Professorial Lecture

INNOVATIVE GREEN TECHNOLOGY THROUGH MULTIDISCIPLINARY APPROACH

Suhaimi Abdul Talib
Professorial Lecture

INNOVATIVE GREEN TECHNOLOGY
THROUGH MULTIDISCIPLINARY APPROACH
Professorial Lecture

INNOVATIVE GREEN TECHNOLOGY
THROUGH MULTIDISCIPLINARY APPROACH

Suhaimi Abdul Talib
# CONTENTS

- List of Figures vii
- List of Tables xiii
- Preface xvii
- Acknowledgement xix

- Introduction 1
- Heavy Metals Removal using Biological Sulphide 17
- Bioremediation of Polyaromatic Hydrocarbon Contaminated Land 35
- Biosorption for Heavy Metal Removal 63
- Wastewater Treatment and Energy Production 101

- Biography 119
- References 121
- Index 147
<table>
<thead>
<tr>
<th>Figure</th>
<th>Description</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Elements of Innovative R&amp;D (Abdul-Talib, 2012)</td>
<td>12</td>
</tr>
<tr>
<td>2</td>
<td>Oxidation and Reduction Processes of Sulphur Compounds</td>
<td>22</td>
</tr>
<tr>
<td>3</td>
<td>The Schematic Diagram for Metal Sulphide Precipitation</td>
<td>26</td>
</tr>
<tr>
<td>4</td>
<td>Experiment Conducted on Biologically Assisted Nickel-sulphide Precipitation</td>
<td>27</td>
</tr>
<tr>
<td>5</td>
<td>DGGE Profiles of PCR-amplified 16S rRNA Genes from Bulk Water and Biofilm Samples</td>
<td>31</td>
</tr>
<tr>
<td>6</td>
<td>Contaminated Land Remediation Technologies in Malaysia (Yin et al., 2006)</td>
<td>37</td>
</tr>
<tr>
<td>7</td>
<td>Mechanism of Bioremediation (Adapted from USEPA, 2001)</td>
<td>42</td>
</tr>
<tr>
<td>8</td>
<td>Effect of Surface Tension and Concentration of Surfactant on the Formation of Surfactant Micelle</td>
<td>46</td>
</tr>
</tbody>
</table>
Figure 9: Mechanism of Biosurfactants Interactions between Microorganism, Soil and Pollutant (Adapted from Volkering et al., 1997) 47

Figure 10: ZVI-Permeable Reactive Barrier System (Adopted from Cook, 2009) 49

Figure 11: ZVI was Pumped into the Contaminants Flow 49

Figure 12: Reactions Involved on the ZVI Surfaces 51

Figure 13: Degradation of Phenanthrene in Liquid Culture and Sand Slurry at Initial Phenanthrene 53

Figure 14: Biodegradation of LMW and HMW PAH 55

Figure 15: Colonies of Microbes Showing Clear Zone on Blood Agar 56

Figure 16: Microplate Assay (a) POME Strain S01, (b) Tween 80, (c) POME Strain R06 (d) Distilled Water 57

Figure 17: Clear Zone Around (a) Tween 80, (b) POME Strain R06, (c) Corynebacterium Uroalyticum (Municipal Strains) 57

Figure 18: Oil Spreading Assay (a) Measurement Method (b) Clear Zone by POME Strain R06 (c) Clear Zone by Tween 80 57

Figure 19: Clear Zone Diameter (a) Water (Control) (b) Pome Strain S08 58
Figure 20: Phenanthrene Degradation at Different ZVI Concentrations. Data are Mean of Triplicate Samples

Figure 21: Correlation between the Degradation Rates Coefficient of Phenanthrene and ZVI Concentrations

Figure 22: Preparative Steps for the Evaluation of PSMC as a Biosorbent for Heavy Metal Removal

Figure 23: The Effect of Biosorbent Concentration on Heavy Metal Biosorption

Figure 24: SEM Micrograph of PSMC (a) before Biosorption; (b) after Pb(II) Biosorption; (c) after Cu(II) Biosorption; and (d) after Ni(II) Biosorption

Figure 25: EDX Spectrum of PSMC (a) before Biosorption; (b) after Pb(II) Biosorption; (c) after Cu(II) Biosorption; and (d) after Ni(II) Biosorption

Figure 26: FTIR Spectrum of PSMC Biosorbent (a) before Biosorption; (b) after Pb(II) Biosorption; (c) after Cu(II) Biosorption; and (d) after Ni(II) Biosorption

Figure 27: Comparison of CPMAS Spectra for PSMC Biosorbent before and after Heavy Metal Biosorption
Figure 28: $^{13}$C ssNMR Spectra of DP SatRec before and after Heavy Metal Biosorption

Figure 29: DP Invrec Experiment for Heavy Metal Ions Paramagnetic Effect Evaluation (a) Pb(II); (b) Cu(II); and (c) Ni(II)

Figure 30: The T1 Time in TORCHIA Experiments for (a) Lignin Carboxyl; and (b) Lignin C-1/ C-4 of Aromatic of Syringyl and Guaiacyl

Figure 31: Effect of Biosorbent Particle Size on the Mass Transfer and Operational Pressure Drop for a Fixed-bed Biosorption Column

Figure 32: A Typical S-shaped Sorption Curve that is Obtained from Fixed-bed Biosorption. As the Heavy Metal Solution Moves Through the Column (Blue), the Biosorbent Behind the Mass Transfer Zone is Fully Saturated with the Heavy Metal Ions (Black)

Figure 33: Biosorption Curves of Pb(II) Containing Solution using PSMC as Biosorbent under Different Flow Rates and Column Length. The x-axis Values for Curves Obtained using Flow Rate of 1ml/min in Scaled 1/10 to Enable Visualization within the Same Graph

Figure 34: Changes in Pb(II) and Ca(II) Concentration as Well as pH during Biosorption of Pb(II) Ions by PSMC
Figure 35: Typical Results Obtained for PSMC Undergoing Three Cycles of Sorption and Desorption. Each Cycle Consist of Sorption with mg/L Pb(II) Ion Solution Followed by Desorption with 0.1N HNO₃. Regeneration was Achieved by Flushing Off Excess Acid with Water After Desorption.

Figure 36: Schematic Diagram of a Typical Two-chamber MFC (Source: Modification from Du et al., 2007)

Figure 37: Two-chambered MFC

Figure 38(a): Biodegradation of COD

Figure 38(b): Current Generation
Table 1: Pollutants Addressed in EQA 1974
Table 2: Chemicals Contained in Consumable Products and Their Effects to Human Health
Table 3: Changes in Concepts and Approaches to Address Environmental Problems Over the Last Five Decades (Adapted from Ujang, 2010; Abdul-Talib, 2012)
Table 4: Disadvantages of Current Methods of Heavy Metals Removal
Table 5: Chemical Reaction and Precipitation Product for Various Precipitants
Table 6: Summary on the Disadvantages of Carbonate and Hydroxide as Precipitants
Table 7: Advantages of Metal Sulphide Precipitation
Table 8: Problems Associated with H₂S in Municipal Wastewater Systems

Table 9: Physiological Groups of Sulphate Reducing Bacteria

Table 10: Identification of Bacterial Community from Wastewater Samples

Table 11: *In-situ* and *ex-situ* Remediation Technologies, Advantages and Disadvantages

Table 12: Advantages and Disadvantages of Bioremediation

Table 13: Advantages of using ZVI

Table 14: Qualitative and Quantitative Screening Method

Table 15: Potential Biosurfactant Producers Screening Results

Table 16: Selected Biosorption Studies on Processes, Functionality

Table 17: Mechanisms in Biosorption and Its Description

Table 18: Heavy Metal Biosorption Studies using Biosorbents

Table 19: Hanes-Woolf Plot of Michaelis Menten Derivation for Half Saturation Constant Determination (Kₘ)
This book addresses sustainability and continuing developments in water and environmental sciences and engineering. The main message is focused on the need for scientists and engineers to adopt both advanced technology platform and multi-disciplinary approach towards providing innovative and sustainable solution to current and future environmental problems.

This book has a humble beginning, with a small group of researchers comprising mainly of postgraduate students working diligently in various laboratories at Universiti Teknologi MARA, Shah Alam, Malaysia. Since then, the small group of researchers has grown in numbers and later became affiliated to myBioRec, a research centre that was set up to explore biological based solutions to environmental problems. Currently myBioRec has created researchers with an understanding of sustainability and the need for multi-disciplinary approach adopting advanced and integrated technologies. This book showcases aspects of research related to management of water resources and water demand, innovative treatment of municipal and industrial wastewater, bioremediation of contaminated soil and potential exploitation of bioenergy.

Finally, it is hope that this book may inspire young scientists and engineers in embracing a multi-disciplinary approach to solve environmental problems as our economic and social well being is very much dependent on the well being of the environment.
First and foremost, I would like express my humble praise and gratitude to the Almighty Allah for His Graciousness and Guidance throughout my life. My most loving gratitude to my parents, wife and daughters for their continuous sacrifice, support and understanding. My heartfelt thanks to my colleagues at UiTM for the support and trust towards me throughout my carrier. Most important to my students whom have always thought that I had help them achieve their success. On the contrary, the experience of supervising them had provided me with the opportunity to learn so much and for this, from the bottom of my heart I thank each and everyone of them.

Last and not least I seek forgiveness from those I have not mentioned in person for their contribution towards this book, for to do so would be impossible as there have been too many individuals and organizations that had contributed in one way or another.
INTRODUCTION

BACKGROUND

The Environmental Quality Act-1974 (EQA-1974) is instrumental in controlling and mitigating pollution in Malaysia. It relies heavily on the uniform emission standards approach rather than the environmental quality objectives approach. The uniform emission standards make use of various general bulk parameters as indicators of pollutants for monitoring and enforcement purposes. Table 1 outlines pollutants including heavy metals, organic or inorganic compounds, oil and grease and their residues regulated under the various regulations under the EQA 1974. Common bulk parameters used include COD, BOD, temperature, pH and TSS. In addition to that, several pollutants such as heavy metals, phenol, free chlorine, sulphite and oil and grease are also used to monitor sewage and industrial effluents.
### Table 1: Pollutants Addressed in EQA 1974

<table>
<thead>
<tr>
<th>Pollutants</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Environmental Quality (Sewage and Industrial Effluents) Regulations 2009</strong></td>
<td>All these pollutants have shown persistent and long term effects to the environment and human health through accumulation in living organisms. The detection limits are generally in ppm level.</td>
</tr>
<tr>
<td>Bulk parameters: COD, BOD, temperature, pH and TSS</td>
<td></td>
</tr>
<tr>
<td>Heavy metals: mercury, cadmium, chromium hexalent, arsenic, cyanic lead, chromium trivalent, copper, manganese, nickel, tin, zinc, boron and iron</td>
<td></td>
</tr>
<tr>
<td>Phenol</td>
<td></td>
</tr>
<tr>
<td>Free chlorine</td>
<td></td>
</tr>
<tr>
<td>Sulphite</td>
<td></td>
</tr>
<tr>
<td>Oil and grease</td>
<td></td>
</tr>
<tr>
<td><strong>Environmental Quality (Scheduled Wastes) Regulations 2005</strong></td>
<td></td>
</tr>
<tr>
<td>Metal and metal-bearing wastes</td>
<td>Scheduled waste is waste from industries containing toxic inorganic and organic compounds, oil and grease, acid and bases solvents. It is treated, disposed of or recovered at approved facilities or incinerators. Leaching of scheduled waste at landfill sites (illegally dumped) to groundwater causes environmental degradation and pose hazards to human health.</td>
</tr>
<tr>
<td>Wastes containing principally inorganic constituents which may contain metals and organic materials</td>
<td></td>
</tr>
<tr>
<td>Wastes containing principally organic constituents which may contain metals and inorganic materials</td>
<td></td>
</tr>
<tr>
<td>Wastes which may contain either inorganic or organic constituents</td>
<td></td>
</tr>
<tr>
<td>Other wastes</td>
<td></td>
</tr>
</tbody>
</table>
Introduction

Many countries are now transforming from industrial based to service or knowledge-based economies with some countries now moving into innovation-based economy. These transformations had led to changing life style and consumer behaviour of societies, which resulted in the use of new products that will eventually find their way back into the environment as wastes. Many of these wastes contain chemical substances that inflicted significant and in some cases irreversible impacts on the carrying capacity of the environment as a life support system. Although much research has been conducted in assessing the newly emerging pollutants in the last ten to fifteen years, these pollutants are not extensively regulated giving rise to concerns on their release into the environment. The Florida Department of Environment (2008) had classified these emerging pollutants into the following categories:

1. Global Organic Contaminants. These include polybrominated diphenyl ethers (PBDEs), hexabromocyclododecanes (HBCDs), perfluorooctane sulfonate (PFOS), perfluorooctanoic acid (PFOA) and siloxanes.

2. Pharmaceuticals and Personal Care Products (PPCPs). These include all prescription and over-the-counter drugs, diagnostic agents, dietary supplements, fragrances, soaps, conditioners, sunscreens, cosmetics, caffeine, nicotine and antibiotics used prophylactically to prevent disease in livestock production operations.

3. Endocrine-Modulating Chemicals (EMCs). These include natural and synthetic hormones, surfactants, pesticides, tributyltin, polychlorinated biphenyls (PCBs) and dioxins/furans.

4. Nano-materials that are widely used in nanotherapeutic pharmaceuticals, drug delivery, cosmetics, personal care products, energy storage products, fabrics, lubricants, and even recreational equipment such as golf balls.
5. Biological metabolites. These include fate and ecotoxicology of emerging pollutants, especially focusing on their metabolites and transformation products (TPs) in the aquatic environment.

These compounds are found widely in common items such as household cleaning products, personal care products, car care products (automotive fluids, paints and adhesives) and office products. All these products are manufactured to protect human health, improve consumer goods, or facilitate daily activities. However, cumulative usage by a large section of the community compounded with continuous and improper disposal of these unused and used products not only causes serious consequences to the environment but also to human health. Table 2 shows basic chemicals used in these products. Improper and unregulated disposal of products containing emerging pollutants through wastewater discharges would lead to negative impact on the wastewater treatment plant, the receiving urban drainage systems and finally the surface and sub-surface ecosystem. In addition, continuous and cumulative release of emerging pollutants to the environment causes bioaccumulation of these pollutants in plants and animals. This phenomenon raises serious concerns on current and future risks to human health. The characteristics of emerging pollutants that can be linked to high tendency for adverse biological effects include (Daughton, 2003; Florida Department of Environmental Protection, 2008);

1. Persistent (structurally stable, compounded by continuous release)
2. Bioaccumulative.
3. Carcinogenic.
4. Lipophilic.
5. Acutely or chronic toxic.
6. Endocrine disruptors.

7. Sized in the nanoscale range.

**Table 2: Chemicals Contained in Consumable Products and Their Effects to Human Health**

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>CHEMICAL</th>
<th>EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bleach</td>
<td>Subtilisin (proteolytic enzyme), Sodium perborate tetrahydrate,</td>
<td><strong>Acute Health Effects</strong></td>
</tr>
<tr>
<td></td>
<td>Benzenesulfonic acid, C10-C16-alkyl derivatives</td>
<td>Eye irritant. Eye contact may result in temporary irritation. Prolonged skin contact may produce irritation. Avoid inhaling dust. Inhalation of large quantities of dust may produce an allergenic response (sensitization). Product may be harmful if swallowed. Do not ingest. No medical conditions are known to be aggravated by exposure to this product. Under directed consumer use conditions, this product is not expected to produce adverse health effects.</td>
</tr>
<tr>
<td>Laundry Detergent</td>
<td>Ethanol/SD Alcohol 40, Sodium dodecylbenzenesulfonate</td>
<td><strong>Acute Health Effects</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhalation: Overexposure to ethanol vapors may cause respiratory tract irritation. Eye Contact: Causes eye irritation upon direct contact. Skin Contact: May cause skin irritation upon prolonged contact. Ingestion: May cause nausea, vomiting and diarrhoea if ingested in large amounts. Medical Conditions Generally Aggravated by Exposure: None given</td>
</tr>
<tr>
<td>Fabric Softener</td>
<td>Fragrance(s)/perfume(s), Biodegradable fabric softening agents, Colorant/Pigment/Dye(s), Quality control agent(s)</td>
<td><strong>Acute Health Effects</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhalation: None given. Eye Contact: Mild eye irritant. Instillation into the eye may result in transient superficial effects similar to those produced by mild toilet soap. Skin Contact: Mild skin irritant. Prolonged skin contact may result in transient superficial effects similar to those produced by mild toilet soap. Ingestion: Oral ingestion may result in mild gastrointestinal irritation with nausea, vomiting or diarrheal. Ingestion of used or unused sheet by a young child or household pet may lead to impaction of the gastrointestinal tract.</td>
</tr>
</tbody>
</table>
### Innovative Green Technology through a Multidisciplinary Approach

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>CHEMICAL</th>
<th>EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Kitchen Cleaner</strong></td>
<td>Ammonium hydroxide</td>
<td><strong>Acute Health Effects</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhalation: Mild exposure to ammonia vapours may cause irritation of the nose and throat, coughing, and sneezing. A more severe exposure may cause respiratory irritation, olfactory fatigue, laboured breathing, and pulmonary edema.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Eye Contact: May cause immediate severe pain, closure of eyelids, and corneal injury.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin Contact: Irritant. Repeated or prolonged contact may cause dermatitis.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ingestion: May cause immediate pain and burns of the mucous membranes. The oesophagus and stomach may be involved with burning pain, vomiting and diarrhoea.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Medical Conditions Generally Aggravated by Exposure: Pre-existing skin conditions and respiratory illnesses (such as asthma).</td>
</tr>
<tr>
<td><strong>Haircolor, Rich Auburn</strong></td>
<td>Ammonium hydroxide, Polyglyceryl-4 oleyl ether, Diethylene glycol monobutyl ether, 4-Amino-2-hydroxytoluene, Behentrimonium chloride,</td>
<td><strong>Acute Health Effects</strong></td>
</tr>
<tr>
<td></td>
<td></td>
<td>HEALTH HAZARDS</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Harmful if swallowed, inhaled, or absorbed through skin. This product may cause serious irritant, respiratory, and/or allergic reactions in sensitive individuals. May cause skin and severe eye irritation. May cause severe irritation of gastric mucous membranes if swallowed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>SIGNES AND SYMPTOMS OF EXPOSURE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Signs of alcohol intoxication may occur following ingestion or prolonged inhalation. Also possible irritation of eyes, skin and mucous membranes. Possible irritant/allergic dermatitis and respiratory signs and symptoms, the onset of which may be delayed.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>MEDICAL CONDITIONS GENERALLY AGGRAVATED BY EXPOSURE</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Existing dermatological conditions, such as eczema, and respiratory conditions, such as bronchial asthma and/or chronic bronchitis, may be exacerbated</td>
</tr>
</tbody>
</table>
Introduction

<table>
<thead>
<tr>
<th>PRODUCT</th>
<th>CHEMICAL</th>
<th>EFFECTS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ink Cartridge Yellow,</td>
<td>Dye(s) (unspecified), Glycerols (unspecified),</td>
<td>Acute Health Effects</td>
</tr>
<tr>
<td>Magenta, Cyan</td>
<td>Diethylene glycol</td>
<td>Routes of Exposure: Eye, skin, inhalation, and oral.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Potential Health Effects</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin: Ink contact with skin may cause irritation, swelling, or redness. It is not expected to cause an allergic skin reaction.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Inhalation: Intentional exposure to ink vapors may cause respiratory irritation.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ingestion: May cause upset stomach.</td>
</tr>
<tr>
<td>Multipurpose Metal</td>
<td>Ammonia, Aliphatic petroleum distillate</td>
<td>Acute Health Effects</td>
</tr>
<tr>
<td>Polish</td>
<td></td>
<td>Inhalation: None known. Eye Contact: Irritating to eyes.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Skin Contact: Not a primary skin irritant. Prolonged contact may cause irritation. Ingestion: Harmful or fatal if swallowed. Medical Conditions Generally Aggravated by Exposure: None given.</td>
</tr>
</tbody>
</table>

Investigations on treatment and remediation of contaminated sites containing these pollutants need to be intensified. For instance, PBDEs and pesticides (and their associated breakdown products) are not only listed as persistent, lipophilic, bio-accumulative, endocrine-disrupting compounds but also listed as carcinogenic. Emerging pollutants should be accorded attention and priority by scientists, technologists, regulators and consumers. Efforts to manage these pollutants and investigation related to risk and effects on human health and the environment are being studied around the world especially in the USA and Europe.

A GENERAL ASSESSMENT OF EMERGING POLLUTANTS

Currently there are more than 26 million organic and inorganic substances documented by the American Chemical Society’s Chemical Abstracts Services. Out of the 26 million known chemicals, nearly 9 million were commercially available, thus making them potential pollutants to be
released to the environment. Out of these, fewer than a quarter million (240,000) were inventoried or regulated by numerous government bodies worldwide, representing less than 3% of those that are commercially available or less than 1% of the known universe of chemicals (Daughton, 2003).

