BIOREMEDIATION OF JERAM SANITARY LANDFILL LEACHATE USING SELECTED POTENTIAL BACTERIA

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ABSTRACT

Over the past decade, generation of municipal solid wastes (MSW) in Malaysia has increased more than 91%. However, MSW management in Malaysia can be considered relatively poor and disorganized. The most preferred of MSW disposal method in Malaysia is through landfilling. The major environmental concern associated with landfill problem is the contamination of leachate into the environment. Due to that problem, this research aimed to characterize leachate and used some selected potential microbes to perform bioremediation on leachate. Utilization of microorganisms such as bacteria in the bioremediation of leachate will help reduce the cost and posed least effect to the environment. Jeram sanitary landfill was used as the source of raw leachate in this study. Leachate was analysed to establish the current characteristics and confirm with previous studies on JSL leachate. The leachate showed deep black colour with a slightly ammoniac odour at pH of 8.38, salinity of 19.30 ppt, conductivity of 35,830 µS/cm and Total Dissolved Solid (TDS) of 20,320 mg/L. BOD_5 and COD values were at 1,050 and 11,031.70 mg/L respectively with ratio of 0.09. Ammoniacal nitrogen content recorded at 6,400 mg/L with oil and grease at 4.4 mg/L. Bacteria used in the study namely *Bacillus salmalaya*, *Lysinibacillus sphaericus*, *Bacillus thuringiensis* and *Rhodococcus wratislaviensis* were previously isolated from the agricultural soil and from a leachate contaminated site in Malaysia. Each strain was grown as a pure culture in NA plates at 33°C for 2 days. The pure strains were used to build up inoculum for leachate remediation. 100 ml of bacteria suspension was added to 900 ml of leachate in each treatment (10% v/v). Leachate were analysed before and after 48 hours of remediation. Results shows that treatment with inoculum which consist of every bacterium used in the study presented a remarkable reducing capacity of oil and grease of 98% and ammoniacal nitrogen at 57% from initial value. On the other hand, the combination of the bacteria also found to be high potential in removing heavy metal in the leachate Pb (86%), Mn (82%), Ba (74%), Al (74%), Zn (73%), As (68%), Ni (66%), Cr (66%) and Fe (63%). In conclusion, the microbial mixtures have showed a good potential in remediating highly heterogeneous and polluted leachate.

Keywords: *Bioremediation, Leachate, Bacteria*

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ABSTRAK

Sejak dekad lalu, penghasilan Sisa Pepejal Perbandaran (SPP) di Malaysia telah meningkat lebih daripada 91% namun pengurusan SPP di Malaysia masih lemah dan tidak tersusun. Kaedah pelupusan SPP yang utama adalah melalui tapak pelupusan sampah. Masalah utama yang dibimbangi akibat pelupusan sisa pepejal adalah pencemaran larut lesapan ke persekitaran. Justeru kajian ini adalah bertujuan bagi mencirikan larut lesapan dan menguji beberapa bakteria terpilih yang berpotensi untuk merawat pencemaran dalam larut lesapan atau bioremediasi. Penggunaan mikroorganisma seperti bakteria di dalam bioremediasi larut lesapan akan membantu mengurangkan kos dan mengurangkan impak negatif terhadap alam sekitar. Tapak pelupusan sanitari Jeram telah digunakan sebagai sumber larut lesapan dalam kajian ini. Larut lesapan dianalisis terlebih dahulu untuk menentukan ciri-cirinya dan disahkan dengan kajian lepas terhadap larut lesapan dari Jeram. Larut lesapan ini mempunyai warna hitam pekat dengan sedikit bau ammonia pada bacaan pH 8.38, kemasinan pada 19.30 ppt, kekonduksian pada 35,830 μS/cm dan jumlah pepejal larut pada 20,320 mg/L. BOD_5 dan COD memberikan bacaan 1,050 dan 11,031.70 mg/L masing-masing dengan nisbah 0.09. Kandungan ammoniakal nitrogen ialah 6,400 mg/L dan minyak dan gris pada 4.4 mg/L. Spesis bakteria *Bacillus salmalaya*, *Lysinibacillus sphaericus*, *Bacillus thuringiensis* dan *Rhodococcus wratislaviensis* yang digunakan adalah diperoleh daripada persampelan tanah pertanian dan tapak larut lesapan yang tercemar di Malaysia. Bakteria ini dibiakkan secara kultur tunggal agar nutrient (NA) pada suhu 33° C selama 2 hari. Baka spesis yang tulen digunakan untuk menghasilkan inokulum bagi merawat larut lesapan. 100 ml larutan bakteria telah ditambah kepada 900 ml larut lesapan dalam setiap rawatan (10% v/v). Larut lesapan telah dianalisa sebelum dan selepas 48 jam bioremediasi. Keputusan menunjukkan bahawa rawatan dengan inokulum yang terdiri daripada setiap bakteria yang digunakan dalam kajian ini memberi impak luar biasa kapasiti dengan mengurangkan minyak dan gris (98%) dan ammoniakal nitrogen (57%). Selain itu, gabungan bakteria ini juga dikesan mempunyai potensi yang tinggi dalam mengeluarkan logam berat di larut lesapan iaitu Pb (86%), Mn (82%), Ba (74%), Al (74%), Zn (73%), As (68%), Ni (66%), Cr (66%) dan Fe (63%). Kesimpulannya, campuran mikrob telah menunjukkan keputusan yang baik dalam proses remediasi air larut lesapan yang tercemar dengan kandungan cemar yang pelbagai.

Kata Kunci : *Bioremediasi, Larut lesapan, Bakteriia*

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percent
$<$	Less Than
$>$	More Than
°C	Celcius Grade
µS/cm	Microsiemens per centimeter
Ag	Silver
Al^{3+}	Aluminium
ANOVA	Analysis of Varience
AOP	Advanced Oxidation Processes
As	Arsenic
Au	Gold
Ba	Barium
BOD	Biochemical Oxygen Demand
Cd	Cadmium
CH_4	Methane
cm	Centimeter
CO_2	Carbon Dioxide
COD	Chemical Oxygen Demand
Cr	Chromium
Cu	Copper
CW	Constructed Wetland
DOE	Department Of Environment
EB	Electron Beam
EDTA	Ethylenediaminetetraacetic Acid
EM	Effective Microorganism
EQA	Environmental Quality Act 1
Fe	Iron
HCO_3^-	Bicarbonate
H_2O_2	Hydrogen peroxide
H_2SO_4	Sulfuric acid
H_3PO_4	Phosphoric Acid
HCl	Hydrochloric acid
HDPE	High Density Polyethylene
Hg	Mercury
K	Pottasium
Kg	Kilogram
L	Liter
MF	Microfiltration
$Mg(OH)_2$	Magnesium hydroxide
mg/L	Miligram/Liter
$MgCl_2$	Magnesium chloride
$MgNH_4PO_4 \cdot 6H_2O$	Magnesium Ammonium Phosphate
MgO	Magnesium oxide
MOH	Ministry Of Health
MSW	Municipal Solid Waste
Na	Sodium
NF	Nanofiltration
NH_3	Ammonia

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NH_3-N	Ammonium Nitrogen
NH_4^+	Ammonium
Ni	Nickel
NO_3^-	Nitrate
NRE	Natural Resources And Environment
O_2	Oxygen
O_3	Ozone
OD	Optical Density
OECD	Organization For Economic Co-Operation And Development
Pb	Lead
PCB	Polychlorinated biphenyls
PO_4	Phosphate
POP	Persistent Organic Pollutant
Ppt	Part Per Thousand
PRB	Population Review Bureau
RCRA	Resource Conservation And Recovery Act
RO	Reverse Osmosis
Se	Selenium
SO_4	Sulphate
SS	Suspended Solids
SWM	Solid Waste Management
TCE	Trichloroethylene
TDS	Total Dissolved Solids
Th	Thorium
TKN	Total Kjeldahl Nitrogen
TOC	Total Organic Carbon
U	Uranium
UF	Ultrafiltration
UNEP	United Nations Environment Programme
US	Ultrasound
USAID	U.S. Agency For International Development
UV	Ultraviolet
VFA	Volatile Fatty Acids
Zn	Zinc

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CHAPTER 1: INTRODUCTION

1.1 Background of Study

Recent data of 2015 has estimated human population had surpassed 7.2 billion mark with more than 53% population living in urban area (PRB, 2015). The growth is accompanied not only by increase in the living standards but also the steady increase in industrial and municipal waste generation due to human activities. Waste generation per capita has increased to more than one kilogram per capita per day in most developing countries comparably as much as or even higher than those of developed countries (UNEP, 2009).

In Malaysia, population growth has also expanded steadily from 13.7 million in 1980 to 28.3 million in 2010 of which 71% of the populations live in urban area (Lian, 2011). Waste generation in Malaysia has increased significantly in recent years, ranging between 0.5 - 2.5 kg per capita per day (or a total of 25000 -30000 tons per day) (Johari *et al.*, 2014). This tremendous amount of waste generation brought not only economic burden to the government but also environmental and social impact to society (Agamuthu, 2001).

Overall waste composition in Malaysia is dominated by municipal solid waste (MSW) (64%), followed by industrial waste (25%), commercial waste (8%) and 3% consists of construction waste (EU-SWMC, 2009). Household area is one of the main primary sources of municipal solid waste in Malaysia, besides institutional and commercial waste (Yousuf & Rahman, 2007). Malaysian solid waste contains a very high concentration of organic waste and consequently has high moisture content and a bulk of density above 200 kg/m^3 (Mohd Armi *et al.*, 2013). A waste characterization study

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found that the main components of Malaysian waste were food, paper, and plastic which comprise 80% of overall weight (Mohd Armi *et al.*, 2013). These characteristics reflect the nature and lifestyle of the Malaysian population.

Municipal solid waste generally consist of around 20 different categories which are food waste, paper (mixed), cardboard, plastics (rigid, film and foam), textile, wood waste, metals (ferrous or non-ferrous), diapers, newsprint, high grade and fine paper, fruit waste, green waste, batteries, construction waste and glass; these categories can be grouped into organic and inorganic (Amin and Go, 2012) as illustrated by Figure 1.1.

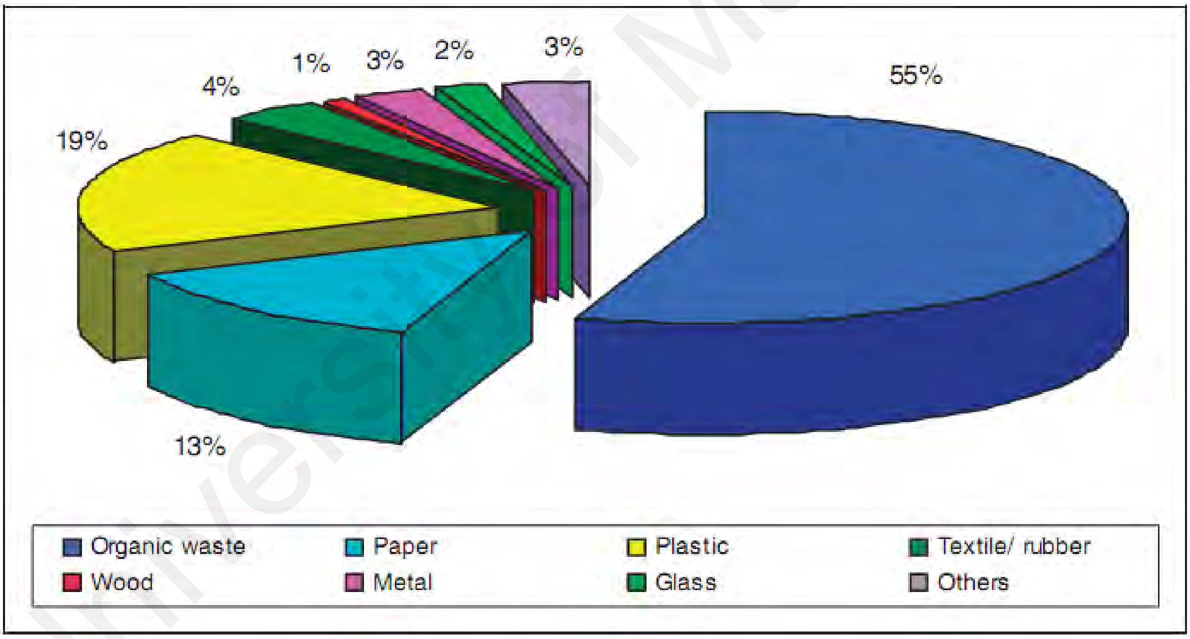

Figure 1.1 Typical municipal solid waste compositions in Malaysia (Fauziah and Agamuthu, 2009).

Although Malaysia has rapid economic and population growth, the environmental awareness on waste management among the people is still very low. There is estimated around 70-80% recyclables material in the household waste but only 5% of population practicing 3R; 'reduce, reuse and recycle' making the waste management problem even worse (Johari *et al.*, 2014; Moh & Manaf, 2014). The latest regulation by Jabatan

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Pengurusan Sisa Pepejal Negara (JPSPN) to make it compulsory for household to separate and disposed recyclables in separate waste container also is not well received and practiced by the population (Dhillon, 2014).

With the advancement of scientific research, capital funding and technologies, there are various methods available for the treatment of waste. Examples of established solid waste treatment technologies are composting, incineration, landfilling and recycling. More advanced technologies utilize methods such as anaerobic digestion, gasification, pyrolysis, and many others. For liquid type of waste or commonly known as waste water, the treatments covers the physical removal of the suspended solids, oil and grease in primary treatment by using sedimentation, filtration and flocculation. Biochemical and/or biological reactions are used to remove dissolved organic material, as well as, nutrients nitrogen and phosphorus in secondary treatment and the tertiary treatment follows with technologies such as micro/ultra-filtration and synthetic membrane. Other technologies are also utilized where necessary namely activated sludge treatment, disinfection to remove pathogenic microorganisms, advance oxidation processing, adsorption, vitrification and chemical treatment for toxic substances.

As to date, the main option of the municipal solid waste (MSW) disposal in Malaysia is landfilling. At present, landfilling is the main waste disposal method (80% usage) and it is still expected to account for 65% of waste in 2020 (Sharifah Norkhadijah & Latifah, 2013). MSW were disposed in uncontrolled dumping sites in earlier days but later more systematic sanitary landfill approach was introduced. There are officially about 230 landfills with different size and age and an estimated three times more illegal dumps are existed in Malaysia (Alkassasbeh *et al.*, 2009).

A landfill is an engineered depression in the ground, or built on top of the ground into which wastes are buried. The purpose is to avoid any connection with surrounding water

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bodies that can pollute the environment (Masirin *et al.*, 2008). The major environmental concern associated with landfill problem is the contamination of leachate into the environment. Due to scarcity of land more often landfills are located on a sloping area where accumulation or contamination of leachate may cause a negative impact.

Leachate is defined as liquid that has percolated through waste which contains dissolved or suspended materials. It arises from the biochemical and physical breakdown of wastes (Lu *et al.*, 1985; Nadiah *et al.*, 2012). Leachate may contain - many different organic and inorganic compounds, suspended solids, heavy metals and other pollutants that can contaminate the ground water and surface water resources. Groundwater pollution can represent a health risk and will create many environmental problems if not properly handled (Kjeldsen *et al.*, 2002). Leachate quality are different and these differences are caused by several factors such as composition and depth of solid waste, availability of moisture and oxygen content, design and operational of the landfill and life expectancy of the solid waste. Leachate resulting from the decomposition of solid waste contain concentrations of COD, BOD, ammonia nitrogen and heavy metals such as zinc, copper, cadmium, lead, nickel, chromium and mercury. The discharge of leachate into the environment is considered under more restrictive views. This is because the risk of groundwater pollution is probably the most severe environmental impact from landfills because in the past, most landfills were built without engineered liners and leachate collection system (Kjeldsen *et al.*, 2002). The larger the size of the landfill site, the more serious the impact of groundwater pollution. Therefore, leachate treatment is important and necessary in order to prevent or minimize these environmental problems.

Leachate treatment is very complicated, expensive and often requires multiple processes. Leachate is treated conventionally in treatment plants built in the landfill compound. It generally utilized biological treatments, mechanical treatment by

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ultrafiltration and treatment with active carbon filters. Many treatment processes were tested and operational ranges and performance levels were established. Several technologies such as oxidation, sedimentation, ion exchange, membrane filtration, chemical precipitation, reverse osmosis, air stripping and adsorption have been applied for leachate treatment (Hamidi, 2015). Another viable option discovered for leachate treatment is by the use of biological processes or bioremediation.

Bioremediation is an organism mediated transformation or degradation of contaminants into nonhazardous or less-hazardous substances. It employs various organisms like bacteria, fungi, algae, and plants for efficient bioremediation of pollutants as exemplified in Figure 1.2.

