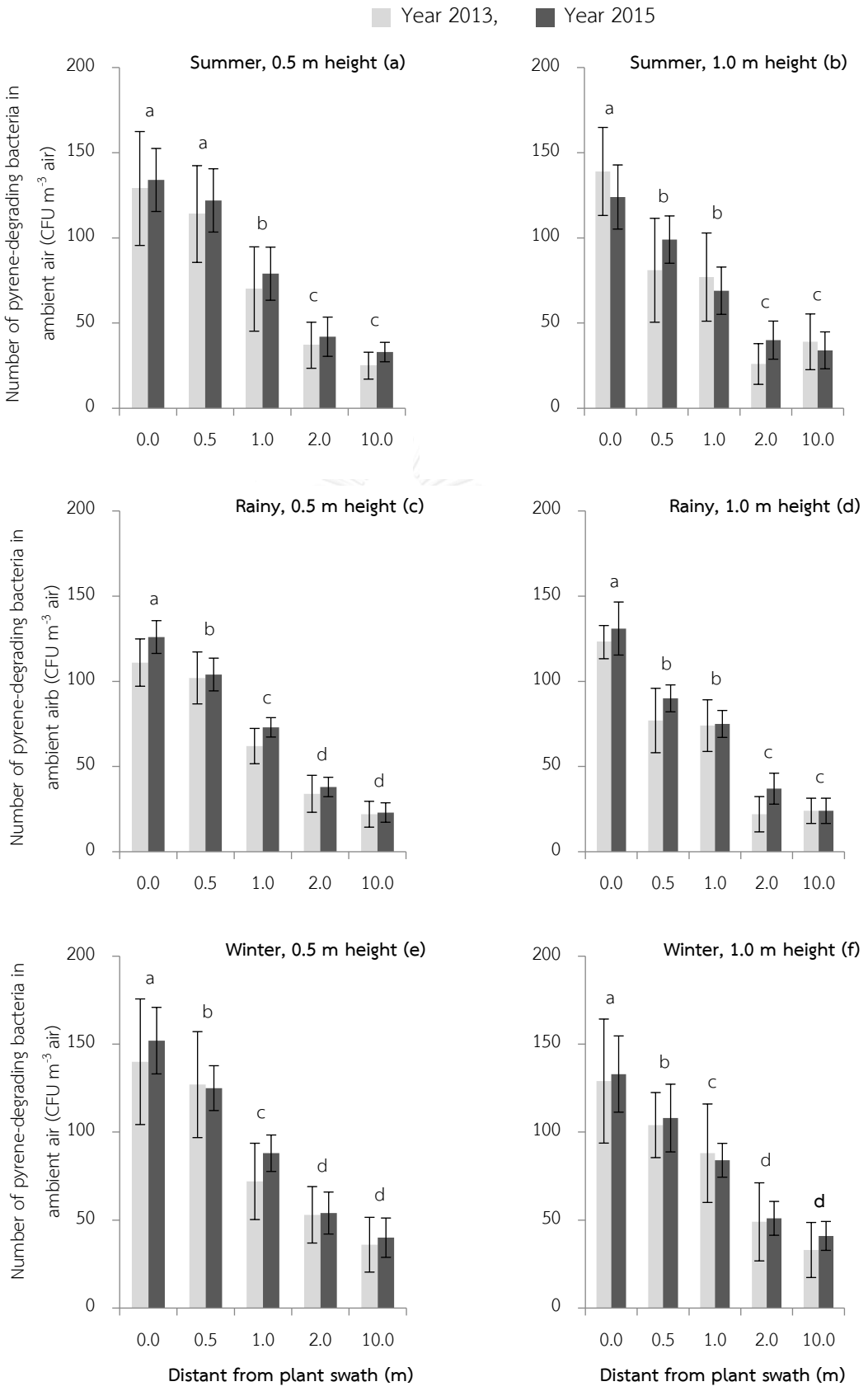

Year 2013,
Year 2015
Summer, 0.5 m height (a)
Summer, 1.0 m height (b)
Rainy, 0.5 m height (c)
Rainy, 1.0 m height (d)
Winter, 0.5 m height (e)
Winter, 1.0 m height (f)
Number of pyrene-degrading bacteria in ambient air (CFU m-3 air)
Number of pyrene-degrading bacteria in ambient airb (CFU m-3 air)
Number of pyrene-degrading bacteria in ambient air (CFU m-3 air)
0
50
100
150
200
0.0
0.5
1.0
2.0
10.0
Distant from plant swath (m)
Distant from plant swath (m)

Figure 4.2 Effect of plant locations on the abundance of airborne pyrene-degrading bacteria. The air samples were collected at 0.5 and 1.0 m height above ground in 2013 (light grey bars) and 2015 (dark grey bars) for summer (a, b), rainy (c, d) and winter seasons (e, f). Error bars represent standard deviation (n = 5). Different letters represent significant differences ($p < 0.05$) between distant from plant swath. The bacterial numbers from 2013 and 2015 at the same distant were statistically analyzed together (n = 10).

The weather conditions during ambient air and leaves sampling were illustrated as Table 4.1. Meteorological conditions of each season between 2013 and 2015 were not much different. The amount of rainfall on each sampling day was lower than the average amount at 223.7 and 188.0 mm of 2013 and 2015, respectively. The leaves and air sampling time were selected to avoid the rainfall during the sampling time because the higher amount of rain fall might wash the bacteria from leaves and ambient air.

Table 4.1 The meteorological condition among leaves and air sampling as shown in below table.

	Meteorological conditions among sampling time					
Season, Year	**Average Temperature (^oC)**	**Average Air pressure (mb)**	**Average Wind speed (km h^{-1})**	**Average Humidity (%)**	**Average Solar radiation (W m^{-2})**	**Amount of rain fall (mm)**
Summer, 2013	31.4	1006.9	9.9	67.6	610.9	no rainfall
Rainy, 2013	29.4	1007.3	6.3	75.7	359.6	6.0 - 42.7
Winter, 2013	25.9	1007.4	3.9	66.9	456.5	no rainfall
Summer, 2015	31.5	1008.7	11.0	76.2	452.4	no rainfall
Rainy, 2015	28.3	1006.5	7.1	82.0	465.3	2.0 - 58.0
Winter, 2015	26.9	1007.8	6.1	78.5	426.4	no rainfall

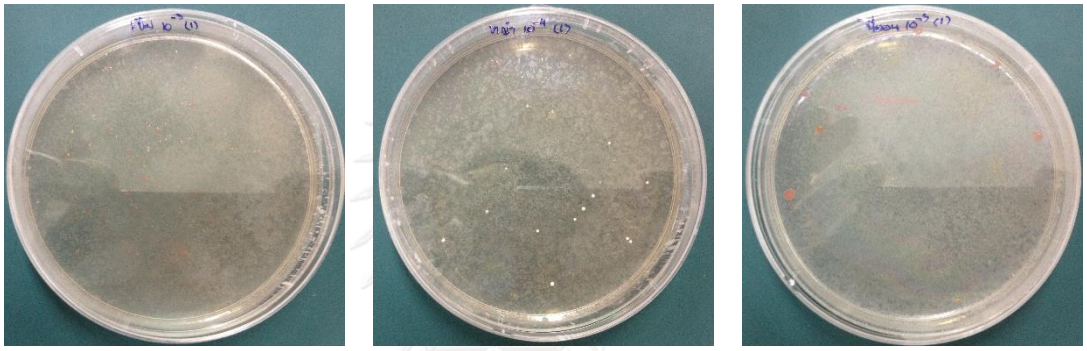
4.1.2 Presence of pyrene-degrading bacteria on plant leaves

Phyllosphere pyrene-degrading bacteria in 2013 and 2015 averaged 5×10^4 CFU g^{-1} on leaves for all tested plant species and accounted for approximately 7% of the total bacterial population (Figure 4.4). The highest amount of pyrene-degrading bacteria was found on *Ixora coccinea* (Ic) leaves at $9.6\pm0.9\times10^4$ and $7.9\pm1.3\times10^4$ CFU g^{-1} in summer and winter seasons, respectively (Figure 4.4b and f). Waight et al. (2007) also found that the highest number of phenanthrene-degrading phyllosphere bacteria was found on *Ixora* sp. in the summer of 2005. They suggest that the high wax content (0.7%) in *Ixora* sp. might be one of factors that promote the colonization of phenanthrene-degrading bacteria. Meanwhile, the *I. coccinea* plants had similar pyrene-degrading bacterial number when compared with other plants except for *Leucophyllum frutescens* (Lf) in rainy seasons of both sampling years (Figure 4.4d). The results confirmed the effects of rainfall on bacterial wash out.

The pyrene-degrading bacteria were isolated from plant leaves and identified as *Bacillus* sp., *Janibacter* sp., *Kocuria* sp., *Methylobacterium* sp., *Microbacterium* sp. and *Staphylococcus* sp. (Table 4.2). *Kocuria* sp. was found in all plant species, thus it was expected as the most prevalent pyrene-degrading bacteria in these plant swaths (Table 4.2). Previously, bacteria in the genus *Kocuria* were isolated from soil and found to efficiently degrade HMW-PAHs such as *Kocuria* sp. P10 (Sun et al., 2014) and *Kocuria* sp. B26 (Zafra, Absalón, Cuevas, & Cortés-Espinosa, 2014). The results suggested that *Kocuria* sp. is a HMW-PAH-degrading bacteria that is associated with the soil and plants.

The amounts of total phyllosphere bacteria from different plant species ranged from 10^5 - 10^6 CFU g^{-1} on leaves (Figure 4.4a, c and e). This study had a higher bacterial number than the phyllosphere under the harsh conditions of the Mediterranean, which was lower than 10^5 CFU g^{-1} on leaves (Vokou et al., 2012). The fluctuated environmental conditions in rainy seasons resulted in the lowest number of total

bacteria with high variability among plant species (Figure 4.4a, c and e). *L. frutescens* leaves had the significantly highest number of total bacteria, with an average of 10^6 CFU g^{-1} on leaves (Figure 4.4a, e). The leaves of *L. frutescens* were covered with short hair settling on adaxial and abaxial surfaces (Table 4.2, Figure 3.1), which could increase the surface area for phyllosphere bacterial colonization. In conclusion, the different numbers of total and pyrene-degrading bacteria on leaf surfaces were influenced by the plant morphology and environmental conditions.



Ixora coccinea *Axonopus compresus* *Leucophyllum frutescens*

Figure 4.3 Colonies characteristic of phyllosphere bacteria from different plant species

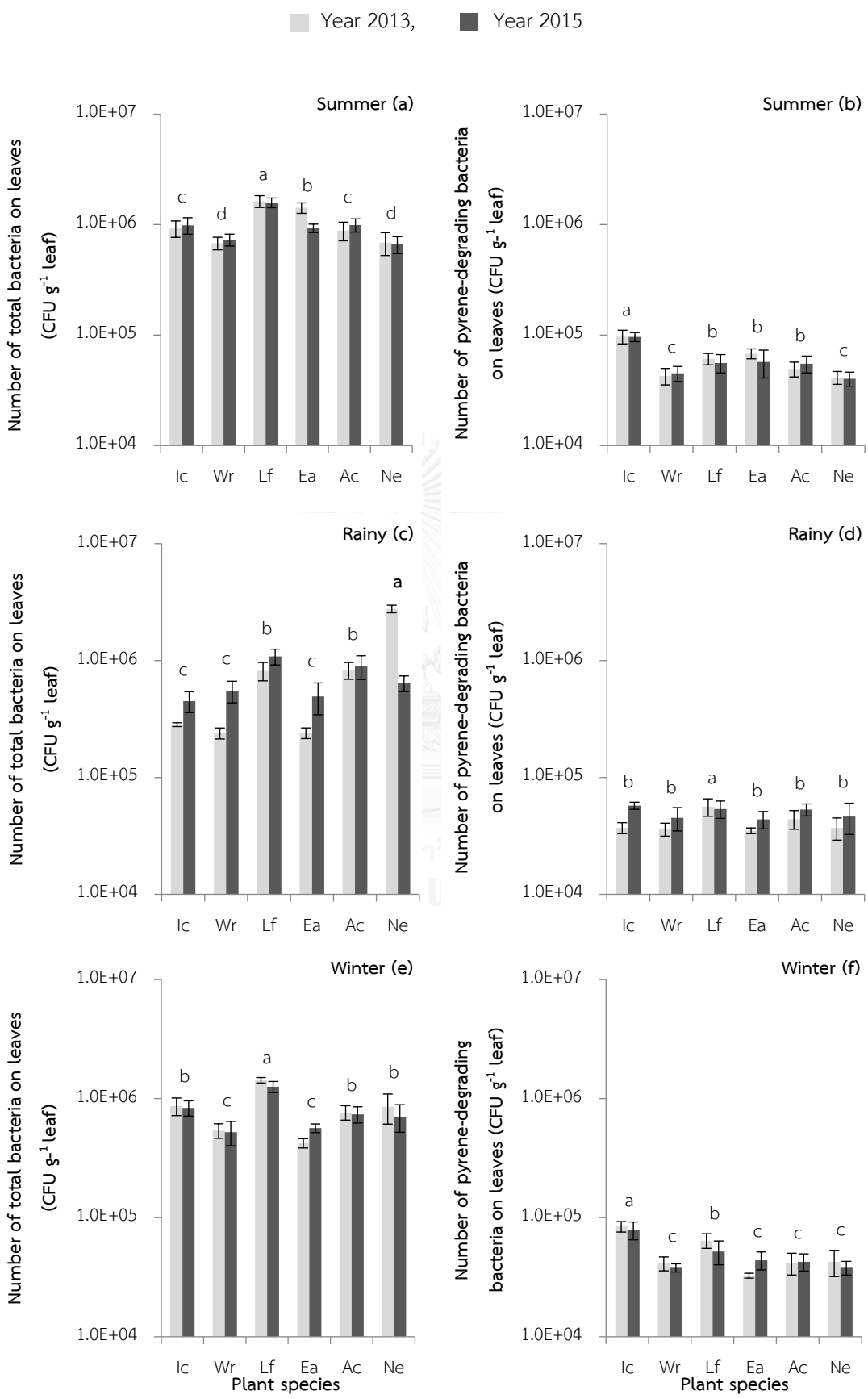

Year 2013,
Year 2015
Summer (a)
Summer (b)
Rainy (c)
Rainy (d)
Winter (e)
Winter (f)
Number of total bacteria on leaves (CFU g-1 leaf)
Number of pyrene-degrading bacteria on leaves (CFU g-1 leaf)
1.0E+07
1.0E+06
1.0E+05
1.0E+04
Ic Wr Lf Ea Ac Ne
Plant species

Figure 4.4 Effect of plant species on the abundance of phyllosphere pyrene-degrading bacteria. The leaf samples were collected in 2013 (light grey bars) and 2015 (dark grey bars) for summer (a, b), rainy (c, d) and winter seasons (e, f). The plant species were *Ixora cocccinea* (Ic), *Wrightia religiosa* (Wr), *Leucophyllum frutescens* (Lf), *Epipremnum aureus* (Ea), *Axonopus compresus* (Ac) and *Nephrolepis exaltata* (Ne). Error bars represent standard deviation (n = 3). Different letters represent significant differences ($p < 0.05$) between distant from plant swath. The bacterial numbers from 2013 and 2015 at the same distant were statistically analyzed together (n = 6).

Table 4.2 Ornamental plants with their leaves properties and isolated pyrene-degrading bacteria.

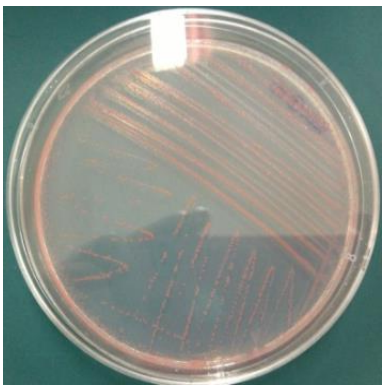
Plant species	Leaves properties		Isolated pyrene-degrading bacteria (Number of isolated strains)
	Area (cm^2 $leaf^{-1}$)	Moisture (% fw.)	
Ixora coccinea	2.1±0.7	34.6±0.5	*Methylobacterium* sp. (1) *Kocuria* sp. (2) *Bacillus* sp. (1)
Wrightia religiosa	13.1±2.2	28.2±0.3	*Methylobacterium* sp. (1) *Kocuria* sp. (1) *Janibacter* sp. (1)
Leucophyllum frutescens	3.4±1.9	38.2±0.6	*Kocuria* sp. (2) *Bacillus* sp. (1) *Microbacterium* sp. (1)
Epipremnum aureus	25.2±2.1	52.2±1.4	*Methylobacterium* sp. (1) *Bacillus* sp. (1) *Staphylococcus* sp. (1)
Axonopus compresus	7.2±2.4	29.7±0.8	*Kocuria* sp. (2) *Bacillus* sp. (1)
Nephrolepis exaltata	3.5±0.4	31.8±1.3	*Methylobacterium* sp. (1) *Kocuria* sp. (1)

4.1.3 Characterization of pyrene-degrading bacteria from *Ixora coccinea* leaves

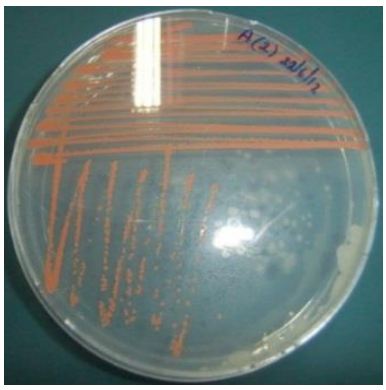
I. coccinea was selected as a model plant for further study due to its high number of pyrene-degrading bacteria. Four strains of pyrene-degrading bacteria, including *Methylobacterium* sp. IC1, *Kocuria* sp. IC2, *Kocuria* sp. IC3 and *Bacillus* sp. IC4, were isolated from *I. coccinea* leaves for use as bacterial models (Figure 4.5 and Table 4.3). After culturing in pyrene containing broth, these bacteria exhibited low cell surface hydrophobicity, with the percentage of cells adhering to n-hexadecane ranging from 16±5 to 30±2% (Table 4.3). The cell surface hydrophobicity allowed the bacterial cells to attach with hydrophobic compounds. Similarly, after grown on fluoranthene and anthracene, 14% and 16% of the *Pseudomonas putida* cells adhered to n-hexadecane, respectively (Rodrigues et al., 2013).

The biosurfactant-producing capability of isolated bacteria was tested by examining the reduction of surface tension compared to culture broth (69±1 mN m^{-1}). The surface tension obtained from *Kocuria* sp. IC2 and IC3 was 53±2 and 50±3 mN m^{-1}, respectively, which was significantly lower than that of *Methylobacterium* sp. IC1 (58±1 mN m^{-1}) and *Bacillus* sp. IC4 (60±4 mN m^{-1}) (Table 4.3). The results indicate that *Kocuria* sp. IC2 and IC3 has the potential to produce biosurfactants, but their abilities were low compared to those of other known biosurfactant producing bacteria (Mnif & Ghribi, 2015). Consequently, a surfactant should be applied to these bacterial cultures to enhance pyrene bioavailability and biodegradability.

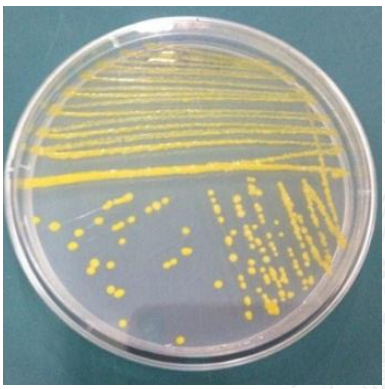
The biofilm formation of isolated bacteria was investigated after cultivating the bacteria in various media including 0.5X NB, 0.5X NB+glycerol and CFMM+pyrene. The results were concluded from all media and showed that these bacteria had weak to moderate biofilm formation (Table 4.3).



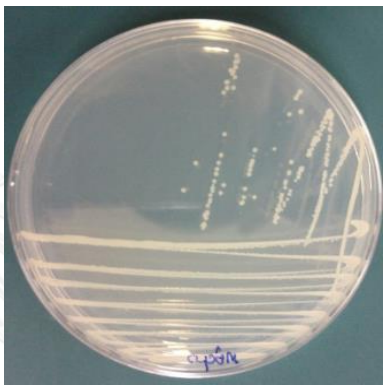
Methylobacterium sp. IC1



Kocuria sp. IC2



Kocuria sp. IC3



Bacillus sp. IC4

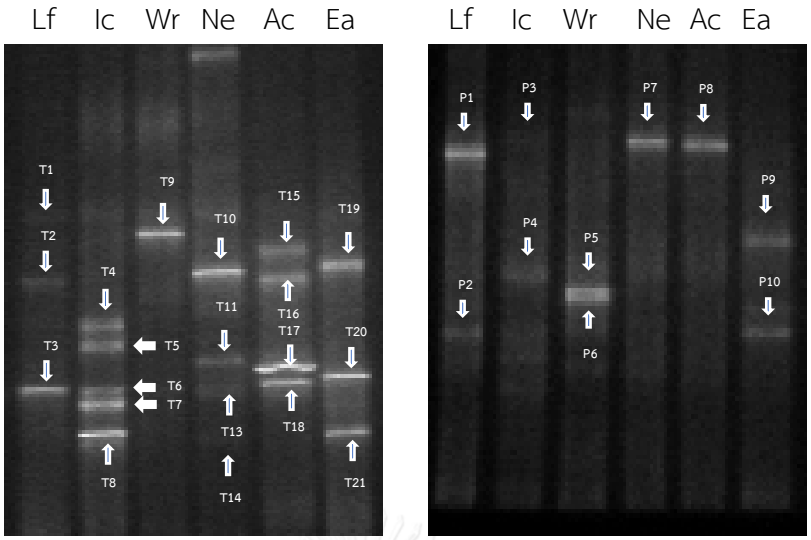
Figure 4.5 Colony characteristic of isolated pyrene-degrading bacteria from *Ixora coccinea* leaves

Table 4.3 Characteristics of pyrene-degrading bacteria isolated from *I. coccinea* leaves. Different letters in the same column represent significant differences ($p<0.05$) between bacterial strains.

Bacterial strain	16S rDNA length (bp) (Percent identity)	NCBI closest match (Accession number)	Cell surface hydrophobicity (%)	Surface tension (mN m^{-1})	Biofilm formation
IC1	1430 (99%)	*Methylobacterium* sp. (NC 010725.1)	25±3[a]	58±1[a]	Weakly
IC2	1453 (98%)	*Kocuria* sp. (NC 010617.1)	24±5[a]	53±2[b]	Moderately
IC3	1410 (100%)	*Kocuria* sp. (KF 306369.1)	30±2[a]	50±3[b]	Weakly
IC4	1446 (99%)	*Bacillus* sp. (NC 016047.1)	16±5[b]	60±4[a]	Moderately

4.1.4 Characterization of bacterial communities by PCR-DGGE

Diversity of total and pyrene-degrading bacterial populations on plant leaves was preliminary analyzed by PCR-DGGE analysis of 16S rDNA. The DGGE profiles of both total and pyrene-degrading bacteria showed different band patterns which compared among plant leaves species (Figure 4.6). A total of 31 DGGE bands migrating to different position. These are labeled T1 to T21 for total phyllosphere bacteria and P1 to P10 for pyrene-degrading bacteria of six ornamental plants. The majority of dominant bands appeared to be unique for each plant sample. The sequence analysis of these 31 DNA samples was not success due to the interference DNA, impurities and insufficient amount of DNA. In addition, the researcher could not clone the dominant DNA bands to *E. coli* and the DNA samples were later degraded.



(a) Total phyllosphere bacteria (b) Pyrene-degrading bacteria

Figure 4.6 Composite 16S rDNA gene directed PCR-DGGE banding profiles of (a) total bacteria and (b) pyrene-degrading bacteria of plant species consists of: *L. frutescens* (Lf), *I. coccinea* (Ic), *W. religiosa* (Wr), *N. exaltata* (Ne), *A. compresus* (Ac), *E. aureus* (Ea). The symbols T1 to T21 bands are total bacteria and P1 to P10 bands are pyrene-degrading bacteria which were indicate dominant bands selected for sequencing. Both gels had equal denaturant gradients of 30-70%.