Prior to establishing regulations and standards to control the release of these pollutants, a sound assessment method must first be established. A strong drive towards adopting risk-based assessment is being introduced by various regulators in the USA and Europe. However, both regulators and scientists are admitting that it is a very complex problem as the number of chemicals currently available is enormous. In addition to the enormous amount of chemicals that needs to be identified and quantified, the problem is compounded further by the fact that current technology poses serious limitations to make the method fully reliable.

ENVIRONMENTAL MILESTONE: A REVISIT

The shift in economic focus globally and the undeniable evidences of environmental degradation had significantly affected the concepts and approaches adopted by engineers and scientists in providing services and solving environmental problems with the main aim of improving the quality of life for human (mostly measured through economic gains). These changes in approaches to include environmental importance are shown in Table 3. In the last two decades intense focus was accorded to carbon and ecological footprints. Carbon footprint is an indirect measure of human activities while the ecological footprint measures the impact on the environment. More efforts were focused on Quantitative Sustainability Assessment in order to have a better estimate of the carbon and ecological footprints.
Introduction

Table 3: Changes in Concepts and Approaches to Address Environmental Problems Over the Last Five Decades (Adapted from Ujang, 2010; Abdul-Talib, 2012)

<table>
<thead>
<tr>
<th>Concept or Approach</th>
<th>Timeline</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cost Benefit Analysis</td>
<td>1970's</td>
</tr>
<tr>
<td>Matrix Selection of Components</td>
<td>1980's</td>
</tr>
<tr>
<td>Sustainability Index</td>
<td>1990's</td>
</tr>
<tr>
<td>Life Cycle Analysis</td>
<td>1990's</td>
</tr>
<tr>
<td>Ecological and Carbon Footprint</td>
<td>1990's</td>
</tr>
<tr>
<td>Quantitative Sustainability Assessment</td>
<td>2000's</td>
</tr>
<tr>
<td>Platforms of advanced and integrated technologies including social security</td>
<td>2010's</td>
</tr>
</tbody>
</table>

Currently, it is essential that engineers and scientists embrace advanced technologies such as nanotechnology, biotechnology, bioinformatics and ICT as the basic platforms in seeking solutions to the present and future problems. In addition to the cutting edge technologies, it is essential that engineers and scientists take into consideration not only the economic well being of the society but also their psychological and emotional needs. The decision makers are now more attuned to these needs than just the technological capability and the economic needs of the society.
SUSTAINABILITY OF THE ENVIRONMENT

Economic development and well-being of society is simply not possible without a sustainable environment. For example, no industry can exist if there is no adequate supply of water in terms of both quantity and quality. The well being of the environment depends very much on the sustainability of the planet to maintain the natural circulation of fresh air, fresh water and fertile land. The sustainable presence of these three basic elements is critical to the survival of the diverse flora and fauna, including human. Whilst there is no denying that development in sciences and technologies had contributed tremendously to the well being of the society, it is also undeniable that development had to some extent impaired the life supporting capability of the planet. Engineers and scientists must not look upon development and environment as contradicting components of societal needs. Instead, both components need to be viewed as the two opposite sides of a coin. The coin has no value if it is only imprinted on one side. Similarly, the services or solutions provided to society are of no value if the technologies or approaches adopted did not adequately balance the need for development and ensuring sustainability of the environment.

THE NEW BASIC KNOWLEDGE PLATFORM

The basic tool or knowledge for engineers and scientists had been based on the fundamental sciences of physics, chemistry and biology. These are then supported by the mathematical and computing tools which over the last three decades had made significant changes in scientific and engineering practices. In the last decade, focus was given to new platforms of emerging technologies founded on nano-sciences, molecular biology and bio-informatics. The emergence of these technologies must not only be taken advantage of but also efforts must be made to ensure that they are guided towards green applications rather than accelerating the deterioration rate of the environment. These new platforms of
knowledge are founded based on multi-, inter- and trans-disciplinary approach. It is now imperative that the training of young scientists and engineers adopts this approach. In addition, the continuous professional development of scientists and engineers must also incorporate a dose of this multi-disciplinary approach in addressing technological and environmental problems.

INNOVATIVE R&D

In the age of innovation-based economy, engineers and scientists need to have strong fundamental knowledge not only in determining the root cause of an environmental problem but in developing green-based, creative and innovative solutions with a full understanding of how the proposed solution affects other components of the environment. The proposed solution must address issues such as:

1. Whether high or low technology is appropriate.
2. Whether costs associated to the solution is affordable by society.
3. Whether the efficiency and effectiveness is acceptable.

The implication of choices related to the above issues must be fully understood. Furthermore, the following four-element approach must be considered in seeking and proposing a solution;

1. Understanding of the fundamental mechanism that is governing the process.
3. Benefits to be transferred to or enjoyed by society.
4. Costs to society in terms of economics, environment and psychological well-being.

An illustration of how these elements are taken onboard is shown in Figure 1.

A strong feature of the innovative approach is the identification of two problematic industrial streams that contain compounds or substances that can be complementary in removing the pollutants. An illustration of this feature is highlighted in the following section.
Introduction

APPLICATION OF THE INNOVATIVE R&D

– Treatment of Municipal and Industrial Wastewater

Rapid industrial development, both locally and globally has increased the amount of effluent containing heavy metals that is being released to the environment. In most wastewaters, the concentration of heavy metals present is much larger than the permissible limits and therefore, requires treatment. Conventional methods for heavy metals treatment include chemical precipitation, ion-exchange, electro-dialysis, membrane filtration and adsorption (Lesmana et al., 2009). However, these treatments are ineffective or expensive in most cases, especially when heavy metals ion concentration in aqueous solution is in the range of 1 to 100 mg/L (Nurchi and Villaescusa, 2008). Moreover, the sludge produced by such treatment modalities only serves to add further burden the environment.

The drastic increase in both volume and type of agricultural wastes has become a burgeoning problem in the wake of population growth. According to UNEP (2009), 140 billion metric tons of agricultural waste is generated every year globally. Agricultural wastes take the form of residual stalk, leaves, roots, husk, nut, waste wood and animal waste (Khan et al., 2004; Park et al., 2010). In general, agricultural waste is carbon rich as it contains high amounts of cellulose, lignin, pectin and tannin. Applying the concept of sustainability and sustainable technology, this widely available, renewable and virtually free agricultural waste can become an important resource through reuse and recycling. Although there is an emerging trend on the utilization of agricultural waste for biofuel and biochar technologies, agricultural waste is still largely under-utilized and left to rot or openly burned in the fields, especially in developing countries.
Agricultural waste management and sustainable heavy metals treatment technology are still major gaps to be filled, as it is usually treated as out of sight is out of mind. There is a lack of awareness in looking for sustainable heavy metals treatment technology by using agricultural waste for treatment and metals recovery. The challenge, therefore, is to generate revenue from the recovered materials and develop a sustainable heavy metals treatment technology.

An alternative method of treating heavy metal waste is through the use of under-utilized agricultural waste with biosorption. It is a passive physical-chemical process that binds heavy metals ions from aqueous solutions to molecules on non-living biological material called biosorbent. This process offers advantages of low cost, being effective for dilute effluent, minimum chemicals usage and reduced toxic sludge generation (Gupta et al., 2009). Furthermore, biosorbent has advantages over live and immobilized micro-organism remediation techniques as it does not depend on nutrient availability, toxicity tolerance and metabolic activities (Iqbal and Saeed, 2007; Krishnani and Ayyappan, 2006; Locci et al. 2008).

There is a wide choice of biosorbent in respective countries which showed that waste from agriculture and industries have high potential to be exploited as biosorbent. The selection criteria of a sustainable biosorbent are availability, cost, removal effectiveness, ion selectivity, the non-toxic nature of the material, reusability and biodegradability (Vieira and Volesky, 2000; Hawari and Mulligan, 2006). Ideally it should be available locally and in large quantity, at low cost from waste materials requiring minimum processing. One of the materials that have been studied at myBioREc is *Pleurotus ostreatus* spent mushroom compost (PSMC). This lignocellulosic material is a waste that is widely available from mushroom farms in Malaysia and the disposal of PSMC is currently handled by open burning or converted onto low commercial value organic fertilizer in order to reduce cost of solid waste disposal.
Introduction

The cost of heavy metal removal with this biosorbent is estimated to be six times lower than the use of ion exchange resin of similar heavy metal removal performance (Tay et al., 2011). The biosorbent can be reused and as biosorbents are degradable, it is environmentally sustainable. Application of biosorbent is divided into two categories, namely, batch reactor treatment and continuous flow column. Batch reactor treatment is easily operated and usually used to screen biosorbents. Meanwhile, continuous flow column shows better process control and recovery.

If chemical treatment is preferred, an alternative, sustainable treatment that can be utilised is the use of biologically produced sulphide to remove heavy metals from industrial wastewater. Currently most industries that deal with heavy metals and sulphide in their effluent are treating their waste stream separately, each with a specific method to remove heavy metals and sulphide. Chemicals are required to effectively remove these contaminants. Thus, treating multiple streams of waste incur additional operational and maintenance cost.

Biologically produced sulphide can replace this industrial-sourced sulphide. To produce sulphide biologically, one need not look far as municipal wastewater is a good source of sulphate, which will undergo reduction to sulphide with the help of sulphate reducing microorganisms if subjected to anaerobic condition.

The use of biologically produced sulphide solves two treatment issue; one for the heavy metal contaminated wastewater, and the other, municipal wastewater. There is a continuous supply of municipal and industrial wastewater as long as the plant is operational. The sulphide which is a nuisance to the sewer system and environment can be used to actually solve the problem of other industries. The sulphide produced from biological processes is used to react with heavy metals and forms metal sulphide precipitates Therefore, heavy metals and sulphide will be both removed from the wastewater.
Innovative Green Technology through a Multidisciplinary Approach

The precipitates formed can be disposed off in a landfill. Thus, instead of separately removing both compounds from the wastewater, chemical wastes from both streams can be mixed in an engineered reactor for mutual treatment.

Using either the physical-chemical approach with PSMC or the biological sulphide approach, heavy metals from industrial effluent can be removed using materials from other waste streams. The success of the approach relies heavily on a strong understanding of the individual waste stream and the mechanism or kinetics of processes in the combined waste streams designed to remove the pollutants. A critical analysis of available and suitable material from different waste streams needs to be conducted prior to adopting this innovative approach. The design of the combined process will result in pollutants being removed from waste streams in a more cost effective and an environmentally accepted manner. The adoption of this approach requires the researcher to be knowledgeable and competent in several knowledge disciplines.

The remaining chapters of this book present results of studies on pollutant removal utilising green-based technology with a multi-disciplinary approach undertaken at the myBioRec laboratory, Faculty of Civil Engineering, Universiti Teknologi MARA.

CONCLUDING REMARKS

The presence of emerging pollutants raises concerns as the present regulations are not adequate to regulate and monitor their discharges into the environment creating hazards to the biotic environment. There is an urgent need for researchers, scientists and engineers to find innovative approaches in dealing with these emerging pollutants. A four-element innovation multi-disciplinary approach focusing on the fundamental processes mechanisms, material availability, transferable benefits to society and costs to society in terms of economics, environment and psychological well-being is proposed.
BACKGROUND

The major problem associated with the automotive and metal-based industry is the presence of heavy metals which constitute a health hazard to the biotic environment. Much effort had been done to address this problem. To some extent the various technology adopted by the industry had been successful in removing heavy metals from the waste streams. However, further improvements are needed to ensure further reduction in the concentrations of heavy metals released into the environment. Currently, the industries are quite content as long as the effluent quality discharged from their premises complies to the standards set by the Regulators. Considering the properties of heavy metals which fit the characteristics of the emerging pollutants highlighted in Chapter 1, it is essential that researchers explore new technology that can further remove heavy metals from the waste streams even at low concentrations. This chapter critically evaluate the available technology for heavy metals removal and highlight an innovative approach utilising biological sulphide derived from municipal wastewater to remove heavy metals from automotive wastewater.
CURRENT METHODS FOR HEAVYS METAL REMOVAL

Various methods are available for the removal of heavy metals including electro-coagulation, nanofiltration, ion exchange and phytoremediation. Whilst they are widely used in the industry, these technologies have several disadvantages associated with their application in the removal of heavy metals. Table 4 shows the disadvantages of current methods of heavy metals removal.

Table 4: Disadvantages of Current Methods of Heavy Metals Removal

<table>
<thead>
<tr>
<th>Method applied</th>
<th>Heavy metals studied</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Electro-coagulation</td>
<td>Cu (II), Ni (II), Mn (II), Zn (II)</td>
<td>Heavy metals removal is only effective at high pH, initial pH adjustment is therefore required before treatment.</td>
<td>Al Aji et al., 2012</td>
</tr>
<tr>
<td>Nanofiltration</td>
<td>As (III), Cu (II), Cd (II), Mn (II), Pb (II)</td>
<td>The presence of negative ions (Cl⁻, OH⁻) in samples may cause fouling to the filter and lead to decreased efficiency in heavy metals removal.</td>
<td>Al-Rashdi et al., 2012</td>
</tr>
<tr>
<td>Ion exchange</td>
<td>Ni (II)</td>
<td>Need to maintain the initial concentration of heavy metals at low range to allow for regeneration of resin due to the cost constraint.</td>
<td>Shaidan et al., 2012</td>
</tr>
<tr>
<td>Phytoremediation</td>
<td>Cd (II), Cu (II), Mn (II), Pb (II), Zn (II)</td>
<td>Requires large number of plants (water hyacinth) to remove heavy metals. A further need to compost the water hyacinth to avoid further contamination of heavy metals to the environment.</td>
<td>Chunkao et al., 2012</td>
</tr>
</tbody>
</table>

In addition to the methods listed in Table 4, chemical precipitation can also be used. It is cheaper compared to ion exchange where resin is needed. Chemical precipitation is commonly adopted by industries to treat wastewater containing heavy metals. Chemical precipitation occurs when two different soluble chemicals react and form precipitates. There
are three common types of precipitants used in chemical precipitation, namely, carbonate, hydroxide and sulphide. A summary on reaction equations and precipitation products for the three types of precipitants is given in Table 5.

<table>
<thead>
<tr>
<th>Type of precipitant</th>
<th>Carbonate</th>
<th>Hydroxide</th>
<th>Sulphide</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chemical reaction</td>
<td>$M^{2+} + CO_3^{2-} \rightarrow MCO_3$</td>
<td>$M^{2+} + OH^- \rightarrow M(OH)_2$</td>
<td>$M^{2+} + S^{2-} \rightarrow MS$</td>
</tr>
<tr>
<td>Forms of precipitants</td>
<td>Calcium carbonate, sodium carbonate</td>
<td>Lime, caustic or magnesium hydroxide</td>
<td>Biological sulphide produced in solution forms or $H_2S$ (g)</td>
</tr>
</tbody>
</table>

**Metal-carbonate and Metal-hydroxide Precipitation**

Both metal carbonate and metal hydroxide precipitation are widely applied in industries to treat and remove heavy metals from the wastewater. Soluble heavy metals ion will be converted to insoluble metal carbonate and metal hydroxide.

Carbonate precipitation is considered to be an effective treatment method to remove dissolved heavy metals from industrial wastewater. This precipitation process can be achieved by adding precipitating agents such as sodium carbonate ($Na_2CO_3$), sodium bicarbonate ($NaHCO_3$), carbon dioxide or calcium carbonate ($CaCO_3$). Feng et al. (2000) reported that $CaCO_3$ is an example of high alkalinity reagents for carbonate precipitation. The use of carbonates to precipitate out heavy metals offers the advantage where the precipitates formed can be easily filtered.

Metal hydroxide precipitation process can be achieved by adding an alkali-precipitating agent such as lime, caustic or magnesium hydroxide. In hydroxide precipitation, pH is an important factor that needs to be controlled to avoid heavy metals removal by coagulation and flocculation.
Innovative Green Technology through a Multidisciplinary Approach

process. The advantage of hydroxide precipitation is the ease of pH control in terms of constant metal value at each pH adjusted (Huisman et al., 2006). Thus, it is easy to quantify the quantity of metal dissolved and metal still in solid form (Heredia and Martin, 2009). However, there are some disadvantages of using both carbonate and hydroxide as shown in Table 6.

### Table 6: Summary on the Disadvantages of Carbonate and Hydroxide as Precipitants

<table>
<thead>
<tr>
<th>Method of precipitation</th>
<th>Example of precipitant</th>
<th>Disadvantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carbonate</td>
<td>Limestone Sodium carbonate</td>
<td>One or more chemical precipitation is required to remove heavy metals with high initial concentrations. Requires pH adjustment of the initial solution to above neutral condition (&gt;7).</td>
<td>M. Silva et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Limestone</td>
<td>Large quantity of limestone is required to remove concentration of 2mg/L heavy metals.</td>
<td>H. A. Aziz et al., 2008</td>
</tr>
<tr>
<td>Hydroxide</td>
<td>Caustic calcine magnesia</td>
<td>Longer contact time is required for the heavy metals removal. The potential of hydroxide precipitates formed to re-dissolve.</td>
<td>Harvey et al., 2012</td>
</tr>
<tr>
<td></td>
<td>Fly ash, lime and carbon dioxide</td>
<td>More than one precipitants are required to effectively remove heavy metals from waste streams. More time is required to establish the optimum pH for effective heavy metals removal.</td>
<td>Chen et al., 2009</td>
</tr>
</tbody>
</table>

**Metal-sulphide Precipitation**

Sulphide precipitation is widely used as an alternative treatment method to carbonate and hydroxide precipitation (Veeken et al., 2003; Lewis and Hille, 2006). This process converts soluble metal compounds into insoluble metal sulphide compounds.
Various chemical sources of sulphide can be applied to remove heavy metals including sodium sulphide (Na$_2$S), iron sulphide (FeS) and calcium sulphide (CaS). Many studies have reported on the advantages of using sulphide to remove heavy metals from industrial waste. The advantages of using sulphide precipitation are listed in Table 7.

**Table 7: Advantages of Metal Sulphide Precipitation**

<table>
<thead>
<tr>
<th>Advantages of sulphide precipitation</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ability to remove toxic metals the low range of 0.01-1mg/L</td>
<td>Huisman et al., 2006</td>
</tr>
<tr>
<td>High degree of metal removal over a broad range of pH</td>
<td>Feng et al. 2000; Huisman et al. 2006</td>
</tr>
<tr>
<td>Effective at low influent pH (&lt;2)</td>
<td>Feng et al., 2000; Lewis and Hille 2006</td>
</tr>
<tr>
<td>Low production of sludge volume (1/10 times lower compared to carbonates and hydroxides)</td>
<td>Hao, 2000; Huisman et al., 2006; Liamleam and Annachhatre 2007</td>
</tr>
<tr>
<td>Sludge produced is more compact, faster settling and less subject to dissolution.</td>
<td>Costa and Duarte 2005</td>
</tr>
<tr>
<td>Metal sulphides are more dense and stable which exhibit better thickening and dewatering characteristics</td>
<td>Lewis and Hille 2006; Hoa et al., 2007; Liamleam and Annachhatre 2007</td>
</tr>
<tr>
<td>Potential of recover metals from the sulphides sludge</td>
<td>Hao, 2000</td>
</tr>
<tr>
<td>High reaction rates results in low hydraulic retention time</td>
<td>Feng et al., 2000; Veeken et al., 2003; Huisman et al., 2006</td>
</tr>
<tr>
<td>Possibility of removing selective metal</td>
<td>Feng et al., 2000; Veeken et al., 2003; Huisman et al., 2006</td>
</tr>
<tr>
<td>Insensitive to the presence of chelating agents and the interference in wastewater is less problematic</td>
<td>Veeken et al., 2003; Huisman et al., 2006</td>
</tr>
<tr>
<td>Economical and attractive</td>
<td>Veeken et al., 2003</td>
</tr>
</tbody>
</table>
Alternatives to the use of sulphide from chemical sources, sulphide present in municipal wastewater can be also be utilised. Sulphide is commonly present in municipal wastewater treatment plants as a result of biological transformation of sulphate. The presence of sulphate in municipal wastewater owes its origin from the use of toiletries and detergents in households.

Sulphate reduction to sulphide occurs under anaerobic condition where sulphate act as the electron acceptor when the oxygen and nitrate are not present (Abdul-Talib, 2002). Sulphate in wastewater can undergo oxidation and reduction process depending on the presence of the electron acceptor and process condition in the sewer. Figure 2 shows the flow of the redox processes between sulphur compounds.

![Reduction and Oxidation Processes of Sulphur Compounds](image)

**Figure 2: Oxidation and Reduction Processes of Sulphur Compounds**

The presence of sulphide in municipal wastewater treatment plant is quite troublesome to the engineers of the plant and residents around the area. Sulphide, in the form of gaseous hydrogen sulphide ($H_2S$) can react with water to produce sulphuric acid which causes corrosion to metals and deterioration of concrete components at wastewater treatment facilities. Gaseous hydrogen sulphide on the other hand is an odorous substance which is the main cause of odour nuisance to the local neighbourhood. In addition at higher concentration in confined spaces, sulphide is toxic.
Heavy Metals Removal using Biological Sulphide

and fatal to human. Table 8 shows the many effects of hydrogen sulphide when it is released in municipal wastewater systems.