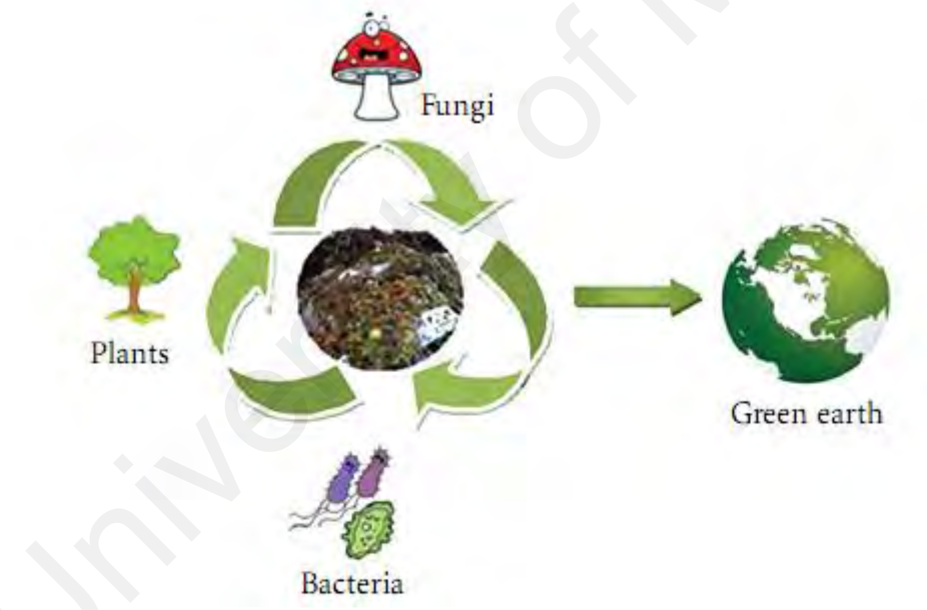

Figure 1.2 Process of bioremediation of waste (Karigar and Rao, 2011)

Bioremediation is the process by which microorganisms are stimulated to rapidly degrade hazardous organic pollutants to environmentally safe levels in soils, sediments, substances, materials and ground water. For bioremediation to be effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products.

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Recently, biological remediation process have also been devised to either precipitate effectively or immobilize inorganic pollutants such as heavy metals (Rathoure, 2015). Stimulation of microorganisms is achieved by the addition of growth substances, nutrients, terminal electron acceptor/donors or some combination thereby resulting in an increase in organic pollutant degradation and bio-transformation (Rathoure, 2015).

The control for bioremediation processes is a complex system of many factors. These factors include the existence of a microbial population capable of degrading the pollutants, the availability of contaminants to the microbial population and the environment factors (type of soil, temperature, pH, the presence of oxygen and nutrients) (Das, 2014).

Microorganisms can be isolated from almost any environmental conditions. Microbes will adapt and grow at subzero temperatures, as well as extreme heat, desert conditions, in water, with an excess of oxygen, and in anaerobic conditions, with the presence of hazardous compounds or on any waste stream. The main requirements are an energy source and a carbon source.

Aerobic: In the presence of oxygen. Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, and Mycobacterium*. These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

Anaerobic: In the absence of oxygen. Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and chloroform (Naik & Duraphe, 2012).

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Ligninolytic fungi: Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants. Common substrates used include straw, saw dust, or corn cobs.

Bioremediation offers advantages over other treatment strategies. Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated material such as soil. Microbes able to degrade the contaminant increase in numbers when the contaminant is present when the contaminant is degraded, the biodegradative population declines (Soni, 2007). The residues for the treatment are usually harmless products and include carbon dioxide, water, and cell biomass (Soni, 2007).

Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants (Rathoure, 2015). Many compounds that are legally considered to be hazardous can be transformed to harmless products (Rathoure, 2015). This eliminates the chance of future liability associated with treatment and disposal of contaminated material. Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible (Rathoure, 2015).

Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation (Goltapeh *et al.*, 2013). Bioremediation can prove to be less expensive than other technologies that are used for clean-up of hazardous waste (Goltapeh *et al.*, 2013).

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1.2 Problem statement

In general, the most typical harmful effect of leachate discharge into the environment is groundwater pollution. Major problems in managing a landfill in a tropical country like Malaysia is managing the leachate that is generated when the water pass through the waste. Malaysia's climate is hot and humid with relative humidity ranging from 80 - 90 percent except for highlands (Abdullah *et al.*, 2011). It is dominated by the effect of two monsoons or "rainy seasons", which affect different parts of Malaysia to varying degrees (Abdullah *et al.*, 2011). Heavier rainfall is experienced when the monsoon changes direction. During this time, large volume of leachate is produced as more precipitates pass through the waste in the landfill. According to Li *et al* (2009), the composition of a leachate depends on a variety of parameter such as the type of waste, climate conditions, mode of operation, and age of the landfill.

Landfill leachate may consist of large amount of dissolved organic matters (alcohols, acids, aldehydes, and short chain sugars), inorganic macro-components (common cations and anions including sulphate, chloride, and ammonium), heavy metals (Pb, Ni, Cu, Hg) xenobiotic organics and polychlorinated biphenyls (Emenike *et al.*, 2012; Ludwig *et al.*, 2012). Moreover, landfill leachate is also characterized by high level of biochemical oxygen demand (BOD), chemical oxygen demand (COD), salts and NH_3-N as well as high organic loading (Christensen *et al.*, 2001; Emenike *et al.*, 2012). According to Tao *et al.* (2007), higher organic loading yields greater substrate availability for planktonic and epiphytic bacteria that may induce inhibitory effects on sedimentary bacteria. More than 200 organic compounds have been identified in municipal landfill leachate (Schwarzbauer *et al.*, 2002), with about 35 of these compounds having the potential to cause harm to the environment and human health (Emenike *et al.*, 2012; Paxus, 2000). On the other hand, according to Emenike *et al.* (2012), high level of ammonia is toxic to many living organisms in surface water

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because it contributes to eutrophication, and dissolved oxygen depletion. Due to its polluted contents, leachate has become more difficult to manage. However, care must be taken with MSW leachate analyses due to the presence of harmful substances.

Earlier studies of landfill leachate in Malaysia in particularly Jeram Sanitary Landfill by Emenike *et al.* (2013b) showed high biochemical oxygen demand (BOD), chemical oxygen demand (COD) and ammonia concentrations at 27 000 mg/L, 51 200 mg/L and 3 032 mg/L, respectively. Toxicological implications of leachate pollution based on the characterized leachate quality, ranged from aquatic life suffocation due to oxygen depletion to tissue lysis caused by ammonia toxicity and bioaccumulation of other toxicants.

Ammoniacal-N is also a significant determinant for the pollution potential of every landfill or waste dump brought about by continued degradation of amino acids and nitrogenous organic matter. A leachate characteristic is a reflection of waste components that manifest after some biological and physico-chemical interactions in the landfill. Some of the components are contaminants which have toxic nature especially in the form of persistent organic pollutants (POPs), monocycyclic aromatic hydrocarbons, heavy metals and etc. (Emenike *et al.*, 2013b).

For that reason, the treatment of leachate is very important before it is discharged into water bodies to avoid pollution to the ground and surface soil and to prevent both severe and continual toxicity (Öman & Junestedt, 2008;Sanphoti *et al.*, 2006; Tatsi & Zouboulis, 2002). As waste sent to landfill increases from day to day, cost of managing the leachate will also increase. Thus, a more cost effective method of leachate treatment before discharging to water body is important to sustain the landfill.

Current method of leachate treatment uses physical and chemical reactions. It is costly and not environmental friendly. One of alternative option is bioremediation using living

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organisms such as microorganism, plant or fungi to degrade the highly polluted leachate before it is discharged to environment. Utilization of microorganisms such as bacteria in the bioremediation of leachate will help reduce the cost and posed least effect to the environment (Kumar *et al.*, 2011).

Previous studies have been performed to isolate several strains of bacteria from local environment that could be of potential as effective microorganisms (EM). Some of them are already screened for landfill leachate bioremediation capabilities including biodegradation of the leachate characteristics and reduction in heavy metals content. The reduction of these leachate characteristics and heavy metal content below the limits are the pre-requisite required for landfill leachate or any other wastewater treatment system before it can be discharged.

However, several species are also not yet tested in bioremediation study especially for landfill leachate remediation. It is also considering the fact that landfill leachate is very heterogeneous and varied in the pollutants contents and characteristics. Therefore, this study is designed to test the abilities of several species of potential bacteria either in single or mixed application to remediate landfill leachate freshly sampled from local site. This will form a fundamental study for future extended laboratory or field test using the potential bacteria before it can be effectively used commercially.

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1.3 Objectives of study

The objectives of this study are as follows:

1. To characterize and evaluate the JSL leachate as the test subject for the use of potential bacterial isolates as its treatment agent.

2. To test the ability of the selected bacteria in the treatment of JSL leachate bioremediation as single and mixed isolate of bacteria.

3. To study the potential of beneficial bacteria to reduces heavy metals in leachate.

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CHAPTER 2: LITERATURE REVIEW

2.1 Population Growth, Urbanization and Waste Generation

Recent published data in 2015 World Population Review estimated that world population had surpassed 7.3 billion mark in 2015. More than 6 billion human population is from less developed or developing countries such as highly populated China, India, Indonesia, Brazil and Pakistan (PRB, 2015). Although estimations and projections had predicted the growth rate will be slowed down in this century, the population still increases at a lower rate especially in less developed countries (Lutz *et al.*, 2001). The recent data also showed that the extreme poverty and child mortality rate have declined steadily across the world indicating improvement of the life in those countries. The population increase is accompanied by urbanization process as more than 53% from world population colonize urban cities area (PRB, 2015). This is expected as life in the urban area offer more jobs, better economic opportunities and is the center for population activities.

The population and economic growth across the world bring not only improvement to the standard of living but also elevated the problems in managing population growth (Thuku *et al.*, 2013). Urbanization and industrialization in cities and surrounding area has provided the source of income to people and nation but the increase of human activities are also accompanied by increase in waste generation. Tremendous amount of both municipal and industrial solid waste production is recorded in urban area due to increasing affluent lifestyles, ongoing rapid industrial and commercial growth (Agamuthu *et al.*, 2007).

Waste generation rates in many developing countries have now crossed the one-kilogram per capita per day mark (UNEP, 2009). In most member countries of Organization for Economic Co-operation and Development (OECD) which are considered as developed nations, municipal solid waste (MSW) generation rates are slightly above one-kilogram per capita. The population growth and urbanization in developing countries is very high in comparison to more developed countries. As a result, overall waste generation amount is also much higher than most developed countries. Industrial waste generation rates is also high as most of the industries are primary industries producing raw materials for industrial production (UNEP, 2009). MSW generation has doubled or tripled in some industrial countries over the last two decades (Agamuthu *et al.*, 2007).

2.2 Waste management in Malaysia

In the context of Malaysia, as one of the 'Asian Tiger' in term of economic growth since 1990s to early 21st century, the population and urbanization growth has also expanded rapidly. The national population had increased from just 13.7 million in 1980 to 28.3 million in 2010 of which 71% of the populations live in urban area in 2010 compared to only 34.2% in 1980 (Department of Statistics Malaysia, 2010). This led to waste generations of around 30,000 tonnes a day in 2013, as compared to 22,000 tonnes of solid waste produced daily in 2012 (Ikram, 2014). According to Masirin *et al*. (2008), the per capita solid waste generated in Malaysia has increased from 0.5 kg/day in the 1980´s to the current volume of more than 1kg/day. This represents a 200% increased in 20 years (Agamuthu, 2001). Solid waste management (SWM) has become an economic, social and environmental responsibilities and also burden to government and society as waste generation grew over time affecting us either directly or indirectly.

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Generally, solid waste management (SWM) in Malaysia involves the participation of varies government agencies from federal, state and local authorities. There are many governmental agencies which involved either directly (temporary storage, collection, landfill management) or indirectly (legal, transport, housing, land management authorities) with SWM (Sakawi, 2011). In Malaysia, solid wastes are generally categorized into three major groups, and each category is under the responsibility of a different government agencies:

i. Municipal solid waste – under Ministry of Urban Wellbeing, Housing and Local Government
ii. Schedule/hazardous waste – under Department of Environment (DOE), Ministry of Natural Resources and Environment (NRE)
iii. Clinical waste – under Ministry of Health (MOH) (Latifah *et al.*, 2009)

Managing MSW has becoming one of the major waste management issues not only in Malaysia but worldwide. The changed characteristics of the solid waste made it more complex for the municipalities to handle (Masirin *et al.*, 2008). More than 28,500 tonnes of MSW are disposed directly into landfills daily (P. Agamuthu & S. Fauziah, 2011). Due to various factors, landfilling is one the most practiced method of MSW disposal in Malaysia. Past 30 to 40 years ago, MSW was disposed off in uncontrolled landfilling or dumping sites scattered across strategic urban areas in the country. Later in the early 20th century, more controlled and systematic landfilling approach was implemented and the sanitary landfill method was introduced to achieve better level of MSW management.

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2.3 Landfill – conventional and modern (sanitary)

A landfill is an engineered depression in the ground, or built on top of the ground, resembling a football stadium, into which wastes are buried. The purpose is to avoid any hydraulic or water-related connection between the wastes and the surrounding environment, particularly groundwater (Masirin *et al.*, 2008). The major environmental concern associated with landfill problem is the contamination of leachate into the environment. Due to scarcity of land more often landfills are located on a sloping area where accumulation or contamination of leachate may cause a negative impact(Sharifah Norkhadijah & Latifah, 2013).

The sanitary landfill method for the final disposal of solid waste material remains to be widely accepted and adopted due to its economic advantages. Studies on the various possible means of removing solid waste namely landfilling, incineration, composting and others have shown that landfilling is the cheapest, in term of exploitation and capital costs (Białowiec, 2011). Besides its economic advantages, landfill method minimizes direct environmental and human impacts, and allows waste to decompose under controlled conditions until its eventual transformation into relatively inert and stabilized material (Renou *et al.*, 2008).

2.4 Characteristics of good landfill practice

Selection of good landfill site is the key step towards proper waste disposal. It ensures environmental protection and promotes public health and quality of life. For the development of new landfill, adoption of this important step will prevent any imminent problems and long-term effects. In general, landfill site which is well-selected will require simple design and has sufficient cover material that leads to eco-friendly and lower cost of operation (Ball, 2005).

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The environmental, economic and sociopolitical aspects are the factors to be considered to locate a landfill. This selection process has become more complex as public environmental awareness increased, new regulation introduced and other developments occurred over time. This leads to the development of new selection procedures and tools (Ball, 2005). Several critical technical factors to be considered to locate a landfill are geology, geohydrology and surface drainage(Sharifah Norkhadijah & Latifah, 2013). Geological investigations are carried out to locate features like dykes, faults and geological contacts (Savage *et al.*, 1998).

Assessment of the water-body system in the area and thickness and properties of the soil in the unsaturated zone, are the geohydrological investigations performed (Savage *et al.*, 1998). Flow and head gradient of the groundwater is also considered, apart from spring and water borehole inventories, depth to the top of aquifers and piezometric levels, water quality and permeability of rock and soil formations (Savage *et al.*, 1998).

In short, the ideal location for landfill should have the following geological characteristics; no geological faults/ dykes, very low permeability strata at the base of the landfill, unsaturated layer of thickness more than 30 m, more than 1000m from the nearest surface water bodies, low hydraulic conductivity of the ground and the nearest aquifer below the landfill should not be used for domestic purposes and downstream of the aquifers (Savage *et al.*, 1998).

Munawar and Fellner (2013) had outlined a good sanitary landfill design which should consist of landfill liners and landfill capping.

i. Landfill liners

In tropical countries like Malaysia, leachate emission from landfilled waste is a problem due to the high organic content and the high volume of rainfall in the country. Therefore

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proper landfill design is required to isolate waste from surrounding environment at low construction and operation costs (Edi & Fellner, 2013; Fauziah & Agamuthu, 2012).

The isolation of waste from the environment at the base of a landfill can be achieved by a base lining system. In developed countries, landfill regulations often require a composite liner at the landfill base. This composite liner usually consists of a clay layer (of 40 to 80 cm thickness) and a high density polyethylene (HDPE) (Edi & Fellner, 2013). The later in particular is expensive and hence often unaffordable for landfill operations in developing countries (Edi & Fellner, 2013).

In developing countries, it is recommended to use a "single" baseliner system consisting of compacted clay. The clay material should preferably be accessible in the vicinity of the landfill site, in order to minimize transportation costs and traffic. Thus, site selection is crucial for the overall costs of landfilling. Requirements for the compaction of the clay and the required hydraulic conductivity can be referred from various international regulations on landfill construction for example EU landfill directive (Edi & Fellner, 2013).

ii. Landfill capping

At the end of landfill operations, the landfill must be covered or capped. The wastes need to be covered first by an intermediate cover layer, which is insensitive to settlements of the landfill surface. This intermediate cover layer of 50 cm soil or compost functions as: prevention of erosion by wind and water, reduction of water infiltration, and gas emissions (at least partial oxidation of generated methane), to promote vegetation and for aesthetic purpose (Edi & Fellner, 2013).

The infiltration of water can be reduced by using a cover material of high water retention capacity such as compost material, using sloped surface or vegetation (Edi &

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Fellner, 2013). The intermediate cover could be replaced after 5 to 20 years and by overlaying top sealing system, for example clay liner of 50 cm and soil layer > 50 cm to further reduce water infiltration (Edi & Fellner, 2013). Final capping with surface slop and intensive vegetation is also recommended for landfills (Edi & Fellner, 2013).

2.5 Practice and Issue of MSW in Malaysia

In Malaysia, the main option of MSW disposal is landfilling. Up to 95% of total MSW collected are disposed off in landfills. There are officially about 230 landfills with different sizes and ages and an estimated three times more illegal dumps are existed in Malaysia (Alkassasbeh *et al.*, 2009). The landfills in Malaysia generally are classified into 4 categories (NAHRIM, 2009):

i. Landfills that are operating at critical stage without any control to prevent pollution into the environment. These landfills will be closed once a new landfill starts to operate.
ii. Landfill sites (open dumpsites) that have capacity of receiving waste and will be allowed to continue accepting waste, but need to be upgraded to manage leachate and methane gas.
iii. Landfills that are already closed (ceased operation) but do not have prepared any safety closure plan.
iv. Landfills designed with up-to-date technologies, for example sanitary landfill.