Although, the identification of dominant bacterial populations was not carried out in this study. Our results indicated that bacterial community structures analyzed by PCR-DGGE from each ornamental plant species presented distinct band patterns. This was similar to Yutthammo et al. (2010), which reported that the distinctiveness of these phyllosphere bacterial communities was relatively due to the variation in leaf morphologies and chemical properties of plant species. To better identify the phyllosphere bacteria, Whipps et al., (2008) proposed the analysis of 16S rDNA cloned directly from leaf samples and they demonstrated that proteobacteria are the dominant group found on leaves.

The isolated pyrene-degrading bacteria from *I. coccinea* leaves consisted of *Methylobacterium* sp. IC1, *Kocuria* sp. IC2, *Kocuria* sp. IC3, *and Bacillus* sp. IC4. These four species were not reported in Yutthammo et al. (2010), which found phenanthrene-degrading populations in the genera of *Acinetobacter*, *Pseudoxanthomonas*, *Mycobacterium*, *Pseudomonas* and various uncultured bacteria. Moreover, this results were not similar to Lors et al., (2011) that investigated the bacterial community during bioremediation of PAHs in a coal tar contaminated soil. They found that PAH-degrading bacterial community structures were mainly Gamma-proteobacteria, in particular, the *Enterobacteria* and *Pseudomonas* genera. The discrepancy might be due to the different between culture and molecular techniques used in each study. Nonetheless, this study later focused on culturable bacteria because they could be used as model for investigating activity in laboratory scale.

4.1.5 Investigation of particulate matter (PM_{10}) in ambient air

Particulate matters (PM_{10}) in ambient air were collected at two opposite sites across the Prachachuen road at Bang Sue, Bangkok in summer, rainy and winter, respectively of 2015 (Figure 4.7). The meteorological conditions (average data) during samplings were illustrated in Table 4.1 (data from Thai Meteorological Department). The PM concentrations were investigated and the results shown at roadside area 1 and 2 that 144.07 ± 0.75 and 72.70 ± 1.92 μg m^{-3} in summer; 82.86 ± 0.26 and 61.70 ± 0.75 μg m^{-3} in rainy; 123.13 ± 3.87 and 110.50 ± 2.27 μg m^{-3} in winter (Figure 4.7). The sampling site 1 was located at the intersection. It, therefore, had the amount of PM on roadside higher than sampling site 2, the avenue. In addition, the sampling site 1 had more PM released time than the sampling site 2 from traffic light control and U-turn point, it could cause more contact time and less air variation on leaf surface. This results related to Sahanavin et al. (2017), which investigated the relationship between PM10 and PM2.5 level in high-traffic areas in Bangkok. They demonstrated that

meteorological conditions e.g. wind speed, temperature, and relative humidity had a direct effect on the particulate level and that the effect of traffic flow were more variable in open areas. They found that PM_{10} concentration was 102.52±38.68 μg m^{-3} and $PM_{2.5}$ concentration was 54.94±23.83 μg m^{-3} in open traffic areas (Sahanavin, Prueksasit, & Tantrakarnapa, 2017). Moreover, Thongsanit et al. (2003) found that the daily PM_{10} concentrations at heavy traffic roadside areas ranged between 30 and 160 μg m^{-3}. The highest PM_{10} level occurred during the winter period (dry season), which similar to this study as higher PM concentration at roadside 2 at 110.50±2.3 and 123.13±3.9 μg m^{-3} at roadside 1, respectively. In addition, they suggested that based on a 1-yr survey, the particulate concentrations are associated with traffic volumes and seasonal factors (temperature and rainfall) which they found the lower PM concentration in rainy season (Thongsanit, Jinsart, Hooper, Hooper, & Limpaseni, 2003).

The PM concentrations at plant swath 1 and 2 were lower than roadside 1 and 2 in every seasons. The PM at concentrations plant swath 2 were lower than the plant swath 1 and there were 103.54 ± 0.98, 60.51 ± 0.49, and 81.21 ± 0.99 μg m^{-3} in summer, rainy, and winter, respectively (Figure 4.7). Similar to the roadside, the sampling point 1 located at the intersection had more PM released and contact time. From this results, the PM concentrations at plant swath were similar to Terzaghi et al. (2013) and Sæbø et al (2012). They recommended that plant leaves play a major role in removing PM and their associated semi-volatile organic compounds (SVOCs) from the atmosphere. In addition, important traits for PM accumulation were leaf properties such as wax cover or hair (Saebo et al., 2012; Terzaghi et al., 2013). From this phenomena, the plant leaves were a sink and sources of PM associated particle-bound air pollutants. For example, Howsam et al. (2000) found higher PAH concentrations in hairy leaves compared to hairless leaves collected in the same area. Hairs increase the leaf surface that is able to capture particulate from the air; in addition hairs favor a boundary layer of stagnant air on the leaf surface, improving particle retention.

The level presented in terms of capturing PM can be used to select plant species for air pollution removal in urban areas. Thus, the efficient plant species and planting designs can shield susceptible areas in urban settings from polluting traffic or anthropogenic activities. However, the importance of leaf benefit in removing PM and air pollutants from the atmosphere has also been highlighted and has contributed to the polluted environments (Saebo et al., 2012).

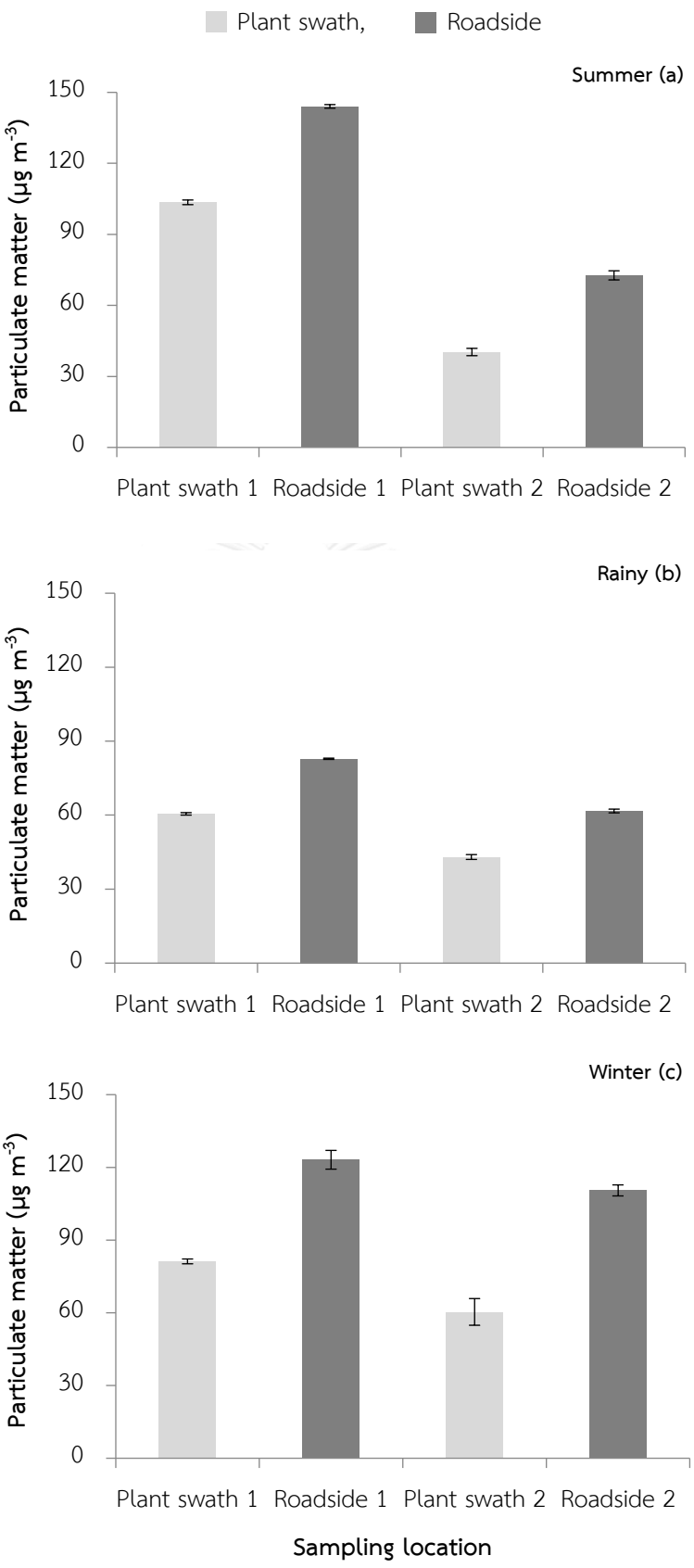

Plant swath,
Roadside
Summer (a)
Particulate matter (µg m-3)
150
120
90
60
30
0
Plant swath 1
Roadside 1
Plant swath 2
Roadside 2
Rainy (b)
Particulate matter (µg m-3)
150
120
90
60
30
0
Plant swath 1
Roadside 1
Plant swath 2
Roadside 2
Winter (c)
Particulate matter (µg m-3)
150
120
90
60
30
0
Plant swath 1
Roadside 1
Plant swath 2
Roadside 2
Sampling location

Figure 4.7 The amount of particulate matter different sampling point. The particulate matter were collected at plant swath (light grey bars) and roadside (dark grey bars) forsummer (a), rainy (b) and winter seasons (c) in 2105.

4.1.6 Investigation of particle-bound PAHs (pPAHs) profile and concentration

Generally, most of PAHs with low vapor pressure in the ambient air are adsorbed on fine particles. More concentrations of PM would provide more concentration of PAHs. Thus, PAHs contained in PM might concentrate more at the roadside areas than within the proximity to plant swath (Figure 4.7). To clarify this phenomenon, the particle-bound PAHs (pPAHs) on filter were extracted and analyzed. This study investigated the pPAHs at four sampling points, which divided into 2 sampling sites at the roadside and 2 sampling sites within the proximity of ornamental plant swath. Moreover, the concentration of pPAHs at each sampling point were compared among summer, rainy, and winter seasons.

The pPAHs concentrations monitored at roadside area 1 and 2 were 20.554 and 14.342 ng m^{-3} in summer; 16.511 and 14.263 ng m^{-3} in rainy; 17.779 and 17.062 ng m^{-3} in winter, respectively (Figure 4.8). This results related to the amount of PM in each point. Thus, higher concentrations of PM presented are also provided higher concentration of pPAHs. The pPAHs concentrations at plant swath 1 and 2 were lower than roadside 1 and 2 in every seasons. The pPAHs concentrations at plant swath 2 were 8.174, 8.658, and 9.021 ng m^{-3} in summer, rainy, and winter, which lower than plant swath 1 (Figure 4.8). Correspondingly, the sampling point 1 had located at the intersection which had more PM released and contact time.

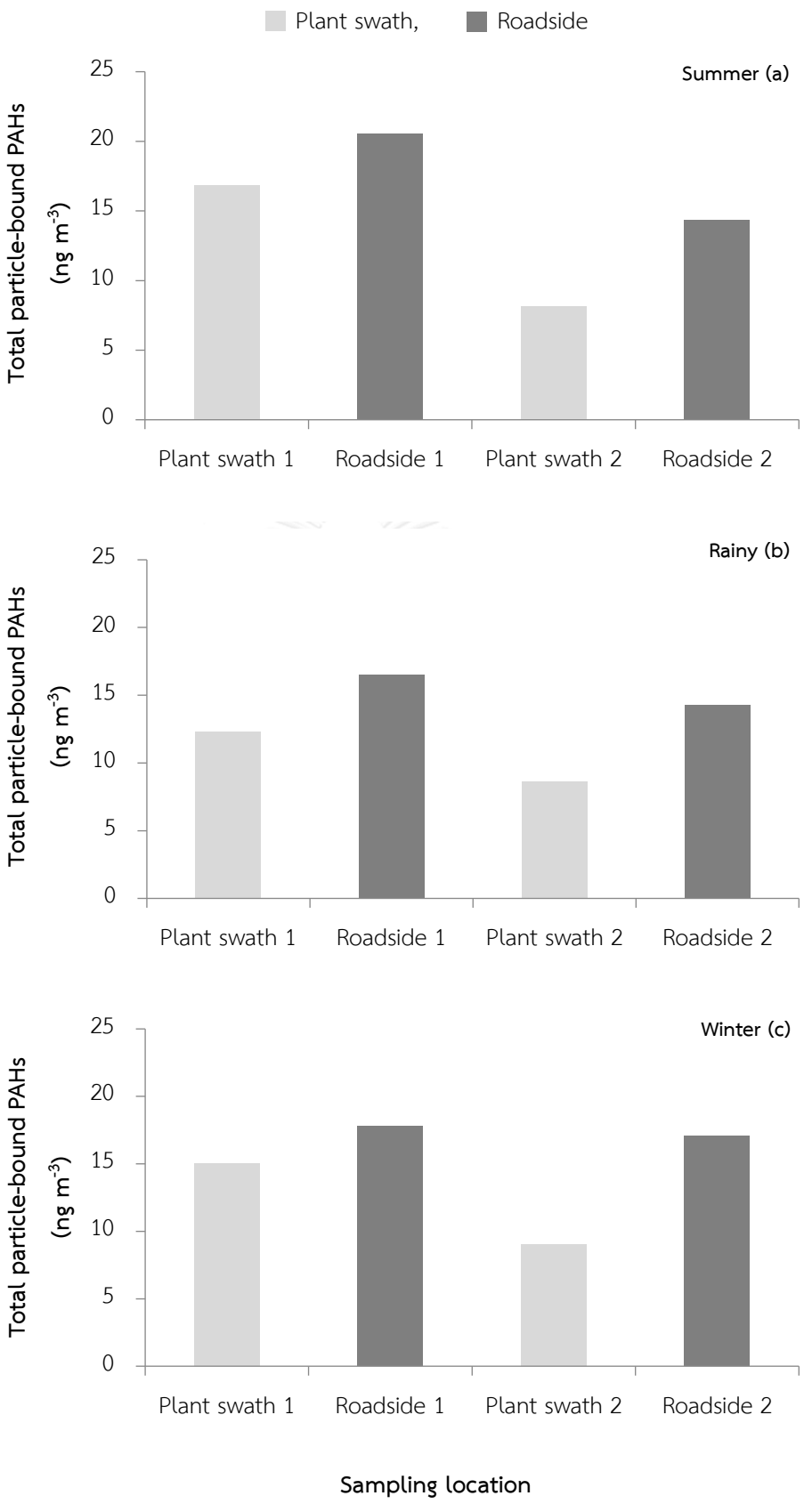
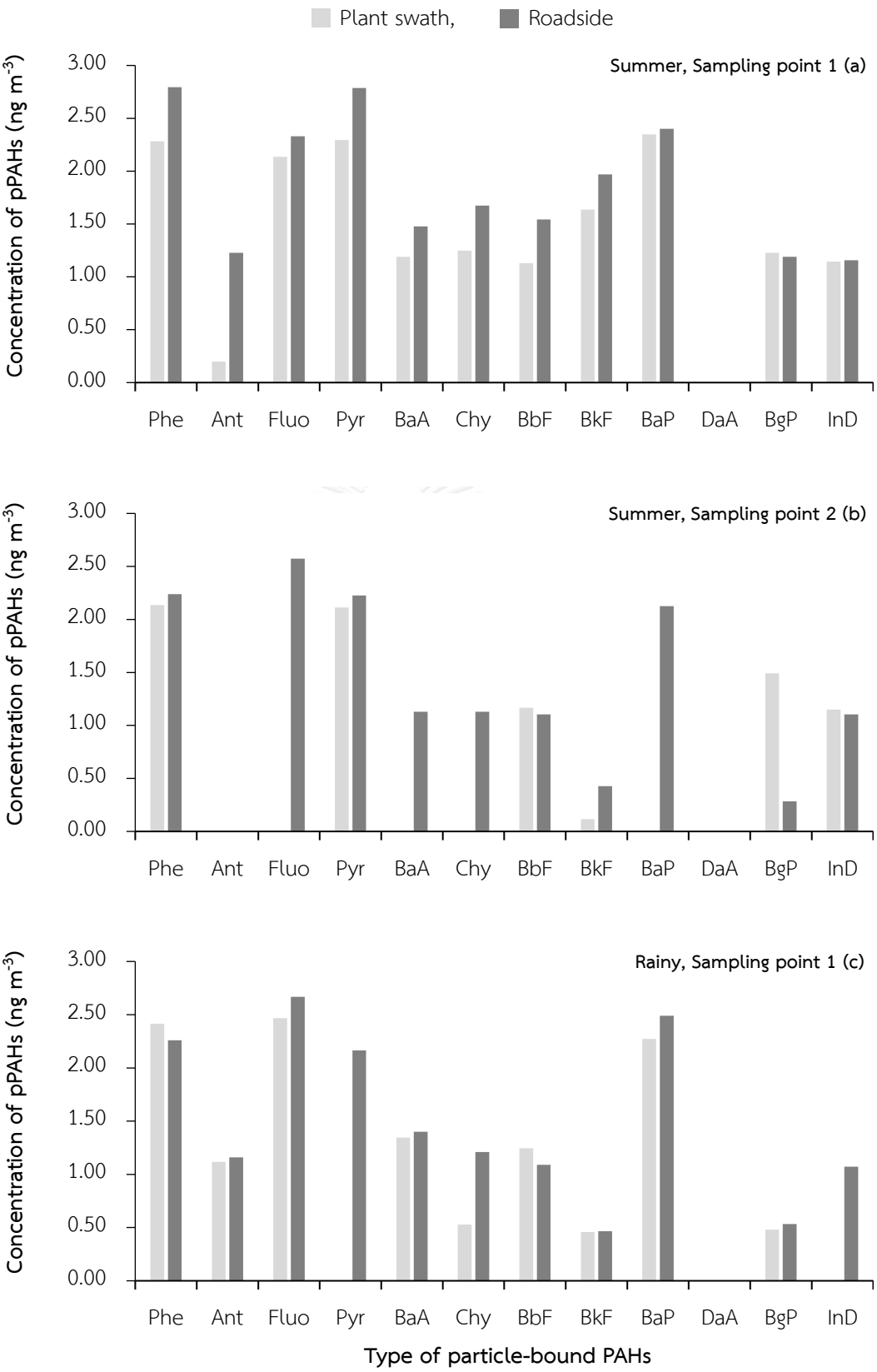

Plant swath,
Roadside
Summer (a)
Rainy (b)
Winter (c)
Total particle-bound PAHs (ng m-3)
25
20
15
10
5
0
Plant swath 1
Roadside 1
Plant swath 2
Roadside 2
Sampling location

Figure 4.8 The amount of total particle-bound PAHs extracted from particulate matter at different sampling season. The total HMW-PAHs were collected at the plant swath (light grey bars) and roadside (dark grey bars) for summer (a), rainy (b) and winter seasons (c) in 2105.

The dominant species of pPAHs were presented in Figure 4.9. The results demonstrated that the predominant species of pPAHs of all sampling points consisted of phenanthrene (Phe), fluoranthene (Fluo), pyrene (Pyr), benzo (a) pyrene (BaP), benzo (ghi) perylene (BgP) and indenol (1,2,3-C,D) pyrene (Ind.). The first four species of pPAHs were selected for simulate the mixed HMW-PAHs in the gas-tight chamber experiment (section 4.3.3). The pPAHs species in this study are similar to Thongsanit et al. (2003), which collected air samples from six sites in Bangkok, Thailand. They found several dominant pPAHs e.g. benzo (ghi) perylene (BgP), indenol (1,2,3-C,D) pyrene (Ind.), benzo (b) fluoranthene (BbF), and benzo (a) pyrene (BaP).


Plant swath,
Roadside
Summer, Sampling point 1 (a)
Summer, Sampling point 2 (b)
Rainy, Sampling point 1 (c)
Concentration of pPAHs (ng m-3)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Phe
Ant
Fluo
Pyr
BaA
Chy
BbF
BkF
BaP
DaA
BgP
InD
Type of particle-bound PAHs

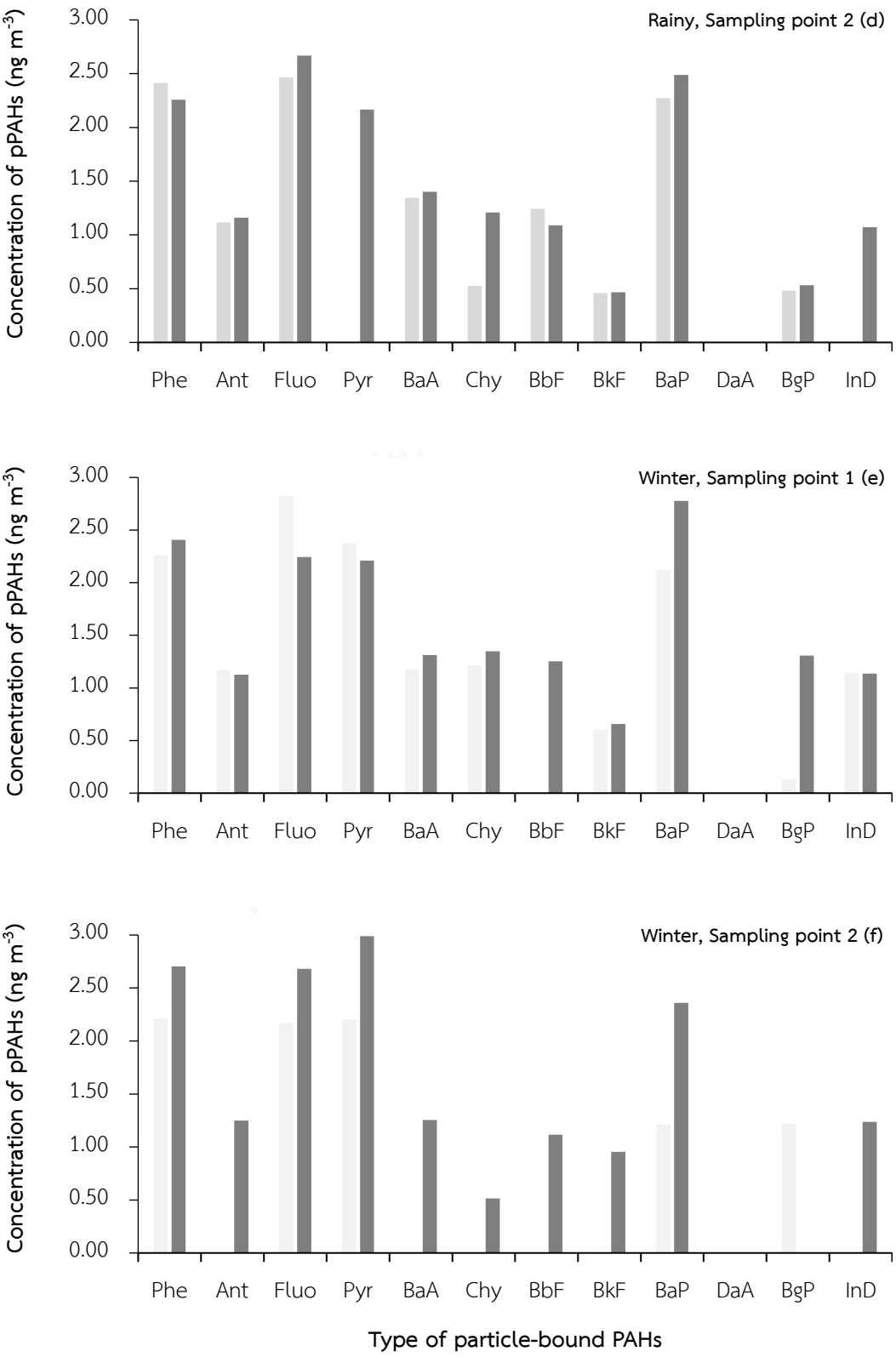

Rainy, Sampling point 2 (d)
Winter, Sampling point 1 (e)
Winter, Sampling point 2 (f)
Concentration of pPAHs (ng m-3)
3.00
2.50
2.00
1.50
1.00
0.50
0.00
Phe
Ant
Fluo
Pyr
BaA
Chy
BbF
BkF
BaP
DaA
BgP
InD
Type of particle-bound PAHs

Figure 4.9 The type of particulate-PAHs extracted from particulate matter at different sampling points. The total particulate-PAHs were collected at plant swath (light grey bars) and roadside (dark grey bars) for summer (a, b), rainy (c, d) and winter seasons (e, f) in 2105.