Table 8: Problems Associated with H$_2$S in Municipal Wastewater Systems

<table>
<thead>
<tr>
<th>Problems associated with sulphide</th>
<th>Description</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrosion</td>
<td>Observed corrosion in sewer pipes within few months with the pH value in the range of 1 -2, indicating presence of H$_2$S at high concentration. Corrosion began close to the water line and within four months the corrosion had moved and corroded the pipe crown. All H$_2$S present in sewer pipes will be oxidized to H$_2$SO$_4$. Based on the oxidation rate, the corrosion rate for sewer pipes was estimated at 3.88 mm/year.</td>
<td>Vollertsen et al., 2008 Nielsen et al., 2008</td>
</tr>
<tr>
<td>Health hazards</td>
<td>Effect at low and high level of exposure to H$_2$S. Exposure to low level of H$_2$S resulted in conjunctivitis, wheezing and upper airway irritation. Exposure to high level of H$_2$S can result in cough, confusion, coma, seizures, loss of consciousness and haemoptysis. Cause irritation to the eyes</td>
<td>Yalamanchili and Smith 2008 Lambert et al., 2006</td>
</tr>
</tbody>
</table>

INNOVATIVE R&D ON SULPHIDE AND HEAVY METALS REMOVAL

The mixing of wastewater from municipal wastewater treatment plants and automotive wastewater treatment plant may simultaneously overcome the problems of corrosion, nuisance and health hazard at the former and removing heavy metals from latter. Sulphide produced in
Innovative Green Technology through a Multidisciplinary Approach

sewers through microbial processes can be used to precipitate out heavy metals from industrial wastewater. Simultaneously heavy metals from industrial wastewater can be used to remove sulphide from municipal wastewater treatment plants.

Municipal wastewater contains sulphate at an average concentration of 40 to 200 mg/L (Zhang et al., 2008). Velasco et al. (2008) established that a minimum sulphate concentration of 40 mg/L is able to produce 8.8 mg/L of sulphide. Thus, it is viable to produce sulphide from sulphate using municipal wastewater.

The process of metal sulphide precipitation will occur in two stages; first the production of biological sulphide followed by biological sulphide precipitation to remove heavy metals. In the first stage, sulphate present in the municipal wastewater will be converted to sulphide under anaerobic condition. The conversion process occurs with the help of sulphate reducing microorganisms (SRM) naturally present in the sewer environment.

SRM are classified as a group of chemoorganotrophic microorganisms. SRM can be divided into two broad physiological groups as shown in Table 9.

<table>
<thead>
<tr>
<th>Table 9: Physiological Groups of Sulphate Reducing Bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Group 1</strong></td>
</tr>
<tr>
<td>SRM that utilize lactate, pyruvate, ethanol and certain fatty acids</td>
</tr>
<tr>
<td>Desulfovibrio, Desulfomonas Desulfobulbus, Desulfomonile Desulfobotulus, Desulfomicrobium, Desulfotomaculum and Desulfobacula</td>
</tr>
</tbody>
</table>

The presence and growth of SRM is very important in biological sulphide metal precipitation process (Lopes et al., 2010). An increase in the population and growth of SRM indicates a much higher sulphide
production which will eventually increase the rate of metal sulphide removal. SRM decomposes simple carbonaceous organic compounds or substrate. Sulphate in the wastewater acts as the terminal electron acceptor and produce sulphide as the end product (Foucher et al., 2001). The governing chemical equation for the production of sulphide in the first stage of the process is given by Equation 1.

$$\text{SO}_4^{2-} + \text{organic matter} + \text{anaerobic bacteria} \rightarrow \text{S}^2^- + \text{H}_2\text{O} + \text{CO}_2$$ (1)

In the second stage, the biological sulphide produced from the first stage was used to precipitate out heavy metals. $M^{2+}$ may represent heavy metals with two electron charges such as $\text{Mn}^{2+}$, $\text{Ni}^{2+}$ or $\text{Zn}^{2+}$. The governing chemical equation for the biological sulphide and metal precipitation is expressed as Equation 2:

$$M^{2+} + S^2^- \rightarrow MS$$ (2)

The biological process producing sulphides is an alternative method which is economical and more environmentally friendly. It reduces the dependency on chemical sources of precipitants such as carbonates, hydroxides and sulphides. If this approach is successful, the dependency on chemicals will eventually be reduced and finally the heavy metals can be removed from the industrial wastewater in a sustainable manner (Cao et al., 2009; McCauley et al., 2009).

A CASE STUDY ON NICKEL-SULPHIDE PRECIPITATION

A study was conducted to observe the removal of nickel using biological sulphide. Figure 3 shows the schematic experimental setup for metal sulphide precipitation. An artificial wastewater prepared by spiking 10 mg/L of nickel into ultra pure water (UPW) was used in the study. The amount of nickel spiked was selected to replicate the condition of industrial wastewater. The experiment was conducted by channelling both
Innovative Green Technology through a Multidisciplinary Approach

waste streams, namely, municipal wastewater and spiked wastewater into 
metal sulphide precipitation reactor. The nickel-sulphide precipitation 
takes place in the metal sulphide precipitation reactor. The experiment 
was conducted over 70 minutes with samples taken at intervals of 10 
minutes.

![Schematic Diagram for Metal Sulphide Precipitation](image)

**Figure 3: The Schematic Diagram for Metal Sulphide Precipitation**

**Results**

From the experiment, an amount of 5.715 mg/L of nickel was 
precipitated out. Based on Equation 2, 1 mol of heavy metals react with 
1 mol of sulphide. Therefore, in this study, 1 mol of nickel will react with 
1 mol of biological sulphide to form metal sulphide precipitates. Thus 
it can be deduced that 3.12 mg/L sulphide was biologically produced to 
precipitate out 5.715 mg/L of nickel. It can be further inferred that the 
reaction was limited by the availability of sulphide as there is still nickel 
remaining in the system. Figure 4 shows the graph on nickel-sulphide 
precipitation.
Heavy Metals Removal using Biological Sulphide

Biologically assisted Ni-S precipitation (10 mg/L)

<table>
<thead>
<tr>
<th>Concentration (mg/L)</th>
<th>0.000</th>
<th>2.000</th>
<th>4.000</th>
<th>6.000</th>
<th>8.000</th>
<th>10.000</th>
<th>12.000</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>Inf</td>
<td>0</td>
<td>10</td>
<td>20</td>
<td>30</td>
<td>40</td>
<td>50</td>
</tr>
</tbody>
</table>

![Figure 4: Experiment Conducted on Biologically Assisted Nickel-sulphide Precipitation](image)

Figure 4 shows that nickel was precipitated at almost half of the initial concentration spiked at 0 minutes. The trend of heavy metals removed was at stable condition after precipitation occurs. From observation, it shows that there is no re-dissolution of heavy metals occurring. Between 10 to 70 minutes, no further reduction of heavy metals was observed due to insufficient amount of sulphide present in the reactor. Sulphide was not detected in the reactor at 10 minutes, it shows that all available sulphide had reacted completely with nickel.

A CASE STUDY ON IDENTIFICATION OF SRM

This study is presented in order to highlight the need for multi- and trans-disciplinary approach in understanding the problem at hand. The identification of the SRM is essential in order to create conditions that favour the presence and growth of the relevant SRM that contribute directly to the production of sulphide. Samples were taken from a reactor containing both the bulkwater and the biofilm phases. The reactor was subjected to alternate anaerobic and anoxic conditions in order to enable
active strains under anaerobic conditions to be identified. Molecular
techniques using a modified linker-PCR as primers to enable complete
sequencing of DGGE bands was adopted in this study.

Molecular Analysis

The changes in microbial community composition of bulkwater
and biofilm were identified using denaturing gradient gel electrophoresis
(DGGE).

DNA Extraction and PCR Amplification

Total DNA of the bacteria from bulkwater and biofilms samples
were extracted as that described by Yeates et al. (1998) but with slight
modifications. Equal volume of extraction buffer (100 ml of 100 mM
Tris-HCl [pH 8.0], 100mM sodium EDTA [pH 8.0], 1.5M NaCl) was
mixed with the samples. Glass beads were added to the mixture and
the sample was vortexed vigorously. 10 ml of 20% sodium dodecyl
sulphate (SDS) was added and vortexed for another 30 s. Samples were
then incubated at 65°C for 1 h in water bath and centrifuged at 10000g
for 10 min. The supernatant was transferred to half volume of 1.5M
sodium chloride (NaCl) and incubated at room temperature for 2 h. Then 1/10 volume of sodium acetate (CH₃COONa) was added. Samples
were transferred to ice for 5 min and centrifuged at 16000g for 30 min.
The aqueous phase was extracted with an equal volume of phenol/
chloroform/isoamyl alcohol (25:24:1) and it was allowed to settle for 1
h at room temperature before centrifugation at 13000 g for 15 min. DNA
was precipitated with equal volume of isopropanol. The reactions were
then left at -20°C for 15 min and centrifuged at 13000g for 30 min. The
supernatant was removed again and 700 µl of cold 75% ethanol (chilled
at 4°C) was added and centrifuged for 5 min at 7000g. The DNA was
allowed to air dry and resuspended in 50 µl TE buffer (10 mM Tris-HCl,
1 mM EDTA [pH 8.0]). The total DNA samples were stored at -20°C
for PCR amplification.
Amplification of DNA

The V3 region of 16S rDNA was amplified with standard bacterial primers 357F-GC (5'-CGCCCGCCGCGCGCGGCGGGCGGGCCGCCCCTACGGGAGGCAGCAG-3') and 518R (5'-ATTACCGGGCTGCTGG-3') (Muyzer et al.,1993). The PCR reaction system (25 µl) contained: 1 x Go Taq Green Master Mix (Promega), 10 µM forward primer, 10 µM reverse primer and genomic DNA extract. PCR amplification were performed under the following conditions: 95°C for 5 min; 10 cycles of 94°C for 30 s, 55°C for 30 s and 72°C for 1 min; 26 cycles of 92°C for 30 s, 52°C for 30 s and 72°C for 1 min; followed by 10 min at 72°C (O’Sullivan et al., 2008). PCR products were confirmed by electrophoresis on 1.5% agarose gel with DNA mass ladder standard.

Denaturing Gradient Gel Electrophoresis (DGGE)

A DCode Universal Mutation Detection System instrument (Bio-Rad) was used to perform DGGE analysis. Electrophoresis was performed in 1 mm thick 8% (w/v) polyacrylamide gel (acrylamide-bisacrylamide of 37.5:1) with a denaturing gradient of urea and formamide ranging from 40% to 65% (100% denaturant is defined as a mixture of 7 M urea and 40% formamide). Gel was run at a constant temperature of 60 °C for 16 h at 37V. Gel was stained with ethidium bromide by gently shaking for 15 min and were captured using a gel documentation system (Bio-Rad).

DNA Sequencing

DGGE bands were excised and re-amplified with the bacterial modified linker-PCR primers 357F-GC -M13R (5’-CAGGAAAAACAGCTATGACGGGCGGGCGGGCCGCGGGGCACGGGGGACCTACGGGAGG CAGCAG-3’) and 518R-AT-M13F (5’-GTAAAAACGACCGCAGTAAATAAAAAT AAAATGT AA AAAAATTACCGGGCTGCTGG-3’) as described by O’Sullivan et al. (2008). The re-amplified products were sequenced with primers M13F
Innovative Green Technology through a Multidisciplinary Approach

and M13R. Sequences were aligned using BioEdit 7.0.5 and BLASTN was used to search for similar sequences of the GenBank at the U.S. National Centre for Biological Information (NCBI).

**Results**

High bacterial diversity of bulk water and biofilm in two conditions, anaerobic and anoxic were characterized using DGGE as shown in Figure 5. In this study, excised DNA from the polyacrylamide gels were amplified using modified linker PCR primers to produce full sequences instead of the standard approach which retrieved on average about 77% of the available sequence (O’Sullivan *et al.*, 2008). In total, approximately 48 to 50 bands were excised and sequenced from the samples and the highest number of bands were obtained from the biofilm in anaerobic condition.

The sequences were compared with GenBank databases, where high similarity was found for some bacterial isolates as shown in Table 10. Most of the bacteria obtained were found to be uncultured bacteria. In bulk water the total number of bands obtained were 13 and 11 in anoxic and anaerobic conditions respectively with six bands showing similar mobility that were found to be closely related to uncultured bacteria, archea bacteria, alpha proteobacterium or *Geobacillus* sp. In bulk water samples, the bands Hn1, Hn3, Hn4, Hn6, Hn7, Hn11 and Hn13 only existed in anoxic condition. Bands Hn4, Hn6 and Hn7 were highly related to bacilli, Hn1 as an alphaproteobacteria and the remaining to be uncultured bacteria. Meanwhile HA14, HA15, HA16, HA17, and HA18 only appeared in bulk water anaerobic samples of which HA16 and HA17 showed high similarity to betaproteobacteria while the rest of the bands were uncultured bacteria.

The presence of large number of bacteria bands in BA (15) showed that the diversity of bacterial community in BA was more compared to Bn (11). Eight identical sequences were found between Bn and
BA which were closely related to Geobacillus sp., uncultured alpha proteobacterium or uncultured bacteria. In the biofilm community, Bn6, Bn19 and Bn20 bands existed only in the anoxic condition and were all found to be uncultured bacteria (Bn19 and 20) or an uncultured alpha proteobacterium (Bn6).

Figure 5: DGGE Profiles of PCR-amplified 16S rRNA Genes from Bulk Water and Biofilm Samples
## Table 10: Identification of Bacterial Community from Wastewater Samples

<table>
<thead>
<tr>
<th>Bands</th>
<th>Closest relative</th>
<th>GenBank accession number</th>
<th>Max ident</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Uncultured gamma proteobacterium isolate DGGE gel band E9</td>
<td>GQ383872.1</td>
<td>88%</td>
</tr>
<tr>
<td>2</td>
<td><strong>Uncultured bacterium clone W29</strong></td>
<td><strong>AY770969.1</strong></td>
<td><strong>100%</strong></td>
</tr>
<tr>
<td>3</td>
<td>Uncultured bacterium clone sfl526</td>
<td>AJ314704.1</td>
<td>76%</td>
</tr>
<tr>
<td>4</td>
<td>Aquamonas Fontana strain: AQ9</td>
<td>AB120963.1</td>
<td>77%</td>
</tr>
<tr>
<td>5</td>
<td><strong>Geobacillus sp. MKK-2005</strong></td>
<td><strong>DQ309334.1</strong></td>
<td><strong>100%</strong></td>
</tr>
<tr>
<td>6</td>
<td>Uncultured alpha proteobacterium clone NL5BD-01-H03</td>
<td>FM252632.1</td>
<td>92%</td>
</tr>
<tr>
<td>7</td>
<td>Bacillus foraminis strain XJSL3-10</td>
<td>GQ903409.1</td>
<td>87%</td>
</tr>
<tr>
<td>8</td>
<td>Uncultured bacterium clone LaYa6-83</td>
<td>GU291703.1</td>
<td>96%</td>
</tr>
<tr>
<td>9</td>
<td>Geobacillus sp. Enrichment culture clone d8m_OTU4</td>
<td>FJ895353.1</td>
<td>98%</td>
</tr>
<tr>
<td>10</td>
<td>Uncultured archaeon clone ma76</td>
<td>GU567434.1</td>
<td>97%</td>
</tr>
<tr>
<td>11</td>
<td><strong>Uncultured bacterium clone nbw117h11c1</strong></td>
<td><strong>GQ008495.1</strong></td>
<td><strong>100%</strong></td>
</tr>
<tr>
<td>12</td>
<td>Uncultured alpha proteobacterium clone 651</td>
<td>EF188320.1</td>
<td>98%</td>
</tr>
<tr>
<td>13</td>
<td>Uncultured bacterium clone A314016</td>
<td>AY907753.1</td>
<td>90%</td>
</tr>
<tr>
<td>14</td>
<td>Uncultured bacterium isolate DGGE gel band X6</td>
<td>GU395564.1</td>
<td>89%</td>
</tr>
<tr>
<td>15</td>
<td>Uncultured bacterium clone H2-2D.69</td>
<td>DQ423661.1</td>
<td>89%</td>
</tr>
<tr>
<td>16</td>
<td>Oxalicibacterium sp. JCN-21</td>
<td>GU295961.1</td>
<td>89%</td>
</tr>
<tr>
<td>17</td>
<td>Uncultured Betaproteobacteria clone QEDT2DB10</td>
<td>CU920167.1</td>
<td>99%</td>
</tr>
<tr>
<td>18</td>
<td>Uncultured bacterium clone nbw638f09c1</td>
<td>GQ114515.1</td>
<td>94%</td>
</tr>
<tr>
<td>19</td>
<td>Uncultured bacterium clone A9M13F</td>
<td>EU795990.1</td>
<td>91%</td>
</tr>
<tr>
<td>20</td>
<td>Uncultured bacterium isolate DGGE 1</td>
<td>AJ520090.1</td>
<td>86%</td>
</tr>
<tr>
<td>21</td>
<td>Uncultured bacterium KM-69-D9</td>
<td>EU686644.1</td>
<td>83%</td>
</tr>
<tr>
<td>22</td>
<td>Uncultured bacterium clone 33-1</td>
<td>AB425314.1</td>
<td>93%</td>
</tr>
<tr>
<td>23</td>
<td>Uncultured bacterium clone SA1-A9</td>
<td>GU083566.1</td>
<td>97%</td>
</tr>
<tr>
<td>24</td>
<td>Uncultured bacterium clone 146-64</td>
<td>GU212561.1</td>
<td>88%</td>
</tr>
<tr>
<td>25</td>
<td>Methylobacterium chloromethanicum CM4</td>
<td>AF198624.1</td>
<td>85%</td>
</tr>
<tr>
<td>26</td>
<td>Uncultured bacterium clone MN46</td>
<td>EU239317.1</td>
<td>90%</td>
</tr>
</tbody>
</table>
CONCLUDING REMARKS

The mixing of two wastewater streams, each of which is problematic to its industry, has presented a unique opportunity for wastewater engineers to solve both problems in one go. Immediate application of this concept may be hindered by infrastructure that had not been designed for this purpose. Nevertheless, a better understanding of the pollutant removal mechanisms and the kinetics of the processes is needed before an engineering design can be put into place. The results of the molecular study have identified organisms that are active under anaerobic conditions, which can be inferred as SRM. However, the findings also pose a challenge in producing this bacteria strain in large quantities to promote a high rate of sulphide production as they fall into the category of uncultured strains, meaning that they cannot be produced in the laboratory. Although much has been established on biological sulphide precipitation, there are still other aspects that need further investigations. Some recommendations for further studies include:

1. A study on the mechanisms involved during the biological sulphate reduction by sulphate reducing microorganisms.

2. A study on the structure and properties of the formed metal sulphide precipitates.

3. A study on the optimization of biological sulphate reduction and metal sulphide precipitation process with various environmental parameters such as temperature and pH.
BIOREMEDIATION OF POLYAROMATIC HYDROCARBON CONTAMINATED LAND

BACKGROUND

The presence of contaminated land is of concern in most developed countries due to scarcity of uncontaminated and arable land for new development as well as public health concerns. With the increasing population and the pressure for a more sustainable urban development, a framework for management provisions which adopt sustainability treatment practices for contaminated land was formulated by the Department of Environment (DOE), Malaysia. Remediation is usually considered after assessment has been made, possibly improving the area through removal or reduction of contaminants. However, one must ensure that all potential impacts of the remediation are taken into account. A risk-based land management approach is the overall guiding principle for contaminated land management framework. This approach ensures that the site is fit for its intended purpose, that the environment is protected and that long term care is an important factor.

As such, new treatment technologies, preferably based on green technology, need to be explored for treating these pollutants. The relative merits of green technology against conventional technology will be discussed. In addition, successful laboratory scale applications of bioremediation using indigenous bacteria isolated from petroleum and
Innovative Green Technology through a Multidisciplinary Approach

Municipal sludge to degrade various polycyclic aromatic hydrocarbons (PAHs) will also be presented. The potential treatment strategies to improve bioavailability by using surfactants and the application of chemical treatment using strong oxidizing agent, zero-valent iron to reduce PAHs will also be further elaborated in this chapter.