At present, landfilling is the only method used for the disposal of MSW in Malaysia, and most of the landfill sites are open dumping areas, which pose serious environmental and social threats (Yunus & Kadir, 2003). Disposal of wastes through landfilling is becoming more difficult because existing landfill sites are filling up at a very fast rate. At the same time, constructing new landfill sites is becoming more difficult because of

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land scarcity and the increase of land prices and high demands, especially in urban areas due to the increase in population.

2.6 Jeram Sanitary Landfill

Jeram Sanitary Landfill, which is located in an oil palm plantation near Mukim Jeram, Kuala Selangor currently is one of the active sanitary landfill in Malaysia. The landfill is 160 acres big and is designed with a capacity to hold 6 million tons of waste (Worldwide Environment, 2015). Jeram sanitary landfill is operated by Worldwide Holdings under a 25 year concession agreement with the Kuala Selangor state government since January 2007. The landfill receives an average 2,500 tonnes of MSW per day thus generates approximately 315,000 L/day leachate (P. Agamuthu & S. H. Fauziah, 2011). The leachate collection and treatment ponds are roughly rectangle in shape and occupied 64.7 hectares of area (Zainab *et al.*, 2013). The leachate collected in several ponds is treated by physico-chemical treatment system on site.

The types of waste received are domestic waste, bulky waste and garden waste only. The landfill caters for seven major municipalities in Klang Valley namely Kuala Selangor, Subang Jaya, Klang, Petaling Jaya, Shah Alam, Ampang Jaya and Selayang. The landfill is estimated to be completely filled by 2017 and current observation in 2015 showed that it is nearly fully filled (Zainab H *et al.*, 2015). Layers of covers have been placed onto most part of the landfill to prevent water seepage into the waste.

2.7 Generation of landfill leachate

Leachate is defined as liquid that has percolated through waste which contains dissolved or suspended materials. It arises from the biochemical and physical breakdown of wastes (Lu *et al.*, 1985; Nadiah *et al.*, 2012). Leachate may contain many different organic and inorganic compounds, suspended solids, heavy metals and other pollutants

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that can contaminate the ground water and surface water resources. Groundwater pollution can represent a health risk and will create many environmental problems if not properly handled (Kjeldsen *et al.*, 2002).

The discharge of leachate into the environment is considered under more restrictive views. This is because the risk of groundwater pollution is probably the most severe environmental impact from landfills because in the past, most landfills were built without engineered liners and leachate collection system (Kjeldsen *et al.*, 2002). The larger the landfill site, the more serious the impact of groundwater pollution. Therefore, leachate treatment is important and necessary in order to prevent or minimize these environmental problems.

Landfill leachate is produced via two main routes namely external water that enters the waste and within the waste cell.

i. Generation of leachate from outside the cells

Most landfill leachate originated from direct external water such as rainwater as it flows into the waste itself. It is formed when excess water percolates through the waste layers, thus removing the contaminant compound from the solid waste (Adhikari *et al.*, 2014). The water leaches and dissolves various constituents until it contains a load of heavy metals, chlorinated organic compounds and other substances (Christensen *et al.*, 2001). Finally, they become polluted liquid or leachate that can harm the nearby surface-water and groundwater. The leachate water quality worsens after mass of rainwater rinsed the landfill. Intensity, regularity and interval of rainfall affects the quantity of leachate production and the humid climate has strong influence on generation of leachate (Ahmed & Lan, 2012).

Malaysia's climate is hot and humid with relative humidity ranging from 80 - 90 percent except for highlands. It is dominated by the effect of two monsoons or "rainy seasons", which affect different parts of Malaysia to varying degrees. Heavier rainfall is experienced when the monsoon changes direction and usually during this time, large volume of leachate is produced as more precipitate pass through the waste in the landfill.

ii. Generation of leachate within the waste cell

When solid waste is disposed of and processed at landfills, it undergoes a combination of physical, chemical and microbial processes (Adhikari *et al.*, 2014). These processes transform waste into various water-soluble compounds and transfer the pollutants from the refuse to the percolating water (Kulikowska & Klimiuk, 2008).

The wet waste contains excess moisture either from its own moisture or the adsorbed moisture from environment (atmosphere or rainwater). Processes which involved compaction and organic decomposition of wet waste in landfill increase the moisture content and also the absorbed moisture (Vaidya, 2002). The waste moisture is produced during waste movement and placement which resulted in leachate generation.

Leachate is also produced by the anaerobic decaying process of organic components inside the waste which becomes heavily polluted liquid (Tengrui *et al.*, 2007). Its production rate is affected by the composition, pH, temperature and type of bacteria present in the waste. Generation of leachate also depends on several factors including quality of wastes, decaying or crumbling rate, techniques of landfilling, degree of waste compaction, age of landfill, and environmental factors such as humidity and precipitation.

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2.8 Process and Characteristics of Leachate

Landfill leachate mainly consists of large amounts of organic matter including dissolved organic matter, phenol, ammoniacal nitrogen, phosphate, heavy metals, sulphide, hardness, acidity, alkalinity, salinity, solids, inorganic salts, and other toxicant (Aziz *et al.*, 2009; Foul *et al.*, 2009; Kang *et al.*, 2002; Renou *et al.*, 2008; Wang *et al.*, 2002). Because of its increasing polluted contents, management of leachate has becoming more difficult for landfill operators and authorities.

Factors that affect the composition of landfill leachate include the composition of the waste which can be determined by knowing the nature of the waste (solid or liquid), the source of the waste (municipal, industrial, commercial or mining) and the amount of precipitation in the waste (Adhikari *et al.*, 2013). Besides that, the age of the landfill also plays important role for the quality of the leachate. The composition of landfill leachates varies greatly depending on the age of the landfill (Baig *et al.*, 1999). Landfilling technique such as waterproof covers, liner requirements such as clay, geotextiles and/or plastics play remains primordial to control the quantity of water entering the tip and so, to reduce the threat of pollution (Lema *et al.*, 1988; Renou *et al.*, 2008). Other factors that also contribute to the quality of leachate include depth of waste, moisture availability, available oxygen and the processed waste (Adhikari *et al.*, 2013).

Municipal waste has great variation in composition and characteristics. The waste composition of refuse determines the extent of biological activity within the landfill (Adhikari *et al.*, 2014). Rubbish, food, garden wastes, and animal residues contribute organic material in leachate (Christensen *et al.*, 2001).

Inorganic components in leachate are often obtained from ash wastes, construction wastes and destruction debris (Christensen *et al.*, 2001). Ahmed and Can (2012) found that increased quantities of paper in solid waste resulted in a decreased rate of waste decomposition. This can be explained from the main component of the paper itself that is lignin. Lignin is resistant to anaerobic decomposition which is the primary means of degradation in landfills. Due to the variability of solid waste, only general assumptions can be made about the relationship between waste composition and leachate quality (Adhikari *et al.*, 2014).

i. The effect of landfilling age on leachate

Leachate is highly variable and heterogeneous. Quality of leachate is greatly influenced by the duration of time too. Leachate will undergo many types of reactions over time. Generally, leachate produced in younger landfills is characterized by the presence of substantial amounts of volatile acids, as a result of fermentation during the acid phase (Adhikari *et al.*, 2013).

In mature landfills, the great portion of organics in leachate are humic and fulvic-like fractions (Kulikowska & Klimiuk, 2008). A young leachate in the acidogenic phase is characterized by a high organic fraction and a Biochemical Oxygen Demand (BOD)/Chemical Oxygen Demand (COD) ratio greater than 0.4 (Tengrui *et al.*, 2007). The ratio will gradually decline during the first 10 years (Adhikari *et al.*, 2014).

Because of biodegradable nature, organic compounds decrease more rapidly than inorganic ones with increasing age of the landfill (Adhikari *et al.*, 2013). An older leachate in the methanogenic phase is not as easily biodegraded as a young leachate (Adhikari *et al.*, 2013). It contains obstinate organic compounds, high concentrations of ammonia and is characterized by higher pH values which will increases with time (Adhikari *et al.*, 2013). It reflects the decrease in concentration of the partially ionized free volatile fatty acids (Adhikari *et al.*, 2013).

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In general, variations in leachate quality due to age are expected throughout the landfill life because organic matter will continue to undergo stabilization (Adhikari *et al.*, 2014). Basically, it can be concluded that there are three types of leachate which are defined according to landfill age (refer Table 2.1).

Table 2.1 Landfill leachate classification vs. age (Alvarez-Vazquez *et al.*, 2004)

Components/ Characteristics	Young leachate	Medium leachate	Old leachate
Age (year)	<1	1-5	>5
pH	<6.5	6.5-7.5	>7.5
COD (g/L)	>15	3.0-15.0	<3.0
BOD_5/COD	0.5-1	0.1-0.5	<0.1
TOC/COD	<0.3	0.3-0.5	>0.5
NH_3-N (mg/L)	<400	400	>400
Heavy metals (mg/L)	>2.0	<2.0	<2.0
Organic compound	80% Volatile fat acids	5-30% Volatile fat acids Humic acids Fulvic acids	Humic acids Fulvic acids

The different landfilling technology also affects the quality and quantity of leachate. Flood control system is useful to assist surface-water discharge. The clay layer on the bottom of landfill used to control the inflow of surface water or groundwater into the landfill. The content of organic matter in the leachate normally is significantly higher than normal wastewater (Liu, 2013). Using normal clay to prevent infiltration of leachate into the groundwater or surface is normally less successful. This situation will reduce the concentrations of leachate but will greatly increase the volume of leachate (Wang *et al.*, 2006).

Based on the research by Tatsi *et al.* (2002), Kang *et al.* (2002) and World Health Organization (2006), greater concentrations of constituents are found in leachate from deeper landfill sites. However, deeper landfills require more water to reach saturation besides it requires a longer time for decomposition, and distribution. Water will travel down through the waste collected in the landfills. In general, when water permeates through the landfill, it come to contacts with the refuse and seeps chemicals from the wastes. Landfills of greater depth offer greater contact times between the liquid and solid phases which increase leachate strength (Tränkler *et al.*, 2005).

According to Barnes *et al*. (2004), moisture addition has demonstrated repeatedly to have a stimulating effect on methanogenesis although some researchers indicate that it is the movement of moisture through the waste of landfill site (Aziz *et al.*, 2010; Zouboulis *et al.*, 2004). Moisture within the landfill functions as a reactant in the hydrolysis reaction. Besides that, it also transports nutrients and enzymes, dissolves metabolites, provides pH buffering, dilutes inhibitory compounds, exposes surface area to microbial attack, and controls microbial cell growth (Aziz *et al.*, 2010). Some of the researchers stated that high moisture flow rates can flush soluble organics and microbial cells out of the landfill (Aziz *et al.*, 2010; Tatsi & Zouboulis, 2002; World Health Organization, 2006). In such cases microbial activity plays a lesser role in determining leachate quality.

Oxygen level in the landfill site can determines the decomposition process that takes place whether in aerobic or anaerobic condition. At the initial stage, aerobic decomposition occurs and it continues at the surface area where oxygen is readily obtainable (Amokrane *et al.*, 1997). Products of aerobic decomposition of wastes differs greatly from those of anaerobic degradation, where microbes degrade organic matter to CO_2, H_2O and release heat. Anaerobic degradation process release organic acids, ammonia, hydrogen, carbon dioxide, methane and water (Adhikari *et al.*, 2014). As level of oxygen reduced, transitional change takes place and anaerobic decomposition occurs as oxygen is depleted.

Physical state of waste greatly affects landfill leachate characteristics. Shredded or baled waste which is highly contaminated during early waste stabilization stage produce higher strength leachate that has high concentrations of pollutants as compared with leachate from un-shredded waste (Adhikari *et al.*, 2014). This could be due to higher surface area of the waste and consequently, increased rates of biodegradation in shredded wastes in the landfill (Robinson, 2007). According to Chu *et al*. (1994), rate of pollutant removal, solid waste decomposition, and cumulative mass of pollutants released per unit volume of leachate was significantly increased when compared to un-shredded waste fills.

Baling of waste will produce leachate which is more diluted as water is drawn out faster and the waste stabilized quicker. Generally, baling of wastes can improve leachate production by diminishing the elapse time before leaching. It likewise reduces the moisture-retention ability of the waste, and increase the general volume of the leachate produced (Aderemi *et al.*, 2011). Nonetheless, once the field limit of the shredded or baled refuse is achieved, the total mass of pollutant evacuation per unit volume of solid waste would be the same (Aderemi *et al.*, 2011).

Definition of compositions in leachate is difficult, diverse and time-consuming (Rowe *et al.*, 2004). The typical data of the composition of leachate from new and mature landfill indicated that the leachate contains pollutant loads larger than many industrial wastes (Tchobanoglous *et al.*, 1993). The conditions within a landfill differ over time from aerobic to anaerobic thus allowing different chemical reactions to take place. The compositions of leachate can be divided into four parts of pollutants; organic matter such as COD and TOC (total organic carbon); specific organic compounds; inorganic compounds; and heavy metals (Christensen *et al.*, 2001). However, the organic content of leachates is often measured through analyzing sum of parameters such as COD, BOD, TOC and dissolved organic carbon. Typical ranges of the concentration of selected parameters in leachate are shown in Table 2.2.

Table 2.2 Typical chemical composition of landfill leachate - concentration ranges (mg/L) (Crutcher & Yardley, 1991).

Parameter	Range (mg/l)
pH (no units)	3.7- 9
Hardness	400- 2,000
Total Dissolved Solids (TDS)	0- 42,300
Chemical Oxygen Demand (COD)	150- 6,000
Biochemical Oxygen Demand (BOD)	0- 4,000
Total Kjeldahl Nitrogen (TKN)	1- 100
Ammonia	5- 100
Nitrate	<1- 0.5
Nitrite	<1
Sulphate (SO_4)	<1- 300
Phosphate (PO_4)	1- 10

ii. Characteristics of Landfill Leachate

The characteristics of the landfill leachate can usually be represented by the basic parameters of COD, BOD, the ratio of BOD/COD, pH, suspended solids (SS), ammonium nitrogen (NH3-N), total Kjeldahl nitrogen (TKN) and heavy metals (Renou *et al.*, 2008).

Leachate is generally found to have pH between pH 4.5 and pH 9 (Christensen *et al.*, 2001). The pH of young leachate is less than pH 6.5 while old landfill leachate has pH higher than pH 7.5 (Abbas *et al.*, 2009). Initial low pH is due to high concentration of volatile fatty acids (VFAs) (Bohdziewicz *et al.*, 2008). Stabilized leachate shows fairly

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constant pH with little variations and it may range between pH 7.5 and pH 9 (Agbozu *et al.*, 2015). Kulikowska and Klimiuk (2008) and Tatsi and Zouboulis (2002) reported similar range of pH from old landfill sites, that is, pH 7.46 to pH 8.61 and pH 7.3 to pH 8.8, respectively.

BOD is a measure of the amount of oxygen used by microorganisms as they feed upon organic matter. The young landfill leachate is commonly characterized by high BOD of 4000 to 13,000 mg/L (W. Li *et al.*, 2010). The BOD will peak up at the early phase of the landfill operation from six months to two years (Dandautiya, 2012). The BOD becomes very deliquescent or more diluted as the leachate absorbs moisture, which is a main characteristic of BOD. The BOD value finally will start to reduce until the landfill is steady through the later six to 15 years (Dandautiya, 2012).

COD refers to a measurement of the quantity of oxygen for oxidation of organic compounds in a leachate by a strong oxidizing agent (Mohd Harun, 2012). Young landfill leachate is characterized by high COD of between 30,000 to 60,000 mg/L (Li *et al.*, 2010). The reduction of COD is slow but the decrease of BOD is fast by time as the leachate was processed. The reduction of BOD_5 or COD leads to reduced biochemical treatability of the leachate (Tyre & Dennis, 1997).

Leachate from MSW landfills typically has high values for total dissolved solids (TDS). TDS comprises mainly of inorganic salts and dissolved organics (Muhammad *et al.*, 2010). TDS is one of the parameters taken into consideration in licensing discharge of landfill leachate in many countries such as the United Kingdom (Koshy *et al.*, 2008). The amount of TDS reflects the extent of mineralization and a higher TDS concentration can change the physical and chemical characteristics of the receiving water (Al-Yaqout & Hamoda, 2003).

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Electrical conductivity or specific conductance of a solution is a measure of the ability of the leachate to convey an electrical current (Mohd Harun, 2012). It is associated with the quantity of dissolved salts present or ionized substances found in the leachate from both inorganic and organic species such as free volatile acids. Since the conductivity of acids depends on degree of dissociation, the conductivity measurement is pH dependant (Chian & DeWalle, 1975). In older leachate, the conductivity is mainly attributed to the presence of Na^+, K^+, and HCO_3^- ions and to a lesser extent to fulvic acids; the measurement becomes, therefore less pH dependent (Chian & DeWalle, 1975).

High concentration of salt in leachate mostly is chloride (200 - 3000mg/l) and phosphate (9 - 1600mg/l) are more serious when rainfall is lower (Dandautiya, 2012). A high concentration of inorganic salts, as well as, organic substances in the leachate indicates complicated equilibria existing between cations and anions (Yimer & Sahu, 2013). Thus we can expect that the majority of calcium, magnesium and iron exists in the form of complexes with various ligands and not as a free cations. This had to be taken under consideration when design an effective treatment system (Yimer & Sahu, 2013). Furthermore, the discharge of leachate with high salts content into fresh water such as river will alter the salinity and thereby affect the aquatics system (Johannessen, 1999).