4.2 Effect of surfactant on enhancing bioavailability and biodegradability of pyrene

4.2.1 Toxicity of surfactants on isolated bacteria and plants

The selected surfactant should not be toxic to both phyllosphere bacteria and plants. Thus, this study first exposed the four isolated bacteria to various concentrations of surfactants, including lipopeptide biosurfactant, 5 types of synthetic surfactants and a dispersant, Slickgone NS. The minimum bactericidal concentration (MBC) of each surfactant is shown in Table 4.4. The MBC of each bacterial strain was dependent on the type of surfactant. However, *Bacillus* sp. IC4 was more sensitive than were the other strains. The biosurfactant and Tween 20 did not inhibit bacterial growth at concentrations lower than 50 g L^{-1} for all bacterial strains, whereas Dehydol LS7TH, Dehydol LS9TH, Triton X-100 and Slickgone NS inhibited each bacterium at different concentrations. Similarly, Aryal and Liakopoulou-Kyriakides (2013) reported that Triton X-100 could inhibit bacterial growth, while Tween 20 was relatively non-toxic. Consequently, the biosurfactant and Tween 20 were selected for phytotoxicity test.

Table 4.4 Minimum bactericidal concentration of surfactants on isolated pyrene-degrading bacteria.

Bacterial strain	Biosurfactant (g L^{-1})	Synthetic surfactants (g L^{-1})					Slickgone NS (g L^{-1})
		SDS	Dehydol LS7TH	Dehydol LS9TH	Triton® X-100	Tween 20	
IC1	> 50.00	0.20	0.05	> 50.00	> 50.00	> 50.00	> 50.00
IC2	> 50.00	0.05	> 50.00	> 50.00	0.39	> 50.00	6.25
IC3	> 50.00	0.05	12.5	25.00	> 50.00	> 50.00	> 50.00
IC4	> 50.00	0.10	1.56	3.13	6.25	> 50.00	25.00

Since the phytotoxicity of surfactants depends on the given surfactant dose, this study investigated the effects of biosurfactant and Tween 20 at 1X CMC (i.e., 1 g L^{-1} and 60 mg L^{-1}, respectively) and 10X CMC (i.e., 10 g L^{-1} and 600 mg L^{-1}, respectively) after sprayed on *I. coccinea* leaves for 7 days. The changes in plant leaf morphology, including the changing of leaf color, number of faded leaves, misshaped or distorted leaves, dead spots or flecks on leaves and modifications in color, were not observed at all surfactant doses (Figure 4.10). The appearance of *I. coccinea* with biosurfactant and Tween 20 was similar to that of the untreated plants. Similarly, Yilmaz and Dane (2012) investigated the phytotoxicity of a surfactant (0.25% BioPower) on *Triticum aestivum* L. and suggested that a low concentration of surfactant would not dissolve epicuticular waxes. The biosurfactant was selected for the next experiment because it has better ecological acceptance, such as low toxicity and a biodegradable nature, compared to synthetic surfactants. Moreover, the biosurfactant is not expensive because its precursors are glycerol waste and palm oil (Khondee et al., 2015).

Biosurfactant (a) Tween-20 (b) DDW (c)

Figure 4.10 Phytotoxicity testing on *Ixora coccinea* plant by biosurfactant (a), Tween-20 (b) and control, DDW (c) at day 7 after exposure.

4.2.2 Effect of surfactant on enhancing pyrene solubilization and desorption

The pyrene solubilization test was conducted with various biosurfactant concentrations. The pyrene solubilized efficiencies were significantly increased when the biosurfactant concentrations were ≥1X CMC (Figure 4.11a). The amounts of solubilized pyrene were 22±1, 24±1 and 28±1 mg L^{-1} in 1X, 5X and 10X CMC biosurfactant solution, respectively (Figure 4.11a). The solubilization behavior is generally attributed to the incorporation or partitioning of organic solute within surfactant micelles formed at ≥1X CMC. Nonetheless, the pyrene solubilization efficiencies of biosurfactant solutions at 0.1X and 0.5X were similar and significantly higher than did water. Das et al. (2008) reported that crude biosurfactant produced by *Bacillus subtilis* DM-04 and *Pseudomanas aeruginosa* NM in the range of 0-0.5 g L^{-1} can solubilize phenanthrene, anthracene and pyrene at 0.5-1.1, 0.04-1.1 and 0.13-1.2 mg L^{-1}, respectively. Compared to this study, the biosurfactant had a higher pyrene

solubilization efficiency, which was probably due to higher biosurfactant concentrations (0.1-10 g L^{-1}) (P. Das, Mukherjee, & Sen, 2008).

The ability of a biosurfactant to desorb pyrene at 500 mg g^{-1} leaf was investigated from sterilized *I. coccinea* leaves. After surface sterilization, the residual total phyllosphere bacteria and pyrene-degrading bacteria were $9.9\pm1.1\times10^{3}$ and $8.5\pm2.0\times10^{2}$ CFU g^{-1} leaf, respectively. Therefore, the loss of pyrene was due more to the desorption process than pyrene biodegradation. The pyrene desorption efficiencies of biosurfactant solution at $\geq$1X CMC were significantly increased with increasing biosurfactant concentrations, and the highest pyrene desorption of 221±10 mg L^{-1} was found at 10X CMC of biosurfactant (Figure 4.11b). Nonetheless, the biosurfactant solution at 0.1X - 0.5X CMC had a significantly higher desorption activity than did water (DDW) and the concentrations of desorbed pyrene ranged from 65±4 mg L^{-1} to 89±5 mg L^{-1}, respectively (Figure 4.11b). Therefore, the low concentration of biosurfactant (0.1X CMC) could be used to desorb the deposited pyrene on plant leaves. Bordoloi and Konwar (2009) and (Gottfried, Singhal, Elliot, & Swift, 2010) also reported that biosurfactant addition effectively enhanced solubilization, bioavailability and biodegradation of phenanthrene, fluorene and pyrene (Bordoloi & Konwar, 2009; Gottfried et al., 2010). The relationship between pyrene solubilization and desorption and pyrene biodegradability after adding the biosurfactant was tested in further experiments.

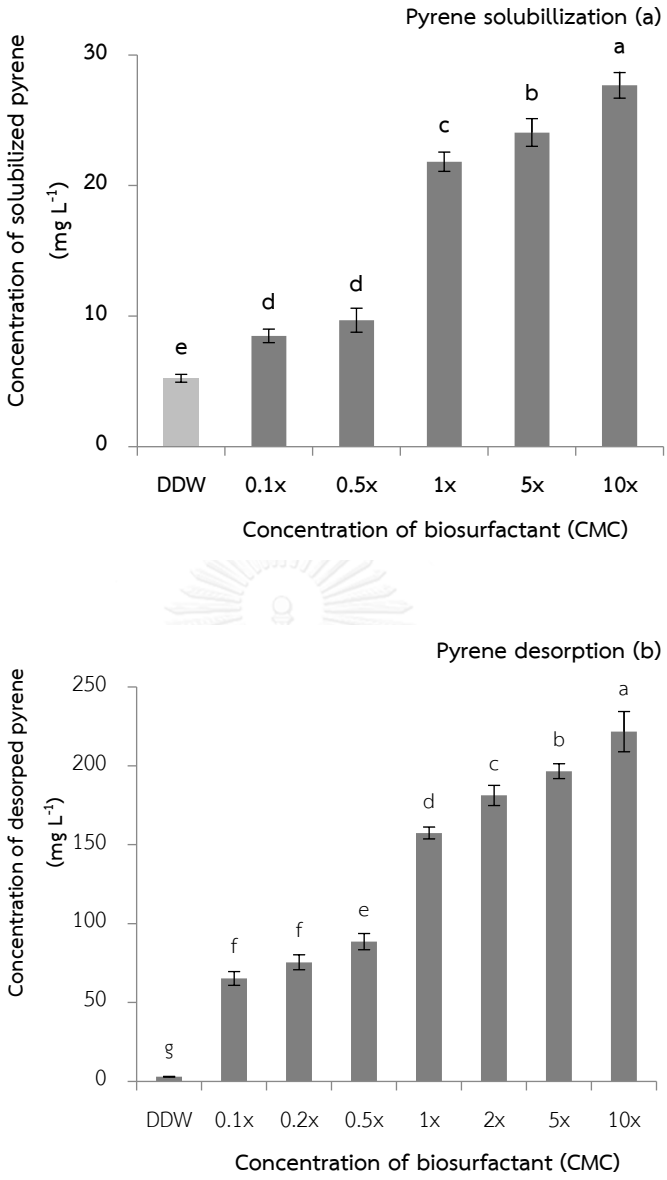


Figure 4.11 Effect of biosurfactant concentrations on pyrene solubilization (a) and pyrene desorption (b) from *I. coccinea* leaves. Error bars represent standard deviation, of which the numbers of sample for solubilization and desorption tests were 3 and 5, respectively. Different letters represent significant differences ($p < 0.05$) among biosurfactant concentrations.

4.2.3 Pyrene biodegradation by isolated phyllosphere bacteria

The ability of a biosurfactant to enhance pyrene biodegradation was investigated with *Methylobacterium* sp. IC1, *Kocuria* sp. IC2, *Kocuria* sp. IC3 and *Bacillus* sp. IC4. The initial pyrene concentrations at 50, 100 and 200 mg L^{-1} were applied to each culture with and without 1 g L^{-1} (1X CMC) of biosurfactant. Regardless of the initial pyrene concentrations, the controls showed that pyrene reduction by an abiotic process was in the range of 10-15% (Figure 4.12). All of the bacterial strains effectively degraded the low concentration of pyrene (50 mg L^{-1}), and the addition of biosurfactant was not required (Figure 4.13). When the initial pyrene concentration was increased, three bacterial strains, including *Methylobacterium* sp. IC1, *Kocuria* sp. IC2 and *Kocuria* sp. IC3, had a significantly higher pyrene degradation efficiency in the presence of the biosurfactant than those in the absence of the biosurfactant ($p < 0.05$). In addition, *Kocuria* sp. IC3 had the highest pyrene degradation efficiency at all tested concentrations (Figure 4.13c). At the initial 100 mg L^{-1} of pyrene, this strain reached 88±1% of pyrene degradation within the shortest time of 21 days and degraded most of the pyrene in 35 days. Moreover, this strain degraded 74±1% of 200 mg L^{-1} of pyrene, whereas the other strains could degrade only 50±1 to 65±1% of pyrene. Therefore, the addition of a biosurfactant at 1X CMC was necessary to enhance the bioavailability and biodegradability of $\geq$ 100 mg L^{-1} of pyrene. Nonetheless, the addition of the biosurfactant could not increase the efficiency of *Bacillus* sp. IC4 (Figure 4.13d), which suggests that other limitations might occur in this bacterium.

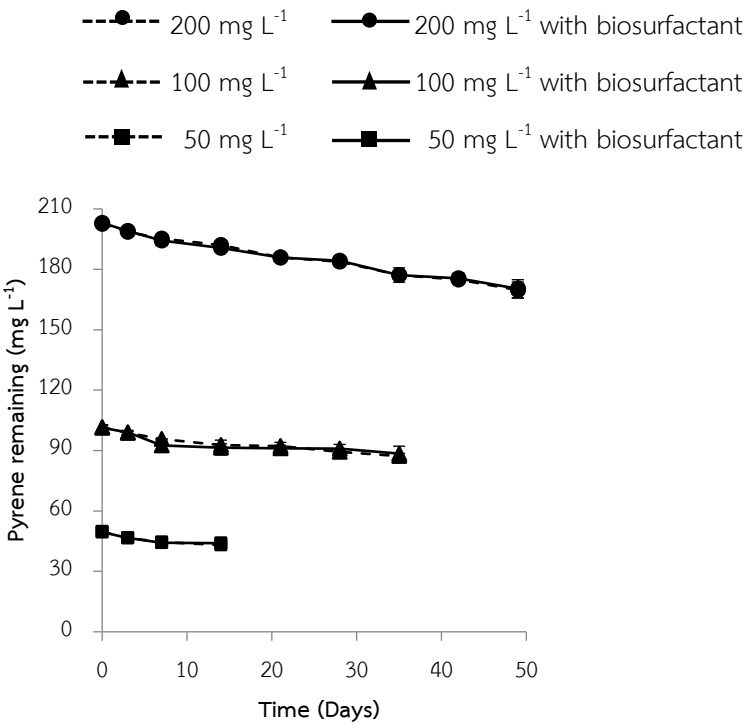


Figure 4.12 Pyrene remaining in the control experiments without bacterial inoculum.

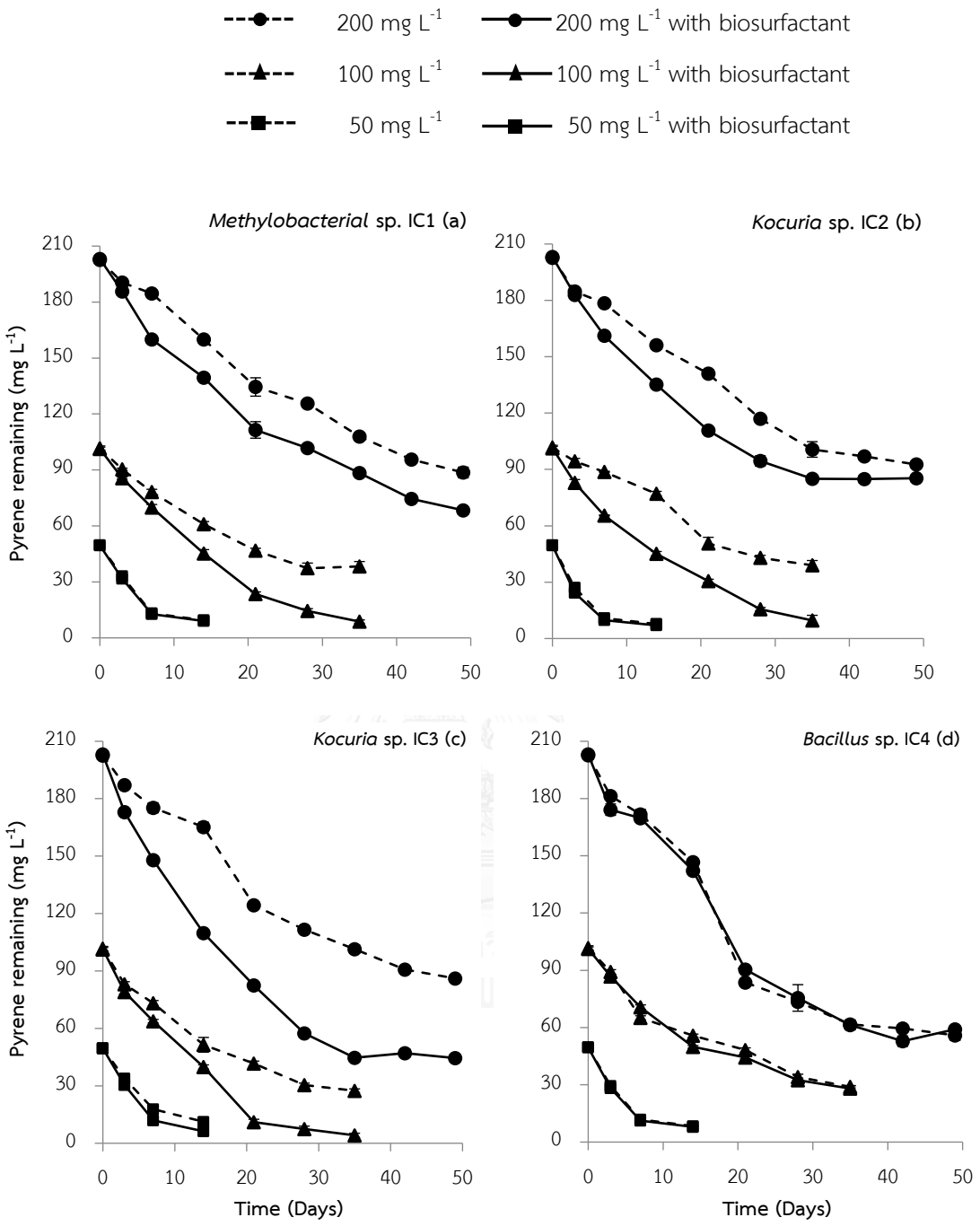


Figure 4.13 Degradation of 50-200 mg L^{-1} pyrene in CFMM broth by *Methylobacterium* sp. IC1 (a), *Kocuria* sp. IC2 (b), *Kocuria* sp. IC3 (c) and *Bacillus* sp. IC4 (d) with and without biosurfactant.

In the culture medium without pyrene or biosurfactant, the number of all bacteria was decreased (Figure 4.14). Thus, the increasing bacterial numbers after incubation indicated that these bacteria could utilize pyrene and/or biosurfactant as a growth substrate (Figure 4.15).

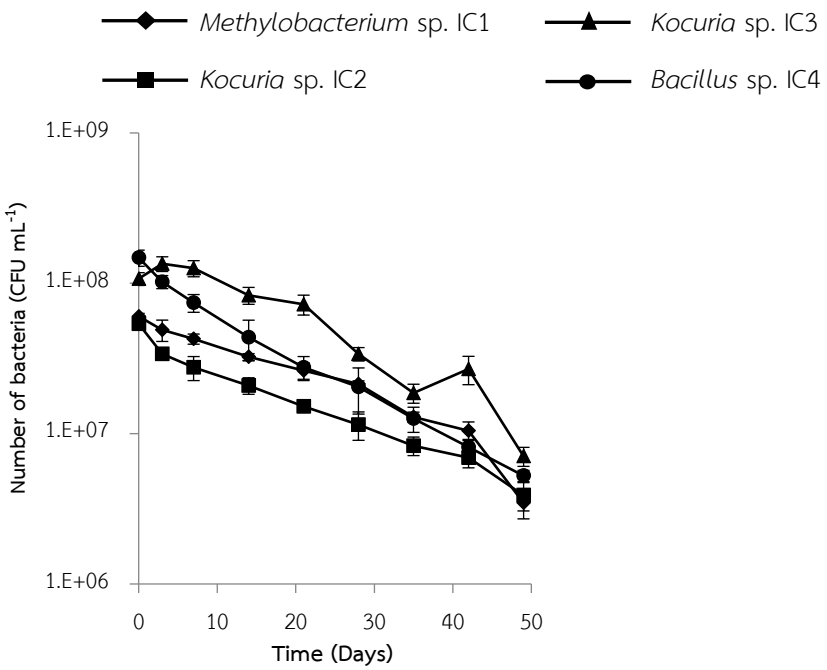


Figure 4.14 Number of *Methylobacterium* sp. IC1, *Kocuria* sp. IC2, *Kocuria* sp. IC3 and *Bacillus* sp. IC4 in CFMM broth without the addition of pyrene or biosurfactant.

The growth of *Methylobacterium* sp. IC1 (Figure 4.15a), *Kocuria* sp. IC2 (Figure 4.15b) and *Kocuria* sp. IC3 (Figure 4.15c) in the presence of a biosurfactant was significantly higher than those observed in the absence of a biosurfactant in all incubation periods ($p < 0.05$). *Kocuria* sp. IC3 had the significantly highest growth, which corresponded to its highest pyrene biodegradation efficiency (Figure 4.13c). Therefore, the solubilized pyrene in biosurfactant micelles was expected as the main carbon source for these three bacterial strains. Meanwhile, *Bacillus* sp. IC4 had a higher

bacterial number in the presence of a biosurfactant than that in the medium with only 50 mg L^{-1} of pyrene. When the initial pyrene concentrations were increased to 100 and 200 mg L^{-1} of pyrene, the bacterium similar growth in both the absence and presence of a biosurfactant (Figure 4.15d). These results suggest that the bacterium was able to use both the biosurfactant and pyrene as carbon sources. Consequently, the remaining biosurfactant molecules were not sufficient to enhance the bioavailability of pyrene at high initial concentrations. This finding corresponds to the low ability of biosurfactant to promote pyrene biodegradation by *Bacillus* sp. IC4 (Figure 4.15d). Ghosh and Mukherji (2016) also reported that the pyrene degradation by *Pseudomonas aeruginosa* is decreased due to the preferential degradation of an added biosurfactant. Since, three of the four phyllosphere bacterial strains had increased pyrene-degrading efficiency after adding the biosurfactant. It is possible to promote pyrene biodegradation on plant leaves by biosurfactant application.

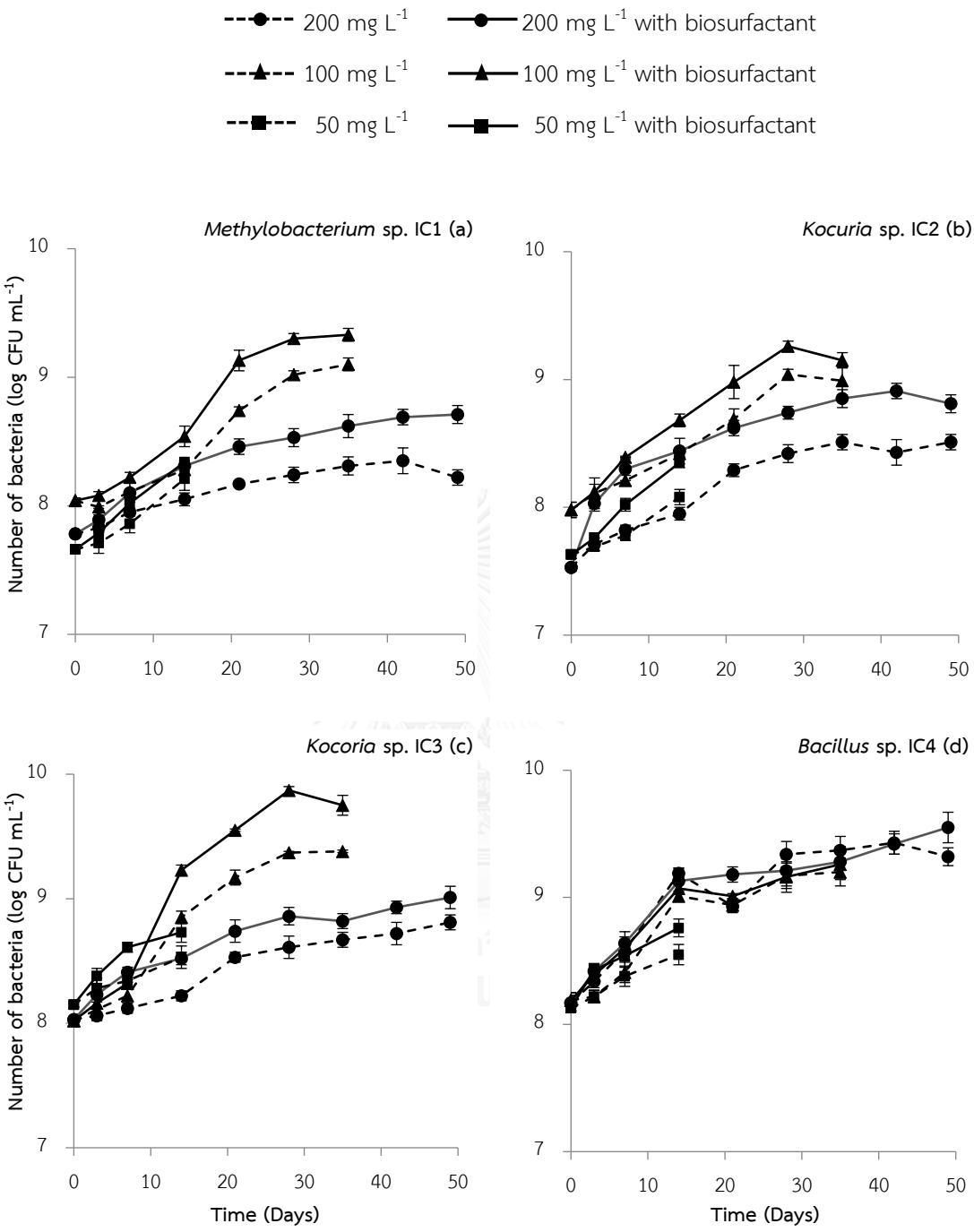


Figure 4.15 Number of *Methylobacterium* sp. IC1 (a), *Kocuria* sp. IC2 (b), *Kocuria* sp. IC3 (c) and *Bacillus* sp. IC4 (d) during the degradation of 50-200 mg L^{-1} pyrene in CFMM broth with and without biosurfactant.