**REMEDIATION TECHNOLOGIES**

Industrial production of chemicals as well as their inappropriate use, improper disposal and accidental leakage has resulted in contamination of many areas. Among man-made substances that cause ecotoxicological problem areas are a variety of aromatic compounds such as halogenated aromatic compounds, polycyclic aromatic hydrocarbons (PAHs) and BTEX compounds (benzene, ethylbenzene, toluene and three isomers of xylene). Apart from the type of pollutants present, the selection of remediation treatment technology for contaminated land is also dependent on factors such as soil and hydro-geological conditions of the sites. In Malaysia, one of the forefront industries that give serious considerations to contaminated land remediation is the petroleum-based industry. Locations such as petrol kiosks, oil depots and petroleum refineries are examples of petroleum-based premises that require contaminated land remediation. Figure 6 shows the list of contaminated land remediation technologies used in Malaysia.
Physical treatment methods such as solidification have been successful in containing pollutants from soil in a very short time. However these processes are highly expensive. Meanwhile, application of ozonation as a chemical treatment method using anaerobically digested sludge has been used to improve the PAH removal rate with the addition of hydrogen peroxide (Bernal-Martinez et al., 2007). However, reduction of PAH by strong oxidizing agents such as ozone, Fenton’s reagent and hydrogen peroxide is becoming undesirable as by-products of incomplete oxidation may produce toxic metabolites. Besides, they can be costly especially on large scale applications (Haapea and Tuhkanen, 2006).
Alternatively, a biological treatment method which utilizes microorganism is becoming one of the highly potential and economical options for the in-situ treatment of hydrocarbon contaminated soil. In the bioremediation process, the microorganisms such as bacteria and fungi will break down or/and mineralize organic pollutants through a complex enzyme reaction and redox process into simple and less hazardous compounds. Some of the native soil micro-floras have exhibited potential in degrading PAHs compounds. Nevertheless, the degradation of PAH through this natural attenuation have several limitations such as insufficient quantity and quality of indigenous strains that are capable of breaking down the PAHs. Besides, the long time taken from natural attenuation may cause risks to the environment (Hamdi et al., 2007). Table 11 summarizes the different types of in-situ and ex-situ remediation treatment approaches, its advantages and limitations.
Table 11: In-situ and ex-situ Remediation Technologies, Advantages and Disadvantages  
(Environment Canada, 2002)

<table>
<thead>
<tr>
<th>Technologies</th>
<th>Contaminant</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
</table>
| In-situ/ Ex-situ              | Solidification/ Stabilization | In solidification or stabilization, contaminants are physically or chemically bound to the medium to produce a non-leachate material. Commonly used binding agents include cement, lime, organic polymers, and silicates. This can be accomplished either in-situ or ex-situ. | Most soils can be treated.  
Less effective for some semi-volatiles, pesticides  
Most soils can be treated.  
Time to complete cleanup is relatively short.  
Low operating and maintenance costs.  
Control vapor migration to structures, therefore inhalation/explosive risk reduced.  
Minimal site disturbance, low capital costs.  
Can be a permanent solution.  
Regulatory and public acceptance is high when off-gas is treated  
Volatilization inhibited by high humic content of soil.  
Best suited for relatively permeable, homogeneous soils.  
Residual liquid from treated air requires disposal.  
Thermal enhancement of volatilization may sterilize the subsurface, killing microbes required for biodegradation.  
High operating and maintenance costs.  
Remediation may take many years. |
| In-situ                       | Soil Vapour Extraction | Soil vapour extraction is a process of inducing air flow through unsaturated soils to remove volatilized contaminants. The air flow is induced by applying a vacuum to the soil through a network of extraction wells. The technology is applicable to volatile compounds with a high vapour pressure. | Permanent immobilization of organic contaminants cannot be assured.  
Organic compounds may interfere with binding agents.  
Volume of contaminated material can increase 50 to 100% due to addition of solidifying agents.  
High capital costs.  
High capital costs.  
High capital costs.  
High capital costs.  
High capital costs.  
High capital costs.  
High capital costs.  
High capital costs.
<table>
<thead>
<tr>
<th>Technologies</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bioremediation</td>
<td>Bioventing is a form of in situ attenuation in which oxygen in the form of air is delivered to contaminated unsaturated soils through a system of extraction and injection wells. Unlike soil vapor extraction, lower air flow rates are used to provide just enough oxygen to enhance biodegradation while minimizing volatilization to the atmosphere.</td>
<td>Natural attenuation describes the processes that act on a contaminant in the natural environment to reduce contaminant concentration. These processes may include dilution, biodegradation, volatilization, adsorption, and chemical reactions. Natural attenuation is slow because it may be perceived as a &quot;do-nothing&quot; option.</td>
</tr>
<tr>
<td>In-situ</td>
<td>Better oxygen delivery to less permeable formations. Less sensitive to formation water. Minimal site disturbance. Low capital and O&amp;M costs. Remediaion may be a permanent solution.</td>
<td>Modeling and long-term monitoring generally required. Degradation products may be more mobile and toxic than the original contaminant. Risk that contaminants may migrate to sensitive receptors before being attenuated. Regulatory and public acceptance is low because it may be perceived as a &quot;do-nothing&quot; option.</td>
</tr>
<tr>
<td>Natural attenuation</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Technologies

| Ex-situ | Soil washing | Soil washing remediates excavated soils by separating contaminants sorbed onto soil particles with an aqueous solution that may contain a basic leaching agent, surfactant, chelating agent, or pH adjustment. The treated soils are returned to the excavation site or removed to an off-site landfill.
| Ex-situ | Chemical Reduction/Oxidation | Reducing/oxidizing agents are used to chemically convert toxic contaminants in excavated waste materials to less toxic compounds that are more stable, less mobile, and/or inert. Commonly used reducing/oxidizing agents are ozone, hydrogen peroxide, hypochlorites, chlorine, and chlorine dioxide.

Contaminant

- Semi-volatiles, fuel hydrocarbons, inorganics.
- Less effective for some volatiles, pesticides.

Advantages

- The process is transportable to the site.
- Can be a permanent solution.
- Time to complete cleanup is relatively short.
- Regulatory and public acceptance is generally high.

Disadvantages

- Silts and clays are difficult to remove from washing fluid.
- Optimizing the washing fluid becomes more difficult when mixtures of metals and organics are present.
- Treatment and disposal of wastewater and sludge are necessary.
- High capital and high O/M costs
MICROBIOLOGY AND BIODEGRADATION

Bioremediation allows natural processes to clean up harmful organic chemicals in the environment. Microbes that are present in soil and groundwater can degrade some harmful chemicals, such as those found in gasoline and oil spills. When microbes completely digest these chemicals, they change them into water and harmless gases such as carbon dioxide (USEPA, 2001). Bioremediation can be conducted in-situ or ex-situ. The most significant parameters affecting bio-remediation are temperature, concentration of nutrients/fertilizers and concentration of oxygen (aeration). Laboratory-scale treatability studies must be carried out prior to actual clean-up. A diagram illustrating the mechanisms in bioremediation is shown in Figure 7.

Contaminated sites containing organic pollutants such as polycyclic aromatic hydrocarbons are considered high risks areas with potential health hazards as contaminants may be ingested through the food chain and bioaccumulate in humans (Knutzen, 1995; Fismes et al., 2002; Xiaojun et al., 2009). PAHs cause concerns because of their carcinogenic and mutagenic characteristics. PAHs are relatively stable and recalcitrant
Innovative Green Technology through a Multidisciplinary Approach

(Othman et al., 2010). Yet, low bioavailability of pollutants to bacteria due to strong sorption of pollutant to soil often resulted incomplete degradation of pollutants in soil media (Mohd-Kamil et al., 2010; Alias et al., 2012).

The use of chemical surfactants can substantially enhance the bioavailability and therefore the biodegradability of PAHs. However, chemical surfactants have been used to some extent with concerns. Apart from the high cost associated with chemical surfactants, there are concerns on the residue remaining on site after the remediation process (Makkar and Cameotra, 2001). Therefore, biosurfactants have considerable potential for surfactant-enhanced remediation application because biosurfactants are naturally occurring, can be synthesized from renewable sources, have lower toxicity, higher biodegradability and better biocompatibility to many contaminated sites. Biosurfactants extracted from microbes have recently received much attention because their potential to become a green technology alternative to conventional surfactants due to their biodegradability, low toxicity, renewable nature and functionality under extreme condition (Bodour & Maier, 2002; Makkar and Cameotra, 2001).

Apart from this, the slow and incomplete biodegradation of pollutants can be overcome with the combination of biological and chemical treatments. Several attempts have been made to address the limitation of individual physical, biological and chemical treatment process by combining treatment methods such as extraction, biological and oxidation treatment (Haapea & Tuhkanen, 2006; Bogolte et al., 2007; Zheng et al., 2007). Ozone and hydrogen peroxide are commonly employed in the hybrid chemical-biological treatment. These hybrid oxidation-bioremediation studies have confirmed that they increase the bioavailability and biodegradability of PAHs. However, relatively the uses of ozone and hydrogen peroxide as an oxidant too have incurred relatively high costs. Furthermore, the by-product from incomplete oxidation of ozone may pose detrimental effects to the existing bacteria.
Bioremediation of Polyaromatic Hydrocarbon Contaminated Land

communities. In a very recent finding, zero-valent iron (Fe⁰ or ZVI) seems promising as an emerging chemical agent for the cleanup of PAHs contaminated soil (Alias et al., 2012; Chang, et al., 2007). Deploying zero-valent iron (ZVI) to remove organic and inorganic pollutants from the environment has drawn scientists' attention as ZVI (Fe⁰) can replace the conventional oxidizing agent, ozone (Rivas et al., 2009; Wang & Tseng, 2009) and hydrogen peroxide (Alderman et al., 2007; Ferrarese et al., 2008). ZVI which can be obtained as waste material of iron scrap from the iron manufacturing sectors can be an economical and effective materials in treating pollutants.

BIOSURFACTANT POTENTIAL IN IMPROVING THE BIOAVAILABILITY OF POLYCYCLIC AROMATIC HYDROCARBONS

The effectiveness of the bioremediation process is typically governed by three important factors, namely, the presence of the degraders, availability of the contaminants to the degraders and a favourable environment for the degradation to occur. Therefore the intensity of biodegradation is highly dependent on microbial activity to degrade these contaminants and their rate limiting kinetics. Although very few microbes have been recognized as hydrocarbon degraders, the persistent characteristics possessed by PAHs such as low aqueous solubility and recalcitrance towards degradation makes it unavailable for microorganism. These conditions can substantially affect the microbial growth and the biodegradation capabilities. This phenomenon is referred to as limited bioavailability. Limited bioavailability is often defined when the uptake rate by organisms is limited by the physicochemical barrier between pollutant and the organism (Volkering et al., 1997). Hence, the performance of the bioremediation process can be improved by increasing the bioavailability of PAHs.
The concentration of surfactants, surface tension and its relation to critical micelle concentration (CMC) are elucidated in Figure 8.

Critical micelle concentration (CMC) is described as a measurement for the efficiency of surfactant. In reducing the surface and interfacial tension, surfactant monomer existed below the CMC. Low CMC indicates less surfactant is needed to saturate interfaces and form micelles. Solubilisation of PAHs will commence once the formation of micelles takes place at higher CMC. Consequently, solubilisation of PAHs leads to mobilisation of the pollutant, which in turn improves their bioavailability.

The interactions between microorganisms, pollutant, soil and surfactants can be further described in Figure 9. PAHs entrapped in soil matrix can be dispersed by the sorption of surfactant molecules onto soil. Surfactant monomers are capable in mobilising the PAHs
Bioremediation of Polyaromatic Hydrocarbon Contaminated Land

into water phase leading toward a micelle formation where PAHs are solubilised into the hydrophobic core. As for microorganisms, once PAHs are available it can be uptake directly by sorption of micelle onto microorganism, partitioning of PAHs between water phase and the micelles, directly from the water phase or directly from the solid phase. On the other hand, these relative interactions are strongly influenced by the physical state of pollutant whether it is adsorbed, absorbed or trapped in soil. Although surfactants play an important role in promoting the biodegradation, it is necessary to understand that the effectiveness of surfactant-aided bioremediation depends on the selection of surfactant and its most conducive working environment.

Figure 9: Mechanism of Biosurfactants Interactions between Microorganism, Soil and Pollutant (Adapted from Volkering et al., 1997)

Surfactants, of both biological and chemical origin, are amphipathic molecules that accumulate at interfaces, decrease interfacial tensions, and forming aggregate structures that allows hydrocarbon solubilisation. Due to these properties, surfactants modify interfacial behaviour and
Innovative Green Technology through a Multidisciplinary Approach

impact on the way other molecules behave at interfaces and in solution. In general, surfactants can be classified into two types namely chemical synthesized surfactants and biological derived surfactants. Nevertheless, the level of chemical synthesized surfactant toxicity that could harm the environment and costly production are among the major drawbacks in their applications. Biosurfactants have considerable potential for remediation application because biosurfactants have better biocompatibility, lower toxicity and higher degradability compared to synthetic surfactants (Ron and Rosenberg, 2002; Volkering et al., 1997). Biosurfactant (lipopeptides, rhamnolipids and surfactin) producing bacteria such as those belonging to the genus *Pseudomonas*, *Brevibacillus* and *Bacillus* have been reported to enhance phenanthrene and anthracene availability (Das et al., 2008; Reddy et al., 2010; Tecon and Van der Meer, 2010). Nevertheless, though biosurfactant could enhance the bioremediation process, incomplete degradation could still occur. Therefore, combination of biological and chemical treatment as an alternative could be an option in removing the remaining PAHs.

**ZERO VALENT IRON: EMERGING OXIDIZING AGENT AN ALTERNATIVE FOR BIOREMEDIATION APPLICATION**

Zero-valent iron particle was first introduced in the early 1990s to remediate groundwater contamination in which the ZVI particles were attached onto the permeable reactive barrier (PRB) system. The structure of PRB system was installed across the polluted groundwater flow as shown in Figure 10. The pollutants may adsorb, precipitate and transform to more simple components when interact with this engineered iron wall technology. The technology provides rapid pollutants removal from the contaminated area over the conventional PRB system. Since then, over 100 of PRB structures were installed in the USA. However, in the late 1990s, the application of ZVI-PRB system seemed not to be cost effective as to construct the system in deep aquifer and relocate the system required high investments (Li et al., 2006). Thus the new remediation technique
Bioremediation of Polyaromatic Hydrocarbon Contaminated Land

was proposed by pumping the ZVI particles into the contaminants flow as shown in Figure 11.

![Permeable Reaction Barrier (PRB)](image1)

**Figure 10: ZVI-Permeable Reactive Barrier System (Adopted from Cook, 2009)**

Employing ZVI has advantages as the possibility of ZVI to generate hydrogen peroxide and Fenton reagent (hydroxyl radical) through series of reduction/oxidation (redox reaction) process on ZVI surfaces (Mylon 49).
et al., 2010; Feitz et al., 2005; Joo et al., 2005). Mylon et al. (2010) found the hydrogen peroxide ($H_2O_2$) had been generated within 3 minutes after the ZVI reaction with the water phase. Meanwhile, Joo et al. (2005) and Feitz et al. (2005) confirmed that hydroxyl radical (OH) was produced during the degradation of organic pollutants. These oxidizing agents promote fast pollutants degradation and mineralization (Mylon et al., 2010; Lin et al., 2008; Feitz et al., 2005).

In the presence of oxygen, ZVI will be oxidized and release two electrons as in Equation 3. The electron will react with oxygen which later generates hydrogen peroxide ($H_2O_2$) Equations 4 through 7. Hydrogen peroxide is decomposed by the ferrous ion, which is previously released from the oxidation of ZVI. The reaction between these two chemicals will produce stronger oxidizing agent known as hydroxyl radical (OH) Equation (8). Hydroxyl radical may oxidize the PAH pollutants through the hydroxylation reaction (Equation 9).

$$\text{Fe}_0 \rightarrow \text{Fe}^{2+} + 2e \quad (3)$$
$$\text{O}_2 + 2e \rightarrow 2\text{O}^2^- \quad (4)$$
$$2\text{H}_2\text{O} \rightarrow 2\text{H}^+ + 2\text{OH}^- \quad (5)$$
$$2\text{H}^+ + 2\text{O}^2^- \rightarrow \text{H}_2\text{O}_2 \quad (6)$$
$$\text{Fe}_0 + \text{O}_2 + 2\text{H}^+ \rightarrow \text{Fe}^{2+} + \text{H}_2\text{O}_2 \quad (7)$$
$$\text{Fe}^{3+} + \text{H}_2\text{O}_2 \rightarrow \text{Fe}^{3+} + \text{OH} + \text{OH}^- \quad (8)$$
$$\cdot\text{OH} + \text{RH} \rightarrow \text{R}^\cdot + \text{H}_2\text{O} \quad (9) \quad \text{(Mylon et al., 2010)}$$

The reaction mechanism of the aromatic compounds can be varied depending on the nature of PAHs and sorption ability of the PAHs (Yap et al., 2011). Apart from the capability of ZVI to oxidize the pollutant, the ZVI are also able to absorb pollutants on the ZVI surfaces. This bi-function demonstrated in the ZVI reaction can reduce significant concentration of pollutant (Kang & Choi, 2009). Figure 12 illustrates the absorption and oxidation reactions involve on the surface of ZVI particles.
The removal of pollutants by ZVI however is dependent on the passivation level at ZVI surfaces or the oxidation stage on the shell layer of ZVI (Tratnyek et al., 2001). Other benefit of employing ZVI includes the possibility of ZVI to be synthesised to nano size. Nano-valent iron, Fe⁰ (ZVI) has been demonstrated to be an efficient reductant to many pollutants and extensively investigated for remediation of organic and inorganic pollutants in water, sediment and soil. The nano ZVI has several advantages such as the nano size of ZVI particles offer large contact area with pollutants thus giving a more rapid reaction rate (Rajan, 2011; Shih et al., 2011; Tiraferri et al., 2008; Wang & Zhang, 1997). ZVI had been found to remove half of the organic and inorganic pollutants in less than 1 hour compared to bioremediation process which normally takes at least 2 days (Alias et al., 2012; Mylon et al., 2010). Besides synthesizing of ZVI, these iron particles can also be obtained as waste material of iron scrap from the iron manufacturing sectors (Oh et al., 2003). The idea of using this scrap iron may help the iron-based industries to reduce their cost in managing iron waste products. Other advantages of using ZVI are shown in Table 13.
Innovative Green Technology through a Multidisciplinary Approach

Table 13: Advantages of using ZVI

<table>
<thead>
<tr>
<th>Advantages</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fast degradation of pollutants</td>
<td>Alias et al., 2012; Mylon et al., 2010; Feitz et al., 2005</td>
</tr>
<tr>
<td>Yields hydrogen peroxide and hydroxyl radical</td>
<td>Mylon et al., 2010; Feitz et al., 2005; Joo et al., 2005</td>
</tr>
<tr>
<td>Provides as electron donor for bacteria</td>
<td>Oller et al., 2011; Cook, 2009; An et al., 2009</td>
</tr>
<tr>
<td>Degradation possible at high pH</td>
<td>Feitz et al., 2005</td>
</tr>
<tr>
<td>Can be synthesis to the nano size which can provide greater surface area</td>
<td>Rajan, 2011; Shih et al., 2011; Tiraferri et al., 2008; Wang &amp; Zhang, 1997</td>
</tr>
<tr>
<td>Can be obtained from waste product of iron manufacturing industry</td>
<td>Oh et al., 2003</td>
</tr>
</tbody>
</table>

LABORATORY STUDIES ON BIOREMEDIATION

Selected studies from the myBioRec laboratory, Faculty of Civil Engineering will be presented in the subsequent subsections.

Polycyclic Aromatic Hydrocarbon Biodegradation in Liquid Culture and Sand Slurry

The potential of Corynebacterium urealyticum in degrading phenanthrene in liquid culture and in sand slurry was evaluated under non-indigenous conditions. The Corynebacterium urealyticum was isolated from municipal sludge by Othman et al. (2010). The tests were conducted in 250 mL volumetric flasks comprising 50 mL artificially contaminated phenanthrene minimal media and 10% bacterial inoculum (v:v ratio). Flasks containing artificially contaminated phenanthrene sand (20 g) were inoculated with 10% bacterial inoculum and 40% minimal media.
The performance of *Corynebacterium urealyticum* was better in liquid culture compared to sand slurry as shown in Figure 13. Degradation trends in both media established in this study were consistent with those reported in the literature. Complete degradation was observed in liquid culture, whereas in solid media such was not the case. In sand slurry, the PAH pollutant (phenanthrene) was entrapped and had strong adsorption to sand particles, contributing to low bioavailability of phenanthrene to the bacterium (Boopathy, 2000; Volkering et al., 1992). Thus, the low bioavailability in sand slurry slowed and to some extent limited the transfer of phenanthrene into the cell and subsequently retarded the degradation process.

![Graph showing degradation of phenanthrene in liquid culture and sand slurry](image)

*Figure 13: Degradation of Phenanthrene in Liquid Culture and Sand Slurry at Initial Phenanthrene*
Biodegradation of High and Low Molecular Weight Polycyclic Aromatic Hydrocarbons

The degradation performance of low molecular weight (LMW) PAH and high molecular weight (HMW) PAH by Corynebacterium urealyticum in sand slurry system was evaluated as shown in Figure 14. Phenanthrene (PHE) and benzo(a)pyrene (BaP) representing respectively a LMW and HMW PAHs were used as the substrates for the bacteria. The experiment was conducted in an artificially contaminated phenanthrene and benzo(a)pyrene sand slurry system (20 g) in reactor flasks of 250 mL.