According to Dandautiya (2012) the colour of leachate is orange brown to dark brown or black. The dark brown color of the leachate is mainly attributed to the oxidation of ferrous to ferric form and the formation of ferric hydroxide colloids and complexes with fulvic or humic substance (Mor et al., 2006). Leachate has malodorous smell, mainly due to the presence of organic acids, which come from the high concentration of decomposed organic matter (Dandautiya, 2012).

Another means for measuring the organic matter present in water is the total organic carbon (TOC) test, which is especially applicable to small concentrations of organic matter. Wastewater content of carbon bound in organic molecules is TOC. Organic carbon comprises nearly all carbon compounds except for a few carbon species which are looked at as inorganic such as carbon dioxide, hydrogen carbonate, carbonate, and cyanide (Mohd Harun, 2012).

iii. Variation in leachate characteristics

Despite all the reported typical leachate characteristics and quality, the actual properties are very well diverse and varied across the landfills. The characteristics cannot be expected to follow certain range or criteria but simple boundaries of range as published by other researchers could be used. The variation in leachate composition is simulated mainly by the heterogeneous composition of waste and different level of water penetration through the top cover of the landfill. The leachate composition for a given landfill cannot be forecasted from literature data since the parameters influencing its quality are difficult to validate (Dandautiya, 2012).

Study has shown that the composition of landfill leachate from the same or different waste source is highly variable. The composition of leachate and its emission rates also vary between the old and the new areas of the fill. The composition of landfill leachate can exhibit considerable spatial and temporal variations depending upon site operations and management practices, refuse characteristics, and internal landfill processes (El-Fadel *et al.*, 2002).

Figure 2.1 summarizes factors that are commonly known to affect the composition of landfill leachate. Refuse age and the corresponding landfill fermentation stage are usually major determinants of leachate composition. In terms of landfill site operation and management, how the refuse pre-treated, the irrigation and recirculation of

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percolation design and existence of liquid waste co-disposed with the refuse determines the leachate composition. This followed by the chemical and biochemical internal processes occurred involving factors such as hydrolysis, adsorption, biodegradation, speciation, dilution, partitioning, precipitation and etc forming the varied composition of leachate produced.

SITE OPERATIONS & MANAGEMENT
Refuse Pretreatment
Irrigation, Recirculation, Liquid Waste Codisposal

REFUSE CHARACTERISTICS
Composition, Age

INTERNAL PROCESSES
Hydrolysis, Adsorption, Biodegradation
Speciation, Dissolution, Dilution
Ion Exchange, Re-dox
Contact Time, Partitioning, Precipitation
Gas & Heat Generation & Transport

⇨ **LANDFILL LEACHATE COMPOSITION**

Figure 2.1 Factor influencing leachate composition in landfill (El-Fadel *et al.*, 2002).

2.9 Metals and Heavy Metals Content in Leachate

Heavy metals are one of the common environmental pollutants with renowned toxic effects on living systems. Because of their toxicities, heavy metals have been singled out for concern as environmental pollutants (Aucott, 2008). Due to the documented toxicity to organisms, certain metals have been specified by the U.S. Resource Conservation and Recovery Act (RCRA) of its groundwater limits.

The heavy metals, also termed as “RCRA heavy metals”, include Arsenic (As), Barium (Ba), Cadmium (Cd), Chromium (Cd), Lead (Pb), Mercury (Hg), Selenium (Se), and Silver (Ag). Other heavy metals such as Nickel (Ni), Copper (Cu), and Zinc (Zn) are also of concern. These metals are apparently not RCRA metals because at low levels they function as nutrients and also because they have not shown human toxicity at the

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same degree as the RCRA metals (Aucott, 2008). However, they can be toxic to other organisms and in some situations to humans as well. Typical heavy metal contents in landfill leachate is listed in Table 2.3 (Crutcher & Yardley, 1991).

Table 2.3 Typical heavy metals content of landfill leachate (Crutcher & Yardley, 1991).

Parameter	Range (mg/l)
Aluminum	<0.01- 2
Arsenic	0.01- 0.04
Barium	0.1- 2
Beryllium	<0.0005
Boron	0.5- 10
Bromide	<1- 15
Cadmium	<0.01
Calcium	100- 1,000
Chloride	20- 2,500
Cobalt	0.1- 0.08
Copper	<0.008- 10
Chromium	<0.01- 0.5
Fluoride	5- 50
Iron	0.2- 5,500
Lead	0- 5
Magnesium	16.5- 15,600
Manganese	0.06- 1,400
Nickel	0.4- 3
Potassium	3- 3,800
Selenium	0.004- 0.004
Sodium	0- 7,700
Zinc	0- 1,350

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Christensen *et al*. (2001) reported that the concentration of heavy metals in landfill leachate is dependent on the ages of the landfill. Concentration of heavy metals in a landfill is generally higher at earlier stages because of higher metal solubility as a result of low pH caused by production of organic acids (Kulikowska & Klimiuk, 2008). As a result of decreased pH at later stages, a decrease in metal solubility occurs resulting in rapid decrease in concentration of heavy metals except lead because lead is known to produce very heavy complex with humic acids (Harmsen, 1983).

The solubility and mobility of metals may increase in the presence of natural and synthetic complexing ligands such as EDTA and humic substances (Jones *et al.*, 2006). Furthermore, colloids have great affinity for heavy metals and a significant but highly variable fraction of heavy metals is associated with colloidal matter (Christensen *et al.*, 2001; Jensen & Christensen, 1999; Moh & Manaf, 2014).

According to Baun and Christensen (2004) , less than 30%, typically less than 10% of the total metal concentration is present in free metal ion forms and the rest is present in colloidal or organic complexes. Jensen and Christensen (1999) found that 10–60% of Ni, 30–100% Cu and 0–95% Zn were constituted in colloidal fractions. The solubility of metals can also increase because of the reducing condition of leachate which change the ionic state of the metals for example Cr (VI) to Cr (III), and As (V) to As (III) (Halim *et al.*, 2004; Jones *et al.*, 2006; Y. Li *et al.*, 2007; Sierra-Alvarez *et al.*, 2005).

2.10 Risks and problems associated with leachate management

In general, the most typical harmful effect of leachate discharge into the environment is groundwater pollution. Major problems in managing a landfill in a tropical country like Malaysia in managing the leachate that is generated when the water pass through the waste (Li *et al.*, 2009). Managing the leachate is the major problem in landfill operation. Leachate is formed when landfill waste degrades and mixes with rainwater running through the waste.

Table 2.4 EQA Standard B limit and the JSL leachate characteristics from previous studies

Parameters	EQA Standard B	(Emenike *et al.*, 2013b)	(Norazela *et al.*, 2014)	(Mansor *et al.*, 2011)
BOD_5	20	27,460	320	15.97
COD	400	51,200	2050	1222
pH	6.0-9.0	7.35	8.78	7.72
TDS	-	1730	-	-
NH_3-N	5.0	880	745	-
Oil&Grease	5.0	48	-	-
Pb	0.10	-	-	13.3
Zn	2.0	828	-	15.2
Fe	5.0	98	-	-
Mn	0.20	541	-	-

*All units in mg/l except for pH; (-) is not available/detected.

The Environment Quality Act (1974) limits were developed to ensure that any effluent must comply with Standard B which is discharged into any other inland water or effluent in downstream. From the Table 2.4 majority of the readings in previous studies were above the permissible limits, including the metals concentrations in the leachate. Even if the municipal solid waste is used for disposal of non-hazardous solid waste, toxic and carcinogenous chemicals have been identified in several landfill leachates (Baig *et al.*, 1999). The composition of leachate made it very toxic and due to that it can have negative impacts at both surface and groundwater environments. Impacts on the water environment are detrimental to human, animal and plants.

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During acetogenic stage of the biodegradation phase in landfill, the leachate has high content of most pollutants such as COD, BOD, sodium, chloride, ammonium and electrical conductivity (Mukherjee *et al.*, 2015). Jones *et al*. (2006) stated in their research that those constituents are toxic to aquatic life and can have serious consequences if leachate enters surface water sources.

Under aerobic condition, ammonium (NH_4^+) in the leachate can be rapidly transformed by nitrification to nitrate (NO_3^-) which is less toxic and can be absorbed by plants. But, at the point when nitrate is consolidated with phosphate, the condition can prompt eutrophication of surface water courses (Jones *et al.*, 2006). Algae blooms deplete oxygen levels in aquatic ecosystems and thus have a detrimental effect on the organisms within the system (Fried *et al.*, 2012).

Major potential environmental impact of leachate release to surface water is ammonia toxicity (Emenike *et al.*, 2013b). Pivato and Gaspari (2006) stressed that the danger of the leachate may rely upon ammonia concentration and that leachate toxicity is much lower in old landfills where ammonia had been degraded. Study by Emenike *et al*. (2013) found that NH_3-N concentrations show no decreasing trend with time and may range from 500 to 2000 mg/L in old landfills. More than 100 mg/L of NH_3-N is considered extremely toxic to aquatic organisms as demonstrated in toxicity tests using zebra fish (Emenike *et al.*, 2013b). The toxic effect is better explained by the fact that at molecular form (NH_3), it can easily permeate tissue membrane once concentration gradient exists (Emenike *et al.*, 2013b).

In other studies on the toxicity of municipal landfill leachate, Sang *et al*. (2006) and Schrab *et al*. (1993) reported that leachate can have genotoxic effects on plants and bacterial cells. Exposure to leachate pollution in an aquatic environment is likely to pose a risk of generation of 'cytogenetic damage' in organisms (Sang *et al.*, 2006). On the other hand, landfill leachate is also unsafe to sanitation as it contains harmful microorganisms. Leachate may contain *E. coli* and *Streptococcus sp.* in amounts of about 10^6 to10^7 per 100 cm^3 (Bodzek *et al.*, 2006). Leachate migration from landfills and the release of pollutants from sediments (under certain conditions) pose a high risk to groundwater resource if not adequately managed (Akinbile & Yusoff, 2011).

Various individual chemical components found in leachate are known to pose health risks and aesthetic concerns for humans if present in drinking water. Phthalate esters and other plasticisers, for example, adipates, leached from plastic products, primarily PVC, under landfill conditions also become main concern to human health (Mersiowsky, 1999). Those plasticisers are currently omnipresent in the environment and are normally reported in fresh waters and industrial discharges (Klinck & Stuart, 1999). The compounds from plasticisers are microbially degraded, either aerobically or under methanogenic conditions to carbon dioxide. However, in the acetogenic phase the degradation has been shown to be slower (Ejlertsson *et al.*, 1996).

The presence of bis (2-ethylhexyl) phthalate in landfill leachate which has shown to be carcinogenic in laboratory animal experiments were detected in leachates of previous researchers (Klinck & Stuart, 1999).

Young leachate which has high volatile fatty acid (VFA) content has pH that is less than pH 7 and also high concentrations of heavy metal as listed in Table 2.1. To some extent, metal content is a function of the waste stream composition. Studies of leachate in Bandung, Indonesia; Bangkok, Thailand; and León, México have found that it contained high chromium level which originated from wastes produced during the manufacture of leather (Klinck & Stuart, 1999). On the other hand, manganese and zinc are also found to be generally high in acetogenic leachates (Klinck & Stuart, 1999).

Once leachate enters the environment it naturally degrades by physical, geochemical and microbial attenuation processes. Leachate will be transported as plume in groundwater by three mechanisms namely diffusion, convection and dispersive transport (Lee & Jones, 1993). Landfill leachate with high content of heavy metal will contaminate nearby groundwater which may be consumed by human, plant and animals.

Moreover, groundwater which is contaminated by landfill leachate may also contain high quantities of organics. Presence of organics can cause taste and odour problems and oxygen depletion in groundwater. Chemicals comprising organics may also affect public health if the water is consumed (Lee & Jones, 1993).

2.11 Current Leachate Treatment Options

Nowadays, landfill regulations in many countries have necessitates the installation of liners and leachates collection system, as well as, a plan for leachate treatment (Schiopu & Gavrilescu, 2010). Christensen *et al*. (1994; 2001) reviewed the characteristics of leachate plumes down gradient of landfills. For that reason, the treatment of leachate is very important before it is discharged into water bodies to avoid pollution to the ground and surface soil and to prevent both severe and continual toxicity (Öman & Junestedt, 2008; Sanphoti *et al.*,2006; Tatsi *et al.*, 2003).

There are several options in treating leachate. The treatment method of choice depends on the composition of the leachate. It also depends on specific bacterial contaminants that may be present in the leachate and the local temperature and its seasonal variation (Grisey *et al.*, 2010; Kjeldsen *et al.*, 2002). As waste sent to landfill increases from day to day, cost of managing the leachate will also increase. Thus, a more cost effective method of leachate treatment before discharging to water body is important to sustain the landfill.

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Many different methods are currently in use to treat landfill leachate. Most of these methods are adapted from wastewater treatment processing and can be divided into two main categories: physical/chemical treatments and biological treatments (Inanc *et al.*, 2000). Current method of leachate treatment uses physical and chemical reactions. It is costly and not environmental friendly. Biological treatments use microorganisms in bioremediate the leachate as it significantly reduces the cost and posed least effect to environment.

Besides that there is also natural treatment system whereby constructed wetland needs to be utilized. In the following section, wetland treatment is discussed, followed by physical/chemical treatments and lastly biological treatments.

2.12 Natural and Constructed Wetland System

Natural wetland systems have often been described as the "earth's kidneys" because they filter pollutants from water that flows through on its way to receiving lakes, streams and oceans. One of their most important functions of natural treatment systems are water filtration (Yilmaz & Akbulut, 2011). As water flows through a wetland, it slows down and many of the suspended solids become trapped by vegetation and settled. Other pollutants are transformed to less soluble forms to be taken up by plants or become inactive (Kadlec & Wallace, 2008).

Engineers and scientists tried to construct systems that replicate the functions of natural wetlands, to improve water quality. Constructed wetlands (CWs) are treatment systems that use natural processes involving wetland vegetation, soils, and their associated microbial assemblages to improve water quality (Kadlec & Wallace, 2008). These systems, mainly comprised of vegetation, substrates, soils, microorganisms and water, utilize complex processes involving physical, chemical, and biological mechanisms to remove various contaminants or improve the water quality. Numerous studies have focused on the design, development, and performance of CWs, and it was also reported that CWs could be efficient for removing various pollutants (organic matter, nutrients, trace elements, pharmaceutical contaminants, pathogens, etc.) from wastewater (Wu *et al.*, 2015).

However, constructed wetland has limitation in treating leachate. The process rates are dependent upon various environmental factors such as temperature, pH, oxygen availability, hydraulic and pollutant loads (DWLC, 1998a). The chemical and biological processes are specifically prone to changes in environmental factors. Under some environmental conditions, process rates may slow down or cease altogether, or even reverse, releasing pollutants (Sundaravadivel & Vigneswaran, 2001).

According to Sundaravadivel and Vigneswaran (2001), the effectiveness of pollutant removal processes that rely on biological activities may be reduced due to decrease in metabolic activities caused by low temperature. Many metabolic and chemical activities are also pH dependent, and are less effective if pH is too high or too low (Sundaravadivel & Vigneswaran, 2001).

Furthermore, hydraulic and pollutant loading rates also limit the capacity of constructed wetland. Hydraulic overloading occurs when the flow exceeds the design capacity, thus reducing the actual hydraulic retention time. Pollutant overload occurs when the influent pollutant loads exceed the process removal rates of the system (Sundaravadivel & Vigneswaran, 2001). Other environmental factors, including excessive organic matter, nutrient or toxins, or lack of oxygen, also have effects on wetland processes.

The salinity of water within wetlands can increase as the water levels drop, and the pollutants may become concentrated depending on the size and design of wetland. Successive high flows may flush pollutants from the system and transporting them to the discharging water bodies (Sundaravadivel & Vigneswaran, 2001).

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2.13 Physical and chemical treatments

Physical-chemical treatment uses physical and/or chemical properties of the contaminants or of the contaminated medium to destroy (i.e., chemically convert), separate, or contain the contamination. In the chemical processes the chemical structure (and then the behavior) of the contaminants is changed by means of chemical reactions to produce less toxic or better separable compounds from the solid matrix (Erdogan & Karaca, 2011).

Physical and chemical processes include reduction of suspended solids, colloidal particles, floating material, color, and toxic compounds by flotation, coagulation/flocculation, adsorption, chemical oxidation and air stripping (Mojiri *et al.*, 2013). Physical/chemical treatments for landfill leachate are used in addition to treatment line (pre-treatment or last purification) or to treat a specific pollutant (ammonia stripping) (Renou *et al.*, 2008). However, physical-chemical processes are generally considered to incur high operating costs and sometimes have lower effectiveness.

i. Adsorption

Adsorption is the physical process through which a substance, originally present in one phase, is removed by accumulation at the interface between that phase and a separate solid phase (Pandhare *et al.*, 2013). The adsorption process is used as a stage of integrated chemical-physical-biological process for landfill leachate treatment, or simultaneously with a biological process (Geenens *et al.*, 2001; Kargi & Yunus Pamukoglu, 2003; Wiszniowski *et al.*, 2006). The most frequently used adsorbent is granular or powdered activated carbon. Renou (2008) stated that the adsorption of pollutants onto activated carbon provides better COD reduction than the chemicals methods.

