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**BIODEGRADATION OF BENZO(A)PYRENE BY FUNGI**

**Mrs. Suphang Chulalaksananukul**

**A Dissertation Submitted in Partial Fulfillment of the Requirements  
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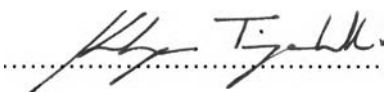
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
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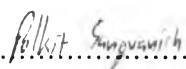
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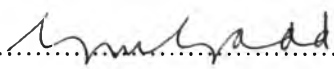
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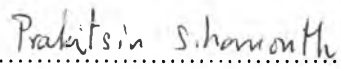
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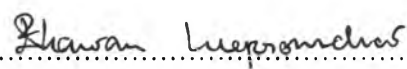
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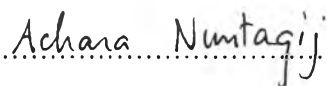
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สุภาวงศ์ จุฬาลักษณ์านุกูล : การย่อยสลายทางชีวภาพของสารเบนโซไพเร็นโดยรา  
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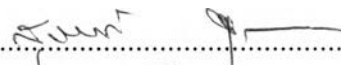
การย่อยสลายทางชีวภาพของสารเบนโซไพเร็นโดยรา ซึ่งคัดแยกมาจากใบและเปลือกของต้น  
ประดู่ที่สัมพันธ์กับควั่นรถยนต์ในบริเวณการจราจรคับคั่ง ผลการคัดแยกได้ราทั้งสิ้น 53 ชนิด ราที่คัดแยกจาก  
ใบประดู่แบ่งเป็นรา epiphyte 40 ชนิด และรา endophyte 7 ชนิด และราที่ได้จากเปลือกประดู่ 6 ชนิด  
ราทั้งหมดนี้ถูกตรวจสอบความสามารถในการย่อยสลายสารเบนโซไพเร็นที่ ความเข้มข้นเริ่มต้น 100 พีพี  
เอ็ม (0.4 มิลลิโมลาร์) บนอาหารแข็ง พบว่ามีรา 13 ชนิดที่มีการตอบสนองทางบวกกับสารนี้ จึงมีการศึกษา  
ต่อในอาหารเหลว เพื่อดูความสามารถในการย่อยสลายสารเบนโซไพเร็น ผลการศึกษาพบว่า มีราเพียง 3  
ชนิดที่สามารถย่อยสลายสารเบนโซไพเร็น 100 พีพีเอ็มในอาหารเหลวที่เติมกลูโคส ที่อุณหภูมิ 32 องศา  
เซลเซียสและ เขย่าด้วยความเร็ว 120 รอบต่อนาที การตรวจหาชนิดของราทั้งหมดโดยเทคนิคทางรูปพรรณ  
สัณฐานและเทคนิคทางพันธุศาสตร์ (การหาลำดับเบส) พบว่าเป็นรา *Aspergillus niger* N003 รา  
*Aspergillus niger* B002 และรา *Fusarium oxysporum* E033

เมื่อทำการศึกษาทางจุลนศาสตร์ของการย่อยสลาย รวมถึงตัวแปรที่มีผลต่อการย่อยสลายและ  
สารมัธยันต์ที่เกิดจากการย่อยสลายในราแต่ละชนิด พบว่ารา *Aspergillus niger* N003 รา *Aspergillus*  
*niger* B002 และรา *Fusarium oxysporum* E033 แสดงประสิทธิภาพการย่อยสลายได้ 80  
เปอร์เซ็นต์ 70 เปอร์เซ็นต์ และ 65 เปอร์เซ็นต์ตามลำดับ สำหรับราทั้ง 3 ชนิด เมื่อเพิ่มอัตราการให้อากาศ  
จะเพิ่มทั้งมวลของราและการย่อยสลาย ที่ความเข้มข้นของสารเบนโซไพเร็นสูงๆ (มากกว่า 300 พีพีเอ็ม)  
พบความเป็นพิษต่อราแต่ยังพบการย่อยสลายได้ ถึงแม้จะพบที่ประสิทธิภาพที่ต่ำกว่า ที่ปริมาณกลูโคสสูงๆ  
(50 มิลลิโมลาร์) ทำให้การย่อยสลายของสารเบนโซไพเร็นลดลงอย่างมีนัยสำคัญ การเพิ่มขีดความ  
สามารถในการถูกใช้ของสารนี้ โดยใช้เอทานอลและเมธานอลเป็นแหล่งคาร์บอนทดแทน ช่วยเพิ่มปริมาณ  
มวลของราแต่การย่อยสลายกลับลดลง

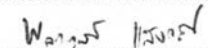
สารมัธยันต์ที่เกิดจากกระบวนการย่อยสลายสารเบนโซไพเร็นของราแต่ละชนิด ซึ่งตรวจหาได้  
โดยการใช้เครื่องลิวติโดกราฟฟีและเทคนิคทางแมสสเปคโตรมิเตอร์ พบว่ารา *Aspergillus niger*  
N003 และรา *Aspergillus niger* B002 เปลี่ยนรูปสารเบนโซไพเร็น เป็นสารไดไฮดรอกซี-ไดไฮโดร  
ไดออลเบนโซไพเร็น ส่วนรา *Fusarium oxysporum* E033 เปลี่ยนรูปสารเบนโซไพเร็นด้วยวิถี  
ทางที่แตกต่างกัน 2 แบบ เกิดสารมัธยันต์ที่แตกต่างกัน คือ สารสกัดภายนอกเซลล์พบสารเบนโซไพเร็น คิว  
โน ในขณะที่พบสารไดไฮโดรไดออลและสารเบนโซไพเร็นไดออลอีปอร์ไกด์ จากส่วนสกัดสารภายในเซลล์


สาขาวิชา การจัดการสิ่งแวดล้อม

ปีการศึกษา 2548

ลายมือชื่อนิสิต..... 