Table 4.5 shown the rate and extent of pyrene degradation by pure culture isolated from *I. coccinea* leaves. All strains of pyrene-degrading bacteria with biosurfactant had higher biodegradation rates, except for *Bacillus* sp. IC4. This result was related to the low ability of biosurfactant to enhance pyrene biodegradation by *Bacillus* sp. IC4 (Figure 4.15d). *Kocuria* sp. IC3 with biosurfactant had the highest biodegradation rate at 0.0047 and 0.0052 mg ml^{-1} day^{-1} at the initial pyrene concentration 100 and 200 mg L^{-1}, respectively.

When compared the pyrene removal efficiency with other studies, the phyllosphere bacteria had lower activity than bacteria isolated from wastewater and polluted sediment such as those in Obayori et al. (2008), Zhong et al. (2012), and Ma et al. (2013) (Table 4.5). This was probably due to the higher pyrene concentration in the wastewater and polluted sediment than on plant leaves. Nonetheless, the presence of pyrene-degrading phyllosphere bacteria showed that there was a potential to remove the deposited pyrene from plant leaves. The bioaugmentation of plant leaves with isolated bacteria was investigated in the next study. The study did not used pyrene-degrading bacteria from somewhere else because those bacteria might not adapt or colonize on plant leaves as a habitat, lose ability to degrade the deposit-PAHs, or antagonist to indigenous microorganisms. Thus, the isolated indigenous bacteria were considered as an appropriate inoculum for pyrene phylloremediation.

Table 4.5 Comparison of the rate and extent of pyrene degradation by pure culture

Bacterial strain	Biodegradation rate (mg ml^{-1} day^{-1}), Time (day)		Degradation (%), Initial pyrene concentration (mg L^{-1}), Time (day)		Reference
	W/O BSF	W/ BSF	W/O BSF	W/ BSF	
Methylobacterium sp. IC1	0.0025	0.0034	58.29%, (100 mg L^{-1}), 28 d	84.15%, (100 mg L^{-1}), 28 d	This study
Kocuria sp. IC2	0.0021	0.0035	52.03%, (100 mg L^{-1}), 28 d	82.91%, (100 mg L^{-1}), 28 d	This study
Kocuria sp. IC3	0.0030	0.0047	66.06%, (100 mg L^{-1}), 28 d	87.91%, (100 mg L^{-1}), 21 d	This study
Bacillus sp. IC4	0.0029	0.0029	61.82%, (100 mg L^{-1}), 28 d	64.36%, (100 mg L^{-1}), 28 d	This study
Methylobacterium sp. IC1	0.0024	0.0035	45.35%, (200 mg L^{-1}), 49 d	57.59%, (200 mg L^{-1}), 42 d	This study
Kocuria sp. IC2	0.0031	0.0038	43.18%, (200 mg L^{-1}), 35 d	52.04%, (200 mg L^{-1}), 35 d	This study
Kocuria sp. IC3	0.0029	0.0052	48.18%, (200 mg L^{-1}), 42 d	74.80%, (200 mg L^{-1}), 35 d	This study
Bacillus sp. IC4	0.0042	0.0039	65.23%, (200 mg L^{-1}), 35 d	69.88%, (200 mg L^{-1}), 42 d	This study

Bacterial strain	Biodegradation rate (mg ml^{-1} day^{-1}), Time (day)		Degradation (%), Initial pyrene concentration (mg L^{-1}), Time (day)		Reference
	W/O BSF	W/ BSF	W/O BSF	W/ BSF	
Pseudomonas sp. LP1	0.1590	ND	68.0 %, (100 mg L^{-1}), 30 d	ND	(Obayori, Ilori, Adebusoye, Oyetibo, & Amund, 2008)
Stenotrophomonas sp. ZQ5	0.0091	ND	91.2 %, (100 mg L^{-1}), 10 d	ND	Ming et al., 2012
Pseudomonas sp. Jpyr-1	0.0015	ND	65.0 %, (200 mg L^{-1}), 20 d	ND	(Ma, He, Chen, Xu, & Rengel, 2010)

Remark: ND = No Data

4.3 Efficiency of selected plant species after spraying with selected surfactant on remediation of HMW-PAHs in box-model

4.3.1 Pyrene removal by *Ixora coccinea* twig, associated phyllosphere bacteria and biosurfactant in flip-top glass-tight jar

I. coccinea was used as a model plant for monitoring pyrene removal in a flip-top glass jar microcosm, which provided a partially gas-tight system to simulate the environment around the plant. Pyrene was sprayed on the leaf surface before closing the lid. The initial deposit-pyrene concentration on the leaves was 28.6±0.9 μg g^{-1} leaf. The sterilized leaves had the most pyrene remaining, at 18.9±3.0 μg g^{-1} leaf, whereas the leaves with *Kocuria* sp. IC3 and biosurfactant addition had the lowest pyrene remaining, with 8.1±2.4, 3.3±0.5 and 0.0±0.0 μg g^{-1} leaf at days 3, 7 and 14, respectively (Figure 4.17). The pyrene removal rates were found in the order of leaves with both biosurfactant and *Kocuria* sp. IC3 > leaves with biosurfactant > leaves > sterilized leaves at 24.1±2.3, 20.9±1.6, 15.2±1.0 and 6.0±1.5 μg day^{-1}, respectively (Table 4.6). On the other hand, the rate of pyrene removal in the microcosm without leaves was 2.8±0.7 μg day^{-1} (Table 4.6), which suggested that abiotic pyrene removal process e.g. photodegradation was minor.

Figure 4.16 *Ixora coccinea* twig in 2.5-L flip-top gas tight jar for removal of pyrene at 30 μg g^{-1} leaf at day 14.

Table 4.6 Pyrene degradation rate and percentage removal of batch experiment by *Kocuria* sp. IC3 and *I. coccinea* leaves with and without biosurfactant

Condition	Degradation rate ($\mu g\ day^{-1}$)	Percent removal (%)
Leaves with both biosurfactant and *Kocuria* sp. IC3	24.1±2.3	100.0±0.0
Leaves with biosurfactant	20.9±1.6	92.9±1.8
Leaves	15.2±1.0	74.2±2.1
Sterilized leaves	6.0±1.5	18.9±2.9
Abiotic	1.3±1.4	17.6±2.2

These results were similar to those of a study by Yutthammo et al. (2007), which compared the activities of phyllosphere bacteria on the reduction of gaseous phenanthrene in a small-scale chamber. They found that the phenanthrene reduction by unsterilized leaves was higher than that by sterilized leaves, and the concentrations of phenanthrene on day 4 were approximately 32-75% of day 1 in all plant species. Our study further showed that the addition of a biosurfactant enhanced the removal of pyrene from leaf surfaces. The leaves with a biosurfactant alone had slightly lower pyrene removal efficiency than the leaves with both *Kocuria* sp. IC3 and a biosurfactant, while its efficiency was significantly higher than normal leaves ($p < 0.05$). The activity of surfactant on promoting phytoremediation has been reported; for example, the addition of alkyl polyglucoside (an environment-friendly surfactant) increased the removal rates of anthracene, phenanthrene, and pyrene from soil cultivated with an aquatic plant, *Scirpus triqueter* (Liu et al., 2013).

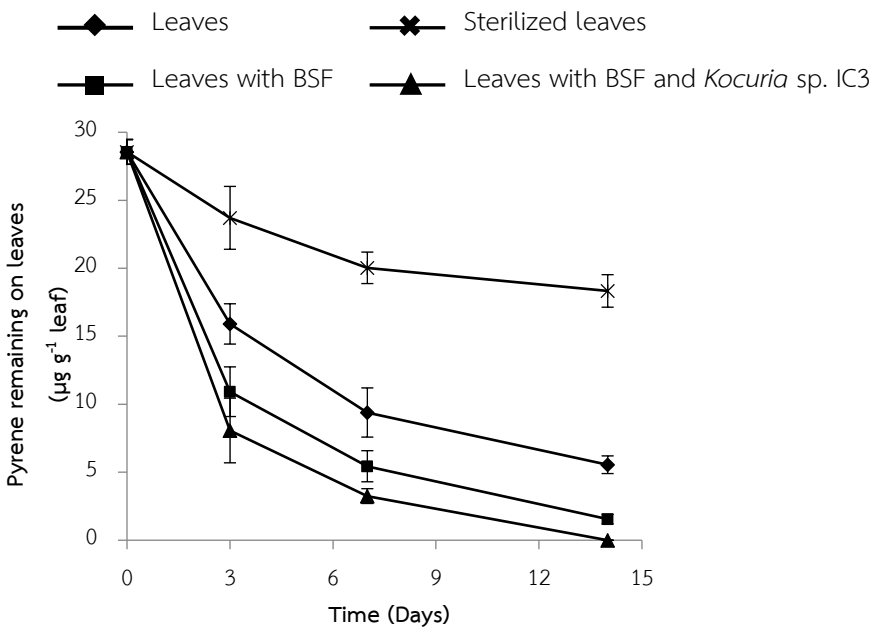


Figure 4.17 Removal of pyrene at 30 µg g^{-1} leaf in a batch experiment by *Kocuria* sp. IC3 and *I. coccinea* leaves with and without biosurfactant.

Without pyrene, the numbers of both total and pyrene-degrading bacteria were almost constant throughout the experiment (Figure 4.18). The numbers of total and pyrene-degrading bacteria were significantly increased in the presence of pyrene ($p < 0.05$) (Figure 4.17). These results suggest that the phyllosphere bacteria were able to utilize pyrene as a carbon source during the experiment. The highest number of pyrene-degrading bacteria was found on leaves with both *Kocuria* sp. IC3 and biosurfactant at $4.4\pm0.8\times10^{7}$ CFU g^{-1} leaf (day 14) (Figure 4.18b), which corresponds to the highest pyrene removal efficiency (Figure 4.17). The pyrene-degrading bacterial number was increased by less than one order of magnitude at the end of the study, which indicated that they could not grow well on leaf surfaces. To select for a better bacterial inoculum, Sorkhoh et al. (2011) isolated phyllosphere bacteria with the abilities to fix nitrogen (diazotrophic) and degrade hydrocarbons (Sorkhoh et al., 2011). They found that these bacteria could reduce volatilized crude oil under the limitation

of nutrients on plant leaves. In contrast, the number of pyrene-degrading bacteria on leaves with only a biosurfactant was significantly increased (p-value < 0.001) from $1.1\pm0.2\times10^5$ to $5.6\pm0.9\times10^6$ CFU g^{-1} leaf, which was higher than leaves without a biosurfactant at $1.6\pm0.3\times10^6$ CFU g^{-1} leaf on the final day of experimentation (Figure 4.18b). The correlation of pyrene-degrading bacteria on total phyllosphere bacteria (r = 0.94, p-value < 0.001) shown that pyrene-degrading bacteria was the major population on leaves during the experiment. The results indicated that the added biosurfactant was sufficient to enhance the growth and pyrene-degrading activity of the indigenous phyllosphere bacteria and the addition of pyrene-degrading bacteria on leaf surfaces might not be necessary. In fact, the cultivation of pyrene-degrading bacteria for application in the real environmental setting would be inconvenient and costly.

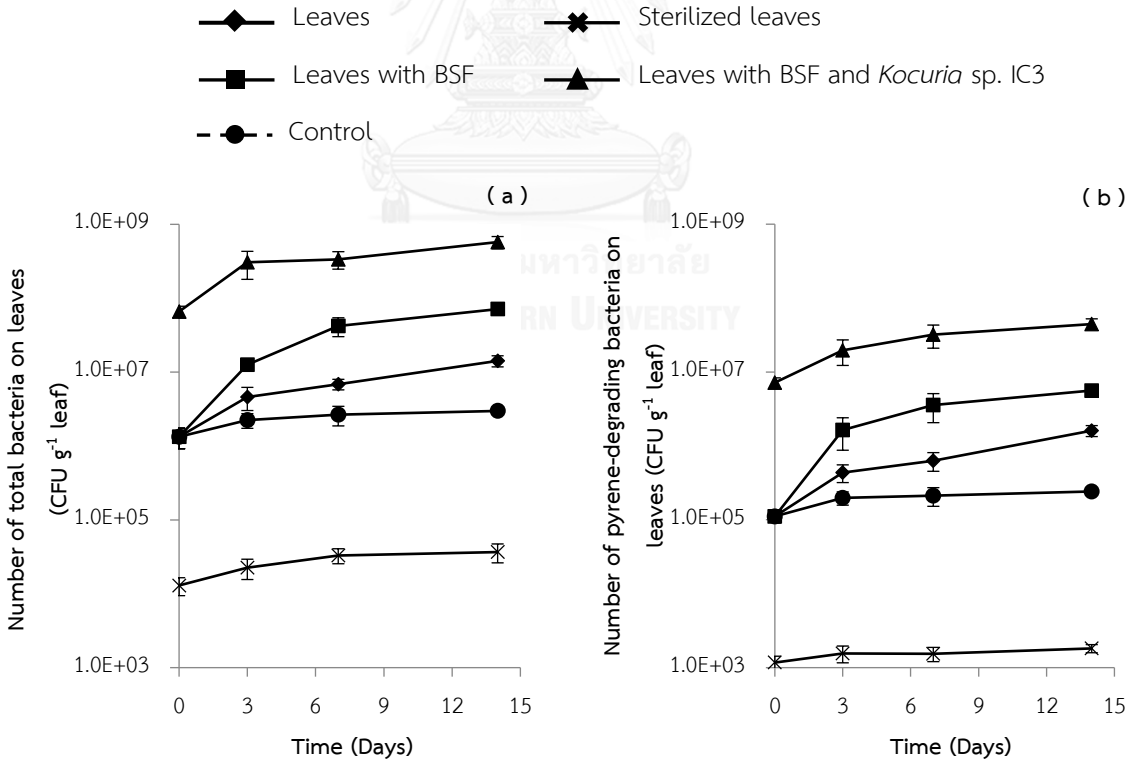


Figure 4.18 Number of total (a) and pyrene-degrading (b) bacteria on leaves during pyrene removal in a batch experiment containing *I. coccinea* leaves with and without biosurfactant.

4.3.2 Pyrene removal by *Leucophyllum frutescens* twig, associated phyllosphere bacteria and biosurfactant in flip-top glass-tight jar

From the results at section 4.1.2, *L. frutescens* had the significantly highest number of total phyllosphere bacteria in both summer and winter of 2013 and 2015 (Figure 4.4a, e). Moreover, the leaves of *L. frutescens* were covered with short hair settling on leaves surfaces (Figure 3.1), which could increase the surface area for phyllosphere bacterial colonization and deposition of particle-bound PAHs. Therefore, *L. frutescens* were selected to compare with the plant model, *I. coccinea.*

L. frutescens was hydroponically cultivation in batch experiment using flip-top glass jar to provide a partially gas-tight environment. Pyrene was sprayed on leaf surface before closing the system. The initial deposit-pyrene concentration on leaves was found at 28.9±0.4 μg g^{-1} leaf. Pyrene concentration in the control set (without leaves) showed about 9% decreasing at the end of incubation period. Meanwhile, pyrene reduction was found in the system with leaves. The pyrene remaining in all treatments with leaves were in range of 17.6±0.6 to 23.9±1.1 μg g^{-1} leaf at day 7. Thus, the plant had low pyrene removal efficiency at 3.6±0.6 to 29.9±1.0%. The sterilized leaves had the highest pyrene remaining at 23.9±1.1 μg g^{-1} leaf. Whereas the leaves with both *Kocuria* sp. IC3 and biosurfactant addition had the lowest pyrene remaining was 17.9±1.1 and 17.6±0.6 μg g^{-1} leaf at day 3 and 7, respectively. Leaves with biosurfactant had similar pyrene remaining at 18.9±0.9 μg g^{-1} leaf, whereas the leaves without biosurfactant had slightly higher pyrene remaining at 21.4±1.0 μg g^{-1} leaf after 7 days (Figure 4.20).

This result was in agreement with the previous experiment (section 4.3.1) that found the highest pyrene removal efficiency on the *I. coccinea* leave with both *Kocuria* sp. IC3 and biosurfactant addition. Moreover, the leaves with biosurfactant addition had higher pyrene removal efficiency, the leaves without biosurfactant had lower pyrene efficiency after 14 days.

However, the pyrene remaining on plant leaves between day 3 and 7 of all treatments was slightly decreased and was not different. This was because the *L. frutescens* leaves has decadence occurred after day 3 especially leaves with *Kocuria* sp. IC3 and biosurfactant addition. Moreover, there was high moisture content inside the glass jar from evapotranspiration. Some water droplets were found on dense-covered short hairs of the leaves and probably caused the decay. This plant cultivation condition might not appropriate for *L. frutescens* growth. Thus, the indigenous and inoculated bacteria could not grow well.

Control (a) Day 3 of treatment (b) Day 7 of treatments (c)

Figure 4.19 *Leucophyllum frutescens* twig in 2.5-L flip-top gas tight jar for removal of pyrene at 30 μg g^{-1} leaf compared among control set and different day of experiment.

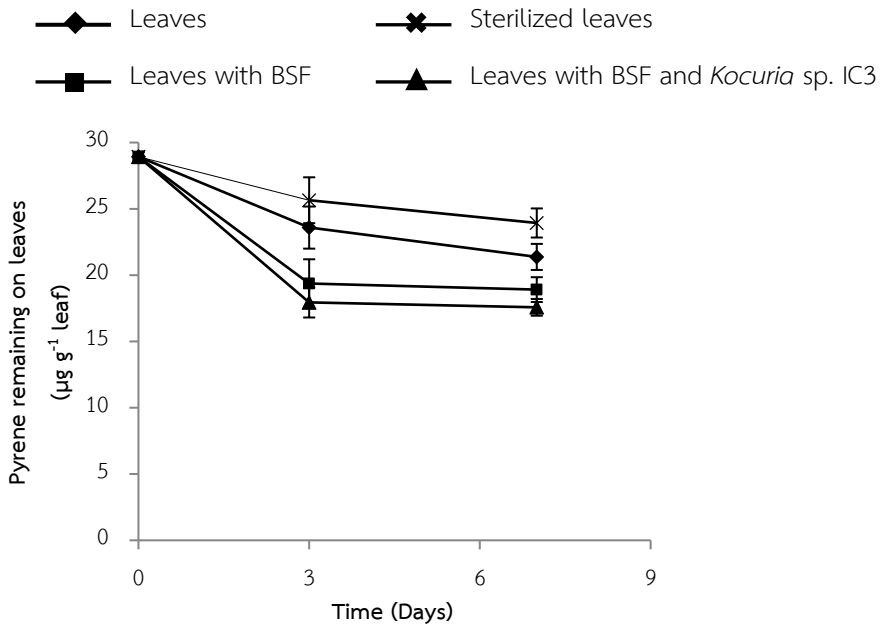


Figure 4.20 Removal of pyrene at 30 µg g^{-1} leaf a batch experiment by *Kocuria* sp. IC3 and *L. frutescens* leaves with and without biosurfactant.

The amounts of total phyllosphere bacteria (Figure 4.21a) on leaves were higher than pyrene-degrading bacteria (Figure 4.21b) in all treatments. The highest number of pyrene-degrading bacteria were found on leaves with *Kocuria* sp. IC3 and biosurfactant at 7.1±0.5×10^6 CFU g^{-1} leaf at initial time and they were significantly decreased to 2.7±0.5×10^5 CFU g^{-1} leaf at day 7. The number of pyrene-degrading bacteria was increased in the experiments with leaves and leaves adding biosurfactant at 1.7±0.4×10^5 and 4.7±0.6×10^5 CFU g^{-1} leaf, respectively at day 3. The results was related to the pyrene removal efficiency on day 3 and indicated that pyrene was used as a carbon source for bacterial growth. However, the number of total phyllosphere and pyrene-degrading bacteria among incubation period was significantly decreased which indicated that the indigenous and the added bacteria could not grow on decayed leaf surface under this experimental conditions.

From section 4.3.1 and 4.3.2, the pyrene degradation was achieved on both *L. frutescens* and *I. coccinae*. However, there is a difference removal efficiency when compared between plant species due to plant morphologies and characteristics. For a long term study, the plant should not cultivate in the gas-tight chamber because the system should have an air flow to reduce the accumulation of carbon dioxide and moisture that trap on the leaves surface.

Moreover, other ornamental plants should be investigated for applying to another areas e.g. smoking room or parking building. Therefore, several plant species might be plant together for removing many kind of air pollutants. Because, the system will contain various phyllosphere bacteria and probably enhance their bioavailability of PAHs by spraying the biosurfactant at time interval. Some researches show that there are methods to improve PAHs removal by using co-planting or multi-cropping. These methods can enhance PAHs or pollutants removal by increase the varieties of plant in the system (Wang et. al., 2013).

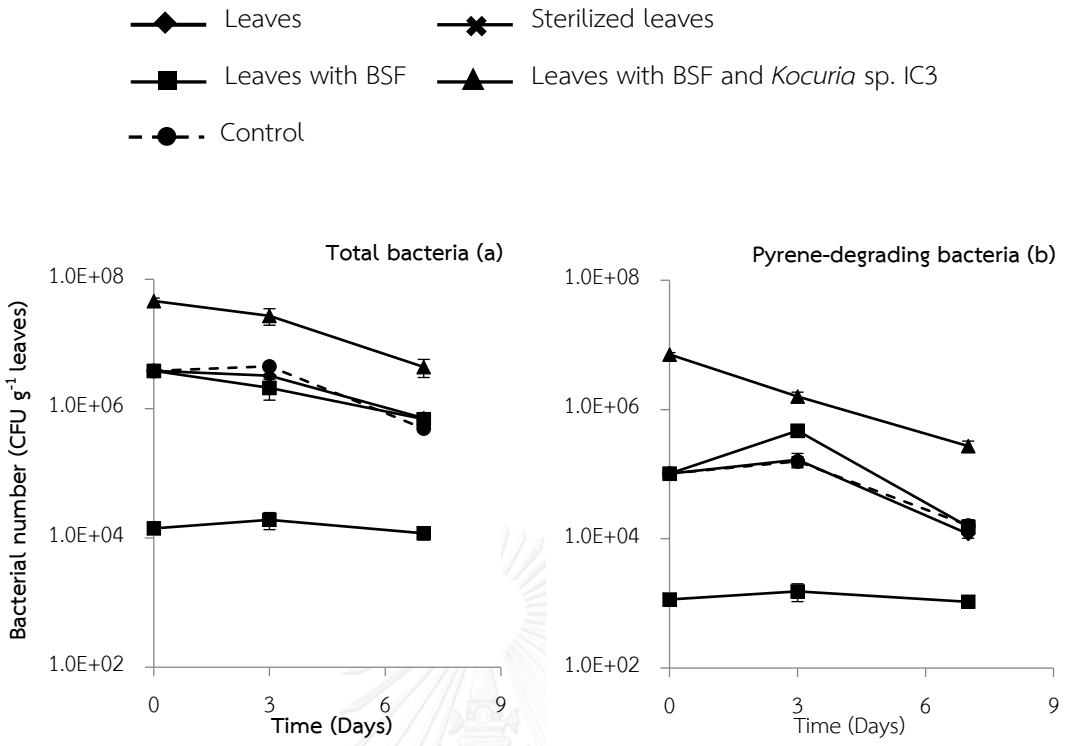


Figure 4.21 Number of total phyllosphere (a) and pyrene-degrading (b) bacteria on leaves during pyrene removal in the batch experiment containing leaves of *L. frutescens* with and without biosurfactant.