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Consequently, activated carbon adsorption aims to (i) make sure final polishing level by removing toxic heavy metals or organics i.e., Adsorbable Organic Halides (AOXs), Polychlorinated Biphenyls (PCB) and (ii) support microorganisms (Wiszniowski *et al.*, 2006). There are also other materials that were tested as adsorbents and have given treatment performances close to those obtained with activated carbon such as zeolite, vermiculite, illite, keolinite, activated alumina and municipal waste incinerator bottom ash (Amokrane *et al.*, 1997).

ii. Chemical Precipitation

Chemical precipitation is defined as the formation of solids in the solution as the result of chemical reaction (Butkovskyi, 2009). In the case of leachate treatment, chemical precipitation is widely used as pre-treatment in order to remove high strength of ammonium nitrogen ($NH4^+$-N) (Renou *et al.*, 2008). In a study, Li *et al.* (1999) confirmed that the performance of a conventional activated sludge process could be significantly affected by a high concentration of NH_4^+-N.

Ammonium is removed in the mineral form of magnesium ammonium phosphate ($MgNH_4PO_4 \cdot 6H_2O$), which is better known as struvite (Butkovskyi, 2009). The magnesium compound ($Mg(OH)_2$, MgO, $MgCl_2$ and phosphoric acid (H_3PO_4) have to be dosed for this reaction to occur, as Mg- and P-containing substances usually occur in very low quantity, comparatively to the ammonium compounds, which have to be removed (Kabdasli *et al.*, 2000). The process is described by the following reaction (Çelen & Türker, 2001):

$$Mg^{2+} + NH4^{+} + HPO_4^{2-} + 6H_2O \rightarrow MgNH_4PO_4 \cdot 6H_2O + H^{+}$$

The pH and temperature of wastewater are also factors in determining the solubility and formation rate of struvite (Ariyanto *et al.*, 2011). Alkaline and increasing pH levels of the wastewater increase the potential of struvite crystallization (Chemtrade, 2014). As with most crystals the buildup of struvite begins with the seeding of eventual growth of the crystal, as long as the condition remains favorable for continual crystal growth (Chemtrade, 2014). Struvite could be applied as the slow-released additive to fertilizer because it doesn't contain any toxic substances (Butkovskyi, 2009). However, struvite precipitation is quite an expensive method due to the high cost of phosphorous and magnesium salts (Butkovskyi, 2009). Another problem is clogging of pipes and connections with precipitated struvite, which has to be removed by pressurized washing, and reduction of service life period of equipment.

Precipitation is the most commonly used technique for phosphorous removal from different types of wastewater. Aluminium, iron salts or lime could be used, preferably Al^{3+}salts which is the most effective for phosphorous precipitation (Panasiuk, 2010). Phosphorous removal is not usually focused while handling leachate. Its concentration is generally neglectable compared to organic and nitrogen concentrations. Still, if the leachate should be released to the environment, particularly into surface water, the discharge limits for phosphorous are strict (0.3 to 0.5 mg/l in Sweden) and phosphorous precipitation could be used (Butkovskyi, 2009).

iii. Ammonium stripping

High levels of ammonium nitrogen are usually found in landfill leachate and stripping can be successful to eliminate it (Marttinen *et al.*, 2002). Due to its effectiveness, ammonium stripping is the most widely utilized treatment for the removal of NH_3-N from landfill leachate. According to Butkovskyi (2009), ammonia stripping is driven by intensive aeration of treated leachate at high pH (10.5 – 11.5). The mechanism of the process is running in the stripping tower, filled with aerated media, which is overflowed by leachate (Butkovskyi, 2009). The treated leachate then is collected at the bottom of the tower and gases raise up to the top. The air polluted with ammonium need to be treated with H_2SO_4 or HCl (Antonello, 2007). Recirculation of treated leachate is often required to achieve discharge limits (Butkovskyi, 2009).

The main concern about ammonia stripping is the release of NH_3 into the atmosphere that cause severe air pollution if ammonia cannot be properly absorbed with either H_2SO_4 or HCl (Wiszniowski *et al.*, 2006). Besides that the treatment itself could be cost-efficient only at very high ammonium concentrations in the leachate (Renou *et al.*, 2008). Costs spent on lime addition for increasing pH before the treatment and acid addition afterwards can be significantly high (Butkovskyi, 2009).

iv. Chemical oxidation

Chemical oxidation is a widely studied method for the treatment of effluents containing refractory compounds such as landfill leachate. Chemical oxidation is required for the treatment of wastewater containing soluble organic non-biodegradable and/or toxic substance (Marco *et al.*, 1997). Growing interest has been recently focused on Advanced Oxidation Processes (AOP). Most of them, except simple ozonation (O_3), use a combination of strong oxidants, e.g. O_3 and H_2O_2, irradiation, e.g. ultraviolet (UV), ultrasound (US) or electron beam (EB), and catalysts, e.g. transition metal ions or photocatalyst (Renou *et al.*, 2008).

Wang *et al*. (2002) confirmed that AOP, adapted to old or well-stabilized leachate, are applied to: (i) oxidize organics substances to their highest stable oxidation states i.e. carbon dioxide and water (i.e. to reach complete mineralization) and (ii) improve the biodegradability of recalcitrant organic pollutants up to a value compatible with subsequent economical biological treatment. The mechanism of AOP usually is mixing the oxidative agent with treated water in treatment chamber. Aqueous hydrogen peroxide usually is easier to mix, than gaseous ozone. Thus, ozone is often difficult to utilize effectively (Butkovskyi, 2009). Most of the time it is necessary to recirculate the leachate several times through the treatment unit to achieve better removal efficiency. As the costs for advanced oxidation are high, it is not used as a main treatment step – easily degradable organic compounds should be preliminary removed in a less expensive biological process (Stegmann *et al.*, 2005).

v. Membrane techniques

Membrane filtration is a physical process defined as the separation of solid particles from a liquid or gas primarily based on size difference (Anand & Singh, 2014). It includes processes such as reverse osmosis (RO), nanofiltration (NF), ultrafiltration (UF) and microfiltration (MF). Nanofiltration (NF) and reverse osmosis (RO) usually concentrate about 25% of initial flow, which has to be either further concentrated and treated as solid waste, or returned to the contaminated leachate (Butkovskyi, 2009).

To prevent clogging, membranes are treated by chemicals, such as combination of acid, caustic soda and hypochlorite solutions (Butkovskyi, 2009). However, there are some drawbacks of membrane process when clogging occurs that chemicals are required to clean the membrane. Besides that, the disintegration and leakage of the membrane may cause pollution of the receiving waters (Butkovskyi, 2009).

2.14 Heavy metals removal from landfill leachate

Landfill leachate contains significant amounts of heavy metals due to disposal of metal-containing waste into sanitary landfills (Cecen & Gursoy, 2000). This arises since metals are solubilised during landfill stabilisation. Metal reduction in leachate can be achieved by physicochemical treatment as a preliminary step to biological treatment or by complete treatment (Cecen & Gursoy, 2000).

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Physicochemical removal processes are needed to reduce the metal concentrations to levels that will not inhibit biological processes (Cecen & Gursoy, 2000). Both the discharge standards into sewers and into receiving waters vary from one country to another. In leachates the major heavy metals reported are Fe, Zn, Pb and Cu. Precipitation, co-precipitation, coagulation, flocculation and adsorption mechanisms are all effective in heavy metal removal, but their application to landfill leachate still presents problems.

2.15 Biological treatments

Biological treatment is a biodegradation processes of leachate carried out by microorganisms, which degrade organic compounds to carbon dioxide and sludge under aerobic conditions and to biogas (a mixture comprising chiefly CO_2 and CH_4) under anaerobic conditions (Lema *et al.*, 1988). Biological treatment whether as suspended or attached growth, is commonly used for the removal of the bulk of leachate containing high concentrations of BOD due to its reliability, simplicity and high cost-effectiveness (Wan Razarinah *et al.*, 2011).

Biological treatment can be divided into two namely aerobic or anaerobic depending on whether or not the biological processing medium requires O_2 supply. In aerobic processing, organic pollutants are mainly transformed into CO_2 and solid biological products (sludge) by using the atmospheric O_2 transferred to wastewater. In anaerobic treatment organic matter is converted into biogas, moisture comprising chiefly CO_2 and CH_4 and in a minor part into biological sludge (Abbas *et al.*, 2009). Organic and nitrogenous matters from immature leachate when the BOD/COD ratio has a high value (> 0.5) can be effectively removed by using biological process (Renou *et al.*, 2008). With time, the major presence of refractory compounds (mainly humic and fulvic acids) tends to limit the process effectiveness (X. Li & Zhao, 2001).

Biodegradation of contaminated substrate such as landfill leachate by living organisms formed one promising treatment method. It is widely studied using various types of organisms such as bacteria, fungi and plant species. Various types and genus of the organisms have been extensively studied, tested and even applied to combat rampant problems arose from environmental pollutions in many places.

Microorganisms that carry out biodegradation in many different environments are identified as active members of microbial consortiums. These microorganisms include: *Acinethobacter, Actinobacter, Acaligenes, Arthrobacter, Bacillins, Berijerinckia, Flavobacterium, Methylosinus, Mycrobacterium, Mycococcus, Nitrosomonas, Nocardia, Penicillium, Phanerochaete, Pseudomonas, Rhizoctomia, Serratio, Trametes* and *Xanthofacter* (Ravindra Singh, 2014).

Microorganisms individually cannot mineralize most hazardous compounds. Complete mineralization results in a sequential degradation by a consortium of microorganisms and involves synergism and co metabolism actions. Natural communities of microorganisms in various habitats have an amazing physiological versatility, they are able to metabolize and often mineralize an enormous number of organic molecules. Certain communities of bacteria and fungi metabolize a multitude molecules that can be degraded is not known but thousands are known to be destroyed as a result of microbial activity in one environment or another. Most bioremediation systems are run under aerobic conditions, but running a system under anaerobic conditions (Colberg & Young, 1995) may permit microbial organisms to degrade otherwise recalcitrant molecules. The consecutive sections discuss the bioremediation of landfill leachate.

2.16 Bioremediation as future treatments

Bioremediation is one of the methods in biological treatment. Bioremediation is defined as use of biological processes to degrade, break down, transform, and/or essentially remove contaminants or impairments of quality from soil and water. It is a natural process which relies on bacteria, fungi, and plants to alter contaminants as these organisms carry out their normal life functions (Pathak, 2011). Metabolic processes of these organisms use chemical contaminants as an energy source, rendering the contaminants harmless or less toxic in most cases (Donlon & Bauder, 2006). Bioremediation technology exploits various naturally occurring mitigation processes including natural attenuation, biostimulation, and bioaugmentation.

Bioremediation uses biological agents, mainly microorganisms, yeast, fungi or bacteria to clean up contaminated soil and water (Strong & Burgess, 2008).This technology relies on promoting the growth of specific microflora or microbial consortia that are indigenous to the contaminated sites that are able to perform desired activities (Agarwal, 1998). Establishment of such microbial consortia can be done in several ways, e.g. by promoting growth through addition of nutrients, by adding terminal electron acceptor or by controlling moisture and temperature conditions, among others (Agarwal, 1998; Hess *et al.*, 1997; Smith *et al.*, 1998). In bioremediation processes, microorganisms use the contaminants as nutrient or energy sources (Agarwal, 1998; Hess *et al.*, 1997; Tang *et al.*, 2007).

Bioremediation has existed in the world since approximately 600BC. Even in the ancient Roman, microorganisms was used to treat wastewater (Le, 2013). However, in 1972 the concept of bioremediation was recognized as the first commercial application upon a case study (Alvarez & Illman, 2005). This concept becomes one of the most significant and useful future prospects in the environmental field. Until now, many methods have been developed to improve bioremediation process to treat pollutants.

The most important thing in bioremediation process is the microorganisms itself. It must be active and healthy for bioremediation to take place. For bioremediation to be

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effective, microorganisms must enzymatically attack the pollutants and convert them to harmless products. As bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate (Rathoure, 2015).

Bioremediation technologies assist microorganisms' growth and increase microbial populations by creating optimum environmental conditions for them to detoxify the maximum amount of contaminants (Le, 2013). The specific bioremediation technology used is determined by several factors including type of microorganisms present, site conditions, and quantity and toxicity of contaminant (Le, 2013). Different microorganisms degrade different types of compounds and survive under different conditions.

Bioremediation approaches are generally classified as *in situ* or *ex situ*. *In situ* bioremediation involves treating the polluted material at the site while *ex situ* involves the treatment of the polluted material elsewhere (Megharaj *et al.*, 2011). *In situ* bioremediation is the application of biological treatment to clean-up hazardous chemicals present in the subsurface (Sharma, 2012).

i. *In-situ* bioremediation

The optimization and control of microbial transformations of organic contaminants require the integration of many scientific and engineering disciplines. The *in-situ* process includes bioventing, biosparging, biostimulation, bioaugmentation and phytoremediation (Vidali, 2001).

i. Bioventing is the most common *in-situ* treatment and involves supplying of air and nutrients through wells to contaminated soil to stimulate the indigenous

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bacteria (Husni, 2008). Bioventing employs low air flow rates and provides only amount of oxygen necessary for the biodegradation while minimizing volatilization and release of contaminants to the atmosphere (Vidali, 2001).

ii. Biosparging involves the injection of air under pressure below the water table to increase groundwater oxygen concentrations and enhance the rate of biological degradation of contaminants by naturally occurring bacteria (Osman, 2013). Biosparging increases the mixing in the saturated zone and thereby increases the contact between soil and groundwater. The ease and low cost of installing small-diameter air injection points allows considerable flexibility in the design and construction of the system (Osman, 2013).

iii. Biostimulation is the addition of substrates, vitamins, oxygen and other compounds that stimulate microorganism activity so that they can degrade the waste faster. Biostimulation of microorganisms by the addition of nutrients because the input of large quantities of carbon sources tends to result in a rapid depletion of the available pools of major inorganic nutrients such as N and P (Lee *et al.*, 2007)

iv. Bioaugmentation is the introduction of a group of natural microbial strains or a genetically engineered variant to treat contaminated soil or water. It is commonly used in municipal wastewater treatment to restart activated sludge bioreactors. Most cultures available contain a research based consortium of microbial cultures, containing all necessary microorganisms (Sharma, 2012).

v. Phytoremediation is an emerging technology that uses plants to remove contaminants from soil and water (Vidali, 2001). Phytoremediation or vegetation- based remediation shows potential for accumulating, immobilizing, and transforming a low level of persistent contaminants. In natural ecosystems, plants act as filters and metabolize substances generated by nature.

ii. *Ex-situ* bioremediation

The contaminated material could also be excavated and treated off site which is often a faster method of decontaminating the area. The techniques that can be used include land farming, composting, biopiles and bioreactors (Vidali, 2001).

"Land farming" involves a simple method of excavating the contaminated soil and spreading over a prepared bed and it is periodically tilled until pollutants are degraded. The idea is to stimulate the growth and metabolism of indigenous biodegradative microorganisms and facilitate aerobic degradation of contaminants (Kulshreshtha *et al.*, 2014). In general, the practice is limited to the treatment of thin layer of 10–35 cm soil only (Vidali, 2001).

Besides that, composting is another technique that involves mixing contaminated soil with nonhazardous organic components such as manure or agricultural wastes. The presence of these organic materials supports the development of a rich microbial population and elevated temperature characteristic of composting (Vidali, 2001).

On the other hand, biopiles are a hybrid between land farming and composting. Essentially, engineered cells are constructed as aerated composted piles. Typically used for treatment of surface contamination with petroleum hydrocarbons, they are an improved version of land farming that aims to control physical losses of the contaminants by leaching and volatilization (Kumar *et al.*, 2011). This method provides a favorable environment for indigenous aerobic and anaerobic microorganisms (Lee *et al.*, 2007).

Furthermore, other technique used is bioremediation in reactor or bioreactor that involves the incubation of contaminated solid material (for example soil, sediment or sludge) or liquid contaminant through an engineered contained vessel system. A slurry

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bioreactor may be defined as a containment vessel and apparatus used to create a three-phase (solid, liquid, and gas) mixing conditions to increase the bioremediation rate of soil bound and water-soluble pollutants. The water slurry of the contaminated soil and biomass usually contains indigenous microorganisms and is capable of degrading target contaminants (Vidali, 2001).

In study by Paisio *et al.* (2014) two bacterial strains isolated from polluted environments were able to remove several phenolic compounds not only from synthetic solutions but also from effluents derived from a chemical industry and a tannery. *Acinetobacter sp. RTE1.4* showed ability to completely remove 2-methoxyphenol (1000mg/L) while *Rhodococcus sp. CS1* not only degrade the same concentration of this compound but also removed 4- chlorophenol, 2,4-dichlorophenol and pentachlorophenol with high efficiency.

In study by Marina *et al.* (2013) a bacterial specie identified as *Bacillus cereus* isolated from oily wastewater of automotive workshop have shown to be able to degrade oily wastewater component in range 3% to 91%. The specie grew optimally in the oily wastewater as the only carbon source.

Bioremediation of municipal wastewater study by Sonune and Garode (2015) have isolated several species of bacteria namely *B. licheniformis NW16, Ps. Aeruginosa NS19, Pseudomonas sp. NS20, P. salinarum NS23, S. maltophilia NS21, Paenibacillus borealis NS3, Paenibacillus sp. NW9* and *Aeromonas hydrophilia NS17* and showed significant degradation of organic matter in term of BOD, COD, nitrate, phosphate, TSS and TDS.