Generally, during the 8 days of experiment, there was no significant difference on the removal of phenanthrene and benzo(a)pyrene by Corynebacterium urealyticum. The PHE degraded drastically within this time meanwhile, a sharp decrease was observed on BaP from day 0 until day 16. The removal of PHE and BaP remained stable at almost around 30% and 49% respectively after day 8 for PHE and day 16 for BaP. The fast degradation of PHE and BaP occurred at early stage due to those PAHs have higher bioavailability to the bacteria. The biodegradation becomes limited when the availability of substrate decreases in the mass transfer rate of substrate (Johnsen et al., 2005).

The result implies that C. urealyticum is capable of degrading LMW and HMW PAHs. However, degradation of HMW PAH was slightly higher than LMW PAH. Similar finding was also observed in a study by Bengtsson et al. (2010) where degradation of BaP was higher than that of PHE. These observations can be explained by the fact that the enzyme produced by C. urealyticum is sufficient and suitable to degrade the BaP. The continuous generation of enzyme in 2 weeks of the experiment improved the degradation of BaP.
Biodegradation of Polycyclic Aromatic Hydrocarbons using Biochemical Treatment Approach-Screening and Isolation of Potential Biosurfactant Producers from Agricultural Waste

Potential biosurfactant producers were isolated from palm oil mill effluent. Isolation from mixed cultures of potential microbes for biosurfactant production was carried out for each dilution from different POME samples. Blood agar was used as an isolation medium for microorganisms obtained from the serial dilution of mixed cultures. Figure 15 illustrates blood agar plate with colonies of microbes.
After isolation process, the potential biosurfactant producers were evaluated by qualitative and quantitative screening methods. Screening was also done for strains isolated from municipal and petroleum sludge. Figures 16 to 19 illustrate the results of the qualitative screening method performed with chemical surfactant and biosurfactant. Table 14 shows the different types of screening method and their typical results to determine the selection of biosurfactant producers.

<table>
<thead>
<tr>
<th>Screening Method</th>
<th>Positive Detection</th>
<th>Negative Detection</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qualitative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drop collapse test</td>
<td>Flat drop</td>
<td>Remain beaded</td>
<td>Jain et.al, 1991</td>
</tr>
<tr>
<td>Microplate assay</td>
<td>Concave surface</td>
<td>Flat surface</td>
<td>Vaux &amp; Cottingham, 2001</td>
</tr>
<tr>
<td>Oil spreading assay</td>
<td>Clear oil displacement activity</td>
<td>No oil displacement activity</td>
<td>Morikawa et. al, 2000</td>
</tr>
<tr>
<td>Blood lysis assay</td>
<td>Clear lysis</td>
<td>No lysis occur</td>
<td>Mulligan et. al, 1984</td>
</tr>
<tr>
<td>Quantitative</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Du Nouy ring method</td>
<td>ST £ 40 mN/m</td>
<td>ST &gt; 40mN/m</td>
<td>Willumsen &amp; Karlson, 1997</td>
</tr>
<tr>
<td>Emulsification assay</td>
<td>Forming emulsion layer</td>
<td>No emulsion formation</td>
<td>Cooper &amp; Goldenberg, 1987</td>
</tr>
</tbody>
</table>
Bioremediation of Polyaromatic Hydrocarbon Contaminated Land

Figure 16: Microplate Assay (a) POME Strain S01, (b) Tween 80, (c) POME Strain R06 (d) Distilled Water

Figure 17: Clear Zone Around (a) Tween 80, (b) POME Strain R06, (c) Corynebacterium Uroalyticum (Municipal Strains)

Figure 18: Oil Spreading Assay (a) Measurement Method (b) Clear Zone by POME Strain R06 (c) Clear Zone by Tween 80
Out of 49 strains screened for biosurfactant production, 11 strains showed biosurfactant producing ability through qualitative and quantitative assessment. Table 15 shows the typical screening results. Further studies have been initiated to identify their properties and consequently determine the potential of their applications particularly to improve the bioavailability of PAHs.

Table 15: Potential Biosurfactant Producers Screening Results

<table>
<thead>
<tr>
<th>Strain</th>
<th>Qualitative Screening</th>
<th>Quantitative Screening</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Microplate Assay</td>
<td>Blood Lysis Assay</td>
<td>Drop Collapse Test</td>
</tr>
<tr>
<td>Chemical Surfactant</td>
<td>Tween 80</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td>Municipal sludge</td>
<td>A1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>F1</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>AN1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td>P1</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>M1</td>
<td>-</td>
<td>✓</td>
</tr>
<tr>
<td>Petroleum sludge</td>
<td>C6</td>
<td>✓</td>
<td>✓</td>
</tr>
<tr>
<td></td>
<td>A6</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

cont.
Degradation of Polycyclic Aromatic Hydrocarbons using Chemical Treatment Approach

The degradation kinetics of phenanthrene at different concentration of ZVI was investigated in phenanthrene contaminated sand. The ZVI at concentrations of 1, 3, 5, 7 and 10% at w:w ZVI:sand ratio was employed in the study. A very fast removal of phenanthrene was exhibited in ZVI-sand water system at all ZVI concentrations as shown in Figure 20. The PAHs was removed at a high rate within 30 minutes and turned almost constant after 1 h at ZVI concentrations of 3, 5, 7 and 10%. More than 36% of PHE was removed in the first 30 minutes of reaction time for all ZVI concentrations. However, only 14.5% of PHE was removed at 1% ZVI concentration. At the end of 48 hours reaction time, 39-54% of PHE was removed. The fast decrease of phenanthrene in the early stage was consistent with the findings by Feitz et al. (2005). The reaction is due to the catalytic reaction of hydrogen peroxide (H2O2) and hydroxyl radical (OH). The oxidation of ZVI particles with time, lead to passivation of ZVI surface which prevents the redox reaction, thus slowed the rate of the PAH removal.
The correlation between the pseudo-first order degradation rates coefficients of phenanthrene ($k_{obs}$) at linear data (in 30 minutes reaction time) and ZVI concentration was observed as shown in Figure 21. The reaction rates increase significantly from 1% to 3% ZVI concentration and then increase steadily up to 10% ZVI concentration. This can be seen where at 3, 5, 7 and 10% ZVI concentrations, almost similar values of the reaction rate coefficient ($k$) were observed which were within the range of 0.015-0.019 min$^{-1}$. The removal rate becomes constant when the binding sites reach maximum capacity or point of saturation.
CONCLUDING REMARKS

*Corynebacterium urealyticum*, a local isolated bacterium showed a high potential to degrade low molecular weight and high molecular weight PAHs. Complete degradation of PAH was observed in liquid culture medium. However, problems associated on the bioavailability may limit the availability of the PAH giving rise to incomplete PAH degradation in sand slurry medium. Potential bacteria producing surfactant had successfully been isolated from Palm Oil Mill Effluent (POME) and petroleum sludge. These bacteria strain might be capable to overcome the incomplete biodegradation of PAH related to bioavailability issues.

The long duration associated with bioremediation can be solved using chemicals in a hybrid biological-chemical treatment. An emerging chemical oxidizing agent, zero-valent iron (ZVI) was found capable
in soils and less easy to degrade compared to other organic compounds. Over the years, bioremediation is gaining wider approval as a feasible alternative treatment technology for the remediation of soil contaminated with persistent organic pollutants such as PAHs. Bioremediation is deemed to be safe, efficient, eco-friendly and economical in removing pollutants from contaminated sites. The method involves biologically catalyzed reduction of complex chemicals or mediates that transform hazardous chemicals to less toxic compounds. In the case of organic pollutants, biodegradation occurs as microorganisms metabolize and mineralize these compounds into inorganic products such as carbon, nitrogen, phosphorus, sulphur and other metabolic waste product. The advantages and disadvantages of bioremediation are shown in Table 12.

<table>
<thead>
<tr>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Remediation can be done in situ.</td>
<td>Slow kinetics.</td>
</tr>
<tr>
<td>Process already implemented for</td>
<td>May require monitoring.</td>
</tr>
<tr>
<td>Biodegradation of organic compounds.</td>
<td>The requirements to eliminate efficiently contaminants may vary from site to site.</td>
</tr>
<tr>
<td>Low environmental impact.</td>
<td>Some contaminants can be present at high concentrations that inhibit microbial activity.</td>
</tr>
<tr>
<td>Compatible with the use of other technologies.</td>
<td>Risk of accumulation of toxic by-products.</td>
</tr>
<tr>
<td>Low cost.</td>
<td>Incomplete degradation often observed in soil system.</td>
</tr>
</tbody>
</table>

Studies have indicated that the use of a competent microbe has efficiently facilitated and sped up the bioremediation of media co-contaminated with hydrocarbons (Alisi et al., 2009). Thus to overcome the problems, potential hydrocarbon degraders were identified and inoculated into the contaminated soil. Studies to identify the potential degraders of pollutants under non-indigenous condition have been widely investigated either in liquid and soil media. Under this condition, complete degradation of PAH was observed in liquid culture medium.
of removing PAH from contaminated soil. Very fast removal of PAH was attained during the ZVI treatment on PAH contaminated sand. The removal capability of PAH increased with the increase of ZVI concentration. The utilization of ZVI to remove PAH in contaminated soil will encourage the reuse of waste materials. This practice is in line with sustainable resource management policies that can help to safeguard the environment, human health and quality of life.

Although much development has been made in enhancing biodegradation of PAHs, recalcitrant behaviour of PAHs has led to incomplete degradation of PAHs. Therefore, it is recommended that the following investigations be carried out in the future:

1. Investigation on the sequence of hybrid treatment with biological-chemical or chemical-biological treatment utilizing PAH-degrading bacteria and ZVI.

2. Investigation on simultaneous treatments with PAH-degrading bacteria, ZVI and biosurfactant producers.

3. Investigations on the application of stabilised nano zero-valent iron (nZVI) to remove remaining PAHs.
Biosorption is a passive physical-chemical process where bio-molecules of non-living biological materials referred to as biosorbents, bind with heavy metal ions from aqueous solutions (Volesky and Holan, 1995). It is classified as an alternative sustainable remediation technology in terms of resources and environmental impacts where the biosorbent, a biodegradable based material and the heavy metal in dilute acid could be extracted via electrolysis (Poliakoff and Licence, 2007; Hashim et al., 2011). Table 16 lists specific studies on biosorption focussing on types of processes, functionality and economic feasibility.
### Table 16: Selected Biosorption Studies on Processes, Functionality and Economic Feasibility

<table>
<thead>
<tr>
<th>Type of study</th>
<th>Parameters</th>
<th>Observation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Process</td>
<td>Biosorbent concentration</td>
<td>Dependent process, maximum dosage or random selection dosage is used in batch study.</td>
</tr>
<tr>
<td></td>
<td>Initial pH</td>
<td>Dependent process, operate under wide range of pH conditions.</td>
</tr>
<tr>
<td></td>
<td>Contact time</td>
<td>Important parameters for kinetic study.</td>
</tr>
<tr>
<td></td>
<td>Initial heavy metal concentration</td>
<td>Important parameters for isotherm study and calculation of $q_{\text{max}}$.</td>
</tr>
<tr>
<td></td>
<td>Temperature</td>
<td>Generally not affected as biosorbent is non-living. Important for thermodynamic study</td>
</tr>
<tr>
<td></td>
<td>Agitation rate</td>
<td>No consistent profile, depends on type of biosorbent.</td>
</tr>
<tr>
<td></td>
<td>Particle size</td>
<td>No consistent profile, depends on type of biosorbent.</td>
</tr>
<tr>
<td>Functionality</td>
<td>Efficiency - percentage</td>
<td>Depends on biosorbent, type of heavy metals and operating condition.</td>
</tr>
<tr>
<td></td>
<td>Effectiveness - uptake</td>
<td>Usually rapid under favourable conditions.</td>
</tr>
<tr>
<td></td>
<td>Versatile</td>
<td>High versatility. The binding sites on biosorbent can bind with a variety of heavy metal ions.</td>
</tr>
<tr>
<td></td>
<td>Selectivity</td>
<td>Generally poor for raw biosorbent. Modification of biosorbent, types and concentration of heavy metal may improve selectivity.</td>
</tr>
<tr>
<td></td>
<td>Recovery</td>
<td>High recovery using dilute acid, alkaline or others solutions.</td>
</tr>
</tbody>
</table>
Biosorption offers advantages of low cost, easily available in large quantity, high effectiveness for dilute effluents, minimum chemical usage and reduced toxic sludge generation (Gupta et al., 2000; Lesmana, 2009). Furthermore, biosorbents have advantages over live and immobilized micro-organism as they do not depend on nutrient availability, toxicity tolerance and metabolic activities (Krishnani and Ayyappan, 2006; Locci et al., 2008).

BIOSORPTION MECHANISMS

Biosorption mechanisms play an important role in the performance evaluation of the biosorbent and are currently the major focus in biosorption related papers. Mechanisms of heavy metal biosorption are influenced by physical and chemical interactions between the biosorbent and heavy metal ions, thus affecting the biosorption efficiency and
Innovative Green Technology through a Multidisciplinary Approach

effectiveness. Biosorption mechanisms can take place in many forms which are complex and several mechanisms may occur simultaneously. Adsorption, ion exchange, chemisorptions, complexation, chelation and microprecipitation are mechanisms intensely discussed in biosorption process (Sud et al., 2008; Park et al., 2010). Table 17 list the mechanisms with its description.

<table>
<thead>
<tr>
<th>Mechanism</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Adsorption</td>
<td>Adhesion of molecules to a surface.</td>
</tr>
<tr>
<td>Ion exchange</td>
<td>Heavy metal ions replacement by heavy metal ions.</td>
</tr>
<tr>
<td>Chemisorptions</td>
<td>Chemical bonds are formed through sharing electron between functional groups and heavy metal ions.</td>
</tr>
<tr>
<td>Complexation</td>
<td>Coordinate bonds are formed through functional groups attached to central metal atom, especially a transition metal atom.</td>
</tr>
<tr>
<td>Chelation</td>
<td>Bonds formed between a centre atom with coordinate bonds.</td>
</tr>
<tr>
<td>Micro-precipitation</td>
<td>A process by which a substance is separated out of a solution as a solid by forming a chemical precipitate fine particles.</td>
</tr>
</tbody>
</table>

(adapted from Nurchi and Villaescusa, 2008; Chojnacka, 2010)

Ion exchange is one of the most popular mechanisms reported for biosorption, whereby the mechanism commonly involves the exchange of heavy metal ions by other metals present on the biosorbent (Slaba and Dlugonski, 2011; Tay et al. 2011a; Blazquez et al., 2011; Vimala and Das, 2011; Tunali Akar et al., 2012). These metal ions can include alkaline metals such as Na\(^+\) and K\(^+\) as well as alkaline earth metals like Mg\(^{2+}\) and Ca\(^{2+}\).

The postulation of this mechanism being involved in the biosorption process is usually obtained through the quantification of heavy metal and other metal ions exchanged using Inductively Coupled Plasma Spectrometry (ICP-OES) or Atomic Absorption Spectroscopy (AAS) and confirmed by the use of FTIR (Fourier Transform Infrared Spectroscopy) and EDX (Energy-Dispersive X-ray Spectroscopy) to detect for the presence and loss of the respective ion species on the biosorbent. In
addition, Murphy et al. (2009) also employed XPS (X-ray photoelectron spectroscopy) as an analysis method besides FTIR to support the role of ion-exchange in the material of study. However, the proposed ion exchange mechanism cannot be correlated directly with the biosorbent’s biosorption performance as there is no direct correlation of the ions or bonds detected using EDX, FTIR or XPS with the amount of metal exchanged in the process.

Chemisorptions can be studied through the use of existing models, namely, pseudo second order kinetic or the mean free energy of Dubinin-Radushkevich isotherm (Yao et al., 2010, Tay et al., 2011a, Subbaiah et al., 2011; Vimala and Das, 2011; Tunali Akar et al., 2012). Obviously, there is lack of evidence from instrumentation analysis to support this mechanism. Therefore, this mechanism needs to be further investigated and supported by equations.

Complexation is characteristically tied to double bonds structures of amide, carboxyl or phosphate functional groups in the material used for sorption (Javaid et al., 2011; Blazquez et al., 2011; Tunali Akar et al., 2012). The formation of complexes in these cases are inferred from the non-saturation of Freundlich and/or the presence of shifting bands in FTIR based on the chemical composition of the materials used. Slaba and Długosński (2011) and Tay et al. (2011a) have both reported that nitrogen derived organic compounds that have such double bonds are involved in metal ion complexation. However, these have not been confirmed by other instrumentation analysis nor evaluated intensively to elucidate the complexes formed. The difficulty lies in the fact that more advanced characterisation methods are required, and in the case of solid biosorbents, the use of solid state NMR to confirm the complexation mechanism.

It can be concluded that recent studies reported in the literature show that further studies on heavy metal biosorption is still needed as there is no conclusive information regarding mechanisms that are responsible
Innovative Green Technology through a Multidisciplinary Approach

for the heavy metal biosorption. Biosorption mechanism is based mainly on functional groups and the affinity between the biosorbent with heavy metal ions, thus mechanisms may differ depending on the biosorbent.

**BIOSORBENT**

Biosorbents consist of dead and metabolically inactive biological material that are involved in the biosorption process and are able to extract or concentrate a desired molecule by its selective retention. In recent years, interest has shifted towards the development of biodegradable and sustainable biosorbents for heavy metal treatment, especially for industrial wastewater laden with heavy metal. Table 18 lists studies in different countries on biosorption of Pb(II), Cu(II) and Ni(II) using various biosorbents. It is evident that there is a wide choice of biosorbent from industrial and agriculture wastes that can be potentially used as biosorbent. The major challenge lies in the selection of a suitable biosorbent that is low in costs and readily available in large quantities (Vieira and Volesky, 2000; Hawari and Mulligan, 2006).

*Pleurotus ostreatus* spent mushroom substrate (PSMC) can be considered as a novel material to be used as biosorbent. There is no published report on it's used as biosorbent or material characterisation studies. PSMC is a lignocellulosic based material that is widely available from mushroom farms in Malaysia. It is mainly made up of rubber tree sawdust and *Pleurotus ostreatus* mycelium. For every 200 g of oyster mushroom produced, 600 g of PSMC would be generated (Singh *et al.*, 2003). The handling and disposal of PSMC at waste disposal sites can be costly for the industry. Thus the usual method of disposal for PSMC adopted by the industry is through open burning or converted into low commercial value organic fertilizer. Studies on PSMC as a potential biosorbent for heavy metal treatment, is yet to be fully explored. Both the lignocellulosic sawdust as well as the fungal biomass of the oyster mushroom has made PSMC to be a potential the heavy metal biosorbent.
Table 18: Heavy Metal Biosorption Studies using Biosorbents from Different Countries

<table>
<thead>
<tr>
<th>Country</th>
<th>Heavy metal species</th>
<th>Biosorbent(s)</th>
<th>Effectiveness (mg/g)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Republic of Iran</td>
<td>Pb(II)</td>
<td>Rice hull</td>
<td>0.84</td>
<td>Asadi et al., 2008</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Papillus sp. sawdust</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cu(II)</td>
<td>Rice hull</td>
<td>0.84</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Papillus sp. sawdust</td>
<td>0.82</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>Rice hull</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Papillus sp. sawdust</td>
<td>0.49</td>
<td></td>
</tr>
<tr>
<td>Thailand</td>
<td>Pb(II)</td>
<td>Spent fungal corn cob based substrate</td>
<td>14.75</td>
<td>Jonglertjunya, 2008</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>Sea grape Caulerpa lentillifera</td>
<td>10.73</td>
<td>Apiratikul et al., 2011</td>
</tr>
<tr>
<td>India</td>
<td>Pb(II)</td>
<td>Peepul leaves</td>
<td>127.34</td>
<td>Gupta et al., 2009</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Banana peels</td>
<td>72.79</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Peanut hulls</td>
<td>69.75</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Coir fibres</td>
<td>52.03</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rice stems</td>
<td>49.57</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Teak sawdust</td>
<td>40.70</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Discard tea leaves</td>
<td>35.89</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Mango leaves</td>
<td>31.54</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Rice husk</td>
<td>31.13</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Grass clippings</td>
<td>29.05</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cu(II)</td>
<td>Pretreated sawdust (Acacia Arabica)</td>
<td>5.64</td>
<td>Meena et al., 2008</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>Acacia leucocephala bark</td>
<td>294.1</td>
<td>Subbaiah et al., 2009</td>
</tr>
</tbody>
</table>
Innovative Green Technology through a Multidisciplinary Approach

<table>
<thead>
<tr>
<th>Country</th>
<th>Heavy metal species</th>
<th>Biosorbent(s)</th>
<th>Effectiveness (mg/g)</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pakistan</td>
<td>Pb(II)</td>
<td>Mango peel waste</td>
<td>99.05</td>
<td>Iqbal et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Cu(II)</td>
<td>Fungus <em>Pleurotus ostreatus</em></td>
<td>8.06</td>
<td>Javaid et al., 2011</td>
</tr>
<tr>
<td></td>
<td>Ni(II)</td>
<td>Fungus <em>Pleurotus ostreatus</em></td>
<td>18.83</td>
<td></td>
</tr>
<tr>
<td>China</td>
<td>Pb(II)</td>
<td>Modified Spent grain</td>
<td>35.04</td>
<td>Li et al., 2009</td>
</tr>
<tr>
<td>Nigeria</td>
<td>Pb(II)</td>
<td>Seed husk <em>(Calophyllum inophyllum)</em></td>
<td>11.47</td>
<td>Lawalefa/., 2010</td>
</tr>
<tr>
<td>Taiwan</td>
<td>Pb(II)</td>
<td>Algae <em>Spirogyra</em></td>
<td>90.91</td>
<td>Lee and Chang, 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Algae <em>Cladophora</em></td>
<td>48.51</td>
<td></td>
</tr>
<tr>
<td>Sweden</td>
<td>Cu(II)</td>
<td>Birch wood <em>Betula</em> sp. sawdust</td>
<td>4.9</td>
<td>Grimm et al., 2008</td>
</tr>
<tr>
<td>Vietnam</td>
<td>Cu(II)</td>
<td>Wheat straw</td>
<td>11.44</td>
<td>Dang et al., 2009</td>
</tr>
<tr>
<td>Turkey</td>
<td>Cu(II)</td>
<td>Fungus <em>Agaricus bisporus</em></td>
<td>3.15</td>
<td>Ertugay and Bayhan, 2010</td>
</tr>
<tr>
<td>Republic of Bulgaria</td>
<td>Cu(II)</td>
<td>Free and immobilised fungus <em>Aspergillus niger</em></td>
<td>0.28 – 0.54</td>
<td>Tseкова et al., 2010</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>Wheat straw</td>
<td>2.5</td>
<td></td>
<td>Baig et al., 2009</td>
</tr>
<tr>
<td></td>
<td>Barley straw</td>
<td>35.8</td>
<td></td>
<td>Thevannan et al., 2010</td>
</tr>
</tbody>
</table>
PSMC which require minimal processing has the potential to be used as the sustainable biosorbent (Tay et al., 2011b). This use of PSMC as a biosorbent is in-line with sustainable remediation technology as well as sustainable waste management concepts.