4.3.3 Mixed-PAHs removal by *Ixora coccinea* plant, associated phyllosphere bacteria and biosurfactant in chamber experiment

I. coccinea was used as a model plant due to the highest number of pyrene-degrading bacteria. This experiment were performed in 96-L acrylic chamber to investigate the mixed-PAHs reduction by plant, their phyllosphere bacteria and 0.1X CMC biosurfactant. The experimental chamber were scale up from 2.5 L flip-top jar in 4.3.1 and 4.3.2 to simulate the real environmental condition. The system contained inner air circulation for reducing moisture trapping on leaves, used a whole plant to have better plant and indigenous bacteria activities, and provided mixed-PAHs to simulate the dominant type of PAHs in urban air. The types of PAHs were selected from the results of PAH profile along roadside in section 4.1.6. However, the

concentration of mixed HMW-PAHs in this study were increased over the real concentrations that found in the ambient air at Prachachuen Road due to the low concentration of each species in the unit of ng m^{-3}. The initial concentration of phenanthrene, fluoranthene, and pyrene diffused in the air were 30.4±3.4, 21.3±0.6, and 21.2±1.0 μg L^{-1}, respectively. Benzo (a) pyrene in the air could not analyzed in this experiment due to its low volatilization property (vapor pressure at 5.49×10^{-9} mm Hg at 25 °C). On the other hand, phenanthrene, fluoranthene, and pyrene had higher volatilization property at 1.21×10^{-4}, 9.22×10^{-6}, and 4.56×10^{-6} mm Hg at 25 °C, respectively. After 14 days, the residual airborne concentrations of phenanthrene, fluoranthene, and pyrene were 5.90±0.8, 6.3±5.9, and 3.6±0.4 μg L^{-1}, respectively. The initial deposited phenanthrene, fluoranthene, pyrene and benzo (a) pyrene on leaves were 445.76±14.86, 429.56±9.26, 451.12±14.56, and 45.79±1.58 μg g^{-1} leaves, respectively. Whereas, the residual of deposited phenanthrene, fluoranthene, pyrene and benzo (a) pyrene were 59.29±13.54, 109.91±11.17, 151.37±20.17, 23.73±4.01 μg g^{-1} leaves, respectively.

The significant PAHs reduction from the air was found in the chamber with biosurfactant spraying on plant leaves (Figure 4.22). Each PAH were decreased along the incubation time. The reduction of phenanthrene, fluoranthene, and pyrene were 80.7±3.7, 63.7±5.9, and 78.9±3.1% in 14 days, respectively. Meanwhile, phenanthrene, fluoranthene, and pyrene in the air of control chamber (without plant leaves) decreased about 19.7±4.7, 18.7±4.7 and 17.0±8.6% of initial concentration along incubation period, respectively (Table 4.7). The depletion of PAHs in abiotic process might occur due to the photodegradation process. In contrast, a significantly higher PAHs reduction was found in the system with *I. coccinea* with biosurfactant spraying.

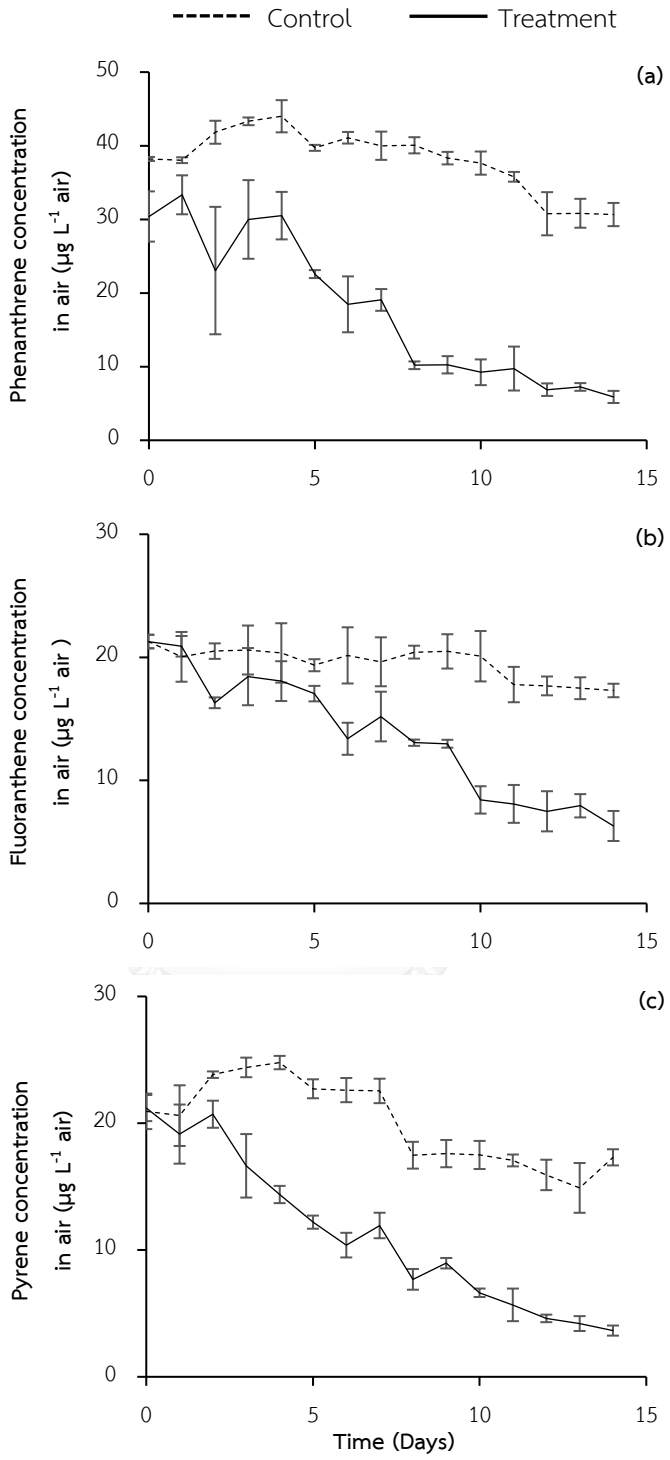


Figure 4.22 Time course of phenanthrene (a), fluoranthene (b), and pyrene (c) remaining in the air of a gas-tight chamber experiment containing leaves of *I. coccinea* with biosurfactant. Control experiment was conducted without plant leaves.

When compared the percentage of degradation with section 4.3.1, the *I. coccinea* twig spraying with biosurfactant had high pyrene removal efficiency at 92.9±1.8%. The higher pyrene removal efficiency was due to the presence of only pyrene, which the bacteria rapidly used as a carbon source. Meanwhile, the whole plant of *I. coccinea* with biosurfactant in a scale-up chamber contained several species PAHs as phenanthrene (a), fluoranthene (b), and pyrene (c) and benzo (a) pyrene, which might compete with each other during biodegradation.

Our results were similar with other researches. For example, *Mycobacterium vanbaalenii* PYR-1 is able to degrade the mixture of phenanthrene, fluoranthene, pyrene and benzo (a) pyrene by approximately 31.2% after 30 days of incubation, while it degrades more than 95% of each PAHs (phenanthrene, pyrene, fluoranthene and benzo (a) pyrene at 150, 100, 20, and 5 μg mL^{-1}) when cultured with single PAH substrates (Kim et al., 2007; Kweon, Kim, & Cerniglia, 2010) . Kim et al. (2015) suggest that, among several possible factors involved in the discrepancy in the metabolism of PAHs, in particular, loss of upregulation of the two type V Nid systems (NidAB and NidA3B3) appeared to have directly affected the metabolism of mixed-PAHs, such as pyrene and fluoranthene.

Moreover, Haritash and Kaushik (2009) concluded that the occurrence of competitive inhibition observed with two different pseudomonas species might be common among PAH-degrading organisms (Haritash & Kaushik, 2009). The presence of phenanthrene is reported to inhibit the degradation of pyrene. In addition, (Dean-Ross, Moody, Freeman, Doerge, & Cerniglia, 2001) suggested that in study with pure denitrifying isolates, the presence of naphthalene enhanced both phenanthrene and pyrene degradation, whereas phenanthrene apparently inhibited pyrene degradation, though the observations were not confirmed with metabolite analysis. Bacterial degradation of anthracene by *Rhodococcus* spp. and pyrene degradation by *Mycobacterium flavescens* has also been reported to be inhibited by the presence of fluoranthene in the medium.

Figure 4.23 shown the amount of deposited PAHs on leaf surface in the chamber. The initial deposit -phenanthrene, -fluoranthene, -pyrene and -benzo (a) pyrene concentration on leaves were found at 445.8±14.9, 429.6±9.3, 451.1±14.6, and 45.8±1.6 μg g^{-1} leaf, respectively. At the end of experiment, the remaining deposit-PAHs were found at 59.3±13.5, 109.9±11.2, 151.4±20.2, and 23.7±4.0 μg/g leaf, respectively (Figure 4.23a). So, the mixed-PAHs removal efficiency of deposited-PAHs by the *I. coccinea* leaves and their phyllosphere bacteria after spraying with biosurfactant were 86.3±2.7, 73.6±3.4, 65.3±5.6, and 46.6±11.1% for phenanthrene, fluoranthene, pyrene and benzo (a) pyrene, respectively (Table 4.7). Normally, the low molecular weight PAHs are easily degraded than high molecular weight or complex molecule. Our results also found that benzo (a) pyrene were hardly degraded in this study (Figure 4.23a). The revolatilization of deposit-PAHs on plant leaves could be continuously released and diffused in the gas-tight chamber. However, the amounts of all PAHs in the air were reduced over time (Figure 4.22). The result suggested that the plant leaves associated with PAHs-degrading bacteria could continuously degrade these PAHs.

Meanwhile, the initial deposit -phenanthrene, -fluoranthene, -pyrene and -benzo (a) pyrene concentrations on GF/C filter membrane in the control chamber were 5405.4±9.7, 5409.0±53.3, 5310.0±124.9, and 519.5±9.7 μg g^{-1} GF/C, respectively. At the end of experiment. The remaining deposit-PAHs were found at 733.8±40.6, 1238.9±108.1, 1074±148.15, and 344.6±19.5 μg g^{-1} GF/C, respectively (Figure 4.23b). So, the removal efficiency of deposited-PAHs, of which phenanthrene, fluoranthene, pyrene and benzo (a) pyrene by abiotic process were 13.5±0.8, 22.9±1.4, 20.0±2.3, and 64.1±4.4%, respectively (Table 4.7). Whereas, their vapor pressure of phenanthrene was the lowest followed by pyrene, fluoranthene and benzo (a) pyrene, respectively. Thus, the remaining of each substance on GF/C filter paper were reduced due to their

volatilization property. In addition, the initial deposited PAHs could volatilize into a gas phase.

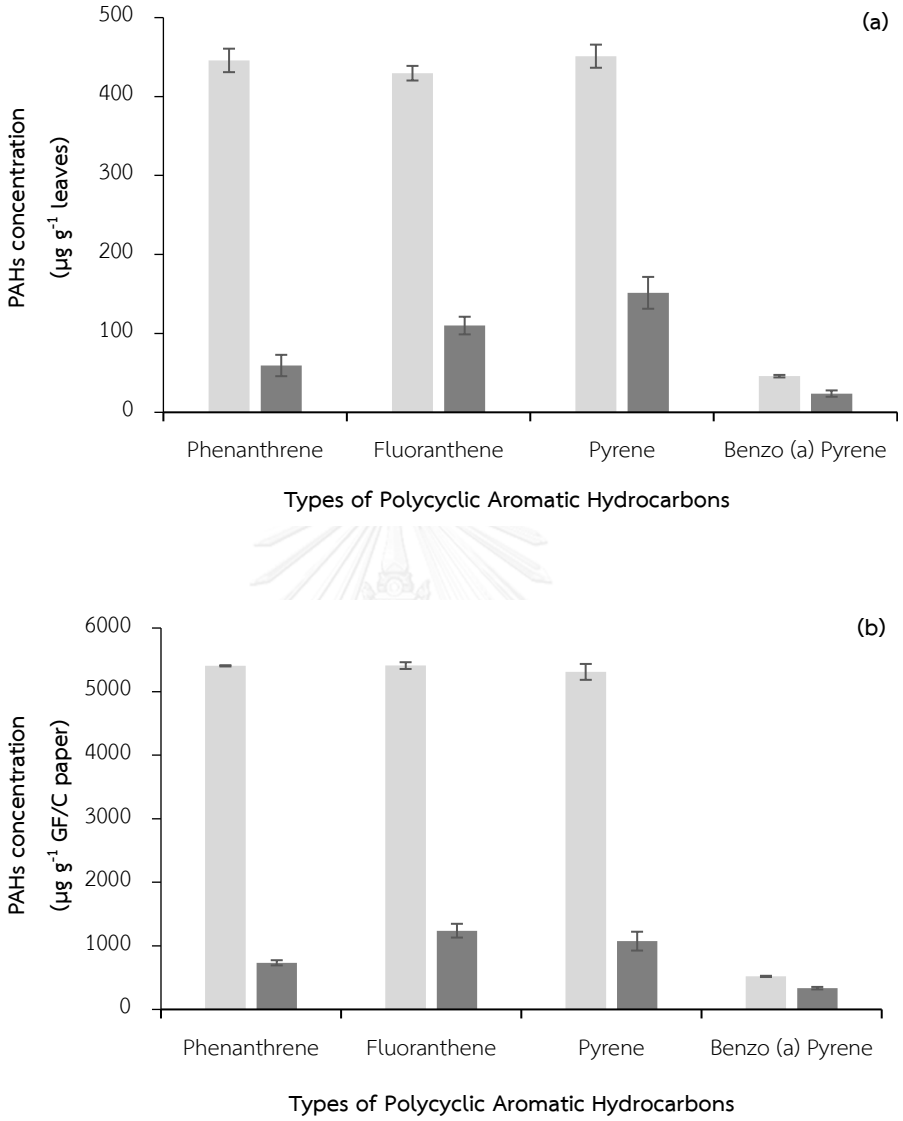


Figure 4.23 Concentration of deposited PAHs on leaves after spraying biosurfactant (a) and deposited on GF/C filter paper (b) compared initial (light-gray) and final (dark-gray) day of experiment.

Table 4.7 The removal percentage of airborne and deposit mixed-PAHs

Type of PAHs	Percent removal (%)			
	Airborne PAHs		Deposit PAHs	
	Control	Treatment	Control	Treatment
Phenanthrene	19.7±4.7	80.7±3.7	13.5±0.8	86.3±2.7
Fluoranthene	18.7±4.7	63.7±5.9	22.9±1.4	73.6±3.4
Pyrene	17.0±8.6	78.9±3.1	20.0±2.3	65.3±5.6
Benzo (a) pyrene	-	-	35.9±4.4	46.6±11.1

The amounts of total phyllosphere bacteria on *I. coccinea* leaves were almost ten-time higher than that of PAHs-degrading bacteria at both initial and final day of the experimental period. The number of total phyllosphere bacteria had grown from $2.0\pm0.4\times10^{6}$ to $2.4\pm0.2\times10^{7}$ CFU g^{-1} leaf; meanwhile, PAHs-degrading bacteria had increased from $1.9\pm0.2\times10^{5}$ to $2.5\pm0.2\times10^{6}$ CFU g^{-1} leaf. (Figure 4.24). These results were similar to the number of total phyllosphere and pyrene-degrading bacteria on *I. coccinea* leaves in the section 4.3.1. The number of total phyllosphere and pyrene-degrading bacteria had grown around ten-time from $1.3\pm0.4\times10^{6}$ to $7.1\pm0.7\times10^{7}$ CFU g^{-1} leaf and from $1.1\pm0.2\times10^{5}$ to $5.6\pm0.2\times10^{6}$ CFU g^{-1} leaf, respectively. (Figure 4.21).

The proportion of PAHs-degrading bacteria on total phyllosphere bacteria were increased from 8.8±1.8 to 10.6±0.2%. Consequently, the increasing number of total phyllosphere bacteria was due to the increasing number of PAHs-degrading bacteria. The results suggested that mixed-PAHs was used as carbon source by the phyllosphere bacteria during the experiment.

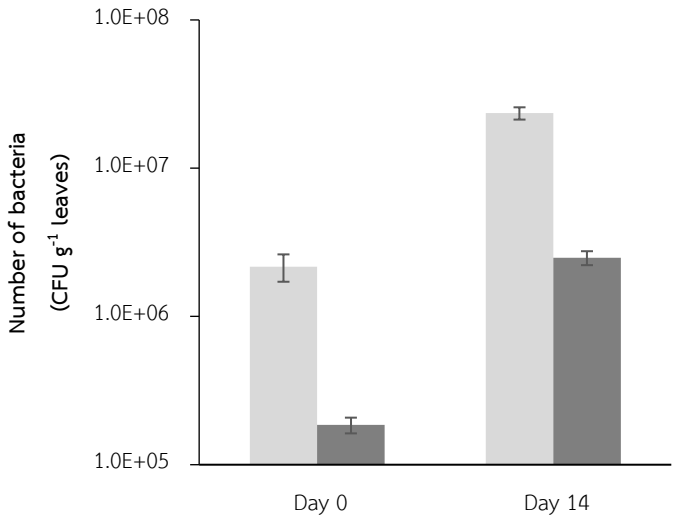


Figure 4.24 Number of total phyllosphere (light-gray) and PAHs-degrading (dark-gray) bacteria on leaves during mixed-PAHs removal in the chamber experiment containing whole plant of *I. coccinea* with biosurfactant addition.

Figure 4.25 *Ixora coccinea* at the first day (a) and the final day (b) in 96-L acrylic chamber to investigate the mixed-PAHs reduction by plant, their phyllosphere bacteria and 0.1X CMC biosurfactant.

These results were similar to the study of Yutthammo et al. (2010), which selected ornamental plants to observe and compare the activities of phyllosphere bacteria. The reduction of gaseous phenanthrene was examined in small-scale chamber (a 60-ml glass vial) containing either sterilized or unsterilized leaves. Phenanthrene reduction was found in the vials with leaves after incubation which the concentration of phenanthrene on day 4 were about 32-75% of day 1 in all plant species and highest removal efficiency on *Wrightia religiosa*. Moreover, phyllosphere bacteria on *W. religiosa* were able to reduce other PAHs such as acenaphthylene, acenaphthene and fluorene in both vial and glass chamber.

Our study further identified the approach to increase the activities of plants and their associated bacteria, which consisted of the addition of biosurfactant by spraying on plant leaves. The degradation of organic compounds by phyllosphere bacteria is an encouraging indication for their bioremediation potential. This new knowledge gap as an environmental-friendly technology could apply to reduce the air pollutants especially from motor vehicle exhaust in the urban environment. For example, the ornamental plant were apply to the carpark or parking building to reduce the PAHs released when preparing a park and spraying the biosurfactant at time interval to enhance the PAHs degradation by both indigenous bacteria and plant mechanisms. Moreover, the indoor ornamental plant species should be investigated when applying to reduce the PAHs in the building e.g. in the smoking room or copy room.

Besides PAHs, the activity of plants and their associated bacteria should be investigated for other pollutants such as volatile organic compounds (VOCs), which are found in indoor air, and many of these can affect human health (Bluyssen, Janssen, van den Brink, & de Kluizenaar, 2011). From other researches, aromatic hydrocarbon degradation by phyllosphere were investigated for their enzyme activity along with the decreasing of aromatic hydrocarbon concentrations (Kannangara S, Undugoda L, & Rajapaksha N, 2016). However, for enhancing the biodegradability, medium might be needed to apply onto leave surface for microbe development. The ornamental plants with the ability to improve air quality might be chosen to decorate the indoor areas.

CHAPTER V
CONCLUSIONS AND RECOMMENDATIONS

5.1 Conclusions

Polycyclic aromatic hydrocarbons (PAHs) are major air pollutants in urban environment. In this study, pyrene was chosen to represent HMW-PAHs in roadside air and street dusts. The air and plant samplings were carried out at Prachachuen Road, Bang Sue, Bangkok in 3 seasons; summer (March - July), rainy (August - October) and winter (November - February) of 2013 and 2015. There were higher amounts of particle-bound PAHs (pPAHs) at roadside areas than plant swath. The results suggested that plants and their associated bacteria might play important role in PAHs removal. This study therefore investigated the biodegradation potential of HMW-PAHs on ornamental plants. However, a major limiting factor for biodegradation of HMW-PAHs is their low bioavailability to the degrading microorganisms. Thus, the application of surfactants to enhance PAH degradation were optimized in this study to overcome this problem. The application of surfactant to increase PAHs bioavailability and enhance biodegradation had never been studied on plant surface.

Initially, pyrene was chosen as a representative of HMW-PAHs. To determine the biodegradation potential of pyrene, this study monitored the abundance of pyrene-degrading phyllosphere bacteria on six ornamental plant leaves and in ambient air around ornamental plant swaths. Plant leaves were found to be the major habitat of airborne and phyllosphere pyrene-degrading bacteria. In addition, the number of airborne pyrene-degrading bacteria were decreased when the distance increases far from ornamental plant swaths. The presence of pyrene-degrading bacteria was dependent on the plant species and was the lowest in the rainy season. The bacterial numbers were varied and the highest amount was found on *Ixora coccinea* leaves.

Four strains of pyrene-degrading bacteria, including *Methylobacterium* sp. IC1, *Kocuria* sp. IC2, *Kocuria* sp. IC3 and *Bacillus* sp. IC4 were later isolated from *I. coccinea* leaves. Both of *I. coccinea* leaves and isolated pyrene-degrading bacteria were used as a plant and also bacterial model for pyrene phylloremediation experiment.

To enhance pyrene degradation, several surfactants were initially investigated for their toxicity on *I. coccinea* leaves and isolated pyrene-degrading bacteria. In addition, the biosurfactant could desorb pyrene from plant leaves. The lipopeptide biosurfactant from *Bacillus* sp. GY19 was found to be non-toxic so it was selected for further study. When applied the lipopeptide biosurfactant to liquid medium, it enhanced the pyrene degradation by most phyllosphere bacteria (3 out of 4 strains). This was due to the ability of the biosurfactant to solubilize pyrene, which was used as bacterial carbon source. However, lipopeptide biosurfactant did not enhanced the activity of some isolated pyrene-degrading bacteria, which might be due to the degradation of biosurfactant by these phyllosphere bacteria. Thus, other comparatively non-toxic surfactant molecule such as Tween-20 should be investigated to enhance the mobility of PAHs on leaf surface for bacterial biodegradation.

Koruria sp. IC3 in a liquid medium adding with biosurfactant shown the highest pyrene removal efficiency and shortage time to biodegradation. Moreover, *Koruria* sp. IC3 exhibited the highest pyrene biodegradation rate with an increasing of initial pyrene concentrations. To examine the efficiency of plant, phyllosphere bacteria (*Koruria* sp. IC3 and indigenous bacteria) and biosurfactant on removal of deposited pyrene, the study placed plant samples in air tight system. In the *I. coccinea* microcosm experiment, the continual depletion of pyrene and mixed-PAHs by plant leaves, along with the increasing number of phyllosphere bacteria, indicated that the major pyrene and mixed-PAHs removal mechanism was biodegradation. The system with

biosurfactant alone enhanced the activities of indigenous phyllosphere bacteria and suggested that the bioaugmentation of leaves was not necessary. Similarly, the pyrene reduction in *L. frutescens* microcosm were the highest after spraying biosurfactant.

The indigenous phyllosphere bacteria on ornamental plant leaves with biosurfactant efficiently degraded the bioavailable pyrene and mixed-PAHs. Accordingly, a plant-bacteria approach for reducing atmospheric PAHs should be carried out by spraying diluted biosurfactant on ornamental plants growing along roadsides at certain time intervals. The process is recommended for summer and winter seasons, when the PAHs removal mechanism by rain is minor. In addition, the effective pyrene-degrading bacteria and also plant species could be selected for planting designs in urban area to improve the PAHs removal. The planting of various ornamental plant species might enhance PAHs or air pollutants removal due to the increasing varieties of plants and phyllosphere bacterial diversity in the system.

