Biodegradation of High Molecular Weight Polycyclic Aromatic Hydrocarbons in Soils by Defined Bacterial and Fungal Cocultures

A Thesis submitted for the degree of

DOCTOR OF PHILOSOPHY

By

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Declaration

I CERTIFY THAT THIS THESIS DOES NOT INCORPORATE WITHOUT ACKNOWLEDGMENT ANY MATERIAL PREVIOUSLY SUBMITTED FOR A DEGREE OR DIPLOMA IN ANY UNIVERSITY; AND THAT TO THE BEST OF MY KNOWLEDGE AND BELIEF IT DOES NOT CONTAIN ANY MATERIAL PREVIOUSLY PUBLISHED OR WRITTEN BY ANOTHER PERSON EXCEPT WHERE DUE REFERENCE IS MADE IN THE TEXT.

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"Everything that lives, lives not alone, nor for itself"
William Blake

Publications

Conference Abstracts

Lease C, Bentham R and McClure N (2002) *Degradation of High Molecular Weight Polycyclic Aromatic Hydrocarbons by Defined Fungal-Bacterial Co-Cultures in* Proceedings of the Environmental Engineering Research Event Blackheath NSW (Poster)

Stewart R, Juhasz AL, Lease C, Dandie C, Waller N and Bentham R (2004) *An Emerging Technology for High Molecular Weight PAH Bioremediation – Bacterial – Fungal Co-Cultures in* Proceedings of Enviro 04 Conference and Exhibition, Sydney, Australia (Poster)

Lease C, Bentham R, Juhasz AL and Stanley G (2004) *Breaking Benzo[a]pyrene: The Case for Cocultures in* Proceedings of the 4th International Conference on the Remediation of Chlorinated and Recalcitrant Compounds, Monterey, United States of America (Platform Presentation – Winner of Student Paper Prize)

Manuscripts in Preparation

Juhasz AL, Waller N, Lease C, Bentham R and Stewart R (*submitted*) *Pilot Scale Bioremediation of Creosote-Contaminated Soil – Efficacy of Enhanced Natural Attenuation and Bioaugmentation Strategies*

Summary

Despite microbial degradation being the primary route of degradation of PAHs in soils, high molecular weight polycyclic aromatic hydrocarbons (such as benzo[*a*]pyrene) have consistently proven to be resistant to microbial attack. However, recent research has demonstrated the potential for bacterial-fungal co-cultures to achieve biodegradation of high molecular weight PAHs. The aim of this research was to determine the efficacy of co-culture bioaugmentation for the remediation of high molecular weight PAH-contaminated soils.

PAH degrading bacteria were enriched on multiple PAHs and isolated on pyrene from both contaminated (soil from a former manufactured gas plant) and uncontaminated (agricultural soil, termite mound matrix and kangaroo faeces) sources. The bacterial isolates were identified using 16SrRNA analysis as *Mycobacterium* sp. Strain BS5, *Mycobacterium* sp. Strain KA5 and *Mycobacterium* sp. Strain KF4 or fatty acid methyl ester (FAME) analysis as *Ralstonia pickettii* and *Stenotrophomonas maltophilia*.

The initial phase of assessment of PAH degradation by fungal and bacterial coculture components was undertaken using liquid media. Two fungal isolates from a previous investigation into the coculture process (*Penicillium janthinellum*) and the American Type Culture Collection (*Phanerochaete chrysosporium*) were assessed for their ability to degrade benzo[*a*]pyrene in minimal media and MYPD. The fungal isolates were found to be able to degrade benzo[a]pyrene cometabolically in MYPD. The bacterial isolates and two others from previous investigations were assessed for their ability to degrade single PAHs (fluorene, phenanthrene, fluoranthene, pyrene and benzo[a]pyrene) in liquid culture. This process was used as an initial screen to select the best bacterial isolates for further investigation of PAH degradation by axenic cultures and cocultures with the fungal isolates using a PAH mixture. Based on the results of these experiments four bacterial isolates (VUN 10,010, *Mycobacterium* 1B, *Mycobacterium* sp. Strain BS5 and *Mycobacterium* sp. Strain KA5) and the two fungal isolates were selected to investigate further using a PAH mixture composed of the previously mentioned PAHs. It was found

that the use of a fungal bacterial coculture increased the degradation of the PAH mixture beyond that of axenic bacterial cultures. Based on these experiments, the coculture composed of *P. janthinellum* and VUN 10,010 was selected for assessment of its ability to degrade the same PAH mixture in spiked soil microcosm experiments.