**BIOSORBENT PREPARATION AND EVALUATION**

The evaluation of the biosorptive potential of any natural material can differ widely as the materials considered are typically heterogeneous and may require several preparation steps before lab-scale studies can be considered. Steps include preliminary treatment such as drying or heat sterilization, mechanical grinding and sizing, washing and even chemical modification if so desired by the study.

For the evaluation of the potential of PSMC biosorbent, minimal processing which involves sterilizing, mechanical grinding and finally washing was carried out as indicated by Figure 22. The rationale behind the sterilization is to ensure that the study focuses on the biosorption characteristics which are inherent of the non-living biomass. Mechanical grinding and sieving provides us with a range of particle sizes thus the effect of the surface to mass ratio is known whilst washing pre-treatment removes contaminants in the sample that may affect the performance of biosorption.
Innovative Green Technology through a Multidisciplinary Approach

Figure 22: Preparative Steps for the Evaluation of PSMC as a Biosorbent for Heavy Metal Removal

The prepared biosorbent is then subjected to biosorption study as well as material characterization both prior to, and post sorption. Material characterization studies include Brunauer Emmett Teller (BET) Surface Area Analysis, Scanning Electron Microscope/Energy Dispersive X-ray Spectroscopy (SEM/EDX) Analysis, X-ray Photoelectron Spectrometer (XPS) Analysis, Fourier Transform Infrared Spectrometry (FTIR) Analysis, and $^{13}$C Solid State Nuclear Magnetic Resonance ($^{13}$C ssNMR).
INNOVATIVE R&D ON BIOSORPTION OF HEAVY METALS

Selected studies on PSMC for the removal of heavy metals from the myBioRec Laboratory, Faculty of Civil Engineering will be presented in the subsequent subsections.

Optimization Study of Biosorbent Concentration

Figure 23 shows the effect of increasing biosorbent concentration on heavy metal biosorption. Basically, heavy metal biosorption process can be divided into three stages, namely, initial rapid stage, slow biosorption stage and equilibrium stage. In this study, only Pb(II) biosorption showed all three stages whilst the equilibrium phase for Cu(II) and Ni(II) biosorption were not observed. The Pb(II) biosorption increased from 15.26 ± 0.12 to 97.10 ± 0.07 % when biosorbent concentration was increased from 0.40 to 20.00 g/L. For the biosorption for Cu(II), an increase from 29.58 ± 0.81 to 88.91 ± 0.03 % was observed when biosorbent concentration was increased from 4.00 to 160.00 g/L. A similar trend was observed for Ni(II) with increase in biosorption of 12.68 ± 0.17 to 88.63 ± 0.07 % when biosorbent concentration was increased from 2.00 to 160.00 g/L. At the initial rapid stage, increase in biosorbent concentration had increased the binding sites and surface area for heavy metal biosorption. Thus, this resulted in an increase of heavy metal biosorption. Following this stage, a much slower biosorption rate was observed. This is attributed to particle aggregation and coagulation of biosorbent when biosorbent concentration increased. Only Pb(II) biosorption attained the equilibrium stage which was attributed to saturation of biosorbent binding sites. Likewise, findings from studies on other agricultural-based biosorbents such as pecan nutshell, Moringa oleifera bark, peanut shell, fungal Mucor hiemalis and deciduous sawdust biosorbent were found to have trends that are consistent with this study (Božić et al., 2009; Vaghetto et al., 2009; Reddy et al., 2010b; Shroff and Vaidya, 2011; Witek-Krowiak et al., 2011).
As the results did not show a clear equilibrium stage for Cu(II) and Ni(II) biosorption, the half saturation constant of biosorbent concentration for heavy metal biosorption was determined in order to ensure that the system is under excess heavy metal ions condition which provides for faster reaction, thus minimising the time of biosorption process and the use of biosorbent. The half saturation constant concentration was calculated via a Hanes-Woolf plot, a derivative of Michaelis Menten. Table 19 shows the Hanes-Woolf plot equation, correlation coefficient \( r^2 \) and the calculated half saturation constant of biosorbent concentration \( K_m \). The calculated half saturation constant concentration \( K_m \) was found to be at 0.08 g for Pb(II) biosorption while 0.7 g for both Cu(II) and Ni(II) biosorption.
Table 19: Hanes-Woolf Plot of Michaelis Menten Derivation for Half Saturation Constant Determination ($K_m$)

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>Equation of Hanes-Woolf plot</th>
<th>$r^2$</th>
<th>$K_m$ (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb(II)</td>
<td>$y = 1.1680x + 0.0888$</td>
<td>0.9956</td>
<td>0.08</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>$y = 1.1928x + 0.8389$</td>
<td>0.9974</td>
<td>0.7</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>$y = 1.1492x + 0.8312$</td>
<td>0.9990</td>
<td>0.7</td>
</tr>
</tbody>
</table>

**Langmuir Isotherm Model**

The Langmuir isotherm model is well established for monolayer biosorption on the surface of biosorbent. Table 20 shows that heavy metal biosorption using PSMC biosorbent was excellently fitted to Langmuir isotherms. The correlation coefficient of Pb(II), Cu(II) and Ni(II) were 0.9988, 0.9695 and 0.9983 respectively. Results specifically point out that heavy metal biosorption occurred in a single layer, involving interaction of heavy metal ions with functional groups on the surface of PSMC biosorbent.

Table 20: The Langmuir Isotherm Parameters for Heavy Metal Biosorption using PSMC Biosorbent

<table>
<thead>
<tr>
<th>Heavy metal</th>
<th>$q_{max}$ (mg/g)</th>
<th>$b$ (L/mg)</th>
<th>$r^2$</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pb(II)</td>
<td>21.10</td>
<td>0.1696</td>
<td>0.9988</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>3.87</td>
<td>0.0218</td>
<td>0.9695</td>
</tr>
<tr>
<td>Ni(II)</td>
<td>3.03</td>
<td>0.0573</td>
<td>0.9983</td>
</tr>
</tbody>
</table>

Table 20 also shows that the calculated maximum uptake for heavy metal ($q_{max}$) were found to be at 21.10 mg/g, 3.87 mg/g and 3.03 mg/g for Pb(II), Cu(II) and Ni(II) respectively. These constitute evidences that PSMC biosorbent is a promising biosorbent for treating wastewater containing heavy metal since PSMC is relatively abundant and effective. The PSMC biosorbent has highest $q_{max}$ for Pb(II), followed by Cu(II) and lastly Ni(II). A heavy metal with high atomic number, standard atomic weight and atomic radius exhibits a better capacity in $q_{max}$. Likewise, Zakhama et al. (2011) reported that algae *Ulva lactuca* has $q_{max}$ value in descending order for Pb(II), Cd(II), Cu(II) and Ni(II). Furthermore,
Innovative Green Technology through a Multidisciplinary Approach

results from studies using chemically modified orange peel, green algae and cork waste biosorbents also are consistent with this finding where Pb(II) remarkably showed highest $q_{\text{max}}$ when compared to Cd(II), Cu(II) or Ni(II) (Feng et al., 2011; Lee and Chang, 2011; López-Mesas et al., 2011; Lasheen et al., 2012). Observations where $q_{\text{max}}$ for Cu(II) is higher than Ni(II) was also documented by Kleinübing et al. (2012).

Taking the Langmuir constant ($b$) into consideration, the PSMC biosorbent demonstrates an obvious selectivity towards Pb(II) at 0.1696 L/mg when compared to 0.0573 L/mg for Ni(II) and 0.0218 L/mg for Cu(II). The result infers that the parameter $b$ for Pb(II) is approximate one order of magnitude higher than Cu(II) and Ni(II). The parameter $b$ of heavy metal biosorption strengthened results from the established half saturation constant concentration and selectivity of heavy metal biosorption in descending order of Pb(II), Ni(II) and Cu(II). Such desirable circumstance leads to effective treatment of the targeted heavy metal in industrial wastewater.

Table 21(a-c) compares the parameters of PSMC Langmuir isotherm heavy metal biosorption with that of the different biosorbents cited in the recent literature. The PSMC biosorbent has a comparable $q_{\text{max}}$ when compared to other raw biosorbents with the exception of fungus Agaricus cerevisiae for Cu(II) as well as bacteria Rhodococcus opacus and waste pomace of olive oil factory for Ni(II). These can be attributed to the fine particle size of 104 μm for fungus Agaricus cerevisiae and 150-250 μm of waste pomace. The fine particles give higher surface area when compared to 710 μm of PSMC biosorbent. The fine particle sizes of biosorbents increase the surface area and thus resulted in improvement of $q_{\text{max}}$.

Additionally, lower $q_{\text{max}}$ values were observed for PSMC biosorbent when compared to modified or high light metal ions biosorbents as shown in Table 21(a-c). To improve the $q_{\text{max}}$ values of a particular biosorbent, many researchers utilised both chemical and heat modifications. Although
these modifications may substantially improve the $q_{\text{max}}$ values, a severe shortcoming in many of these modified biosorbents is the lack of an environment impact on carbon footprint assessment of the modifications made on the biosorbents. The impact of chemical and heat modification may very well diminish the sustainability value of reusing agricultural waste as an environmentally friendly biosorbent. On the other hand, algae, moss and Ca-alginate based biosorbents are rich in light metal ions such as Ca(II), Mg(II), Na and K (Stokke et al., 2000; Reimann et al., 2001; Davis et al., 2003). This circumstance favours the ion exchange mechanism. Hence, a better performance in heavy metal biosorption was documented.

### Table 21(a): Comparison of Pb(II) Langmuir Isotherm Parameters with Other Recent Studies using PSMC Biosorbent

<table>
<thead>
<tr>
<th>Biosorbent</th>
<th>Langmuir parameters</th>
<th>Reference(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>$q_{\text{max}}$ (mg/g), [mmol/g]</td>
<td>$b$ (L/mg), [L/mmol]</td>
</tr>
<tr>
<td><strong>Raw biosorbents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walnut sawdust</td>
<td>15.90</td>
<td>0.01</td>
</tr>
<tr>
<td>Spent fungal corn cob based substrate</td>
<td>14.75</td>
<td>0.0007</td>
</tr>
<tr>
<td>Fungus <em>Pleurotus ostreatus</em></td>
<td>14.75</td>
<td>0.0873</td>
</tr>
<tr>
<td>Shell of hazelnut <em>Corylus avellana</em> and almond <em>Prunus dulcis</em></td>
<td>28.18, 8.08 [0.136, 0.039]</td>
<td>4.1752, 0.3441 [865.052, 71.304]</td>
</tr>
<tr>
<td>Crushed de-oiled residue of allspice (<em>Pimenta dioica</em> L. Merril)</td>
<td>12.09</td>
<td>0.468</td>
</tr>
<tr>
<td>Fungus <em>Mucor indicus</em> for mostly filamentous, purely filamentous, purely yeast-like and mostly yeast-like</td>
<td>15.63, 22.07, 12.12, 14.68</td>
<td>0.069, 0.187, 0.077, 0.1142</td>
</tr>
<tr>
<td><strong>PSMC</strong></td>
<td>21.10</td>
<td>0.1596</td>
</tr>
<tr>
<td><strong>Modified biosorbents</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Formaldehyde treated Meranti tree sawdust</td>
<td>37.04</td>
<td>0.02</td>
</tr>
</tbody>
</table>

*This study*
Advanced Material Characterization

A multi-approach utilising advanced characterisation techniques was employed to analyse the biosorbent in order to ascertain its potential as a biosorbent and most probable mechanism of heavy metal removal.

Brunauer Emmett Teller (BET) Surface Area Analysis

BET surface area were measured to determine the surface area value \((a\text{BET})\) and mean apparent diameter \((d\text{BET})\) of PSMC biosorbent. The \(a\text{BET}\) and \(d\text{BET}\) were found to be at \(5.5544 \times 10^{-1}\) m\(^2\)/g and 20.997 nm respectively. At the same time the total pore volume was measured to be \(2.9156 \times 10^{-3}\) cm\(^3\)/g \((p/p_0 = 0.990)\). Results give an indication that the physical texture of PSMC biosorbent can be categorized a meso-porous biomaterial and have high surface area. Hence, PSMC has potential as a biosorbent for biosorption process. However, result needs to be investigated and confirmed by SEM analysis.

SEM-EDX Analysis

SEM analysis on PSMC prior to biosorption revealed a heterogenous, rough and porous surface on the biosorbent. (Figure 24a). This provides confirmation to the BET analysis conducted. Post-biosorption SEM micrograph of PSMC revealed subtle morphological changes, namely ‘muddy deposits’ on the porous surface of the biosorbent (Figure 24b-d). These slight changes could be caused by chemisorptions processes or sudden changes in external bulk fluid environment, which can have a significant impact on its morphology.

The EDX analysis, as shown in Figure 25, identified the peak of Mg(II) in the PSMC, that was not detected post-biosorption. As expected, the respective heavy metal peaks were detected after biosorption process, which signify the attachment of heavy metal ions onto PSMC biosorbent. The disappearance of light metal Mg(II) peak and the presence of heavy metal peaks as revealed by EDX indicated the occurrence of ion exchange
during biosorption process. Similar observation also has been reported by Tay et al. (2011a) and other researchers (Bueno et al., 2008; Mata et al., 2008; Qaiser et al., 2009; Kamal et al., 2010).

Figure 24: SEM Micrograph of PSMC (a) before Biosorption; (b) after Pb(II) Biosorption; (c) after Cu(II) Biosorption; and (d) after Ni(II) Biosorption

XPS Analysis

Shifts of binding energy of coordination atoms in XPS deconvoluted spectra before and after heavy metal biosorption can provide us with information of the functional groups that are involved in biosorption. The samples before heavy metal biosorption indicate that carboxyl, hydroxyl from ether and alcohol as well as amide groups were present in lignocellulosic based PSMC biosorbent.
Innovative Green Technology through a Multidisciplinary Approach

This result provides an indication that the acidic groups of carboxyl, hydroxyl and amide, are functional groups involved in heavy metal biosorption.

After heavy metal biosorption, it was observed that carboxyl peak of C Is and O Is were absent. The shift of nitrogen N Is after heavy metal biosorption is assigned to amide group of organic nitrogen compound. These indicated that carboxyl from lignocellulosic and amide from chitin of *Pleurotus ostreatus* mycelia, were the major functional groups and components in heavy metal biosorption.

**FTIR Analysis**

Figure 26 shows the FTIR analysis on PSMC before and after biosorption of Pb(II), Cu(II) and Ni(II) ions. Results indicate that peaks at 1640 cm\(^{-1}\), 1321/1371 cm\(^{-1}\) and 1060/1111 cm\(^{-1}\) were shifted after heavy metal biosorption. These suggest that the free carboxyl groups have changed into carboxylate, a phenomenon which normally occurs during the reaction between heavy metal and a carboxyl group. The identified
carboxyl group, i.e., weak acidic functional groups confirms the XPS. Chen et al. (2008) and Akmar Zakaria et al. (2009) noted that carboxyl is the main functional groups in heavy metal biosorption process for fungus *Lentinus edodes* and rubber tree sawdust respectively.

PSMC contains mycelia of *Pleurotus ostreatus*, and like any higher fungi, contains cell walls that consist of chitin molecules (Ginterova and Maxiaitová, 1975). The present study also indicates that the chitin present in PSMC also contribute to the biosorptive properties of PSMC as vibrational peaks at 3400 cm\(^{-1}\), 1321/1371 cm\(^{-1}\) and 612 cm\(^{-1}\) of hydroxyl and amide groups, which can be attributed to chitin, were also shifted after biosorption. The identified hydroxyl and amide groups from organic nitrogen compounds of chitin concur with the results obtained from XPS analysis. This infers that it is a principal contributor to the formation of chitin-heavy metal complexes. Li et al. (2010) and Tay et al. (2011b) suggested that, based on variations of the bands, FTIR spectra peaks indicate the prevalence of chelating characteristics of heavy metal biosorption through complexation mechanism.

![Figure 26: FTIR Spectrum of PSMC Biosorbent](image)

**Figure 26:** FTIR Spectrum of PSMC Biosorbent (a) before Biosorption; (b) after Pb(II) Biosorption; (c) after Cu(II) Biosorption; and (d) after Ni(II) Biosorption
It can be concluded that characterisation analysis using XPS and FTIR support that the carboxyl, hydroxyl and amide were the functional groups involved in heavy metal biosorption with possibility of complexation mechanism. However, further investigation through the use of $^{13}$C ssNMR was conducted to substantiate this observation, particularly for carboxyl group which found in lignocellulosic compound.

**SOLID STATE NMR STUDIES**

Several solid state NMR methodologies were applied in the attempt to furnish evidence on the mechanism of biosorption by PSMC. This includes four types of $^{13}$C solid state NMR experiments, namely, Cross-Polarization Magic Angle Spinning (CPMAS), Direct Pulse Saturation Recovery (DP SatRec), Direct Pulse Inversion Recovery (DP Invrec) and TORCHIA.

There were no significant differences in the $^{13}$C anisotropic chemical shift before and after heavy metal biosorption in the CPMAS (Figure 27). This observation is attributed to the similar matrix of biosorbent and the signal filtering effects on CPMAS experimental design. However, the CPMAS results support the fact that cellulose and lignin were major components found in PSMC biosorbent with carboxyl and hydroxyl present as surface functional groups of the lignocellulosic based biosorbent.
When compared with CPMAS, the spectra of DP SatRec $^{13}$C ssNMR for PSMC before and after heavy metal biosorption showed less filtering effect (Figure 28). However, the result did not show significant changes in chemical shift before and after heavy metal biosorption although experiments took longer time due to very weak magnetization at the extreme tail of the free induction decay (FID).

Direct pulse inversion recovery (DP Invrec) results (Figure 29) indicate strong paramagnetism effect from PSMC with Cu(II) and Ni(II), making it not possible to separate the effects of paramagnetic atoms apart in these samples. Only Pb(II) ions did not have paramagnetic effect and
further $^{13}$C ssNMR approach for heavy metal biosorption evaluation on functional groups and mechanism were only conducted for Pb(II) due to the lack of interfering nuclear paramagnetism.
Figure 29: DP Invrec Experiment for Heavy Metal Ions Paramagnetic Effect Evaluation (a) Pb(II); (b) Cu(II); and (c) Ni(II)

TORCHIA experimental design is a novel approach introduced to evaluate mechanism of Pb(II) biosorption via functional groups determination. As previously explained, experiments were not conducted for Cu(II) and Ni(II) experiments due to their paramagnetic effect that interfere with the results. Figure 30 shows the changes in $T1$ time that represents structural changes that occurred on lignin carboxyl and lignin C-1/C-4 of aromatic units of syringyl and guaiacyl for PSMC after Pb(II) biosorption process. Longer $T1$ of PSMC after Pb(II) biosorption sample indicated that the sample had undergone “stiffening”, i.e., the sample becomes more rigid. This is possibly ascribed to the formation of complexes. In TORCHIA experiments, the decrease in mobility of molecules with longer $T1$ infers the structural changes of molecules which play a pivotal role in complexation. The results obtained from $^{13}$C
ssNMR analysis was integratively analysed with the results of XPS and FTIR analysis that lead to the confirmation of complexation between the Pb(II) and the carbonyl of the carboxylic functional group.