Finally, this research was the first to integrate the knowledge from bioremediation, plant-microbe interactions, and surfactant technology for air pollution abatement.

5.2 Recommendations for future study

Based on this study, some recommendations for further study are proposed as followed;

1. Semi-continuous or continuous PAHs adding's experiment should be performed in order to demonstrate that the whole plant is efficient for removing PAHs that continuously released from the sources e.g. traffic congestion and parking areas.

2. The in-depth study of PAHs biodegradation should be investigated to identify the mechanisms of how biosurfactant interact with phyllosphere degrading-bacteria on

plant leaves. The acquired knowledge will help design a more efficient phylloremediation approach for PAHs as well as other pollutants.

3. Other ornamental plant species especially indoor plants should be conducted to confirm the efficiency of this approach and for further application in rooms and buildings containing indoor air pollutants.

4. The efficient plant species (i.e. high PAH-degrading phyllosphere bacteria numbers, easily for plantation and low maintenance) should be grown for susceptible areas with high concentration of PAHs such as polluting traffic, parking building, or parking areas. Moreover, co-planting, multi-cropping and planting designs should be investigated to improve the PAHs removal. These methods might enhance PAHs or air pollutants removal due to the increasing varieties of plant and thereby phyllosphere bacterial diversity in the system.

5. The results were obtained from laboratory scale and short time experiments, thus it should be carried out in a large pilot scale or real polluted areas to ensure that the suggested condition by spraying the biosurfactant on plant leaves at time interval is valid in the natural environment.

6. The biodegradation of other HMW-PAHs found on particle-bound PAHs, such as benzo(b)fluoranthene (Bbf), benzo(ghi)perylene (BgP), and indenol (1,2,3-C,D) pyrene (Ind.) and volatile organic compounds (VOCs) by plants and their associated bacteria should be investigated as a single compound or as a mixture pollutants. These results will help in the selection of suitable plants for the application with different air pollutants and for using the co-plants or multi-cropping to improve the mixture of air pollutants.

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APPENDIX

APPENDIX A
CULTURE MEDIUM PREPARATION

Nutrient broth (NB)

Beef extract	3 g
Bacto peptone	5 g
Glycerol	25 mL

Adjust the final volume to 1,000 mL by distilled water, and autoclave at 15 psi, 121°C for 15 min.

For solid medium, 1.5% agar was added.

Carbon free mineral medium (CFMM)

Solution A

NH_4NO_3	3 g
KH_2PO_4	2.2 g
$Na_2HPO_4 \cdot 12H_2O$	0.8 g

Adjust the final volume to 1,000 mL by distilled water, pH 7.0, and autoclave at 15 psi, 121°C for 15 min.

Solution B

$FeCl_3 \cdot 6H_2O$	0.05 g mL^{-1}
$CaCl_2 \cdot H_2O$	0.05 g mL^{-1}
$MgSO_4 \cdot 7H_2O$	0.1 g mL^{-1}

Sterilize the solution by using 0.22 μm of CA membrane and added the solution B (1 mL each of solution was added into solution A (1,000 mL).

For solid medium, 1.5% bacto-agar was added.

Hoagland's solution

Macronutrients

Stock A	KH_2PO_4	136.09 g L^{-1}	1 M
Stock B	KNO_3	101.11 g L^{-1}	1 M
Stock C	$Ca(NO_3)_2 \cdot 4H_2O$	236.15 g L^{-1}	1 M
Stock D	$MgSO_4 \cdot 7H_2O$	246.48 g L^{-1}	1 ml
Stock E	$FeCl_3 \cdot 6H_2O$	27.0 g L^{-1}	5 mg mL^{-1}

Micronutrient

H_3BO_3	2.86 g L^{-1}	0.5 mg B mL^{-1}
$MnCl_2 \cdot 4H_2O$	1.81 g L^{-1}	0.5 mg Mn mL^{-1}
$ZnSO_4 \cdot 7H_2O$	0.22 g L^{-1}	0.05 mg Zn mL^{-1}
$CuSO_4 \cdot 5H_2O$	0.08 g L^{-1}	0.02 mg Cu mL^{-1}
$H_2MoO_4 \cdot 2H_2O$	0.02 g L^{-1}	0.01 mg Mo mL^{-1}

The nutrient solution was prepared by adding 1 mL of Stock A, 5 mL of Stock B, 5 mL of Stock C, 2 mL of Stock D, 1 mL of Stock E and 1 mL of micronutrient into a volumetric flask. Then deionized water was added until it reach 1,000 mL. The pH was adjusted to 6.0.

APPENDIX B
SOLUTION PREPARATION

PAH solution at final concentration 10,000 mg L^{-1} (for addition in CFMM broth)

PAH	100 mg
Dimethylformamide	10 mL

PAH was dissolved in dimethylformamide, filtrated by 0.2 µm PTFE syringe filter, and kept at - 20°C.

Phosphate buffer solution

Stock solution

1M of K_2HPO_4 (at 174.18 g mol^{-1})	174.18 g in distilled water 1,000 mL
1M of KH_2PO_4 (at 136.09 g mol^{-1})	136.09 g in distilled water 1,000 mL

Preparation of 0.1 M potassium phosphate buffer at pH =7.0

Volume of 1 M K_2HPO_4	61.5 mL
Volume of 1 M KH_2PO_4	38.5 mL

Dilute the 1 M stock solution to 1,000 mL with distilled water, autoclaved at 15 psi, 121°C for 15 min.

0.85% NaCl solution

NaCl	8.5 g
Distilled water	1,000 mL

The solution was autoclaved at 15 psi, 121°C for 15 min.

Nystatin solution

Nystatin	50 mg

Dissolved in DMSO 10 mL, filtrated by 0.2 µm PTFE syringe filter, and kept at -20°C.

APPENDIX C
STANDARD CURVE

Standard curve of pyrene analyzed by HPLC

Pyrene was dissolved in methanol at final concentration (5,000 mg L^{-1}). Then, pyrene was diluted to final concentration at 10, 50, 100, 150, and 200 mg L^{-1}. The solution was filtrated through 0.22 µm PTFE syringe filter into HPLC vial. Finally, PAHs were analyzed by HPLC using 80% methanol as mobile phase. The calculation to determine concentration of pyrene in sample is follow:

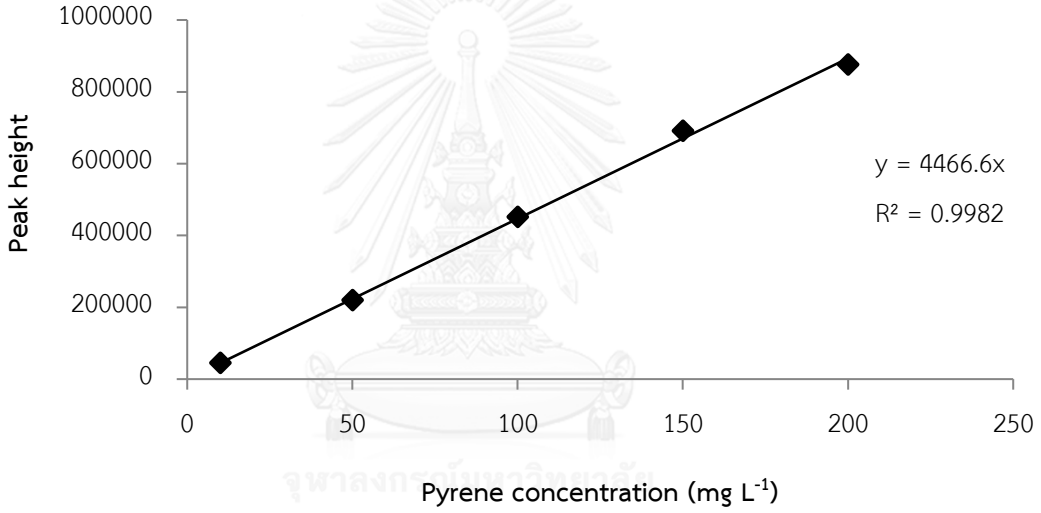

Fig. C1 Standard curve of pyrene from HPLC.

APPENDIX D
NUCLEOTIDE SEQUENCING

1. 16S rDNA of *Methylobacterium* sp. IC1

AGTCGCTGACCCTACCGTGGTCGCCTGCCTCCTTGCGGTTGGCGCAGCGCCGTCGGGTAAGAC
CAACTCCCATGGTGTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGTGGCATGCT
GATCCACGATTACTAGCGATTCCGCCTTCATGCACTCGAGTTGCAGAGTGCAATCCGAACTGAG
ACGGCTTTTGGAGATTGGCTCAGGGTCACCCCTTCGCGTCCCACTGTCACCGCCATTGTAGCAC
GTGTGTAGCCCATCCCGTAAGGGCCATGAGGACTTGACGTCATCCACACCTTCCTCGCGGCTTA
TCACCGGCAGTCTCCCCAGAGTGCCCAACTGAATGATGGCAACTGAGGACGTGGGTTGCGCTC
GTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAGCCATGCAGCACCTGTGTGC
AGGTCCCCGAAGGGAACGACCGATCTCTCGGACAAGCCTGCCATGTCAAAGGATGGTAAGGTTC
TGCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATTCCTTTG
AGTTTTAATCTTGCGACCGTACTCCCCAGGCGGAATGCTTAATGCGTTAGCTGCGCTACTGCGG
TGCATGCACCCCAACAGCTAGCATTCATCGTTTACGGCGTGGACTACCAGGGTATCTAATCCTG
TTTGCTCCCCACGCTTTCGCGCCTCAGCGTCAGTAATGGTCCAGTTGGCCGCCTTCGCCACCGG
TGTTCTTGCGAATATCTACGAATTTCACCTCTACACTCGCAGTTCCACCAACCTCTACCATACTC
AAGCGTCCCAGTATCGAAGGCCATTCTGTGGTTGAGCCACAGGCTTTCACCCCCGACTTAAAAC
GCCGCCTACGCGCCCTTTACGCCCAGTGATTCCGAGCAACGCTAGCCCCCTTCGTATTACCGCG
GCTGCTGGCACGAAGTTAGCCGGGGCTTATTCCTCCGGTACCGTCATTATCGTCCCGGAGAAAA
GAGCTTTACAACCCTAAGGCCGTCATCACTCACGCGGCATGGCTGGATCAGGCTTGCGCCCATT
GTCCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCT
GATCATCCTCTCAGACCAGCTACTGATCGTCGCCTTGGTAGGCCGTTACCCCACCAACAAGCTA
ATCAGACGCGGGCCGATCCTCCGGCAGTAAACCTTTCTGCCAAAGCACGTATCCGGTATTAGCC
CTAGTTTCCCAGGGTTATCCCAGACCGGAGGGCACGTTCCCACGTGTTACTCACCCGTCTGCCA
CTCATCCCGAAGGATGCGTTCGACTTGCATGTGTTAAGCCTGCCGCCAGCGTTCGCTCTGAGCC
ATA

2. 16S rDNA of *Kocuria* sp. IC2

TTAGTCCCAATCGCCAGTCCCACCTTCGACGGCTCCCCCCACAAGGGTTAGGCCACCGGCTTCG
GGTGTTACCAACTTTCGTGACTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCA
GCGTTGCTGATCTGCGATTACTAGCGACTCCGACTTCACGTGGTCGAGTTGCAGACCACGATCC
GAACTGAGACCAGCTTTTTGGGATTAGCTCCACCTCACGGTATCGCAACCCATTGTACTGGCCA
TTGTAGCATGCGTGAAGCCCAAGACATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCT
CCGAGTTGACCCCGGCAGTCTCCTATGAGTCCCCACCATCACGTGCTGGCAACATAGAACGAGG
GTTGCGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCA
CCTGTACACCAGCCCCACAAGGAGGAAAACCCATCTCTGAGCCGATCCGGTGTATGTCAAGCCT
TGGTAAGGTTCTTCGCGTTGCATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCCCCCGT
CAATTCCTTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCACTTAATGCGTTAGCTA
CGGCGCGGAAAACGTGGAATGTCCCCCACACCTAGTGCCCAACGTTTACGGCATGGACTACCAG
GGTATCTAATCCTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAGTAACAGCCCAGAGACCT
GCCTTCGCCATCGGTGTTCTTCCTGATATCTGCGCATTTCACCGCTACACCAGGAGTTCCAGTC
TCCCCTACTGCACTCAAGTCTGCCCGTACCCACTGCACACCCGGGGTTAAGCCCCGGGCTTTCA
CAGCAGACGCGACAAACCGCCTACGAGCTCTTTACGCCCAATAATTCCGGACAACGCTTGCGCC
CTACGTATTACCGCGGCTGCTGGCACGTAGTTAGCCGGCGCTTCTTCTGCACGTACCGTCACTT
TCGCTTCTTCCGTGCTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCT
GCATCAGGCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGT
GTCTCAGTCCCAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCC
ATTACCCCACCAACAAGCTGATAGGCCGTGAGCCCATCCACAACCAGTACAAACCCTTTCCACC
CCCCACCATGCGACAGGAGGTCATATCCAGTATTAGACCCAGTTTCCCAGGCTTATCCCAGAGT
CAAGGGCAGGTTACTCACGTATTACTCACCCGTTCGCCACTCATCCACCCAGTGCAAGCACCAA
GCTTCAGCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTC

3. 16S rDNA of *Kocuria* sp. IC3

CAATCGCCAGTCCCACCTTCGACGGCTCCCCCCACAAGGGTTAGGCCACCGGCTTCGGGTGTTA
CCAACTTTCGTGACTTGACGGGCGGTGTGTACAAGGCCCGGGAACGTATTCACCGCAGCGTTGC
TGATCTGCGATTACTAGCGACTCCGACTTCACGTGGTCGAGTTGCAGACCACGATCCGAACTGA
GACCAGCTTTTTGGGATTAGCTCCACCTCACGGTATCGCAACCCATTGTACTGGCCATTGTAGC
ATGCGTGAAGCCCAAGACATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCCGAGTT
GACCCCGGCAGTCTCCTATGAGTCCCCACCATCACGTGCTGGCAACATAGAACGAGGGTTGCGC
TCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTGTAC
ACCAGCCCCACAAGGAGGAAAACTCATCTCTGAGCCGATCCGGTGTATGTCAAGCCTTGGTAAG
GTTCTTCGCGTTGCATCGAATTAATCCGCATGCTCCGCCGCTTGTGCGGGCCCCCGTCAATTCC
TTTGAGTTTTAGCCTTGCGGCCGTACTCCCCAGGCGGGGCACTTAATGCGTTAGCTACGGCGCG
GAAAACGTGGAATGTCCCCCACACCTAGTGCCCAACGTTTACGGCATGGACTACCAGGGTATCT
AATCCTGTTCGCTCCCCATGCTTTCGCTCCTCAGCGTCAGTAACAGCCCAGAGACCTGCCTTCG
CCATCGGTGTTCTTCCTGATATCTGCGCATTTCACCGCTACACCAGGAGTTCCAGTCTCCCCTA
CTGCACTCAAGTCTGCCCGTACCCACTGCACACCCGGGGTTAAGCCCCGGGCTTTCACAGCAGA
CGCGACAAACCGCCTACGAGCTCTTTACGCCCAATAATTCCGGACAACGCTTGCGCCCTACGTA
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TTCCGTGCTGAAAGAGGTTTACAACCCGAAGGCCGTCATCCCTCACGCGGCGTCGCTGCATCAG
GCTTGCGCCCATTGTGCAATATTCCCCACTGCTGCCTCCCGTAGGAGTCTGGGCCGTGTCTCAG
TCCCAGTGTGGCCGGTCACCCTCTCAGGCCGGCTACCCGTCGTCGCCTTGGTAGGCCATTACCC
CACCAACAAGCTGATAGGCCGTGAGCCCATCCACAACCAGTACAAACCCTTTCCACCCCCCACC
ATGCGACAGGAGGTCATATCCAGTATTAGACCCAGTTTCCCAGGCTTATCCCAGAGTCAAGGGC
AGGTTACTCACGTATTACTCACCCGTTCGCCACTCATCCACCTAAGTGCAAGCACCAAGCTTCA
GCGTTCGACTTGCATGTGTTAAGCACGCCGCCAGCGTTCGT

4. 16S rDNA of *Bacillus* sp. IC4

CCCCAATCATCTGTCCCACCTTCGGCGGCTGGCTCCTAAAAGGTTACCTCACCGACTTCGGGTG
TTACAAACTCTTCGTGGTGTGACGGGCGGTGTGTACAAGGCCCCGGGAACGGTATTCACCCGCG
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ACTGAGAACAGATTTGTGGGATTGGCTTAACCTCGCGGTTTCGCTGCCCTTTGTTCTGTCCATT
GTAGCACGTGTGTAGCCCAGGTCATAAGGGGCATGATGATTTGACGTCATCCCCACCTTCCTCC
GGTTTGTCACCGGCAGTCACCTTAGAGTGCCCAACTGAATGCTGGCAACTAAGATCAAGGGTTG
CGCTCGTTGCGGGACTTAACCCAACATCTCACGACACGAGCTGACGACAACCATGCACCACCTG
TCACTCTGCCCCCGAAGGGGACGTCCTATCTTCTAGGATTGTCAGAGGATGTCAAGACCTGGTA
AGGTTCTTCGCGTTGCTTCGAATTAAACCACATGCTCCACCGCTTGTGCGGGCCCCCGTCAATT
CCTTTGAGTTTCAGTCTTGCGACCGTACTCCCCCAGGCGGAGTGCTTAATGCGTTAGCTGCAGC
ACTAAGGGGCGGAAACCCCCTAAACACTTAGCACTCATCGTTTACGGCGTGGACTACCAGGGTA
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TCGCCACTGGTGTTCCTCCACATCTCTACGCATTTCACCGCTACACGTGGAATTCCACTCTCCT
CTTCTGCACTCAAGTTCCCCAGTTTCCAATGACCCTCCCCGGTTGAGCCGGGGGCTTTCACATC
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GTATTACCGCGGCTGCTGGCACGTAGTTAGCCGTGGCTTTCTGGTTAGGTACCGTCAAGGTACC
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ACTCACGCGGCGTTGCTCCGTCAGACTTTCGTCCATTGCGGAAGATTCCCTACTGCTGCCTCCC
GTAGGAGTCTGGGCCGTGTCTCAGTCCCAGTGTGGCCGATCACCCTCTCAGGTCGGCTACGCAT
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TTTCCCGGAGTTATCCCAGTCTTACAGGCAGGTTACCCACGTGTTACTCACCCGTCCGCCGCTA
ACATCAGGGAGCAAGCTCCCATCTGTCCGCTCGACTGG

APPENDIX E

RAW DATA

Table E1 Effect of plant locations on the abundance of airborne pyrene-degrading bacteria. The air samples were collected at 0.5 and 1.0 m height above ground for summer, rainy and winter seasons in 2013 and 2015.

Season, Year	Height	Distance	Number of airborne pyrene-degrading bacteria (CFU m^{-3} air)						
			Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Avg.	SD
Summer, 2013	0.5 m	0.0 m	140	180	95	105	125	129	33.4
		0.5 m	90	155	115	125	85	114	28.4
		1.0 m	40	80	70	55	105	70	24.7
		2.0 m	30	35	20	55	45	37	13.5
		10.0 m	25	15	20	35	30	25	7.9
	1.0 m	0.0 m	125	110	155	130	175	139	25.8
		0.5 m	125	65	45	75	95	81	30.5
		1.0 m	75	70	90	110	40	77	25.9
		2.0 m	10	25	35	40	20	26	11.9
		10.0 m	35	60	45	15	40	39	16.4
Rainy, 2013	0.5 m	0.0 m	115	90	120	125	105	111	13.9
		0.5 m	115	90	100	85	120	102	15.2
		1.0 m	75	70	60	55	50	62	10.4
		2.0 m	25	40	50	25	30	34	10.8
		10.0 m	30	15	20	15	30	22	7.6

Season, Year	Height	Distance	Number of airborne pyrene-degrading bacteria (CFU m^{-3} air)						
			Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Avg.	SD
Rainy, 2013	1.0 m	0.0 m	130	120	135	120	110	123	9.7
		0.5 m	70	85	55	105	70	77	18.9
		1.0 m	55	65	75	80	95	74	15.2
		2.0 m	35	10	15	30	20	22	10.4
		10.0 m	25	35	25	20	15	24	7.4
Winter, 2013	0.5 m	0.0 m	185	165	95	120	135	140	35.7
		0.5 m	95	145	170	110	115	127	30.1
		1.0 m	75	105	65	70	45	72	21.7
		2.0 m	40	55	60	35	75	53	16.0
		10.0 m	25	40	60	20	35	36	15.6
	1.0 m	0.0 m	150	115	95	180	105	129	35.2
		0.5 m	90	115	85	100	130	104	18.5
		1.0 m	45	115	80	90	110	88	28.0
		2.0 m	55	40	35	85	30	49	22.2
		10.0 m	20	30	60	30	25	33	15.7
Summer, 2015	0.5 m	0.0 m	160	140	125	110	135	134	18.5
		0.5 m	130	145	115	125	95	122	18.6
		1.0 m	75	105	80	65	70	79	15.6
		2.0 m	40	35	30	45	60	42	11.5
		10.0 m	25	30	35	35	40	33	5.7

Season, Year	Height	Distance	Number of airborne pyrene-degrading bacteria (CFU m^{-3} air)						
			Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Avg.	SD
Summer, 2015	1.0 m	0.0 m	105	125	120	115	155	124	18.8
		0.5 m	110	95	115	80	95	99	13.9
		1.0 m	65	50	85	80	65	69	13.9
		2.0 m	55	25	35	40	45	40	11.2
		10.0 m	25	45	20	40	40	34	10.8
Rainy, 2015	0.5 m	0.0 m	140	130	120	115	125	126	9.6
		0.5 m	110	105	90	100	115	104	9.6
		1.0 m	75	70	75	65	80	73	5.7
		2.0 m	35	30	40	45	40	38	5.7
		10.0 m	25	20	15	30	25	23	5.7
	1.0 m	0.0 m	130	120	135	155	115	131	15.6
		0.5 m	90	95	85	100	80	90	7.9
		1.0 m	65	70	85	80	75	75	7.9
		2.0 m	45	30	40	25	45	37	9.1
		10.0 m	25	25	35	15	20	24	7.4
Winter, 2015	0.5 m	0.0 m	125	160	140	165	170	152	18.9
		0.5 m	110	135	125	140	115	125	12.7
		1.0 m	95	75	90	100	80	88	10.4
		2.0 m	55	60	45	40	70	54	11.9
		10.0 m	35	55	45	25	40	40	11.2

Season, Year	Height	Distance	Number of airborne pyrene-degrading bacteria (CFU m^{-3} air)						
			Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Avg.	SD
Winter, 2015	1.0 m	0.0 m	160	135	140	130	100	133	21.7
		0.5 m	105	130	85	95	125	108	19.2
		1.0 m	90	70	95	85	80	84	9.6
		2.0 m	45	40	55	65	50	51	9.6
		10.0 m	30	45	50	35	45	41	8.2

Table E2 Effect of plant species on the abundance of phyllosphere pyrene-degrading bacteria. The leaf samples were collected in 2013 and 2015 for summer, rainy and winter seasons.