However, like other technologies, bioremediation has its limitations. Some of the contaminants, such as chlorinated organic or high aromatic hydrocarbons are resistant to microbial attack and this will slow the degradation of contaminants degraded (Vidali,

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2001). Hence it is not easy to predict the rates of clean-up for a bioremediation exercise since there are no rules to predict if a contaminant can be degraded (Vidali, 2001). Of all the limitation, bioremediation is still the most economical compared to the traditional method such as incineration (Kumar *et al.*, 2011). This method can be the most acceptable technology as it based on natural attenuation. Moreover, it also can be the best method to treat landfill leachate.

2.17 Heavy metal bioremediation by bacteria

Metals play an integral role in the life processes of living organisms. Heavy metals defines as metals with densities of higher than 5 g/cm^3 (Abbas *et al.*, 2009; J.-Z. Chen *et al.*, 2005; X. C. Chen *et al.*, 2005; Kumar *et al.*, 2010). Some metals (Ca, Co, Cr, Cu, Fe, K, Mg, Mn, Na, Ni and Zn) are essential, serve as micronutrients and are used for redox-processes, to stabilize molecules through electrostatic interactions; as components of various enzymes; and regulation of osmotic pressure (Rathoure, 2015). While many other metals (Ag, Al, Cd, Au, Pb, and Hg) have no biological role and they are nonessential. Furthermore, these kind of metals have high potential to be toxic to living organism specially microorganisms (Rathoure, 2015). Toxicity of nonessential metals occurs through the displacement of essential metals from their native binding sites or through ligand interactions. Heavy metals in waste water come from industries and municipal sewage, and they are one of the main causes of water and soil pollution (Lloyd & Lovley, 2001).

Low concentrations of certain metals such as Zn, Cu, Co and Ni are essential for the metabolic activity of bacterial cells. Other metals like Pb, Cd, Hg and Cr have no known effects on cellular activity and are cytotoxic (Abou-Shanab *et al.*, 2007; J.-Z. Chen *et al.*, 2005; X. C. Chen *et al.*, 2005). It is known that microbial activity plays an important role in the metal speciation and transport in the environment (Pires, 2010). In

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high concentrations, heavy metal ions become toxic to cells (Pires, 2010). Due to the fact that some heavy metals are necessary for enzymatic functions (e.g. Zn) and growth, the cell has different mechanisms for metal uptake, this can be accomplished by bioaccumulation or biosorption (Pires, 2010).

The primary goal of metal remediation is to remove the metal from the waste or to decrease metal mobility and toxicity within the sample. Numerous microbially-mediated reactions can achieve these goals, including metal methylation, oxidation–reduction reactions and metal complexation (Kumar *et al.*, 2010). The diverse nature of microbial metabolic activities has long been exploited for human purposes, for example in extraction of precious metals from ores in bioleaching (Kumar *et al.*, 2010). Understanding metal–microbe relationships has led to advances in bioremediation (Bruins *et al.*, 2000; Malik, 2004). Metals are toxic to all biological systems from microbial to plant and animal, with microorganisms affected more so than other systems, due, in part, to their small size and direct involvement with their environment (Giller *et al.*, 1999; Patel *et al.*, 2007; Sarret *et al.*, 2005). Metal toxicity negatively impacts all cellular processes, influencing metabolism, genetic fidelity and growth (Kumar *et al.*, 2010). Loss of bacterial populations in metal-contaminated soils impacts elemental cycling, organic remediation efforts, plant growth and soil structure.

Bacterial surface structures are of extreme importance to understand their interactions with the surrounding environment, especially with metals. Bacteria can be Gram-negative or Gram-positive depending on the composition of the cell wall membrane. Gram-negative cell walls are a multilayered structure with an outer membrane containing lipopolysaccharide (e.g. lipopolysaccharide layer [LPS]), phospholipids and a small peptidoglycan layer. On the other hand, Gram-positive cells have as much as 90 % of the cell wall consisting of peptidoglycan in several layers, with small amounts of teichoic acid usually present (Guiné *et al.*, 2007). These structures are negatively

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charged and can interact with metal ions (Guiné *et al.*, 2007). Bioaccumulation is a substrate specific process, driven by ATP (Pires, 2010) and is an active process of heavy metal uptake. Three mechanisms of metal transport into the bacterial cell are known to be passive diffusion, facilitated diffusion and active transport. Some of the active transport systems are metal selective but with some exceptions. Cd can be transported by the same transporters as Zn (McEldowney *et al.*, 1993). A disadvantage of bioaccumulation is the recovery of the accumulated metal which has to be done by destructive means leading to damage of the biosorbent structural integrity (Ansari & Malik, 2007).

Biosorption refers to other mechanisms that are driven by the chemiosmotic gradient across the cell, not requiring ATP and it is primarily controlled by physicochemical factors. These include adsorption, ion-exchange and covalent bonding and may occur either in living or dead biomass and is considered as an alternative to conventional methods of metal recovery from solutions (J.-Z. Chen *et al.*, 2005; X. C. Chen *et al.*, 2005), being a passive metal uptake system. Both Gram-negative and Gram-positive bacteria have their cell wall charged with a negative charge. This is due to carboxyl, hydroxyl and phosphyl groups, thus in the presence of positive heavy metal cations these groups are very important in cation sorption (Pires, 2010).

Biosorption has a possible application as a process for the removal and concentration of heavy metals from wastewater (Errasquın & Vazquez, 2003). However, the cost of the biomass plays an important role in determining the cost of a biosorption process, thus a low-cost biomass is an important factor when considering practical application of biosorption (J.-Z. Chen *et al.*, 2005; X. C. Chen *et al.*, 2005). Various microorganisms show different responses to toxic heavy metal ions that confer them with a range of metal tolerance (Valls & De Lorenzo, 2002). Bacteria may achieve this in different ways either through biological, physical or chemical mechanisms that include

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precipitation, complexation, adsorption, transport, product excretion, pigments, polysaccharides, enzymes, and specific metal binding proteins (Hetzer *et al.*, 2006).

From a metabolic point of view a group of metal-chelating proteins called metallothioneins, are very important in bacterial metal tolerance (Valls & De Lorenzo, 2002). Metallothioneins are small cystein-rich polypeptides that can bind essential metals (e.g. Zn), and non-essential metals (e.g. heavy metals) (Pires, 2010). Other resistance mechanisms include active efflux, complexation, reduction and sequestration of the heavy metal ions into a less toxic state (Pires, 2010). These tolerance mechanisms are generally plasmid driven, which greatly contributes to dispersion from cell to cell (Valls & De Lorenzo, 2002), chromosome resistance was also related in some bacterial species (Abou-Shanab *et al.*, 2007).

The interest in heavy metal uptake by bacteria has increased in recent years, especially because of the biotechnological potential that microorganisms have for the removal and/or recovery of metal contaminants (Errasquın & Vazquez, 2003; Valls & De Lorenzo, 2002). Bacteria are good biosorbents and with the proper R&D may be in the near future a good alternative for the removal of metals from the environment (Errasquın & Vazquez, 2003).

Some examples of microorganisms having biodegradation potentials for heavy metals are listed in the Table 2.5.

Table 2.5 Examples of microorganisms having biodegradation potentials for heavy metals.

Organisms	Heavy Metals	Reference
Pseudomonas spp	U, Cu, Ni	Sar *et al.* (1999); Sar and D'Souza (2001)
Bacillus spp	Cu, Zn	Kapley *et al.* (1999)
Aspergilus niger	Cd, Zn, Ag, Th, U	Rajendran *et al.* (2003)

As tabulated in Table 2.5, studies have shown that some species of bacteria shows good removal of heavy metal. Rajendran et al. (2003) reported the use of mycelia of *Aspergilus niger* in removal of nickel, zinc, cadmium and lead in large scale fermenters by bioadsorption while studies by Sar and D'Souza (2001) indicate the suitability of the *Pseudomonas* sp biomass as biosorbent for uranium removal from aqueous waste streams.

2.18 Current practice and future prospects

Bioremediation as general practice in pollutants removal is still in its infancy. It is minimally tested and proved in large scale application. Therefore, could not pave its way to be widely accepted in commercial applications as to date yet It has enormous potentials that could help at least improved or complement the current technologies used in contaminants degradation such as landfill or wastewater leachate. Thus, it is the aim of this study to investigate and provide some basis of bioremediation using selected potential bacteria for further research in this field.

CHAPTER 3: METHODOLOGY

3.1 Sample collection

Leachate was collected from Jeram Sanitary Landfill (JSL) located in Mukim Jeram, Kuala Selangor, Selangor Darul Ehsan Malaysia as shown in Figure 3.1. Samples were collected in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2012) and were filled into containers and tightly capped. The samples were brought back to the laboratory at ambient temperature and were analyzed, prepared and used for characterization and treatments.

Figure 3.1: Location of Jeram sanitary landfill in Selangor

Leachate samples were collected monthly from January 2015 to March 2015 for at least 3 times on different days.

Leachate was collected in 30L HDPE sampling bottles for the study from the pipes directly linked to the landfill cells as shown in Plate 3.1. Fresh sample of leachate was collected for each set of treatment and duly replicated to ensure coherence in analysis.

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Plate 3.1 Pond collecting leachate in Jeram Sanitary Landfill

3.2 Characterization of raw leachate

To investigate the physico-chemical parameters of raw leachate, the freshly collected raw samples were analyzed to evaluate its initial colour, odour, ammoniacal nitrogen, oil and grease, pH, total dissolved solid (TDS), salinity, and conductivity. Heavy metal components of the leachate were analyzed using inductively coupled plasma mass spectrometry (ICP-MS). The biological component (BOD_5) and organic compound (COD) was determined using APHA Standard Methods (APHA, 2012). Each parameter was analyzed in triplicates to ensure accuracy of the analysis and due to the limitation of budget in the study. The summarization of analysis for the leachate characterization and methods used are given in Table 3.1.

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Table 3.1: Analysis of Leachate for leachate characterization.

List of analysis	Methods
pH, conductivity, salinity, Total Dissolved Solid	pH, conductivity and salinity probe (YSI Professional Plus handheld multiparameter).
Oil and Grease	Analyzed according to Standard Methods APHA 5520B (APHA, 2012)
BOD_5	Analyzed according to Standard Methods APHA 5210B (APHA, 2012)
COD	Analyzed according to Standard Methods APHA 5220D (APHA, 2012)
Ammoniacal Nitrogen	Analyzed according to Standard Methods APHA 4500-NH_3 (APHA, 2012)
Heavy metals	Analyzed according to Standard Methods ASTM D5673 (ASTM, 2010) using inductively coupled plasma mass spectrometry (ICP-MS).

3.3 Selection of bacteria and treatment design

To study the bioremediation potential of leachate, a few species of identified bacteria were used. Four bacteria were used in the treatment as shown in Plate 3.2. The *Bacillus salmalaya* is a novel soil bacteria locally isolated and named specie that has been extensively studied previously for potential applications as various roles such as bioremediation (Dadrasnia *et al.*, 2015; Dadrasnia & Salmah, 2015; Dadrasnia *et al.*, 2016; Salmah & Dadrasnia, 2015; Usman *et al.*, 2016). The specie *Lysinibacillus sphaericus, Bacillus thuringiensis* and *Rhodococcus wratislaviensis* were first isolated from landfill leachate soil and evaluated by Emenike *et al.* (2016) for bioremediation. The bacteria showed good potential to degrade landfill leachate soil when test in mixed

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isolates of bacteria. Therefore in this study, *Bacillus salmalaya* is tested in single isolate and also in combination with the mixed bacterial culture to test the bioremediation capability and its synergism.

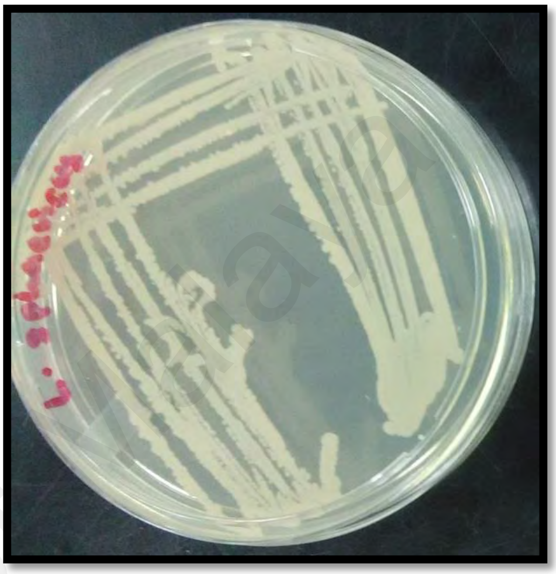
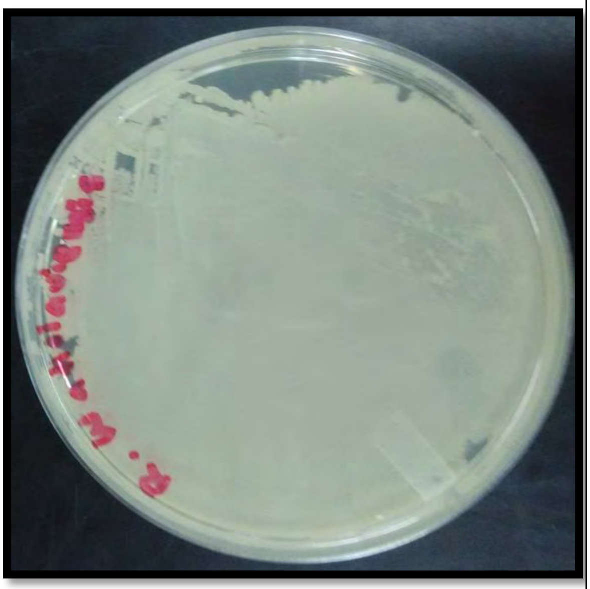
1. *Bacillus salmalaya* 	2. *Lysinibacillus sphaericus* 
3. *Bacillus thuringiensis* 	4. *Rhodococcus wratislaviensis* 

Plate 3.2: Bacteria used in the treatment set-up

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3.4 Inoculum preparation

Each strain of bacteria was grown as a pure culture in nutrient agar (NA) plates at 33°C for 2 days (Emenike *et al.*, 2016). To prepare the bacteria inoculum for the treatment purposes, an enrichment medium was prepared. Nutrient broth E (refer to Appendix H) was used as the medium for all the four bacteria. The broth prepared by dissolving 13 g of the powder in 1 liter ionized water. It then was sterilized and was left to cool down before the introduction of bacteria. Bacteria concentration was monitored by measuring optical density (O.D.) at 600 nm until minimum of 0.6 ABS was obtained.

The inoculum then was incubated in the incubator shaker at 35°C and 150 rpm. The OD reading was taken every 24 hours in order to check the bacterial growth. Once the OD reading was stable, the cocktail of the bacteria were used for the leachate treatment.

3.5 Bioremediation analysis

The bioremediation was divided into three treatments and a control group. Refer Table 3.2 below.

Table 3.2 Bacterial species (single and mixed) used for the bioremediation study

Experiment	Treatment 1	Treatment 2	Treatment 3	Control
Microbial cocktail	*Bacillus salmalaya*	NU	*Bacillus salmalaya*	NU
	NU	*Lysinibacillus sphaericus,*	*Lysinibacillus sphaericus,*	NU
	NU	*Bacillus thuringiensis*	*Bacillus thuringiensis*	NU
	NU	*Rhodococcus wratislaviensis*	*Rhodococcus wratislaviensis*	NU

* NU means not used (such bacteria was not used in the treatment)

** Control contain no specific isolated bacterial strain; only residential species (if any available) as the sample was not autoclaved.

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The group of treatment in treatment 1 was chose because the *Bacillus salmalaya* has suspected to have novel ability in bioremediation as studied by Dadrasnia *et al.* (2015), Dadrasnia & Salmah (2015), Dadrasnia *et al.*(2016), Salmah & Dadrasnia (2015) and Usman *et al.*(2016). On the other hand, the combination of bacteria chose in treatment 2 was based on previous studies by Emenike *et al.* (2016). Furthermore, the combination of bacteria in treatment 3 is to look at the synergistic effects (if any) of the bacterial population perform bioremediation on leachate. Other combination of bacteria was not planned due to the limitation of budget for the study.

Approximately 1L of fresh leachate was poured into a flask for all the bioremediation set mentioned as shown in Plate 3.3. It was added with 10% (v/v) of bacteria in triplicate where *Bacillus salmalaya* for Treatment 1, a mixture of *Lysinibacillus sphaericus, Bacillus thuringiensis* and *Rhodococcus wratislaviensis* for Treatment 2, and the mixture of *Bacillus salmalaya, Lysinibacillus sphaericus, Bacillus thuringiensis* and *Rhodococcus wratislaviensis* for Treatment 3.