Figure 30: The T1 Time in TORCHIA Experiments for (a) Lignin Carboxyl; and (b) Lignin C-1/ C-4 of Aromatic of Syringyl and Guaiacyl
Furthermore, the TORCHIA experiments were conducted to differentiate the components of Pb(II) biosorption functional groups, namely, cellulose and lignin. The identified PSMC functional groups indicate that biosorptive characteristics of PSMC were attributed to lignin and not cellulose. The obtained result was in accordance to XPS and FTIR analysis reported by Krishnani et al. (2008), where investigation on biomatrix from rice husk, suggested that the retention of heavy metal occurs through a complexation reaction involving carboxylate moieties of lignin or lignocellulosic.

The lignin carboxyl group was bonded to Pb(II) ion via two oxygen atom. This mechanism was also proposed by Figueira et al. (1999) for carboxyl-heavy metal complex. In addition to results from XPS and FTIR analysis, this mechanism is proven through the stiffening of the C-C bond in the sample from $^{13}$C ssNMR TORCHIA analysis that implies the formation of complexes between the Pb(II) and the biosorbent functional groups. Consequently, the available binding sites on the biosorbent decreased and these structural changes occurring in the biosorbent was observed through spectra shifts in XPS, FTIR and $^{13}$C ssNMR.

In conclusion, SEM analysis confirmed the structure of biosorbent that were established through BET surface area analysis. The $^{13}$C ssNMR analysis results confirmed that lignin is the component involved in heavy metal biosorption rather than cellulose. Combined results from XPS, FTIR and $^{13}$C ssNMR indicate that weakly acidic functional groups of carboxyl, hydroxyl and amide are involved in heavy metal biosorption process.

Applications of PSMC - Continuous Flow Fixed-Bed Biosorption

The use of biosorbents would be limited if sorption were to be carried out under batch conditions. The biosorbent material would add to the amount of toxic solid wastes after sorption, or require complicated
solid/liquid separation if recovery of the biosorbent is desired. Also, the use of biosorbent in a batch reactor would require mixing, which increases the energy requirement for the process and contribute to sorbent attrition (Table 22). An option to overcome this is to pack the biosorbent into a bed, i.e. to have a fixed bed for the uptake of heavy metal from the wastewater. This allows for a continuous sorption process, which is deemed more efficient for wastewater treatment when compared to a batch process (Mukhopadhyay et al., 2007; Dwivedi et al., 2008). This is because high adsorption capacities in equilibrium with the effluent concentration rather than the influent concentration can be achieved. In continuous operating mode, the saturated solid sorbent zone gradually extends throughout the column where the sorbate is adsorbed (Aksu et al., 2006). Thus, studies on biosorption in fixed-bed columns would be useful in the realization of a commercially viable biosorbent.

Biosorption columns can operate on cycles consisting of loading, regeneration and rinsing. Packed-bed column reactors are commonly used in both industry and research and have been used for separation of molecules, determination of relative molecular mass and identification of substances or purification purposes (Wheelwright, 1991). Industrial packed-bed columns consist of the container and the bed contained within. The target molecule or ion is then passed through the packed-bed, with the hope that it will attach to the assigned ligand or substrate.

For biosorption, the optimal configuration for continuous-flow sorption is with the packed-bed column which gets gradually saturated from the feed to the solution exit end (Volesky and Prasetyo, 1994; Saeed and Iqbal, 2003). Such setup would provide ease of operation, attains a high yield and is easier to scale up from a laboratory-scale procedure. The stages in the separation protocol can also be automated and high degrees of purification can often be achieved in a single step process. A packed bed column also serves as an effective arrangement for cyclic sorption and desorption. It allows efficient utilization of the sorbent capacity and results in a better quality of the effluent (Aksu et al., 2006). Desorption
of the column not only allows the recovery of the metals adsorbed, it also allows the conservative use of raw materials (Diniz et al., 2008). At the end of the usage lifespan of the column, biomass from a fully desorbed biosorption column can then be disposed off without posing a hazard to the environment. This minimizes the generation of toxic solid wastes that require special handling and disposal.

Table 22: Advantages and Disadvantages of Sorption Modes

<table>
<thead>
<tr>
<th>Mode</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch</td>
<td>Easy to set up</td>
<td>Requires mixing</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Requires solid/liquid separation</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Low concentration difference driving force</td>
</tr>
<tr>
<td>Fixed Bed</td>
<td>Effective configuration i.e. highest possible</td>
<td>Cannot handle suspension</td>
</tr>
<tr>
<td></td>
<td>concentration difference driving force</td>
<td>Sensitive to pressure drop</td>
</tr>
<tr>
<td></td>
<td>Continuous flow operation</td>
<td>Scale up by multiplying units</td>
</tr>
<tr>
<td></td>
<td>Unlimited scale-up</td>
<td>Requires column alternation</td>
</tr>
<tr>
<td></td>
<td>In situ regeneration</td>
<td></td>
</tr>
<tr>
<td>Fluidised</td>
<td>Gravity separation of particles possible</td>
<td>Increase reactor volume (large footprint)</td>
</tr>
<tr>
<td>Bed</td>
<td>No preliminary pretreatment necessary</td>
<td>Higher energy consumption to keep bed fluidised</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sorption particle attrition</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Less effective sorption and complicated desorption</td>
</tr>
</tbody>
</table>

Adapted from Volesky, 2003; Volesky & Naja, 2005; Aksu et al., 2006; Mukhopadhyay et al., 2007; Dwivedi et al., 2008.

The design of such a biosorption column is governed by several factors that determine the flow configuration and the flow-rates used, as well the dimensions of the column. Particle size is a factor that affects
mass transfer of heavy metal to the biosorbent. With smaller particle size, particle mass transfer improves, which improves the uptake of metals by the continuous flow column. However, smaller particle size would result in higher pressure drop in the column and increase its sensitivity to clogging. Thus a balance between pressure drop and mass transfer must be sought in the design of the sorption column as shown in Figure 31 (Volesky & Naja, 2005). Hence, the design and optimization of biosorption processes for dynamic flow for commercialization purposes are required (Vieira et al., 2007).

Figure 31: Effect of Biosorbent Particle Size on the Mass Transfer and Operational Pressure Drop for a Fixed-bed Biosorption Column (Adapted from Volesky & Naja, 2005)

Fixed-bed Biosorption Curves

Typically biosorption exhibits an S-shaped curve when the effluent concentration is plotted against time or bed volume as shown in Figure 32. Initially the heavy metal ions in the influent are completely removed by the biosorbent, thus the effluent is free from heavy metal contamination. As biosorption progresses, the mass transfer front slowly progresses down
the column. Breakthrough occurs when the un-adsorbed heavy metal appears in the effluent. This concentration of heavy metal then rises as the bed capacity of the sorbent is used up. At the end of the sorption, the heavy metal concentration in the effluent will be the same as the influent, which marks the total exhaustion of the bed’s sorption capacity.

A steeper S-shaped curve would mean that the bed is better utilised (Svecova et al., 2006), as once the breakthrough concentration approaches the limit of permissible level, the column would have to be replaced and sorption operation on the column must stop. The steepness of the sorption curves is very much dependent on the material as well as the operational conditions, for example, flow rates. An optimal flow rate creates a sharp, narrow mass transfer zone which helps the sorbent to overcome mass transfer resistance, and improve uptake of heavy metal, a condition which is difficult to create under batch sorption conditions. Hence studies on the effect of flow rates are frequently carried out for continuous flow biosorption.

Figure 33 shows the PSMC biosorption curve of Pb(II) ions under different flow rates and column length which clearly shows that low flow rates tend to generate a broad S-shape sorption curve as opposed to higher flow rates for a particular column height. Thus at higher flow rates, the steeper breakthrough curve is an indication that there is better overall utilization of the sorbent within the column and a higher extent to which the bed can be utilized (Ghosh, 2006). The better utilization of the sorption bed can be due to better external mass transfer as higher flow rates have been shown to reduce film resistance (Ko et al., 2001).
Figure 32: A Typical S-shaped Sorption Curve that is Obtained from Fixed-bed Biosorption. As the Heavy Metal Solution Moves Through the Column (Blue), the Biosorbent Behind the Mass Transfer Zone is Fully Saturated with the Heavy Metal Ions (Black)
Mechanism of PSMC Biosorption in a Fixed Bed Column

The biosorption of heavy metal by PSMC appears to be tied to the ion-exchange mechanism as suggested by studies on the leaching of light metals and pH changes that occur during fixed-bed biosorption of Pb(II) ions as shown in Figure 34. At the beginning of the biosorption process, Ca(II) ions were leached out from the biosorbent, and pH of the eluent remained constant. As the sorption progresses towards breakthrough, the pH of the solution began to drop. This is subsequently followed by the drop in leached Ca(II) ions, with the concurrent rise in Pb(II) ion concentration of the effluent.
Innovative Green Technology through a Multidisciplinary Approach

This observation suggests that at the onset of the sorption process, Ca(II) is preferentially exchanged with Pb(II) ions. As the sorption approaches the breakthrough point, the reduction in exchangeable Ca(II) ions appears to be compensated by the release of protons, resulting in the drop of pH that had earlier remained constant. The sorption is then characterised by the drop in exchangeable Ca(II) and proton from the biosorbent, which results in the rise of Pb(II) ions in the effluent during the post breakthrough point of the sorption process. As the sorption curve progresses towards exhaustion, the pH of the effluent slowly rises towards the pH of the influent Pb(II) solution (4.5 – 4.9). Thus it can be deduced that an ion-exchange mechanism is responsible for the uptake of Pb(II) ions by PSMC.

![Figure 34: Changes in Pb(II) and Ca(II) Concentration as Well as pH during Biosorption of Pb(II) Ions by PSMC](image-url)
DESORPTION AND REUSE OF BIOSORBENT

Once a biosorption column has been exhausted, the bed can be desorbed as to recover the adsorbed metal and also to allows for the biosorbent material to be regenerated, usually in situ, for subsequent use. Commonly use desorption solutions include diluted acid (Zulfadhly et al., 2001; Saed et al., 2005; Han et al., 2006; Areco et al., 2012; Oliveira et al., 2012), Group I and II metal ions (Ali Khan et al., 2012) and even alkaline solution (Ali Khan et al., 2012; Akber Hussain et al., 2012), depending on the type of biosorbent and the sorption mechanism. For PSMC, the predominantly ion-exchange mechanism exhibited during biosorption would suggest that displacement of the adsorbed ions can be easily achieved through the use of protons or light metal elements, i.e. via treatment with diluted acid or light metal ion solutions such as Ca\textsuperscript{2+} (Akber Hussain et al., 2012).

After desorption, an ideal fixed bed column should be reusable after regeneration, preferably with minimal loss of biosorbent and sorption capacity. The sorption and desorption curves obtained for PSMC indicate the possibility of reuse, albeit some loss of uptake capacity of around 30%, as shown in Figure 35. Desorption of heavy metal from the biosorbent was rapid, with > 98% of the adsorbed heavy metal being removed from PSMC after 5.4 min from the onset of desorption using 0.1N HNO\textsubscript{3} for all the three desorption cycles. Hence the use of diluted acid allows for the rapid desorption and reuse of the PSMC biosorbent in a fixed-bed setup.
Innovative Green Technology through a Multidisciplinary Approach

Figure 35: Typical Results Obtained for PSMC Undergoing Three Cycles of Sorption and Desorption. Each Cycle Consist of Sorption with 50 mg/L Pb(II) Ion Solution Followed by Desorption with 0.1N HNO₃. Regeneration was Achieved by Flushing Off Excess Acid with Water After Desorption

In conclusion, studies on PSMC have highlighted the possibility of putting to use an agricultural waste, thus putting both monetary as well as environmental value in this discarded material to treat heavy metal contaminated wastewater.
CONCLUDING REMARKS

The applications of biosorption for heavy metal treatment from various wastewaters have been widely established. However, further improvement in both performance and costs can be expected in future. Few recommendations are proposed below for future work:

1. Hybrid biosorption technology for pollutants removal should be developed that can demonstrate more complex biosorption systems. Unlike laboratory solutions, industrial effluents contain various pollutants such as metals, dyes, organics, inorganics etc. Therefore, there is a need to investigate the simultaneous removal of many coexisting pollutants. It is necessary to develop multi-purpose biosorbents that can remove a variety of pollutants. One such possibility would be the use of multi biosorbents consisting more than one type of biomass. Although multi biosorbents would tend to further complicate characterization of these biosorption systems, but it may represent a more realistic approach to the design of biosorption systems.

2. For optimization of biosorption parameters, modelling tools such as Response Surface Methodology (RSM) and SPSS should also be conducted to offer optional tools for heavy metal biosorption modelling. Application of both models also possible to see a comparison data between two models.

3. Investigation on optimisation parameters of particle size, agitation rate and cations interferences should be conducted. Such studies will give information that presents the overall spectrum of independent parameters in fundamental heavy metal biosorption process.
4. Investigation on advanced characterisation of biosorbent after heavy metal biosorption should be extended to deepen the knowledge of the heavy metal biosorption mechanisms. Furthermore, proposed complexation mechanisms also need to be investigated and evaluated by $^{13}$C ssNMR experiments in order to confirm the structure of complexes formed.

5. A comparative study on biosorption capacity among mushroom genera can be considered in the future. The selection of biosorbent with most effective (high sorption capacity) and workable types of biomass is most preferred.

6. A study on cost implications on heavy metal biosorption compared to conventional methods, namely, precipitation and ion exchange resins should be conducted in order to establish the economic viability study.
WASTEWATER TREATMENT AND ENERGY PRODUCTION

BACKGROUND

There are various methods for biological wastewater treatment. These treatment conditions can be categorised as either aerobic or anaerobic, depending on the electron acceptor available for decomposition. Each type of treatment method has its advantages and disadvantages. Table 23 summarises the advantages and disadvantages of each treatment method.

Though each treatment method has its advantages, treatment methods under anaerobic condition are favourable compared to treatment under aerobic condition because of the higher efficiency in the organic removal, relatively low energy requirement and low production of sludge and most important of all is the benefit from energy production in the form of methane gas (Angenet et al., 2004).

Due to the expected scarcity of the conventional fuel and the need for a sustainable environment, treatment methods which produce bioenergy are given intense attention and focus in current research.
## Table 23: Advantages and Disadvantages of Various Wastewater Treatment Methods

<table>
<thead>
<tr>
<th>Treatment Methods</th>
<th>Treatment Conditions</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Activated Sludge</td>
<td></td>
<td>• Highly efficient treatment method</td>
<td>• Requires essential nutrients supply</td>
<td>Prabu et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Requires little land area</td>
<td>• Requires sludge disposal area</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Produces consistent quality effluents</td>
<td>• High energy requirements for aeration</td>
<td></td>
</tr>
<tr>
<td>Trickling Filter</td>
<td></td>
<td>• Simple design and operation</td>
<td>• Unsuitable for high organic loading</td>
<td>Prabu et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Low operation and maintenance cost</td>
<td>• High construction cost (ventilation duct for air supply)</td>
<td></td>
</tr>
<tr>
<td>Oxidation Pond</td>
<td>Aerobic</td>
<td>• Simple operation and less maintenance</td>
<td>• Requires a large land area</td>
<td>Prabu et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Produces effluent with high suspended solids</td>
<td>• May produce undesirable odour</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Easy to operate and maintain</td>
<td>• Effluent requires further treatment</td>
<td></td>
</tr>
<tr>
<td>Aerated Lagoon</td>
<td></td>
<td>• Simple operation and maintenance</td>
<td>• Requires energy to aerate the basin</td>
<td>Prabu et al., 2011</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Low capital cost</td>
<td>• Produces effluent with high suspended solids</td>
<td></td>
</tr>
<tr>
<td>Septic Tank</td>
<td>Anaerobic</td>
<td>• Easy to operate and maintain</td>
<td>• Requires more space</td>
<td>Chan and Chooi (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Rate of digestion is very slow</td>
<td>• Must be pumped occasionally</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Must be pumped occasionally</td>
<td>•Requires further treatment before discharge (low treatment efficiency)</td>
<td></td>
</tr>
<tr>
<td>Anaerobic Pond</td>
<td></td>
<td>• Low capital, operating &amp; maintenance cost,</td>
<td>• Large volume for digestion,</td>
<td>Chan and Chooi (1984)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Able to tolerate big range of organic loading rates,</td>
<td>• Long retention time,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Recovered sludge cake can be sold as fertilizer</td>
<td>• No facilities to capture biogas,</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Small reactor volume,</td>
<td>• Lower methane emission.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Produce high quality effluent,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Short hydraulic retention time,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Able to tolerate shock loadings,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Retains high biomass concentration in the packing.</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Clogging at high organic loading rates,</td>
<td></td>
<td>Poh and Chong (2009)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• High media and support cost,</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Unsuitable for high suspended solids wastewater.</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*cont.*
Wastewater Treatment and Energy Production

<table>
<thead>
<tr>
<th>Treatment Methods</th>
<th>Treatment Conditions</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluidised Bed Reactor</td>
<td></td>
<td>- Suitable for high strength wastewater&lt;br&gt;- Able to handle high organic loading rate&lt;br&gt;- Short hydraulic retention time&lt;br&gt;- Most compact of all high rate processes,&lt;br&gt;- Well-mixed conditions in reactor,&lt;br&gt;- Large surface area for biomass attachment,&lt;br&gt;- No channeling/plugging/ gas hold-up,&lt;br&gt;- Faster start-up&lt;br&gt;- High treatment efficiency</td>
<td>- High power requirements,&lt;br&gt;- High cost for carrier media,&lt;br&gt;- Not suitable for high suspended solid wastewater,&lt;br&gt;- Normally does not capture generated biogas.</td>
<td>Poh and Chong (2009) Leslie Grady et al. (1999)</td>
</tr>
</tbody>
</table>

| Upflow Anaerobic Sludge Blanket (UASB) |                      | - Efficiently retains the complex microbial consortium<br>- Efficiently convert wastewater organic compounds into methane<br>- Suitable for industrial wastewater | - Interference of suspended solids in the incoming wastewater | Angenent et al. (2004) Poh and Chong (2009) |

WASTEWATER TREATMENT TECHNOLOGY - WASTEWATER TO ENERGY

Several biological wastewater treatment technologies are capable of treating wastewater and at the same time produce energy. These technologies include the methanogenic anaerobic digestion and the fermentative hydrogen production (Cheng, 2010). Table 24 summarises the advantages and disadvantages of the two wastewater treatment technologies.
Table 24: Wastewater Treatment Technology - Wastewater to Energy

<table>
<thead>
<tr>
<th>Wastewater Treatment Technology</th>
<th>Advantages</th>
<th>Disadvantages</th>
<th>Reference</th>
</tr>
</thead>
</table>
| Methanogenic Anaerobic Digestion | - Established and reliable technology  
- Able to treat various types of organic waste  
- Production of methane gas (energy recovery)  
- Low sludge production  
- Residual sludge can be used as seeding material for the start-up of another process  
- Low energy requirement | - Long start-up period  
- Effluent still contains high level of organics  
- Requires heat combustion for conversion of methane gas to electricity  
- May produce odour | Cheng (2010); Angenent et al. (2004) |
| Fermentative Hydrogen Production | - Significant industrial demand for renewable energy recovery | - Low hydrogen recovery  
- Restricted to only pre-treatment step in a larger biochemical production  
- Less suitable for transportation and storage due to its properties (lower density compared to methane and high diffusivity)  
- Requires further treatment (purification and pressurization) for conversion to electricity which is energy intensive | |

Though these technologies produce energy in the form of methane or hydrogen, they require energy for the conversion to electricity. An alternative to these technologies is the Microbial Fuel Cell (MFC).
INTRODUCTION ON MICROBIAL FUELCELL

Microbial Fuel Cell (MFC) constitutes a new approach for wastewater treatment and electricity generation. It is similar to a Chemical Fuel Cell (CFC) as both cells convert chemical energy into electricity. However, the major difference lies in the catalyst used to speed up the oxidation process. A CFC uses an elemental catalyst to accelerate the process while the MFC uses life bacteria to catalyse the fuel oxidation (Seop et al., 2006).

MFC is a bioreactor which converts chemical energy into electrical energy through catalytic reactions of microorganisms under anaerobic conditions (Kim et al., 2007). It is a promising technology in wastewater treatment as it can address the issue of bioenergy and wastewater treatment concurrently with reduction in the sludge production (Moon et al., 2006). Treating wastewater using MFC can reduce the amount of sludge production substantially due to the fact that only a small fraction of the energy is consumed by the microorganisms for growth (sludge production) whereas a large fraction is used for bioenergy conversion (Kim et al., 2007).