Season, Year	Plant species	Type of bacteria	Number of abundance of phyllosphere bacteria (CFU g^{-1} leaf)				
			Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Summer, 2013	Ic	Tot. bact.	1.1E+06	7.6E+05	9.4E+05	9.2E+05	1.6E+05
		Pyr.-deg.	1.1E+05	9.4E+04	8.5E+04	9.7E+04	1.4E+04
	Wr	Tot. bact.	6.5E+05	7.8E+05	6.1E+05	6.8E+05	8.9E+04
		Pyr.-deg.	3.9E+04	3.8E+04	5.1E+04	4.3E+04	7.2E+03
	Lf	Tot. bact.	1.8E+06	1.6E+06	1.4E+06	1.6E+06	2.0E+05
		Pyr.-deg.	5.5E+04	5.9E+04	6.9E+04	6.1E+04	7.2E+03
	Ea	Tot. bact.	1.6E+06	1.4E+06	1.3E+06	1.4E+06	1.6E+05
		Pyr.-deg.	7.5E+04	6.8E+04	6.1E+04	6.8E+04	7.0E+03
	Ac	Tot. bact.	8.4E+05	1.1E+06	7.4E+05	8.8E+05	1.7E+05
		Pyr.-deg.	5.7E+04	4.2E+04	4.9E+04	4.9E+04	7.5E+03
	Ne	Tot. bact.	8.7E+05	5.7E+05	6.2E+05	6.9E+05	1.6E+05
		Pyr.-deg.	4.7E+04	4.1E+04	3.6E+04	4.1E+04	5.5E+03
Rainy, 2013	Ic	Tot. bact.	2.9E+05	2.7E+05	2.9E+05	2.8E+05	1.1E+04
		Pyr.-deg.	3.7E+04	3.3E+04	4.1E+04	3.7E+04	4.0E+03
	Wr	Tot. bact.	2.4E+05	2.6E+05	2.1E+05	2.4E+05	2.6E+04
		Pyr.-deg.	4.0E+04	3.1E+04	3.7E+04	3.6E+04	4.6E+03
	Lf	Tot. bact.	6.6E+05	8.4E+05	9.5E+05	8.2E+05	1.5E+05
		Pyr.-deg.	6.1E+04	4.5E+04	6.2E+04	5.6E+04	9.5E+03
	Ea	Tot. bact.	2.1E+05	2.4E+05	2.6E+05	2.4E+05	2.5E+04
		Pyr.-deg.	3.3E+04	3.7E+04	3.5E+04	3.5E+04	2.0E+03

Season, Year	Plant species	Type of bacteria	Number of abundance of phyllosphere bacteria (CFU g^{-1} leaf)				
			Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Rainy, 2013	Ac	Tot. bact.	8.1E+05	7.0E+05	9.7E+05	8.3E+05	1.4E+05
		Pyr.-deg.	3.6E+04	4.4E+04	5.2E+04	4.4E+04	8.0E+03
	Ne	Tot. bact.	2.9E+06	2.5E+06	2.9E+06	2.8E+06	2.1E+05
		Pyr.-deg.	3.4E+04	4.6E+04	3.1E+04	3.7E+04	7.9E+03
Winter, 2013	Ic	Tot. bact.	8.2E+05	7.5E+05	1.0E+06	8.7E+05	1.5E+05
		Pyr.-deg.	7.8E+04	8.1E+04	9.4E+04	8.4E+04	8.5E+03
	Wr	Tot. bact.	5.5E+05	4.6E+05	6.1E+05	5.4E+05	7.5E+04
		Pyr.-deg.	4.1E+04	3.6E+04	4.7E+04	4.1E+04	5.5E+03
	Lf	Tot. bact.	1.4E+06	1.5E+06	1.4E+06	1.4E+06	7.5E+04
		Pyr.-deg.	6.3E+04	7.4E+04	5.6E+04	6.4E+04	9.1E+03
	Ea	Tot. bact.	4.4E+05	4.5E+05	3.8E+05	4.2E+05	3.8E+04
		Pyr.-deg.	3.4E+04	3.1E+04	3.3E+04	3.3E+04	1.5E+03
	Ac	Tot. bact.	8.8E+05	6.7E+05	7.5E+05	7.7E+05	1.1E+05
		Pyr.-deg.	5.1E+04	4.0E+04	3.4E+04	4.2E+04	8.6E+03
	Ne	Tot. bact.	1.1E+06	6.5E+05	7.9E+05	8.5E+05	2.4E+05
		Pyr.-deg.	5.4E+04	4.1E+04	3.3E+04	4.3E+04	1.1E+04
Summer, 2015	Ic	Tot. bact.	8.4E+05	9.5E+05	1.2E+06	9.9E+05	1.7E+05
		Pyr.-deg.	8.8E+04	9.5E+04	1.1E+05	9.6E+04	9.1E+03
	Wr	Tot. bact.	6.4E+05	7.3E+05	8.2E+05	7.3E+05	9.0E+04
		Pyr.-deg.	3.8E+04	4.5E+04	5.2E+04	4.5E+04	7.0E+03
	Lf	Tot. bact.	1.5E+06	1.5E+06	1.8E+06	1.6E+06	1.6E+05
		Pyr.-deg.	4.8E+04	5.2E+04	6.8E+04	5.6E+04	1.1E+04

Season, Year	Plant species	Type of bacteria	Number of abundance of phyllosphere bacteria (CFU g^{-1} leaf)				
			Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Summer, 2015	Ea	Tot. bact.	8.5E+05	9.3E+05	1.0E+06	9.3E+05	8.0E+04
		Pyr.-deg.	4.2E+04	5.5E+04	7.4E+04	5.7E+04	1.6E+04
	Ac	Tot. bact.	8.5E+05	1.0E+06	1.1E+06	9.9E+05	1.4E+05
		Pyr.-deg.	4.6E+04	5.4E+04	6.5E+04	5.5E+04	9.5E+03
	Ne	Tot. bact.	5.5E+05	6.6E+05	7.8E+05	6.6E+05	1.2E+05
		Pyr.-deg.	3.6E+04	3.8E+04	4.7E+04	4.0E+04	5.9E+03
Rainy, 2015	Ic	Tot. bact.	3.7E+05	5.5E+05	4.3E+05	4.5E+05	9.2E+04
		Pyr.-deg.	5.8E+04	6.1E+04	5.3E+04	5.7E+04	4.0E+03
	Wr	Tot. bact.	5.4E+05	6.7E+05	4.4E+05	5.5E+05	1.2E+05
		Pyr.-deg.	3.4E+04	4.7E+04	5.4E+04	4.5E+04	1.0E+04
	Lf	Tot. bact.	9.5E+05	1.0E+06	1.3E+06	1.1E+06	1.7E+05
		Pyr.-deg.	5.5E+04	6.2E+04	4.4E+04	5.4E+04	9.1E+03
	Ea	Tot. bact.	6.4E+05	5.0E+05	3.4E+05	4.9E+05	1.5E+05
		Pyr.-deg.	4.1E+04	5.2E+04	3.8E+04	4.4E+04	7.4E+03
	Ac	Tot. bact.	7.2E+05	8.4E+05	1.1E+06	8.9E+05	2.1E+05
		Pyr.-deg.	4.8E+04	5.1E+04	6.0E+04	5.3E+04	6.2E+03
	Ne	Tot. bact.	7.5E+05	6.1E+05	5.6E+05	6.4E+05	9.8E+04
		Pyr.-deg.	6.2E+04	3.6E+04	4.1E+04	4.6E+04	1.4E+04
Winter, 2015	Ic	Tot. bact.	7.3E+05	8.1E+05	9.7E+05	8.4E+05	1.2E+05
		Pyr.-deg.	6.9E+04	7.3E+04	9.4E+04	7.9E+04	1.3E+04
	Wr	Tot. bact.	4.1E+05	5.1E+05	6.5E+05	5.2E+05	1.2E+05
		Pyr.-deg.	3.5E+04	3.8E+04	4.1E+04	3.8E+04	3.0E+03

Season, Year	Plant species	Type of bacteria	Number of abundance of phyllosphere bacteria (CFU g^{-1} leaf)				
			Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Winter, 2015	Lf	Tot. bact.	1.2E+06	1.2E+06	1.4E+06	1.3E+06	1.3E+05
		Pyr.-deg.	4.2E+04	4.9E+04	6.5E+04	5.2E+04	1.2E+04
	Ea	Tot. bact.	5.3E+05	5.5E+05	6.2E+05	5.7E+05	4.7E+04
		Pyr.-deg.	3.7E+04	4.3E+04	5.2E+04	4.4E+04	7.5E+03
	Ac	Tot. bact.	6.5E+05	7.0E+05	8.7E+05	7.4E+05	1.2E+05
		Pyr.-deg.	3.6E+04	4.2E+04	5.0E+04	4.3E+04	7.0E+03
	Ne	Tot. bact.	5.5E+05	6.6E+05	9.1E+05	7.1E+05	1.8E+05
		Pyr.-deg.	3.3E+04	3.8E+04	4.3E+04	3.8E+04	5.0E+03

Remark:

Ic = *Ixora coccinea*,

Wr = *Wrightia religiosa*

Lf = *Leucophyllum frutescens*

Ea = *Epipremnum aureus*

Ac = *Axonopus compresus*

Ne = *Nephrolepis exaltata*

Tot. bact. = Total phyllosphere bacteria

Pyr.-deg. = Pyrene-degrading bacteria

Table E3 Effect of biosurfactant concentrations on pyrene solubilization

Concentration of biosurfactant (CMC)	Concentration of solubilized pyrene (mg L^{-1})				
	Rep. 1	Rep. 2	Rep. 3	Avg.	SD
0.0x CMC (DDW)	5.51	5.28	4.92	5.24	0.30
0.1X CMC	8.94	8.61	7.92	8.49	0.52
0.5X CMC	10.72	9.41	8.95	9.69	0.92
1X CMC	22.65	21.61	21.22	21.83	0.74
5X CMC	25.14	24.04	23.02	24.07	1.06
10X CMC	28.62	27.75	26.67	27.68	0.98

Table E4 Effect of biosurfactant concentrations on pyrene desorption

Concentration of biosurfactant (CMC)	Concentration of desorped pyrene (mg L^{-1})						
	Rep. 1	Rep. 2	Rep. 3	Rep. 4	Rep. 5	Avg.	SD
0.0x CMC (DDW)	12.88	12.54	12.05	11.29	10.88	11.93	0.84
0.1X CMC	72.75	65.38	63.44	62.39	62.34	65.26	4.37
0.2X CMC	79.59	78.87	76.85	74.50	67.83	75.53	4.73
0.5X CMC	95.26	90.48	90.34	84.57	82.38	88.61	5.15
1X CMC	161.57	159.73	159.17	153.63	153.25	157.47	3.79
2X CMC	189.57	185.38	179.31	178.98	172.99	181.24	6.39
5X CMC	202.03	198.21	197.74	195.97	189.19	196.63	4.71
10X CMC	240.69	227.04	219.68	212.08	208.85	221.67	12.76

Table E5 Pyrene remaining in the control experiments without bacterial inoculum.

A. At initial pyrene 50 mg L^{-1}

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	50.40	49.60	49.21	49.73	0.61
	W/ BSF	50.40	49.41	48.80	49.53	0.81
Day 3	W/O BSF	47.29	46.56	46.01	46.62	0.64
	W/ BSF	47.22	46.56	46.21	46.66	0.51
Day 7	W/O BSF	45.22	44.60	43.78	44.53	0.73
	W/ BSF	45.20	44.19	43.25	44.21	0.98
Day 14	W/O BSF	43.62	43.07	42.64	43.11	0.49
	W/ BSF	44.62	43.79	43.64	44.02	0.53

B. At initial pyrene 100 mg L^{-1}

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	103.04	101.06	100.90	101.66	1.19
	W/ BSF	102.37	100.55	100.97	101.30	0.95
Day 3	W/O BSF	99.21	99.02	97.95	98.73	0.68
	W/ BSF	100.19	98.66	98.19	99.01	1.05
Day 7	W/O BSF	95.92	95.71	95.45	95.69	0.24
	W/ BSF	94.16	93.44	89.97	92.52	2.24
Day 14	W/O BSF	93.23	92.96	91.98	92.72	0.66
	W/ BSF	94.11	93.15	87.28	91.51	3.70
Day 21	W/O BSF	92.56	92.38	91.83	92.26	0.38
	W/ BSF	93.18	92.54	87.85	91.19	2.91

B. At initial pyrene 100 mg L^{-1} (Continued)

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 28	W/O BSF	89.97	89.35	88.94	89.42	0.52
	W/ BSF	91.85	92.43	88.42	90.90	2.17
Day 35	W/O BSF	88.56	87.33	85.92	87.27	1.32
	W/ BSF	90.02	91.19	84.28	88.50	3.70

C. At initial pyrene 200 mg L^{-1}

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	205.03	202.06	200.82	202.63	2.17
	W/ BSF	204.13	202.64	202.56	203.11	0.88
Day 3	W/O BSF	200.22	198.21	197.37	198.60	1.46
	W/ BSF	200.19	198.70	198.13	199.01	1.06
Day 7	W/O BSF	196.91	194.56	194.14	195.20	1.49
	W/ BSF	196.13	195.22	191.47	194.27	2.47
Day 14	W/O BSF	192.31	192.12	191.83	192.09	0.24
	W/ BSF	192.13	190.41	189.52	190.69	1.33
Day 21	W/O BSF	188.45	185.15	184.25	185.95	2.21
	W/ BSF	187.32	186.23	184.02	185.86	1.68
Day 28	W/O BSF	185.94	183.97	181.35	183.75	2.30
	W/ BSF	185.82	185.41	181.52	184.25	2.37
Day 35	W/O BSF	178.26	177.33	175.71	177.10	1.29
	W/ BSF	180.11	178.21	173.22	177.18	3.56

C. At initial pyrene 200 mg L^{-1} (Continued)

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 42	W/O BSF	176.84	174.77	173.35	174.99	1.76
	W/ BSF	177.52	176.41	172.52	175.48	2.63
Day 49	W/O BSF	173.66	169.83	165.71	169.73	3.98
	W/ BSF	173.11	172.81	165.22	170.38	4.47

Remark:

W/O BSF = without biosurfactant

W/ BSF = with biosurfactant

Table E6 Degradation of 50-200 mg L^{-1} pyrene in CFMM broth by *Methylobacterium* sp. IC1, *Kocuria* sp. IC2, *Kocuria* sp. IC3 and *Bacillus* sp. IC4 with and without biosurfactant.

A. At initial pyrene 50 mg L^{-1} of *Methylobacterium* sp. IC1

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	50.40	49.60	49.21	49.73	0.61
	W/ BSF	50.40	49.41	48.80	49.53	0.81
Day 3	W/O BSF	35.41	33.03	30.91	33.12	2.25
	W/ BSF	33.34	30.91	30.74	31.66	1.45
Day 7	W/O BSF	14.17	13.21	12.36	13.25	0.90
	W/ BSF	13.34	12.36	12.30	12.67	0.58
Day 14	W/O BSF	10.12	9.44	8.83	9.46	0.64
	W/ BSF	9.53	8.83	8.78	9.05	0.42

B. At initial pyrene 50 mg L^{-1} of *Kocuria* sp. IC2

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	50.40	49.60	49.21	49.73	0.61
	W/ BSF	50.40	49.41	48.80	49.53	0.81
Day 3	W/O BSF	28.22	27.47	25.66	27.12	1.31
	W/ BSF	25.00	24.61	23.44	24.35	0.81
Day 7	W/O BSF	11.29	10.99	10.27	10.85	0.52
	W/ BSF	10.13	9.84	9.38	9.78	0.38
Day 14	W/O BSF	8.06	7.85	7.33	7.75	0.37
	W/ BSF	7.24	7.03	6.70	6.99	0.27

C. At initial pyrene 50 mg L^{-1} of *Kocuria* sp. IC3

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	50.40	49.60	49.21	49.73	0.61
	W/ BSF	50.40	49.41	48.80	49.53	0.81
Day 3	W/O BSF	34.93	33.53	33.15	33.87	0.93
	W/ BSF	33.23	29.38	28.58	30.39	2.49
Day 7	W/O BSF	18.05	18.01	17.34	17.80	0.40
	W/ BSF	13.29	11.75	11.03	12.02	1.15
Day 14	W/O BSF	11.12	11.01	11.61	11.25	0.32
	W/ BSF	6.49	6.39	5.88	6.26	0.33

D. At initial pyrene 50 mg L^{-1} of *Bacillus* sp. IC4

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	50.40	49.60	49.21	49.73	0.61
	W/ BSF	50.40	49.41	48.80	49.53	0.81
Day 3	W/O BSF	30.01	29.21	29.00	29.41	0.53
	W/ BSF	28.71	28.50	27.38	28.20	0.71
Day 7	W/O BSF	12.00	11.69	11.60	11.76	0.21
	W/ BSF	12.55	10.60	10.29	11.15	1.23
Day 14	W/O BSF	8.57	8.35	8.29	8.40	0.15
	W/ BSF	8.97	7.57	7.35	7.96	0.88

E. At initial pyrene 100 mg L^{-1} of *Methylobacterium* sp. IC1

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	103.04	101.06	100.90	101.66	1.19
	W/ BSF	102.37	100.55	100.97	101.30	0.95
Day 3	W/O BSF	90.97	90.35	89.84	90.39	0.57
	W/ BSF	86.17	85.62	85.03	85.61	0.57
Day 7	W/O BSF	79.77	77.95	76.94	78.22	1.43
	W/ BSF	71.51	69.93	68.37	69.94	1.57
Day 14	W/O BSF	62.37	60.93	59.51	60.94	1.43
	W/ BSF	47.34	45.13	43.04	45.17	2.15
Day 21	W/O BSF	48.17	46.35	45.91	46.81	1.20
	W/ BSF	24.76	23.17	22.43	23.45	1.19
Day 28	W/O BSF	39.91	37.67	34.35	37.31	2.80
	W/ BSF	15.83	13.98	13.43	14.41	1.26
Day 35	W/O BSF	40.91	38.35	35.67	38.31	2.62
	W/ BSF	9.71	8.55	7.92	8.73	0.91

F. At initial pyrene 100 mg L^{-1} of *Kocuria* sp. IC2

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	103.04	101.06	100.90	101.66	1.19
	W/ BSF	102.37	100.55	100.97	101.30	0.95
Day 3	W/O BSF	95.91	94.29	92.81	94.34	1.55
	W/ BSF	84.85	82.34	81.57	82.92	1.72
Day 7	W/O BSF	89.04	88.65	88.57	88.75	0.25
	W/ BSF	65.83	65.37	65.31	65.50	0.28
Day 14	W/O BSF	77.87	77.75	75.74	77.12	1.20
	W/ BSF	46.34	45.13	44.04	45.17	1.15
Day 21	W/O BSF	54.36	49.14	48.51	50.67	3.21
	W/ BSF	31.67	30.35	29.91	30.64	0.92
Day 28	W/O BSF	44.45	42.35	41.91	42.90	1.36
	W/ BSF	16.23	15.98	14.43	15.55	0.98
Day 35	W/O BSF	41.91	37.67	37.35	38.98	2.55
	W/ BSF	12.71	8.62	7.55	9.63	2.72

G. At initial pyrene 100 mg L^{-1} of *Kocuria* sp. IC3

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	103.04	101.06	100.90	101.66	1.19
	W/ BSF	102.37	100.55	100.97	101.30	0.95
Day 3	W/O BSF	83.93	83.74	81.57	83.08	1.31
	W/ BSF	79.75	79.47	77.74	78.99	1.09
Day 7	W/O BSF	74.76	72.51	72.44	73.24	1.32
	W/ BSF	64.83	63.38	63.05	63.75	0.95
Day 14	W/O BSF	55.85	49.55	48.91	51.44	3.84
	W/ BSF	40.63	40.34	38.64	39.87	1.08
Day 21	W/O BSF	42.64	42.13	40.24	41.67	1.26
	W/ BSF	12.43	11.23	9.48	11.05	1.48
Day 28	W/O BSF	31.54	30.04	29.48	30.35	1.07
	W/ BSF	8.71	7.84	5.82	7.46	1.48
Day 35	W/O BSF	28.26	27.73	26.38	27.46	0.97
	W/ BSF	5.21	4.23	3.02	4.15	1.10

H. At initial pyrene 100 mg L^{-1} of *Bacillus* sp. IC4

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	103.04	101.06	100.90	101.66	1.19
	W/ BSF	102.37	100.55	100.97	101.30	0.95
Day 3	W/O BSF	90.37	89.05	88.04	89.15	1.17
	W/ BSF	87.62	86.87	85.73	86.74	0.95
Day 7	W/O BSF	66.18	65.02	64.05	65.08	1.07
	W/ BSF	71.56	71.14	69.31	70.67	1.20
Day 14	W/O BSF	56.71	55.29	55.14	55.71	0.87
	W/ BSF	50.36	50.14	49.03	49.84	0.71
Day 21	W/O BSF	49.14	48.63	47.04	48.27	1.10
	W/ BSF	45.13	44.66	43.42	44.40	0.88
Day 28	W/O BSF	35.65	33.87	32.91	34.14	1.39
	W/ BSF	33.67	32.04	31.48	32.40	1.14
Day 35	W/O BSF	29.53	28.68	28.53	28.91	0.54
	W/ BSF	29.78	27.63	26.73	28.05	1.57

I. At initial pyrene 200 mg L^{-1} of *Methylobacterium* sp. IC1

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	205.03	202.06	200.82	202.63	2.17
	W/ BSF	204.13	202.64	202.56	203.11	0.88
Day 3	W/O BSF	190.97	190.35	189.84	190.39	0.57
	W/ BSF	186.17	185.62	185.03	185.61	0.57
Day 7	W/O BSF	185.77	183.95	183.94	184.55	1.05
	W/ BSF	161.51	159.93	158.37	159.94	1.57
Day 14	W/O BSF	161.21	160.23	158.51	159.98	1.37
	W/ BSF	141.34	140.13	137.04	139.50	2.22
Day 21	W/O BSF	138.17	136.35	128.91	134.48	4.91
	W/ BSF	114.76	113.17	106.43	111.45	4.42
Day 28	W/O BSF	127.47	125.31	123.91	125.56	1.79
	W/ BSF	102.83	101.98	100.43	101.75	1.22
Day 35	W/O BSF	108.57	108.35	106.91	107.94	0.90
	W/ BSF	89.71	88.55	86.92	88.39	1.40
Day 42	W/O BSF	97.67	95.35	93.91	95.64	1.90
	W/ BSF	75.83	73.98	73.43	74.41	1.26
Day 49	W/O BSF	91.67	88.35	85.91	88.64	2.89
	W/ BSF	69.71	68.55	66.92	68.39	1.40

J. At initial pyrene 200 mg L^{-1} of *Kocuria* sp. IC2

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	205.03	202.06	200.82	202.63	2.17
	W/ BSF	204.13	202.64	202.56	203.11	0.88
Day 3	W/O BSF	185.87	184.67	183.54	184.69	1.17
	W/ BSF	184.85	182.34	181.57	182.92	1.72
Day 7	W/O BSF	179.04	178.65	177.57	178.42	0.76
	W/ BSF	162.83	160.37	160.31	161.17	1.44
Day 14	W/O BSF	157.87	156.75	153.74	156.12	2.14
	W/ BSF	136.34	135.13	134.04	135.17	1.15
Day 21	W/O BSF	143.36	140.14	139.51	141.00	2.07
	W/ BSF	111.67	110.35	109.91	110.64	0.92
Day 28	W/O BSF	118.45	117.35	114.91	116.90	1.81
	W/ BSF	96.23	95.98	91.43	94.55	2.70
Day 35	W/O BSF	103.67	102.35	95.91	100.64	4.15
	W/ BSF	85.71	84.62	84.55	84.96	0.65
Day 42	W/O BSF	98.45	97.35	94.91	96.90	1.81
	W/ BSF	86.23	85.98	82.43	84.88	2.13
Day 49	W/O BSF	93.67	92.35	91.91	92.64	0.92
	W/ BSF	86.71	84.62	84.55	85.29	1.23

K. At initial pyrene 200 mg L^{-1} of *Kocuria* sp. IC3

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	205.03	202.06	200.82	202.63	2.17
	W/ BSF	204.13	202.64	202.56	203.11	0.88
Day 3	W/O BSF	188.83	186.76	185.57	187.05	1.65
	W/ BSF	173.47	172.75	172.74	172.99	0.42
Day 7	W/O BSF	177.76	175.44	172.51	175.24	2.63
	W/ BSF	150.23	147.38	146.05	147.89	2.14
Day 14	W/O BSF	167.41	165.85	162.55	165.27	2.48
	W/ BSF	110.63	110.34	108.64	109.87	1.08
Day 21	W/O BSF	125.64	125.13	122.24	124.34	1.83
	W/ BSF	83.48	82.43	81.73	82.55	0.88
Day 28	W/O BSF	113.54	111.48	110.04	111.69	1.76
	W/ BSF	58.82	57.84	55.71	57.46	1.59
Day 35	W/O BSF	103.73	101.26	99.38	101.46	2.18
	W/ BSF	45.21	44.73	44.02	44.65	0.60
Day 42	W/O BSF	91.54	91.04	89.48	90.69	1.07
	W/ BSF	48.82	47.84	44.71	47.12	2.15
Day 49	W/O BSF	87.73	85.26	85.38	86.12	1.39
	W/ BSF	45.21	44.23	44.02	44.49	0.64

L. At initial pyrene 200 mg L^{-1} of *Bacillus* sp. IC4

Time	BSF addition	Pyrene remaining (mg L^{-1})				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Day 0	W/O BSF	205.03	202.06	200.82	202.63	2.17
	W/ BSF	204.13	202.64	202.56	203.11	0.88
Day 3	W/O BSF	183.13	181.20	179.67	181.33	1.73
	W/ BSF	176.27	175.42	170.73	174.14	2.98
Day 7	W/O BSF	174.56	171.14	169.31	171.67	2.66
	W/ BSF	171.02	170.18	168.05	169.75	1.53
Day 14	W/O BSF	148.29	146.14	145.71	146.71	1.38
	W/ BSF	143.44	142.46	140.74	142.21	1.37
Day 21	W/O BSF	85.04	83.14	82.63	83.60	1.27
	W/ BSF	90.66	90.42	90.13	90.40	0.27
Day 28	W/O BSF	75.65	73.87	70.91	73.48	2.39
	W/ BSF	83.53	72.24	70.83	75.53	6.96
Day 35	W/O BSF	62.53	61.68	60.53	61.58	1.00
	W/ BSF	63.81	61.73	58.63	61.39	2.61
Day 42	W/O BSF	60.91	59.87	57.65	59.48	1.67
	W/ BSF	55.87	52.24	50.48	52.86	2.75
Day 49	W/O BSF	58.68	55.53	53.53	55.91	2.60
	W/ BSF	60.58	58.83	57.83	59.08	1.39

Table E7 Removal of pyrene at 30 μg g^{-1} leaf in a batch experiment by *Kocuria* sp. IC3 and *I. coccinea* leaves with and without biosurfactant.