Natural attenuation, axenic *P. janthinellum*, axenic VUN 10,010 and a coculture of these two organisms were assessed for PAH degradation in soil microcosms over a 100 day period. Inoculation of microcosms with the coculture resulted in the removal of benzo[a]pyrene by 11 mg/kg (± 1.21 mg/kg) (30%) over the 100 day incubation period. Substantial PAH degradation was also observed in the microcosms assesing natural attenuation

Using an alternative sequential inoculation method, initially inoculating with *P. janthinellum* then 50 days later with VUN 10,010 significantly enhanced the removal of benzo[*a*]pyrene. After 100 days incubation, benzo[*a*]pyrene was degraded below detection limits in two of three microcosms, compared to a 4.95 mg/kg (± 4.64 mg/kg) (14.7 %) reduction in soil microcosms inoculated using an alternative inoculation process of VUN 10,010 followed by *P. janthinellum*.

Attempts were made to optimise the process using sequential inoculation and soil amendments intended to enhance the performance of the fungal component using distilled water and 1% glucose. The addition of distilled water was not observed to substantially influence the ability of the coculture to degrade PAHs, whereas the addition of 1% glucose was found to inhibit PAH degradation.

Symbols and Abbreviations

%	Percent
BaP	Benzo[*a*]pyrene
BSM	Basal Salts Medium
BSMY	Basal Salts Medium with Yeast Extract
BSMY3	Basal Salts Medium with Yeast Extract (3%)
^{o}C	Degree Celsius
cfu	Colony Forming Unit
DCM	Dichloromethane
DMF	Dimethylformamide
DNA	Deoxyribonucleic Acid
dNTP	Deoxynucleotide triphosphate
EDTA	Ethylenediaminetetra-acetic Acid
EPA	Environment Protection Authority (Australia)
FID	Flame Ionisation Detector
g	Gram
GC	Gas Chromatography
$HgCl_2$	Mercuric Chloride
K_{ow}	Octanol/Water partition coefficient
kg	Kilogram
l	Litre
LB	Luria-Bertani
LiP	Lignin Peroxidase
LOI	Loss on ignition
MnP	Manganese Peroxidase
MGP	Manufactured Gas Plant
MW	Molecular Weight
µm	Micrometre
µmols/mL	Micromoles per milliliter
µg	Microgram
mg	Milligram
mM	Millimolar
ml	Millilitre
MPN	Most Probable Number
MYPD	Malt Yeast Peptone Dextrose Broth
NA	Nutrient Agar
NB	Nutrient Broth
nm	Nanometre
NSWEPA	New South Wales Environment Protection Agency
PAHs	Polycyclic Aromatic Hydrocarbons
PBS	Phosphate Buffered Saline
PCR	Polymerase Chain Reaction
PDA or PDB	Potato Dextrose Agar or Potato Dextrose Broth
pH	Hydrogen Ion Concentration (minus log of)
rpm	Revolutions per Minute

rDNA or rRNA	ribosomal Deoxynucleic Acid or ribosomal Ribonucleic Acid
SDS	Sodium dodecyl sulphate
Tris	Tris (hydroxymethyl) aminoethane
USEPA	United States Environment Protection Authority
UV	Ultraviolet
VUN	Victoria University Strain Number (Gram negative bacterium)
v/v	volume per volume
w/v	weight per volume
WHC	Water Holding Capacity
x g	times gravity

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