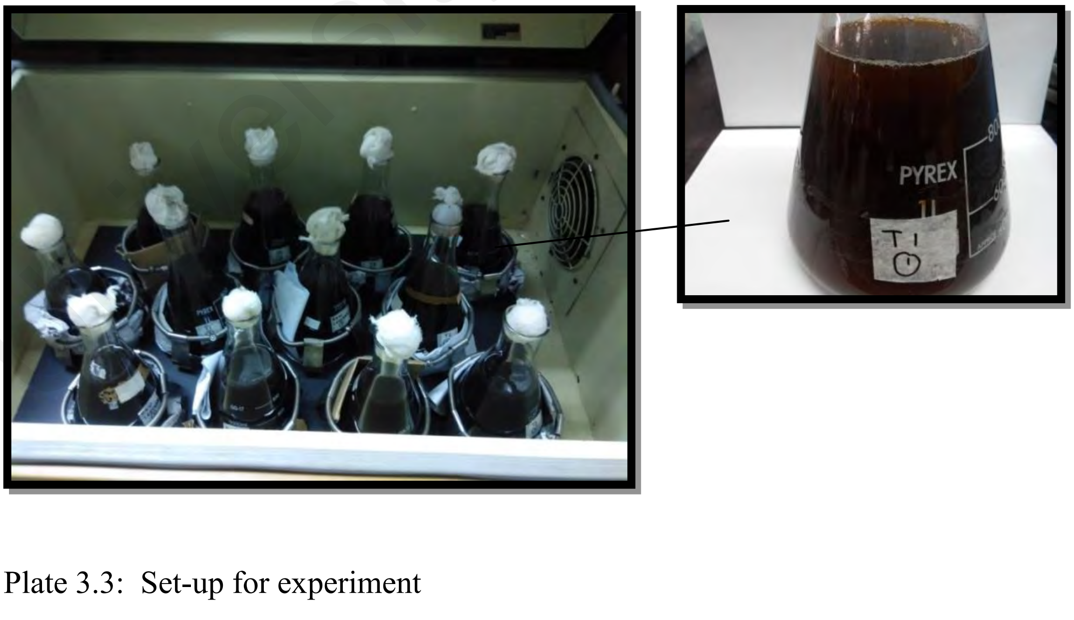

Plate 3.3: Set-up for experiment

All set-up was left in incubator shaker for 48 hours at 35°C and agitation of 200 rpm. Leachate samples were analyzed at 12 hours interval for 48 hours for analysis of the

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treatments by the various bacteria introduced (Emenike *et al.*, 2016; Emenike *et al.*, 2013a, 2013b; Sonune & Garode, 2015).

The leachate was analyzed 12 hour for the rapid analysis and after 48 hour for the complete analysis. The analysis for the rapid analysis and complete analysis are given in Table 3.3.

Table 3.3: Analysis of Leachate for Leachate Bioremediation.

Partial analysis (12 hourly within 48 hours)	Complete Analysis (48 hours)
Determination of physical parameter 1. pH 2. Total dissolved solid 3. Salinity 4. Conductivity Determination of organic pollutant (COD) Determination of nirogenous pollutant	Determination of physical parameter 1. pH 2. Total dissolved solid 3. Salinity 4. Conductivity 5. Oil and grease Determination of organic pollutant (BOD_5 & COD) Determination of nirogenous pollutant Heavy metals content analysis

Analysis for treatments was performed in triplicates. The efficiency for organic load reduction and the percentage of reduction of pollutant was measured using the following equation.

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$$\text{Reduction percentage} = \left(\frac{C_i - C_f}{C_i}\right) \times 100\ \%$$

Where C_i is initial reading and C_f is final reading. Each set of these experiments was done in triplicates.

Although the final aim was to test on total reduction not the incremental trend of the parameters, the rapid analysis was done to observe any significant results and for evaluation purpose. For the sake of more objective result discussion, results of 12, 24 and 36 hours were not included in section 4 but only the 48 hours results reported.

Oil and grease and heavy metal were not analyzed in partial analysis because the aim was to test on total reduction not the incremental trend of reduction. Only TDS was analyzed in the study. The colour was only reported as seen in visual appearance. Those two parameters chosen based on method from the research that has been done to JSL leachate by Emenike *et al* (2011). On the other hand, the ICP-MS screened for common metals and list of metals reported are the metals that found in the JSL leachate. Removing the metals aim at testing the metal remediation capability of the strains therefore achieving bioremediation objective of the study.

Due to research limitation, methods were chosen only to fulfill the objectives. Future research can be done to evaluate the results and elucidate the bioremediation process.

3.6 Statistical Analyses

To evaluate the statistical results, a general linear model (SPSS 19) was used for the ANOVA between the means of the treatments. In addition, Tukey HSD multiple range test was performed to test of significance ($p < 0.05$).

CHAPTER 4: RESULTS & DISCUSSIONS

4.1 Raw leachate characteristics

Prior to treatment, the raw leachate was first analyzed to obtain the physico-chemical characteristic. The characteristic of raw leachate is shown in Table 4.1. In general, the results indicated that leachate has a characteristic of a stabilized to old leachate as JSL has been operated for more than 8 years since 2007. JSL still receives MSW and is subjected to deposition of water soluble compounds. The JSL leachate showed deep black colour accompanied with a slightly ammoniac odour. This obvious leachate colour could be due to dissolved components of the waste. Colour is an important parameter in water quality and effluent discharge considerations (Emenike *et al*., 2013b).

Table 4.1 Characteristic of raw leachate of JSL.

Characteristics (unit)	Average Value	Standard
Apparent colour	Deep black	-
Odour	Slightly ammoniac	-
Conductivity (µS/cm)	35,829.70 ± 293.30	-
pH	8.38 ± 0.08	5.5-9.0 (EQA B)
Salinity (ppt)	19.27 ± 0.02	-
TDS (mg/L)	20,321.17 ± 9.90	-
BOD_5 (mg/L)	1,046 ± 154.50	50 (EQA B)
COD (mg/L)	11,031.67 ± 153.70	100 (EQA B)
BOD_5 / COD	0.09	-
Ammoniacal Nitrogen (mg/L)	6,400 ± 624.50	1 (EPA)
Oil and Grease (mg/L)	4.43 ± 0.03	10 (EQA B)

* n = average of 3 samples from 3 different sampling; (-) value of limits not available

The electrical conductivity (EC) recorded averaged at 35,829.70 ± 293.30 µS/cm. It is similar to the values of previous leachate studies from JSL (Zainab *et al.*, 2013). EC value indicates the ability of solution to convey an electrical current and is associated to the quantity of dissolved salts present and ionized substances found in the leachate. The high EC reading indicates the amount of mineral and organic ions (anions and cations) present in the leachate. TDS recorded was 20,321.20 ± 9.90 mg/L while salinity averaged at 19.30 ± 0.02 ppt. The high values of TDS in leachate samples indicate the presence of inorganic materials in the samples (Nagarajan *et al.*, 2012).

The pH value of the leachate averaged at pH 8.38 ± 0.08 indicating a typical pH of a mature landfill. This result is consistent with those published by previous authors (Zainab *et al.*, 2013) which is in the same range at pH 8.17, pH 8.5, pH 7.6, pH 8.4 and pH 8.28. Stabilized leachate shows fairly constant pH with little variations and it may range between pH 7.9 and pH 9 (Muhammad *et al.*, 2010).

Higher pH values observed might be due to mineralization of carbonates, bicarbonates and hydroxides. These chemical type might have contributed towards higher alkalinity (Maqbool *et al.*, 2011). As the landfill age increased, further increase in pH values occurred, caused by a certain decrease in metal solubility (Kulikowska & Klimiuk, 2008). However, the pH values still remained within the permissible limit (6.0-9.0) set in the Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009, Malaysian Environmental Quality Act 1974 (Act 127).

The average of BOD_5 value for Jeram's landfill leachate recorded was 1,046.00 ± 154.50 mg/L. It means that the leachate has high organic strength. According to Rathod *et al*. (2009), high value of BOD_5 indicates high content of organic pollutants dissolved in the leachate. On the other note, the value of BOD_5 was lower than that reported by Emenike *et al*. (2011 & 2013b). This is due to the process of degradation in the landfill's leachate. A decrease in BOD_5 is often reported with increase in age of the landfill (Muhammad *et al.*, 2010).

It was observed that COD value from Jeram's landfill leachate was 11,031.70 ± 153.70 mg/L. The COD were higher than the permissible limit which means that the leachate was highly polluted with the chemical that may be originated from wastes in the landfill itself.

Organics in leachate are characterized by different levels of biodegradation. In this study, the BOD_5/COD ratios for the collected leachate samples are 0.09. The present BOD_5/COD ratio shows that the age of the landfill was intermediate that is about 5 to 10 years (Amokrane *et al.*, 1997; Renou *et al.*, 2008). Generally, the BOD_5/COD ratio describes the degree of biodegradation and gives information on the age of a landfill. The low BOD_5/COD ratio shows high concentration of non-biodegradable organic compounds and the increased difficulty to be biologically degraded (Ntampou *et al.*, 2006). However, the BOD/COD ratio estimation is not a reflection of whether bioremediation is suitable or not to engage for the sample but rather it is used to estimate landfill maturation. Most findings indicated that low ratio of BOD/COD leads to slow and hardly degradable hence not suitable for biological process.The work intends to study organic compounds degradation by other possible ways such as synergistic effects of the microbial organisms.

Biodegradability which is represented by the mass concentration ratio of BOD/COD is the ability of a substance to be broken down into simpler substances by bacteria. Lower ratios ($<$0.1) reveal the presence of large portions of hard-biodegradable COD, which is composed of non-biodegradable organic molecules, essentially humic and fulvic acids in the landfill leachate. Although the low ratio indicated the hardly biodegradable nature of the leachate and suggesting the slow biodegradation ability, it does not rule out of other possible mechanisms. There organic compounds degradation may happen by synergistic effects of the microbial organisms and by products with the leachate.

Oil and grease in JSL leachate averaged at 4.43 ± 0.03 mg/L. This almost reaches the permissible limit (5.0 mg/L) set in the Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulations 2009, Malaysian Environmental Quality Act 1974 (Act 127). The content of oil and grease recorded differ from the study by Emenike (2013b), which recorded 48±5 mg/L oil and grease content. It may be due to the varied and different composition of waste at that particular time. Oil and grease are considered as hazardous pollutants particularly in the aquatic environments, since they are highly toxic to the aquatic organisms and can completely damage the ecology of the aquatic ecosystem (Bala *et al.*, 2015). When discharged into the environment, it may have objectionable odour, cause undesirable appearance, burn on the surface of receiving water creating potential hazards and consume dissolved oxygen (Jameel & Abass Olanrewaju, 2011).

Ammoniacal nitrogen was found to be very high in the JSL leachate average at 6,400 ± 624.50 mg/L. This may due to the age of the stabilized landfill. Raw leachate from the stabilized landfill is commonly characterized by high strength of ammoniacal nitrogen (NH_3-N)(Davis, 2006). The presence of high amount of NH_3-N in JSL leachate indicates degradation of soluble nitrogen due to the decomposed waste. As a result, the concentration of NH_3-N increases with the increase in age of the landfill which was due to hydrolysis and fermentation of nitrogenous fractions of biodegradable refuse substrate (Muhammad *et al.*, 2010). NH_3-N is known as one of the major aquatic pollutant where it is highly toxic to fish and other aquatic life and it was one of the problems normally faced by landfill operators. Slow leaching of wastes and no significant mechanism for transformation of NH_3-N in the landfills causes a high concentration of ammoniacal nitrogen in leachate over a long period of time (H. A. Aziz *et al.*, 2004).

Metals analysis of the JSL leachate performed according to method testing for elements in water by Inductively Coupled Plasma - Mass Spectrometry, American Society for Testing Materials (ASTM) 2010. The major metals found in the JSL leachate namely Al, Cr, Mn, Fe, Ni, Zn, As, Ba and Pb were analyzed in this study. Table 4.2 denotes the concentration of the metals obtained from the leachate analysis. From the results, most of the metal values were relatively low, i.e. below the limit permitted by Environmental Quality (Control of Pollution from Solid Waste Transfer Station and Landfill) Regulation 2009. This is mainly due to the age of the landfill. As the landfill age increased, further increase in pH values caused a certain decrease in metal solubility and this drastically bring down the heavy metal concentration (Kulikowska & Klimiuk, 2008).

Table 4.2: Metal contents in JSL Leachate

Metal	Value (mg/L)	EQA Standard Limit (mg/L)
Aluminium	0.538 ± 0.06	5.0
Chromium	0.073 ±0.01	0.005
Manganese	0.018 ± 0.001	0.20
Iron	0.669 ± 0.10	5.0
Nickel	0.028 ±0.002	0.20
Zinc	0.076 ± 0.03	2.0
Arsenic	0.012 ± 0.002	0.05
Barium	0.203 ± 0.09	1.0
Lead	0.005±0.003	0.10

The low level of metal contents in the leachate did not negate the intended objective of testing the potential of beneficial bacteria in reduction of metals from the leachate. Landfill leachate is heterogenous and known to have varied level of metals/heavy metals across time, age and source of waste, as showed by previous studies by Kulikowska and Klimiuk, 2008. The low level of metals detected was expected due to the aging of JSL. Malaysia guideline should not be regarded as definitive safe limits but as some basis figure. Heavy metals reduction is the second main objective in testing the bioremediation potential of the bacteria, irregardless of the initial value. Bioremediation in the condition closest to the natural condition and as highly similar as possible for onsite application is the main aim on this setting.

The characteristics of JSL raw leachate indicated high content of non-biodegradable organic compounds and also very high ammoniacal nitrogen composition in the leachate. The oil and grease value also almost reaches the permissible limit although a lot lower than previous study. Due to these reasons, conventional treatment methods of JSL leachate are not suitable to treat the pollutants effectively at economical cost. Hence, the potential of bioremediation with bacteria was looked into to find alternative ways of treating the leachate.

Further study is carried out to investigate the potential of the selected bacteria to remediate the leachate and improved the quality of the leachate treatment before it can be discharged to the environment. In each of the treatments (Treatment 1, Treatment 2 and Treatment 3), the physicochemical parameters of the leachate and the heavy metals content were analyzed. Conventional treatment is costly and could not remove certain contaminants at once. Hence, the potential of bioremediation with bacteria was looked into to find alternative ways of treating the leachate.

4.2 Treatment with *Bacillus salmalaya* (Treatment 1)

Treatment 1 is leachate samples inoculated with *Bacillus salmalaya* (10% v/v) for the potentials of the bacteria to remedy pollutants in the leachate. In general, Treatment 1 results showed reduction in physicochemical parameters after 48 hours of incubation with the bacteria. There were also reductions in the heavy metal content.

4.2.1 Physico-chemical characteristics of leachate in Treatment 1

Table 4.3 summarizes the physico-chemical characteristics of leachate before and after 48 hours.

Table 4.3 Physico-chemical characteristics of leachate before and after Treatment 1 (*Bacillus salmalaya*).

Parameter	Unit	Initial	Final	Reduction percentage (%)
Conductivity	µS/cm	35,830	30,840	13.9
Salinity	ppt	19	17	10.1
TDS	mg/L	20,320	18,400	9.5
Oil and Grease	mg/L	4	1	73.0
BOD_5	mg/L	1,050	1,200	-14.9
COD	mg/L	11,030	7,180	34.9
Ammoniacal Nitrogen	mg/L	6,400	3,900	39.1

Initial conductivity of the leachate showed a value of 35,830 µS/cm and decreased to 30,840 µS/cm after the treatment. This translates to reduction percentage of 13.9%. Salinity of the leachate showed a decrease from initial value of 19 ppt to 17 ppt final value after treatment 1. It is an approximately 10.1% reduction. Similar to conductivity and salinity, total dissolved solid (TDS) of the leachate after Treatment 1 also decreased from 20,320 mg/L at the initial reading to 18,400 mg/L at the final reading with 9.5% reduction. The reduction in conductivity, salinity and TDS of the treatment system

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showed that the bacteria metabolize the organic content of the leachate to form stabilized by-products. Ionics and dissolved matter are used up in the process contributing to the slight decrease. Oil and grease content in Treatment 1 has an initial value of 4.40 mg/L and it decreased to 1.20 mg/L after the treatment, or 73% reduction.

Further analysis of the treatment 1 showed that BOD_5 value recorded an initial value of 1050 mg/L before it increased to 1200 mg/L after the treatment. This is a 14.9 % increase in percentage. The increase indicated that some of the bacteria introduced in the treatment may have acclimatized and the population started to grow and this make the bacteria community increased in abundance after that the biochemical demand for oxygen required by organic matter decomposition decreased. The reason for this trend was the consumption of oxygen by the bacteria increased (Salmah & Dadrasnia, 2015). Therefore, decrease in dissolved oxygen supply due to utilization by the growing populations contributed to higher BOD_5 value. Nevertheless, from the Table 4.3, the COD values in treatment 1 showed an overall decrease from initial reading of 11,030 mg/L to final reading of 7,180 mg/L after 48 hours. The COD decrease may be due to the utilization of organic compounds in the leachate by the bacterial population reflecting the biodegradable components of the soluble and particulate organic matter in the leachate. Ammoniacal nitrogen value in treatment 1 showed a 39.1 % decrease from initial reading. At 0 hours, ammoniacal nitrogen value was 6,400 mg/L and decrease to 3,900 mg/L after the 48^{th} hour.

Figure 4.1 shows the comparison of Treatment 1 and control experiment in the reduction percentage of the physico-chemical properties of leachate. From the result of this study, *B. salmalaya* shows a great potential in remediating oil and grease as the reduction percentage was more than 70% as compared to the control which only reduced less than 10% oil and grease. It might due to the ability of bacteria to utilize hydrocarbons as their source of energy and further reduce their concentration in

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Treatment 1. Similar observation with the same strain was found in previous study by Dadrasnia and Salmah (2015) whereby *B. salmalaya* was employed in the treatment of water polluted with crude oil. *B. salmalaya* showed high potential for oil and grease degradation with 88% reduction after 42 days of incubation period (Dadrasnia & Salmah, 2015).