Conditions	Time	Pyrene remaining on leaves (μg g^{-1} leaves)				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Leaves	Day 0	29.61	28.03	28.03	28.55	0.91
	Day 3	17.59	15.29	14.83	15.91	1.48
	Day 7	11.08	9.61	7.49	9.39	1.81
	Day 14	6.18	5.60	4.88	5.55	0.65
Leaves with BSF	Day 0	29.61	28.03	28.03	28.55	0.91
	Day 3	12.86	10.66	9.24	10.92	1.82
	Day 7	6.75	4.91	4.65	5.44	1.14
	Day 14	1.70	1.81	1.15	1.55	0.35
Leaves with BSF + *Kocuria* sp. IC3	Day 0	29.61	28.03	28.03	28.55	0.91
	Day 3	10.71	7.42	6.09	8.07	2.38
	Day 7	3.73	3.35	2.66	3.25	0.54
	Day 14	0.00	0.00	0.00	0.00	0.00
Sterilized leaves	Day 0	29.61	28.03	28.03	28.55	0.91
	Day 3	26.33	22.82	21.97	23.71	2.31
	Day 7	21.07	20.24	18.78	20.03	1.16
	Day 14	19.71	17.59	17.70	18.33	1.19

Table E8 Number of total and pyrene-degrading bacteria on leaves during pyrene removal in a batch experiment containing *I. coccinea* leaves with and without biosurfactant.

A. Total bacteria

Conditions	Time	Number of total bacteria on leaves (CFU g^{-1} leaf)				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Leaves	Day 0	1.0E+06	1.2E+06	1.8E+06	1.3E+06	4.2E+05
	Day 3	2.9E+05	3.4E+05	5.1E+05	3.8E+05	1.2E+05
	Day 7	7.7E+06	5.6E+06	7.3E+06	6.9E+06	1.1E+06
	Day 14	1.6E+07	1.1E+07	1.5E+07	1.4E+07	2.4E+06
Leaves with BSF	Day 0	1.0E+06	1.2E+06	1.8E+06	1.3E+06	4.2E+05
	Day 3	2.9E+05	3.4E+05	5.1E+05	3.8E+05	1.2E+05
	Day 7	2.9E+07	4.4E+07	5.3E+07	4.2E+07	1.2E+07
	Day 14	6.4E+07	7.3E+07	7.7E+07	7.1E+07	6.7E+06
Leaves with BSF + *Kocuria* sp. IC3	Day 0	5.3E+07	7.0E+07	7.5E+07	6.6E+07	1.2E+07
	Day 3	1.5E+07	2.0E+07	2.2E+07	1.9E+07	3.4E+06
	Day 7	2.8E+08	2.9E+08	4.4E+08	3.4E+08	8.9E+07
	Day 14	4.5E+08	6.7E+08	6.1E+08	5.8E+08	1.1E+08
Sterilized leaves	Day 0	1.0E+04	1.2E+04	1.7E+04	1.3E+04	3.5E+03
	Day 3	2.9E+03	3.4E+03	4.8E+03	3.7E+03	1.0E+03
	Day 7	2.6E+04	3.3E+04	4.1E+04	3.3E+04	7.6E+03
	Day 14	4.8E+04	3.5E+04	2.7E+04	3.7E+04	1.0E+04
Control	Day 0	1.0E+06	1.2E+06	1.8E+06	1.3E+06	4.2E+05
	Day 3	2.9E+05	3.4E+05	5.1E+05	3.8E+05	1.2E+05
	Day 7	2.0E+06	2.4E+06	3.5E+06	2.7E+06	7.9E+05
	Day 14	3.0E+06	3.5E+06	2.5E+06	3.0E+06	4.9E+05

B. Pyrene-degrading bacteria

Conditions	Time	Number of pyrene-degrading bacteria on leaves (CFU g^{-1} leaf)				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Leaves	Day 0	9.7E+04	1.0E+05	1.3E+05	1.1E+05	2.0E+04
	Day 3	3.3E+05	4.1E+05	5.6E+05	4.4E+05	1.2E+05
	Day 7	6.4E+05	4.5E+05	8.1E+05	6.3E+05	1.8E+05
	Day 14	1.9E+06	1.4E+06	1.5E+06	1.6E+06	2.7E+05
Leaves with BSF	Day 0	9.7E+04	1.0E+05	1.3E+05	1.1E+05	2.0E+04
	Day 3	1.1E+06	1.3E+06	2.5E+06	1.6E+06	7.6E+05
	Day 7	2.5E+06	2.9E+06	5.3E+06	3.6E+06	1.5E+06
	Day 14	4.7E+06	5.5E+06	6.6E+06	5.6E+06	9.3E+05
Leaves with BSF + *Kocuria* sp. IC3	Day 0	6.1E+06	7.0E+06	8.4E+06	7.2E+06	1.1E+06
	Day 3	1.2E+07	2.0E+07	2.7E+07	2.0E+07	7.4E+06
	Day 7	2.3E+07	2.9E+07	4.4E+07	3.2E+07	1.1E+07
	Day 14	4.1E+07	3.9E+07	5.3E+07	4.4E+07	7.9E+06
Sterilized leaves	Day 0	9.1E+02	1.2E+03	1.4E+03	1.2E+03	2.6E+02
	Day 3	1.1E+03	1.7E+03	1.9E+03	1.6E+03	4.0E+02
	Day 7	1.3E+03	1.5E+03	1.9E+03	1.5E+03	3.4E+02
	Day 14	1.8E+03	2.1E+03	1.6E+03	1.8E+03	2.3E+02
Control	Day 0	9.7E+04	1.0E+05	1.3E+05	1.1E+05	2.0E+04
	Day 3	1.6E+05	1.9E+05	2.4E+05	2.0E+05	4.0E+04
	Day 7	1.5E+05	2.1E+05	2.7E+05	2.1E+05	6.0E+04
	Day 14	2.7E+05	2.3E+05	2.3E+05	2.4E+05	2.1E+04

Table E9 Removal of pyrene at 30 μg g^{-1} leaf a batch experiment by *Kocuria* sp. IC3 and *L. frutescens* leaves with and without biosurfactant.

Conditions	Time	Pyrene remaining on leaves (μg g^{-1} leaves)				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Leaves	Day 0	28.71	28.73	29.36	28.93	0.37
	Day 3	21.80	24.14	24.82	23.59	1.59
	Day 7	20.31	21.56	22.25	21.37	0.98
Leaves with BSF	Day 0	28.71	28.73	29.36	28.93	0.37
	Day 3	17.78	18.91	21.38	19.36	1.84
	Day 7	17.94	18.99	19.80	18.91	0.93
Leaves with BSF + *Kocuria* sp. IC3	Day 0	28.71	28.73	29.36	28.93	0.37
	Day 3	16.65	18.38	18.79	17.94	1.13
	Day 7	17.03	17.43	18.27	17.57	0.63
Sterilized leaves	Day 0	28.71	28.73	29.36	28.93	0.37
	Day 3	23.77	26.00	27.18	25.65	1.73
	Day 7	22.67	24.62	24.52	23.93	1.10

Table E10 Number of total phyllosphere and pyrene-degrading bacteria on leaves during pyrene removal in the batch experiment containing leaves of *L. frutescens* with and without biosurfactant.

A. Total phyllosphere bacteria

Conditions	Time	Number of total phyllosphere bacteria on leaves (CFU g^{-1} leaves)				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Leaves	Day 0	3.4E+06	3.9E+06	4.3E+06	3.8E+06	4.5E+05
	Day 3	2.4E+06	3.5E+06	3.8E+06	3.2E+06	7.6E+05
	Day 7	6.2E+05	7.2E+05	8.0E+05	7.1E+05	9.4E+04
Leaves with BSF	Day 0	3.4E+06	3.9E+06	4.3E+06	3.8E+06	4.5E+05
	Day 3	1.5E+06	1.9E+06	2.9E+06	2.1E+06	7.5E+05
	Day 7	6.2E+05	6.8E+05	7.7E+05	6.9E+05	7.7E+04
Leaves with BSF + *Kocuria* sp. IC3	Day 0	4.1E+07	4.7E+07	5.1E+07	4.6E+07	5.0E+06
	Day 3	2.0E+07	2.6E+07	3.5E+07	2.7E+07	7.8E+06
	Day 7	2.9E+06	4.7E+06	5.6E+06	4.4E+06	1.4E+06
Sterilized leaves	Day 0	1.1E+04	1.5E+04	1.6E+04	1.4E+04	2.6E+03
	Day 3	1.3E+04	2.0E+04	2.4E+04	1.9E+04	5.6E+03
	Day 7	9.3E+03	1.2E+04	1.4E+04	1.2E+04	2.5E+03
Control	Day 0	3.4E+06	3.9E+06	4.3E+06	3.8E+06	4.5E+05
	Day 3	4.1E+06	4.7E+06	4.8E+06	4.5E+06	3.4E+05
	Day 7	4.4E+05	4.8E+05	5.5E+05	4.9E+05	5.4E+04

B. Pyrene-degrading bacteria

Conditions	Time	Number of pyrene-degrading bacteria on leaves (CFU g^{-1} leaves)				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Leaves	Day 0	9.2E+04	1.0E+05	1.1E+05	1.0E+05	9.4E+03
	Day 3	1.3E+05	1.6E+05	2.1E+05	1.7E+05	4.2E+04
	Day 7	1.0E+04	1.2E+04	1.3E+04	1.2E+04	1.7E+03
Leaves with BSF	Day 0	9.2E+04	1.0E+05	1.1E+05	1.0E+05	9.4E+03
	Day 3	4.3E+05	4.4E+05	5.4E+05	4.7E+05	6.0E+04
	Day 7	1.3E+04	1.4E+04	1.9E+04	1.5E+04	3.5E+03
Leaves with BSF + *Kocuria* sp. IC3	Day 0	6.7E+06	7.0E+06	7.6E+06	7.1E+06	4.7E+05
	Day 3	1.3E+06	1.7E+06	1.8E+06	1.6E+06	2.7E+05
	Day 7	2.2E+05	2.6E+05	3.3E+05	2.7E+05	5.3E+04
Sterilized leaves	Day 0	9.0E+02	1.2E+03	1.4E+03	1.1E+03	2.3E+02
	Day 3	9.9E+02	1.8E+03	1.8E+03	1.5E+03	4.7E+02
	Day 7	9.6E+02	1.0E+03	1.2E+03	1.1E+03	1.3E+02
Control	Day 0	9.2E+04	1.0E+05	1.1E+05	1.0E+05	9.4E+03
	Day 3	1.3E+05	1.6E+05	1.8E+05	1.6E+05	2.8E+04
	Day 7	1.3E+04	1.6E+04	2.0E+04	1.6E+04	3.5E+03

Table E11 Time course of phenanthrene, fluoranthene, and pyrene remaining in the air of a gas-tight chamber experiment containing leaves of *I. coccinea* with biosurfactant. Control experiment was conducted without plant leaves.

A. Phenanthrene

Conditions	Time	Phenanthrene concentration in air (μg L^{-1} air)			
		Rep. 1	Rep. 2	Avg.	SD
Control	Day 0	38.40	38.01	38.2	0.3
	Day 1	37.76	38.31	38.0	0.4
	Day 2	42.94	40.73	41.8	1.6
	Day 3	43.69	42.95	43.3	0.5
	Day 4	45.55	42.46	44.0	2.2
	Day 5	39.43	40.00	39.7	0.4
	Day 6	41.64	40.53	41.1	0.8
	Day 7	38.65	41.36	40.0	1.9
	Day 8	40.84	39.29	40.1	1.1
	Day 9	38.92	37.71	38.3	0.9
	Day 10	38.76	36.53	37.6	1.6
	Day 11	35.31	36.25	35.8	0.7
	Day 12	32.84	28.69	30.8	2.9
	Day 13	29.44	32.22	30.8	2.0
	Day 14	29.54	31.77	30.7	1.6
Treatment	Day 0	32.80	27.98	30.39	3.41
	Day 1	31.47	35.21	33.34	2.64
	Day 2	16.93	29.17	23.05	8.65
	Day 3	33.77	26.21	29.99	5.34

A. Phenanthrene (Continued)

Conditions	Time	Phenanthrene concentration in air (μg L^{-1} air)			
		Rep. 1	Rep. 2	Avg.	SD
Treatment	Day 4	32.79	28.22	30.51	3.23
	Day 5	22.95	22.19	22.57	0.54
	Day 6	15.78	21.15	18.46	3.80
	Day 7	20.10	18.02	19.06	1.48
	Day 8	9.82	10.55	10.18	0.52
	Day 9	9.42	11.08	10.25	1.17
	Day 10	8.00	10.47	9.24	1.75
	Day 11	7.63	11.85	9.74	2.98
	Day 12	6.28	7.48	6.88	0.85
	Day 13	7.61	6.88	7.24	0.52
	Day 14	6.47	5.31	5.89	0.82

B. Fluoranthene

Conditions	Time	Fluoranthene concentration in air (μg L^{-1} air)			
		Rep. 1	Rep. 2	Avg.	SD
Control	Day 0	20.90	21.68	21.3	0.5
	Day 1	18.61	21.47	20.0	2.0
	Day 2	20.06	20.95	20.5	0.6
	Day 3	22.00	19.19	20.6	2.0
	Day 4	18.65	22.06	20.4	2.4
	Day 5	19.70	19.01	19.4	0.5
	Day 6	18.54	21.77	20.2	2.3
	Day 7	18.23	21.04	19.6	2.0

B. Fluoranthene (Continued)

Conditions	Time	Fluoranthene concentration in air (μg L^{-1} air)			
		Rep. 1	Rep. 2	Avg.	SD
Control	Day 8	20.05	20.79	20.4	0.5
	Day 9	21.47	19.49	20.5	1.4
	Day 10	21.54	18.64	20.1	2.0
	Day 11	18.80	16.77	17.8	1.4
	Day 12	18.22	17.14	17.7	0.8
	Day 13	18.11	16.86	17.5	0.9
	Day 14	17.69	16.92	17.3	0.5
Treatment	Day 0	20.89	21.67	21.28	0.55
	Day 1	20.31	21.49	20.90	0.84
	Day 2	16.61	15.99	16.30	0.44
	Day 3	16.79	20.07	18.43	2.32
	Day 4	19.21	16.92	18.07	1.62
	Day 5	16.60	17.49	17.05	0.63
	Day 6	14.30	12.46	13.38	1.30
	Day 7	16.61	13.76	15.19	2.02
	Day 8	12.88	13.24	13.06	0.25
	Day 9	12.75	13.20	12.98	0.32
	Day 10	9.20	7.63	8.41	1.11
	Day 11	7.00	9.18	8.09	1.54
	Day 12	8.64	6.33	7.49	1.63
	Day 13	8.60	7.27	7.93	0.95
	Day 14	7.16	5.43	6.29	1.22

C. Pyrene

Conditions	Time	Pyrene concentration in air (μg L^{-1} air)			
		Rep. 1	Rep. 2	Avg.	SD
Control	Day 0	19.95	21.93	20.9	1.4
	Day 1	22.29	18.91	20.6	2.4
	Day 2	24.01	23.65	23.8	0.3
	Day 3	23.85	24.95	24.4	0.8
	Day 4	25.16	24.41	24.8	0.5
	Day 5	23.24	22.19	22.7	0.7
	Day 6	23.29	21.94	22.6	1.0
	Day 7	23.23	21.86	22.5	1.0
	Day 8	18.22	16.73	17.5	1.1
	Day 9	18.36	16.84	17.6	1.1
	Day 10	18.28	16.71	17.5	1.1
	Day 11	17.40	16.73	17.1	0.5
	Day 12	15.07	16.77	15.9	1.2
	Day 13	13.51	16.29	14.9	2.0
	Day 14	17.76	16.86	17.3	0.6
Treatment	Day 0	20.47	21.93	21.20	1.03
	Day 1	17.49	20.79	19.14	2.33
	Day 2	19.95	21.46	20.71	1.07
	Day 3	18.41	14.87	16.64	2.50
	Day 4	13.90	14.86	14.38	0.68
	Day 5	12.56	11.83	12.20	0.52
	Day 6	11.07	9.70	10.39	0.97
	Day 7	11.22	12.65	11.93	1.01

C. Pyrene (Continued)

Conditions	Time	Pyrene concentration in air ($\mu g\ L^{-1}$ air)			
		Rep. 1	Rep. 2	Avg.	SD
Treatment	Day 8	8.27	7.11	7.69	0.82
	Day 9	9.25	8.67	8.96	0.41
	Day 10	6.39	6.86	6.63	0.34
	Day 11	4.77	6.58	5.67	1.29
	Day 12	4.40	4.82	4.61	0.30
	Day 13	3.79	4.61	4.20	0.59
	Day 14	3.36	3.93	3.65	0.40

Table E12 Concentration of deposited PAHs on leaves after spraying biosurfactant and deposited on GF/C filter paper compared initial and final day of experiment.

A. Concentration of deposit PAHs on leaves

Types of PAHS	Time	PAHs concentration on leaves (μg g^{-1} leaves)			
		Rep. 1	Rep. 2	Avg.	SD
Phenanthrene	Day 0	435.25	456.27	445.76	14.86
	Day 14	49.72	68.86	59.29	13.54
Fluoranthene	Day 0	436.11	423.02	429.56	9.26
	Day 14	102.01	117.81	109.91	11.17
Pyrene	Day 0	440.76	461.48	451.12	14.65
	Day 14	165.64	137.11	151.37	20.17
Banzo (a) Pyrene	Day 0	46.90	44.67	45.79	1.58
	Day 14	20.90	26.56	23.73	4.01

B. Concentration of deposit PAHs on GF/C filter paper

Types of PAHS	Time	PAHs concentration on GF/C filter paper (μg g^{-1} GF/C filter paper)			
		Rep. 1	Rep. 2	Avg.	SD
Phenanthrene	Day 0	5412.22	5398.52	5405.37	9.69
	Day 14	762.48	705.13	733.80	40.56
Fluoranthene	Day 0	5446.67	5371.28	5408.97	53.31
	Day 14	1315.29	1162.40	1238.85	108.11
Pyrene	Day 0	5221.65	5398.35	5310.00	124.95
	Day 14	969.48	1179.00	1074.24	148.15
Banzo (a) Pyrene	Day 0	526.33	512.64	519.49	9.68
	Day 14	320.83	348.41	334.62	19.51

Table E13 Number of total phyllosphere and PAHs-degrading bacteria on leaves during mixed-PAHs removal in the chamber experiment containing whole plant of *I. coccinea* with biosurfactant addition.

Types of bacteria	Time	Number of bacteria on leaves (CFU g^{-1} leaves)				
		Rep. 1	Rep. 2	Rep. 3	Avg.	SD
Total bacteria	Day 0	2.4E+06	1.6E+06	2.5E+06	2.2E+06	4.5E+05
	Day 14	2.1E+07	2.4E+07	2.5E+07	2.4E+07	2.2E+06
PAHs-degrading bacteria	Day 0	1.7E+05	1.8E+05	2.1E+05	1.9E+05	2.3E+04
	Day 14	2.2E+06	2.5E+06	2.7E+06	2.5E+06	2.7E+05

APPENDIX F

JOURNAL PUBLICATION, CONFERENCES AND PROCEEDING

I. JOURNAL PUBLICATION

Siriratruengsuk, W., Furuuchi, M., Prueksasit, T., & Luepromchai, E. (2017). Potential of Pyrene Removal from Urban Environments by the Activities of Bacteria and Biosurfactant on Ornamental Plant Leaves. Water, Air, & Soil Pollution, 228(7), 264.

II. CONFERENCE

Siriratruengsuk, W., Furuuchi, M., Prueksasit, T., & Luepromchai, E. (2013). Pyrene-Degrading Bacteria on Ornamental Plant Leaves along Urban Roadsides. Poster presentation on International Conference on Environmental and Hazardous Substances Management. 21-23 May 2013. Bangkok, Thailand.

Siriratruengsuk, W., Furuuchi, M., Prueksasit, T., & Luepromchai, E. (2015). Phylloremediation of Airborne Pyrene and Its Enhancement by Lipopeptide-Biosurfactant. Poster presentation on The 27th Annual Meeting of the Thai Society for Biotechnology and International Conference. 17-20 November 2015. Bangkok, Thailand.

Luepromchai, E., Siriratruengsuk, W., Furuuchi, M., & Prueksasit, T. (2017). Abundance of Airborne and Phyllosphere Pyrene-Degrading Bacteria in the Urban Environment and the Role of Biosurfactant on Enhancing Pyrene Removal. Oral presentation on International Union of Microbiological Societies. 17-21 July 2017. Singapore.

III. PROCEEDING

Pinthong, J., Paorach, N., Khondee, N., Siriratruengsuk, W., & Luepromchai, E. (2015). Reduction of Formaldehyde in Air by *Epipremnum auream* and Its Phyllosphere Bacteria. Proceeding of the 3rd Environmental Asia International Conference on Towards International Collaboration for an Environmentally Sustainable World. 17-19 June 2015. Bangkok, Thailand.

Pinthong, J., Siriratruengsuk, W., Paorach, N., & Luepromchai, E. (2015). Utilization of Phyllosphere Bacteria for Formaldehyde Removal. Proceeding of the 1st International Conference on Environment, Livelihood, and Services: Environment for Life. 2-5 November 2015. Bangkok, Thailand.

VITA

Mr. Weerayuth Siriratruengsuk was born on September 25, 1982 in Songkhla, Thailand. He graduated with a bachelor of science in Environmental Health from Institute of Medicine, Suranaree University of Technology, Thailand. Later, he pursued his master's degree majoring in Environmental Sanitation from Faculty of Public Health, Mahidol University, Thailand since 2004 - 2006. After that he started his work as a lecturer in School of Health Science, Mae Fah Luang University, Thailand. In 2011, he started his Ph.D. in the international Program in Environmental Management, Center of Excellence on Hazardous Substance Management (HSM) Graduate School, Chulalongkorn University, Thailand and complete his doctoral degree in 2016.

The publication and conferences were shown in Appendix F.