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KEY WORD: BENZO(A)PYRENE /BIODEGRADATION /ASPERGILLUS SP./  
METABOLITE

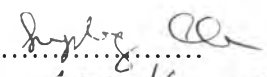
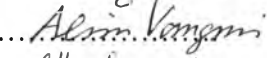
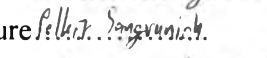
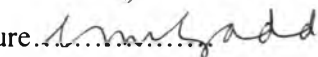
SUPHANG CHULALAKSANANUKUL: BIODEGRADATION OF BENZO  
(A)PYRENE BY FUNGI. THESIS ADVISOR: ASSISTANT PROFESSOR  
ALISA VANGNAI, Ph.D., THESIS CO-ADVISOR: ASSISTANT  
PROFESSOR POLKIT SANGVANICH, Ph.D., AND PROFESSOR  
GEOFFREY M. GADD, Ph.D., 132pp. ISBN 974-14-2922-3.

Biodegradation of Benzo(a)pyrene (BaP) by fungi which isolated from leaves and barks of *Pterocarpus macrocarpus* Kurz. long exposed to the heavy traffic smoke. Fifty three fungi were obtained in total. The fungi isolated from leaves could be classified as 40 epiphytic fungi and 7 endophytic fungi, while 6 fungal isolates were obtained from barks. These fungal isolates were tested for their ability to degrade 100 ppm. BaP (0.4 mM) on solid medium. There were 13 fungal isolates which have the positive response towards BaP. These isolates were then tested in liquid medium in order to investigate BaP biodegradation ability. Three fungal isolates were finally obtained having relatively high BaP degradation ability when grown on glucose-containing medium supplemented with 100 ppm. BaP (0.4 mM) at 32°C, with 120 rpm-shaking conditions. These three fungal isolates were identified morphologically and genetically to be *Aspergillus niger* N003, *Aspergillus niger* B002 and *Fusarium oxysporum* E033.

Then, for each three isolates, BaP degradation kinetics, factors affecting the biodegradation as well as the biodegradation intermediates formed during the biodegradation process were investigated. *Aspergillus niger* N003, *Aspergillus niger* B002 and *Fusarium oxysporum* E033 exhibited 80%, 70% and 65% BaP biodegradation efficiency, respectively. To all fungal isolates, when the aeration rate was increased, the fungal biomass was increased as well as their biodegradation. Higher concentrations of BaP (up to 300 ppm) become toxic to cells; however, they maintained to have BaP biodegradability, although with less efficiency. Higher glucose concentration (50 mM) promoted fungal biomass, but the biodegradation was significantly repressed. Ethanol or methanol was supplemented as an alternative carbon source and to enhance BaP bioavailability in liquid medium. They were successfully increased fungal biomass, but had adverse effect to BaP biodegradation.

The intermediates from BaP biodegradation of each fungal isolate were revealed using liquid chromatography and mass spectrometry. *Aspergillus niger* N003 and *Aspergillus niger* B002 biotransformed BaP to dihydroxy-dihydrodiol, whereas *Fusarium oxysporum* E033 biotransformed BaP via 2 different pathways forming different biodegradation intermediates. While the extra-cellular BaP biodegradation by *Fusarium oxysporum* E033 yielded BaP quinone, BaP dihydrodiol and BaP diol epoxide were detected via intra-cellular BaP biodegradation.

Field of study Environmental Management  
Academic year 2005

Student's signature.....  
Advisor's signature...  
Co-advisor's signature   
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**LIST OF ABBREVIATIONS**

ATSDR	=	Agency for Toxic Substances and Disease Registry
BaP	=	Benzo(a)pyrene
BPDE	=	Benzo(a)pyrene Diol Epoxide
CERCLA	=	The Comprehensive Environmental Response, Compensation and Liability Act
DCM	=	dichloromethane
HPLC	=	High Performance liquid chromatography
IARC	=	The International Agency for Research on Cancer
ITS	=	Internal Transcribed Spacer
K <sub>ow</sub>	=	Octanol-water partition coefficients
LC-MS	=	Liquid Chromatography- Mass Spectrometry
mM	=	milli-molar
MEA	=	Malt Extract Agar
MSM	=	Mineral Salts Medium
PAHs	=	Polycyclic Aromatic Hydrocarbons
PCR	=	Polymerase Chain Reaction
ppm	=	part per million
USEPA	=	United State of the Environmental Protection Agency