Concept on MFC

A typical MFC consists of an anode and a cathode chamber. The organic matter from the substrate/wastewater which is placed in the anaerobic anode chamber is oxidized by the bacteria, causing electrons and protons to be generated in the process. Carbon dioxide (CO₂) is the oxidation product. The resulting electrons are transferred to the electrode of the anode chamber and subsequently to the electrode of the aerobic cathode chamber via an external resistance while the protons are diffused through a Proton Exchange Membrane (PEM). This transfer of electrons is caused by the difference in potential between the two electrodes. The oxidation-reduction process which takes place at the cathode utilises the electrons, protons and oxygen to produce water. The oxidation and reduction processes are governed by Equation 10 and Equation 11.
Innovative Green Technology through a Multidisciplinary Approach

\[(CH_2O)n + nH_2O \rightarrow nnCO_2 + 4ne^- + 4nH^+ \quad \text{(Anode: Oxidation Reaction)}\]  
\[4e^- + 4H^+ + O_2 \rightarrow 2H_2O \quad \text{(Cathode: Reduction Reaction)}\]  

The end results of the overall reaction is the degradation of the organic matter and the production of electricity (Seop et al., 2006). At the anode chamber, the substrate acts as the electron donor (ED) while the anode (electrode) is the electron acceptor (EA). At the cathode chamber, the cathode (electrode) is the electron donor whereas the oxygen is the electron acceptor. The electron transfer process is shown in Figure 28.

![Schematic Diagram of a Typical Two-chamber MFC](Source: Modification from Du et al., 2007)

**Principles and Thermodynamics of MFC**

In an MFC, the electron transport involves the transfer of electrons from the substrate in the anode chamber to the final electron acceptor in the cathode chamber. In order for electrical energy to be spontaneously generated, the overall reaction must be thermodynamically favourable.
Table 25 shows the redox potential for selected organic and inorganic redox couple compared to the Standard Hydrogen Electrode (SHE) potential at pH7.

<table>
<thead>
<tr>
<th>Oxidation/Reduction Pair</th>
<th>E° (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO₂/Glucose</td>
<td>-430</td>
</tr>
<tr>
<td>H⁺/H₂</td>
<td>-420</td>
</tr>
<tr>
<td>NAD/NADH</td>
<td>-320</td>
</tr>
<tr>
<td>CO₂/Acetate</td>
<td>-280</td>
</tr>
<tr>
<td>S⁰/H₂S</td>
<td>-280</td>
</tr>
<tr>
<td>S⁰/HS⁻</td>
<td>-270</td>
</tr>
<tr>
<td>CO₂/CH₄</td>
<td>-240</td>
</tr>
<tr>
<td>SO₄²⁻/H₂S</td>
<td>-220</td>
</tr>
<tr>
<td>Pyruvate²⁻/Lactate²⁻</td>
<td>-185</td>
</tr>
<tr>
<td>Methylene Blue OX/RED</td>
<td>+11</td>
</tr>
<tr>
<td>Fumarate²⁻/Succinate²⁻</td>
<td>+31</td>
</tr>
<tr>
<td>Thionine OX/RED</td>
<td>+64</td>
</tr>
<tr>
<td>Ubiquinone OX/RED</td>
<td>+113</td>
</tr>
<tr>
<td>O₂/H₂O₂</td>
<td>+275</td>
</tr>
<tr>
<td>NO³⁻/NO²⁻</td>
<td>+421</td>
</tr>
<tr>
<td>NO²⁻/NH₄⁺</td>
<td>+440</td>
</tr>
<tr>
<td>O₂/H₂O</td>
<td>+820</td>
</tr>
</tbody>
</table>

(Source: Du et al., 2007)

Air Cathodes Vs Biocathodes

Cathodes which use oxygen as the terminal electron acceptor are known as air cathodes. Oxygen is the most frequently used for an MFC mainly because of its high redox potential, plentiful in the air, readily available and only produces water as the end product, making it a very sustainable option for the environment. However, due to poor oxygen reduction kinetics, air cathodes need to employ catalyst to overcome the problem. The most common type of cathode catalyst for oxygen reduction
is platinum. Nevertheless, the application of platinum is limited as it is expensive, especially if it is to be applied on a large scale basis (He and Angenent, 2006). As a result, researchers are now embarking on the concept of applying biocathodes in MFCs.

**Biocathodes** basically means utilising bacteria as catalyst for the cathode instead of platinum. Unlike air cathodes which are half biological as wastewater is being placed only in the anode chamber, biocathodes are fully biological due to the fact that wastewater is being used in both chambers as electrolytes. The main advantage of biocathodes over abiotic cathodes is the low operational cost for not having to use platinum as the cathode catalyst (He and Angenent, 2006). As a substitute for the platinum, wastewater which is available in abundance will be used as a catholyte to provide the biocatalyst needed for the electron transfer. Biocathodes can be classified as aerobic or anaerobic biocathodes, depending on the terminal electron acceptor.

For aerobic biocathodes, oxygen will be invariably used as the terminal electron acceptor. On the other hand, anaerobic biocathodes with the absence of oxygen, other electron acceptors (NO$_3^-$, NO$_2^-$, SO$_4^{2-}$, CO$_2$, fumarate) will be used, depending on which electron acceptors are available (He and Angenent, 2006).

**Application of MFC**

Generally, all types of wastewater can be used as substrate for an MFC. Table 26 shows some of the examples of wastewater use in studies related to MFC that have been reported in the literature.
Wastewater Treatment and Energy Production

Table 26: Examples of Wastewater used as Electrolytes in the Anaerobic Anode Chamber of MFCs

<table>
<thead>
<tr>
<th>Types of Wastewater</th>
<th>Researcher</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palm Oil Mill Effluent (POME)</td>
<td>Cheng et al., 2010</td>
</tr>
<tr>
<td>Brewery Wastewater</td>
<td>Zhang et al., 2009</td>
</tr>
<tr>
<td>Confectionery Wastewater</td>
<td>Sun et al., 2009</td>
</tr>
<tr>
<td>Chocolate Industry Wastewater</td>
<td>Patil et al., 2009</td>
</tr>
<tr>
<td>Starch Processing Wastewater</td>
<td>Lu et al., 2009</td>
</tr>
<tr>
<td>Sewage Sludge</td>
<td>Jiang et al., 2009</td>
</tr>
<tr>
<td>Landfill Leachate</td>
<td>Greenman et al., 2009</td>
</tr>
<tr>
<td>Swine Wastewater</td>
<td>Min et al., 2005</td>
</tr>
<tr>
<td>Municipal Wastewater</td>
<td>Liu et al., 2004</td>
</tr>
</tbody>
</table>

Combining Wastewater Treatment and Bioenergy

Wastewater treatment processes require an extensive amount of capital investment and maintenance cost due to the large volume of wastewater to be treated and large volume of sludge production (Tsai et al., 2009). In Malaysia, the effluent from the palm oil processing industry had been identified as one of the major threats to the environment due to its very high organic content. Since this industry is one of the major economic contributors to Malaysia, efforts must be made to explore how the wastes produced can be effectively treated or used in a manner that is beneficial to the industry and the environment. The use of Palm Oil Mill Effluent (POME) in Microbial Fuel Cells (MFCs) would be advantageous as the organic matter can be the source of electron donor for the MFCs. Such an application would not only treat the POME but also contribute to electricity generation.
INNOVATIVE R&D ON MICROBIAL FUEL CELL

-Palm Oil Mill Effluent (POME) Treatment and Harnessing of Electricity

A two-chambered bench scale MFC was used in this study to establish the operating conditions for the MFC. Subsequently studies were conducted to investigate the potential of the MFC for treating POME while simultaneously generating electricity.

Laboratory Study to establish operating conditions for the MFC

The studies were carried out on a batch mode basis using a salt bridge which functions as a proton exchange membrane. The set-up was made up of two 500 mL Erlenmeyer Flasks, connected with a glass tube with diameter of 30 mm and 200 mm long, filled with agar salt which acted as the salt bridge. The agar salt was prepared by adding 2 g of agar and 2 g of KCl powder to a 100 mL ultra pure water. The mixture was allowed to dissolve in a microwave oven for 3 minutes.

Carbon electrodes with surface areas of 7 cm² were used for both anode and cathode. The electrodes were connected with a copper wire through a resistance of 3300 Ω. The anode chamber was filled with 500 mL of POME which was deoxygenated with N₂ gas for 2 minutes before starting the experiment to ensure an anaerobic condition. The MFC experiments were conducted at different operating conditions to determine the best set-up which would produce the highest voltage. The operating conditions for each batch is tabulated in Table 27.

For each batch, the voltage across the external resistance in the circuit between the two electrodes was measured every 5 minutes using a digital multimeter (Fluke 289). The maximum values during the process for each batch was recorded.
Table 27: MFC's Operating Conditions for Each Batch

<table>
<thead>
<tr>
<th>BATCH</th>
<th>Anolyte</th>
<th>Catholyte</th>
<th>Anode Chamber</th>
<th>Cathode Chamber</th>
<th>Maximum Voltage (mV)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st. Batch</td>
<td>POME</td>
<td>POME</td>
<td>No Mixing</td>
<td>Mixing</td>
<td>$V_{\text{max}} = 4.288$</td>
</tr>
<tr>
<td>2nd. Batch</td>
<td>POME</td>
<td>Municipal Wastewater</td>
<td>No Mixing</td>
<td>Mixing</td>
<td>$V_{\text{max}} = 12.805$</td>
</tr>
<tr>
<td>3rd. Batch</td>
<td>POME</td>
<td>Municipal Wastewater</td>
<td>No Mixing</td>
<td>Aeration</td>
<td>$V_{\text{max}} = 14.281$</td>
</tr>
<tr>
<td>4th. Batch</td>
<td>POME</td>
<td>Municipal Wastewater</td>
<td>Mixing</td>
<td>Aeration</td>
<td>$V_{\text{max}} = 6.872$</td>
</tr>
</tbody>
</table>

Table 27 clearly shows that using the same electrolyte for anode and cathode chamber would produce a low voltage, as in Batch 1 ($V_{\text{max}} = 4.288$ mV). Changing the catholyte from POME to Municipal Wastewater in Batch 2 managed to increase the $V_{\text{max}}$ from 4.288 mV to 12.805 mV, which might be due to the increase in potential difference between the two chambers. Providing aeration at the cathode chamber in the 3rd Batch managed to further increase the voltage to 14.281 mV. Oxygen is reduced in the cathode chamber when electrons and protons are consumed. Aeration at the cathode chamber supplied the oxygen needed as electron acceptor/oxidant (Ghangrekar et al., 2010). For the 4th Batch, mixing the substrate at the anode chamber reduced the voltage significantly. This finding is consistent with the findings made by Min et al. (2005) stating that it might be due to increase in the oxygen flux into the reactor. However, this situation was not examined further. Based on the study of different batches, the set-up for Batch 3 was adopted for further experiments.
Laboratory Study to Investigate the Potential of the MFC for Simultaneous POME Treatment and Electricity Generation

Full strength raw POME (initial COD of 67,800 mg/L) without any additions of nutrient or treatment was utilised as the substrate for the MFC while the municipal wastewater was used as the electrolyte in the cathode chamber in order to determine the possibility of treating the high organic content wastewater and also the feasibility of harnessing bioelectricity.

A two-chambered reactor as shown in Figure 29 was fabricated using perplex glass with volumes of 10 cm³ for each chamber. The anode chamber is anaerobic, with ports for sampling and gas purging whereas the cathode chamber is aerobic. The two chambers are separated by a Proton Exchange Membrane (PEM, Nafion 117), which is clamped between the chambers. Prior to use, the membrane was treated sequentially in 30% H₂O₂ (to remove organic contaminants from the PEM surface), deionised water, 0.5M H₂SO₄ (to remove metal ion contaminants) and again in deionised water, for 1 hour at each stage. The anode chamber was filled with 900 mL of POME and was purged with N₂ gas for 10 minutes before starting the experiment and after each withdrawal of sample to ensure an anaerobic condition. The cathode chamber was filled with 900 mL of municipal wastewater and provided with aeration. Carbon plates (5 cm x 5 cm; 10 mm thickness) which had been soaked for 24 hours in deionised water were used as electrodes for both chambers. The electrodes were connected with copper wire through a 100 Ω resistance.
The feasibility on harnessing bioenergy from POME was evaluated by measuring the voltage generated. The voltage across the external resistance in the circuit between the two electrodes was measured every hour, using a digital multimeter (Fluke 289). The current was calculated according to Equation 12.

\[ I = \frac{V}{R} \]  

(12)

where \( I \) is the current measured in Ampere (A), \( V \) is the voltage measured in Volt (V), and \( R \) is the resistance measured in Ohm (\( \Omega \)).

Figure 30(a) shows that the percentage removal for COD is approximately 46%. This percentage removal may seemed to be lower...
Innovative Green Technology through a Multidisciplinary Approach

cmpared to studies conducted by Lu et al. (2009) and Patil et al. (2009), which used starch wastewater (98% removal) and chocolate industry wastewater (75% removal), respectively. However, the initial values of COD in the wastewater used are much lower than the POME used in this study, i.e., 4852 mg/L (starch wastewater) and 1459 mg/L (chocolate industry wastewater). The percentage removal in this study is comparable to that conducted by Jiang et al. (2009) (46.4%), which used sewage sludge as a substrate. However, the initial COD was only 10,850 mg/L. Therefore, the COD percentage removal in this study can be considered good, considering its extremely high organic content (67,800 mg/L). In this study, the substrate degradation rate for COD was roughly 1,750 mg/L.day (1.75 kg/m³.day). This value is also comparable to a study conducted by Mohan et al. (2008), with synthetic wastewater which reported a substrate degradation rate ranged between 0.93 – 1.52 kg/m³.day.

**Figure 38(a): Biodegradation of COD**

<table>
<thead>
<tr>
<th>COD (mg/L)</th>
<th>Time (min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>80,000</td>
<td>0</td>
</tr>
<tr>
<td>70,000</td>
<td>5000</td>
</tr>
<tr>
<td>60,000</td>
<td>10000</td>
</tr>
<tr>
<td>50,000</td>
<td>15000</td>
</tr>
<tr>
<td>40,000</td>
<td>20000</td>
</tr>
<tr>
<td>30,000</td>
<td>25000</td>
</tr>
<tr>
<td>20,000</td>
<td>30000</td>
</tr>
<tr>
<td>10,000</td>
<td>35000</td>
</tr>
<tr>
<td>0</td>
<td>40000</td>
</tr>
</tbody>
</table>
Figure 30(b) shows that energy could be generated from a full strength raw POME but at a comparatively low current, as compared to other researches. Though POME has high organic content, a large portion of it is insoluble. The complex organic compounds in the POME need to be hydrolysed prior to utilisation. Besides that, other operating conditions, such as the use of a costly chemical catalyst and unsustainable catholytes, such as ferricyanide used by other researchers (Zhang et al., 2009; Hernandez et al., 2013) contributed to the enhanced electricity generation reported in the literature.

CONCLUDING REMARKS

This study shows that MFC is capable of degrading the palm oil mill effluent at a relatively reasonable rate. However, since POME is initially high in COD, it still requires further treatment before it can be released to the environment. In terms of electricity generation, it is also possible to harness electricity from this high organic content wastewater. However, further enhancements have to be made to improve on the performance of the MFC in generating electricity.
Innovative Green Technology through a Multidisciplinary Approach

It is recommended that the following aspects be explored in the future:

1. Optimization of various operating parameters such as pH and concentration of POME.

2. Modification on the anode to provide a more specific surface area of anode.

CONCLUSION

The presence of emerging pollutants which are not fully regulated by the current regulations in Malaysia has raised awareness and concerns amongst researchers. The need to establish the presence and the concentrations in the environment has now become a necessity. Following that, treatment methods for these pollutants need to be explored and established in order to safeguard the biotic and abiotic environments.

The results of innovative studies on two major groups of emerging pollutants are presented in this book, namely, polyaromatic hydrocarbons (PAH) and heavy metals. The innovation of these studies lies on the pairing of two different problematic waste streams or two different problems in a manner that they become complementary solutions. This deviates from the conventional studies that address a problem in isolation and mainly using solutions that are not environmentally sustainable. The studies highlighted in this book emphasise on the use of biological approach of either making use of a biological process or making use of a bio-base material.

The used of new equipment made available through advanced technologies is very helpful in ascertaining mechanisms taking place during transformation processes as evidently shown in the study on removal of heavy metals using biosorbents.
Studies on biological sulphide precipitation of heavy metals has highlighted the use of complementing waste streams to remove the sulphide problem in municipal wastewater and heavy metals in automotive wastewater. Studies on degradation of PAH in various media using microbes illustrate not only the potential of bio-degradation using microbes but also the value of POME and municipal wastewater sludges as reservoirs rich in microbes that could provide both PAH degraders and surfactant producers. On the other hand, studies on removal of heavy metals using biosorbents elucidate how waste from agriculture can be used to remove pollutants, in this case heavy metals, through physical-chemical processes. Finally, the use of microbial processes in simultaneously treating wastewater (POME) and generate electricity was shown through studies on microbial Fuel Cells. The four-elements approach incorporating the need to have a strong understanding of the fundamental mechanism that is governing the process, need to assess material availability, need to ensure that benefits are transferred to or enjoyed by society and need to evaluate costs to society in terms of economics, environment and psychological well-being was evident in the examples provided in the various chapters of this book.

All the studies discussed in this book require a multi-disciplinary approach from microbiology, bio-chemistry, molecular biology to various fields of engineering, thus emphasising the need for researchers to collaborate or develop competency in at least two disciplines of knowledge. The training of future researchers must incorporate this aspects as solutions to future to problems will most certainly not lie in a domain defined by a single discipline.

The findings from these studies address fundamental issues that lead to better understanding of the processes and mechanisms. Further efforts are needed to translate the fundamental knowledge established in these studies into industrial applications. Although much had been established in the studies presented in this book, much more is still unknown.
Suhaime Abdul Talib was born in Perlis, Malaysia on August 13, 1964. In 1986, he obtained his B.E. (CIVIL) (Hons) from the University of Melbourne, Australia. In 1996, he obtained a M.Sc. in Water and Environment Management from Loughborough University, U.K. and subsequently, his PhD. from Universiti Teknologi Malaysia in 2002. His research interests include in-sewer processes, environmental management, pollution control and bioremediation. Currently his work focuses on microbial transformation, bioremediation and the development of sustainable treatment technologies. He joined Universiti Teknologi Mara (UiTM) in 1988 and currently is a Professor in the Faculty of Civil Engineering, UiTM. Since October 2011, he has been entrusted by the university to serve as the Assistant Vice Chancellor of Development, Facilities Management and ICT of Universiti Teknologi MARA (UiTM). Prof. Sr. Ir. Dr. Suhaime Abdul Talib is a professional member of both Board of Engineers Malaysia (BEM) and Institute of Surveyors Malaysia (MISM), as well as a corporate member of Institution of Engineers Malaysia (MIEM). An advocate of multidisciplinary research, he has published his research findings in various ISI and Scopus indexed journals including Water Science Technology, Korean Journal of Chemical Engineering, Research Journal of Chemistry and Environment to name a few.


Innovative Green Technology through a Multidisciplinary Approach


References


Innovative Green Technology through a Multidisciplinary Approach


References


References


References


References


Innovative Green Technology through a Multidisciplinary Approach


References


References


Innovative Green Technology through a Multidisciplinary Approach


References


INDEX

**B**

Biocathodes 107, 108, 130
Bioremediation 35, 42, 43, 122, 126, 137
Biosorbent 64, 69, 70, 72, 73, 74, 75, 77, 78, 79, 83, 85, 92, 94, 95, 97
Biosurfactant 48, 55, 58

**C**

Corynebacterium urealyticum 52, 54, 61, 122

**D**

Degradation 52, 53, 59, 60, 61, 122, 131

**E**

emerging pollutants 3, 4, 16, 17, 116

**L**

Langmuir Isotherm 75, 77, 78, 79

**M**

Metal Sulphide Precipitation 21, 26
Microbial Fuel Cells 109

**P**

Pleurotus ostreatus 14, 68, 70, 77, 78, 82, 83, 128, 130, 134, 140, 141, 144
Polyaromatic Hydrocarbon 35

**Z**

ZERO VALENT IRON 48
The book illustrates the current scenario on the presence of persistent micro-pollutants in the environment and introduces the use of alternative green technology approaches to address this concern. The alternative approach includes novel systems that utilised unwanted components from two different waste streams as a complementing wastewater engineering solution. A multi-disciplinary approach was introduced to develop bioremediation techniques with the use of microbes for degradation of polyaromatic hydrocarbon. In addition, a bio sorbent was developed for heavy metal removal through the utilisation of local agricultural waste. Furthermore, utilization of organic waste from the palm oil industry subjected to microbial activity was also investigated for energy production. This book emphasises the need for interdisciplinary approaches in converting waste to wealth.