Besides that, Treatment 1 also showed good removal for ammoniacal nitrogen which is 39.1% removal than that of only 15% in control experiment. It showed the ability of *B. salmalaya* to use ammonical nitrogen as their only nitrogen source and further degrade it into benign manner. This is lower but positive result as compared to results reported by Yu *et al.* (2012) whereby incorporation of *Bacillus* sp. in industrial wastewater successfully degraded almost 90% of the initial ammoniacal nitrogen content in the wastewater. According to Hong and Cutting (2005) *Bacillus* species are important candidates for developing commercial biological agents for nitrogen removal and water quality enhancement. Several studies on Bacillus species have been proven of its ability to remove nitrite (Chen & Hu, 2011; Lalloo *et al.*, 2007; Meng R, 2009).

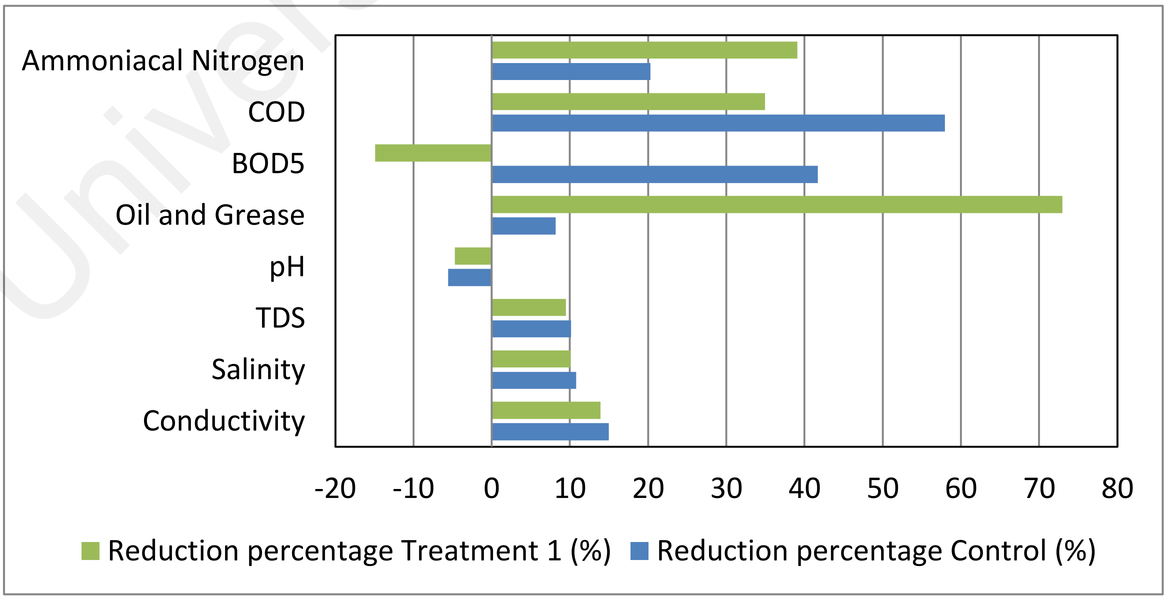

Figure 4.1 Comparison of reduction percentage between Treatment 1 and Control experiments

Similar activity was observed in both Treatment 1 and control in the reduction of TDS, salinity and conductivity. It means that the employment of *B. salmalaya* have no significant effect in improving these properties of leachate. According to Lefebvre *et al.* (2006), saline wastewater are conventionally treated through physico-chemical means, as biological treatment is strongly inhibited by salts mainly NaCl. Conductivity measurements usually can be used to monitor the processes in wastewater treatment that causes changes in conductivity (Levlin, 2010). The processes that occur in many treatment plants that cause changes in conductivity are mainly biological nitrogen removal (Levlin, 2010).

The addition of external bacteria into the system has a positive effect on the reduction of COD. However, lower reduction of COD was observed in Treatment 1 (35%) than that of control experiment (58%). The rapid growth and death of bacteria will resulted in the increased in the overall organic content of Treatment 1 thus resulting in lower reduction of COD. Apart from that, the mass of the dead bacteria in the system retard the degradation and oxidation of organic pollutant hence contribute to higher COD value in Treatment 1 as compared to the control treatment.

On the other hand, increase in BOD_5 value to was observed in Treatment 1 as opposed to control experiment. This is mainly due to the rapid growth and death of bacteria that used up the available oxygen in the treatment system. Thus, sudden decrease in dissolved oxygen supply will contributes to higher BOD_5 value in Treatment 1. Moreover, the low ratio of BOD_5/COD of the leachate may be due to the recalcitrant organic matter which leads to the higher BOD_5 value after the treatment. Generally, organic matters in the leachate are degradable but another substance possibly leads to inhibition of bacteria that uses organic matter makes the BOD_5 value became higher.

pH value showed no significant change across the treatment, therefore not included in the result.It is a worthy note to mention that the control experimental setups have also showed some reduction and positive results of bioremediation. Control set up contained only raw leachate with residential bacteria as it was not autoclaved. It may be the reason of pollution reduction results during the experimental works. The indigenous bacteria existing in the municipal waste or from the surrounding environmental may have acclimatized to the leachate and survived the harsh condition in the leachate pond thus were affecting the results of the experiment.

4.2.2 Heavy metals reduction of leachate in Treatment 1

Furthermore, the study evaluated the potentials of Treatment 1 to remediate heavy metals concentration of the raw leachate. Figure 4.2 reflects the degree of reduction of metals concentration when *B. salmalaya* was introduced as remediation agent to fresh raw leachate. The result showed a higher degree of remediation of Manganese (73%), Barium (72%) and Zinc (68%) after 48 hours of treatment with *B. salmalaya* as against Aluminium (60%), Nickel (60%), Chromium (59%), Iron (57%), Arsenic (55%) and Lead (46%).

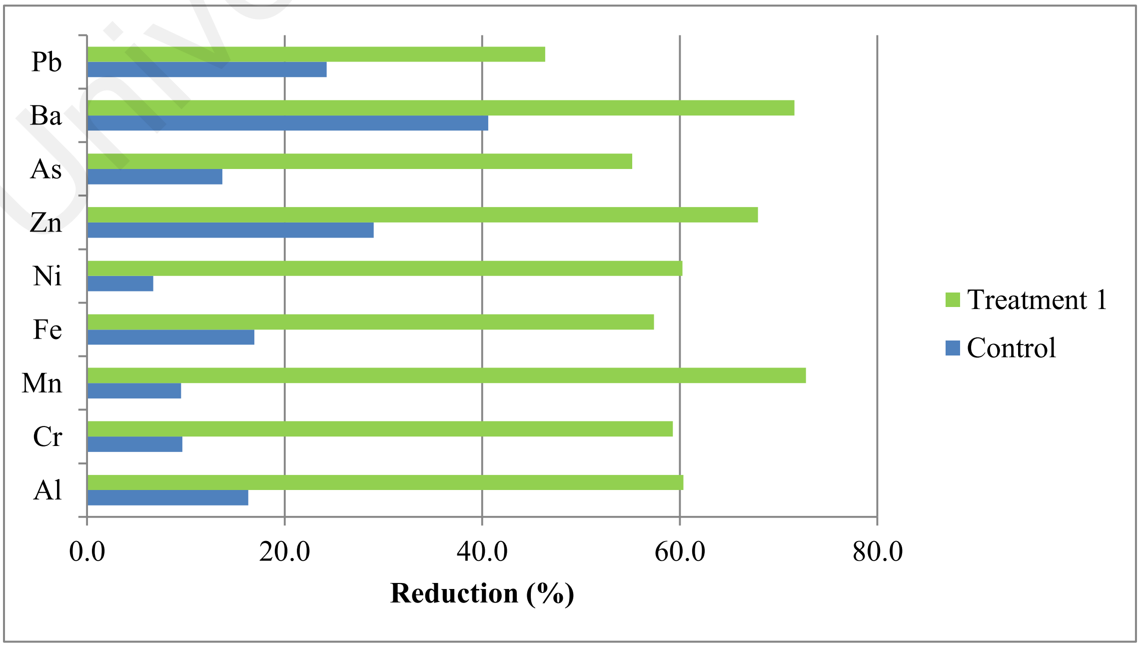

Figure 4.2 Heavy metals reduction of leachate in Treatment 1

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Treatment with *B. salmalaya* showed a reduction of at least 60% for five metals as the other four heavy metals recorded at least 40% reduction. This indicated that the treatment has potential to remedy all heavy metal analyzed to nearly half from its initial content in the fresh leachate after only 2 days of incubation. Incorporation of *Bacillus* sp. has been previously stated to have a high removal potential of heavy metals compound (Krishna *et al*., 2013). Previously, Kumar *et al*. (2010) reported high removal efficiency of *Bacillus* sp. in reducing heavy metals compound namely Cu and Ni in wastewater. On top of that, the initial concentration of heavy metals in the raw leachate was relatively low than the allowable limit by EQA. Thus, presence of additional bacteria in the treatment system provides greater surface area hence successfully reduced the heavy metals concentrations in Treatment 1.

4.3 Treatment with *Lysinibacillus sphaericus, Bacillus thuringiensis and Rhodococcus wratislaviensis* (Treatment 2)

Leachate samples were inoculated with a concoction of 3 bacteria mixture namely *Lysinibacillus sphaericus, Bacillus thuringiensis* and *Rhodococcus wratislaviensis* (10% v/v) in Treatment 2 to study the ability to treat pollutants in the leachate.

In general, Treatment 2 recorded a similar trend of reducing conductivity, salinity and TDS against time. The same case also observed for BOD_5, COD, ammoniacal nitrogen and oil and grease content.

4.3.1 Physico-chemical characteristics of leachate in Treatment 2

The physico-chemical characteristics of treated leachate using Treatment 2 are shown in Table 4.4.

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Table 4.4 Physico-chemical characteristics of leachate before and after Treatment 2.

Parameter	Unit	Initial	Final	Reduction percentage (%)
Conductivity	µS/cm	35,830	30,350	15.3
Salinity	ppt	19	17	9.8
TDS	mg/L	20,320	18,230	10.3
Oil and Grease	mg/L	4	2	43.7
BOD_5	mg/L	1,050	1,210	-15.3
COD	mg/L	11,030	6,250	43.3
Ammoniacal Nitrogen	mg/L	6,400	3,500	45.3

It was found that, ammoniacal nitrogen showed the highest reduction from 6,400 mg/L to 3,500 mg/L at 45.3%. The oil and grease content in the treated leachate reduced from 4 mg/L to 2 mg/L that reflected to 43.7 % reduction. COD value recorded a significant reduction from 11,030 mg/L to 6,250 mg/L which contributes to 43.3% reduction. A minor reduction was observed in several parameters namely conductivity, salinity and TDS values which records a reduction of 15.3%, 9.8% and 10.3% respectively.

On the other hand, a notable increase in the BOD_5 value was observed in the treated leachate (1,210 mg/L) from 1,050 mg/L in the raw leachate.

Figure 4.3 shows the comparison of reduction percentage of physico-chemical properties of leachate between Treatment 2 and control experiment. Similarly to that of Treatment 1, no variations were observed in the reduction percentage of TDS, salinity, as well as, conductivity in both Treatment 2 and control experiment. It confirmed that these parameters will slowly degrade with or without the presence of additional bacteria in the treatment system.

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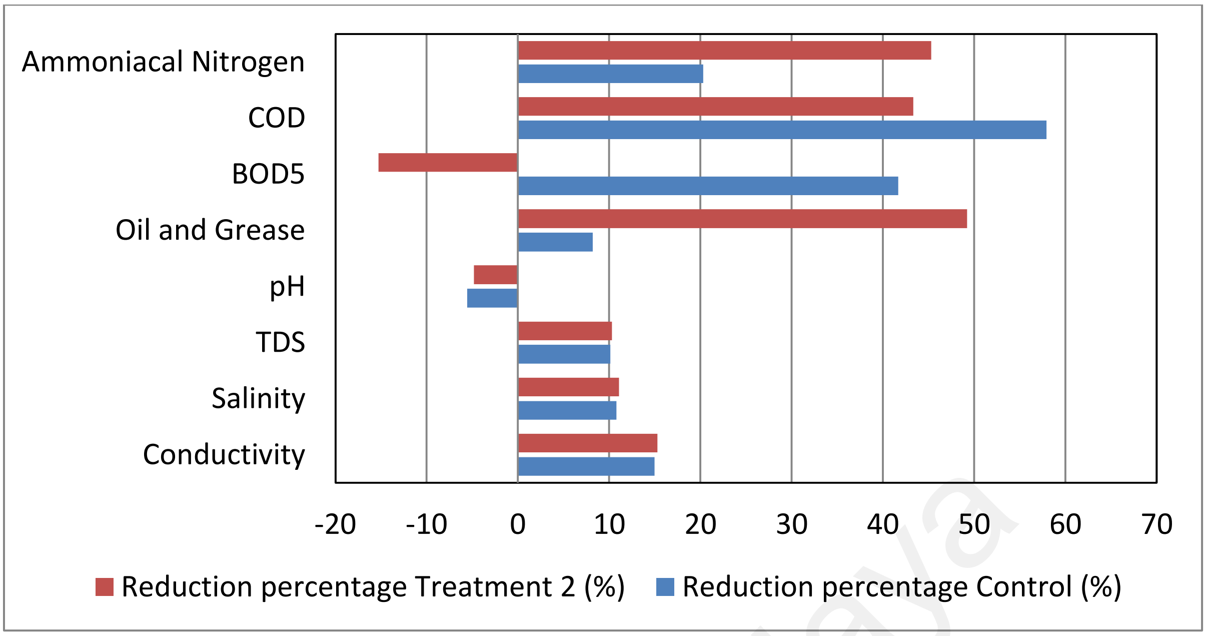

Figure 4.3 Comparison of reduction percentage between Treatment 2 and Control experiments

The application of mixed culture of bacteria in Treatment 2 achieved highest reduction in oil and grease content in the treated leachate with 49% compared to only 8% in control experiment. *B. thurigiensis* share the same genus as the aforementioned *B. salmalaya* as stated in Treatment 1. Wide numbers of *Bacillus* sp. were studied for their ability in degrading oil and grease including *B. salmalaya*, *B. cereus* and *B. sublilis (*Bala *et al., 2015)*. The results obtained from this study showed that, *B. thuregiensis* has high potential in the degradation of oil and grease content in leachate. Also, considering that *Rhodococcus* sp. and *Lysinibacillus* sp. retained similar degradation capability on oil and grease, their presence in Treatment 2 enhanced the overall reduction of oil and grease (Auffret *et al.*, 2009; Pizzul *et al.*, 2007). In other word, mixed culture bacteria consortium significantly improved the degradation of oil and grease component in leachate.

On top of that, Treatment 2 presented significant removal of ammoniacal nitrogen with 45% reduction as compared to only 20% found in control experiment. It was found that,

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mixed culture of bacteria in Treatment 2 is able to convert the ammoniacal nitrogen to different form of gas such as nitrate-nitrogen and release to the atmosphere. *Bacillus* sp. has been widely known for its capacity in reducing ammoniacal nitrogen content (Hong & Cutting, 2005). Strains belonging to several *Bacillus* species, such as *Bacillus subtilis*, *Bacillus cereus*, *Bacillus licheniformis*, *Bacillus pumilus* were isolated and evaluated for their potential as biological agents for water quality enhancement and from there several strains with good nitrogen removal properties were thus found (Xie *et al.*, 2013). Organic and inorganic nitrogen in wastewater can be further reduced by means of chemical and biochemical reaction (Yu *et al.*, 2012). On the other hand, the results may reflect the potentials of *Lysinibacillus* sp. to remedy the ammonical nitrogen and this can be supported by Reghuvaran *et al*. (2012) for its ability in the reduction of ammonia nitrogen content in wastewater. Apart from that, the results also might be due to the ability of *Rhododoccus* sp. in the removal of ammoniacal nitrogen and this can be supported by Li (2013). The combined effect of mixed culture bacteria enhanced the removal of ammoniacal nitrogen in leachate.

Conversely, a negative removal of BOD_5 (-15%) in Treatment 2 denoted the significant increase in the BOD_5 value in the treated leachate. Higher BOD_5 value indicates high content of organic matter in Treatment 2 due to the aforementioned rapid growth and death of bacteria consortium in Treatment 2. Hence, low oxygen availability to microbial population thus affecting the degradation of organic material in the leachate. There is no oxygen level detection performed but the increase in BOD_5 was the indicator that may suggest the low level of dissolved oxygen in the treatment. Lower COD removal was observed in Treatment 2 with 43% removal as compared to around 58% removal in control experiment. pH value showed no significant change across the treatment, therefore not included in the result.

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Furthermore, it is a worthy note to mention that the control experimental setups have also showed some reduction and positive results of bioremediation. Control set up contained only raw leachate with residential bacteria as it was not autoclaved. It may be the reason of pollution reduction results during the experimental works. The indigenous bacteria existing in the municipal waste or from the surrounding environmental may have acclimatized to the leachate and survived the harsh condition in the leachate pond thus were affecting the results of the experiment.

4.3.2 Heavy metals reduction of leachate in Treatment 2

Treatment 2 evaluated the potentials of the bacteria mixture isolated from previous study to remedy heavy metals in raw leachate. Figure 4.4 reflects the degree of reduction of metals concentration when *Lysinibacillus sphaericus, Bacillus thuringiensis* and *Rhodococcus wratislaviensis* was introduced as remediation agent to fresh raw leachate.

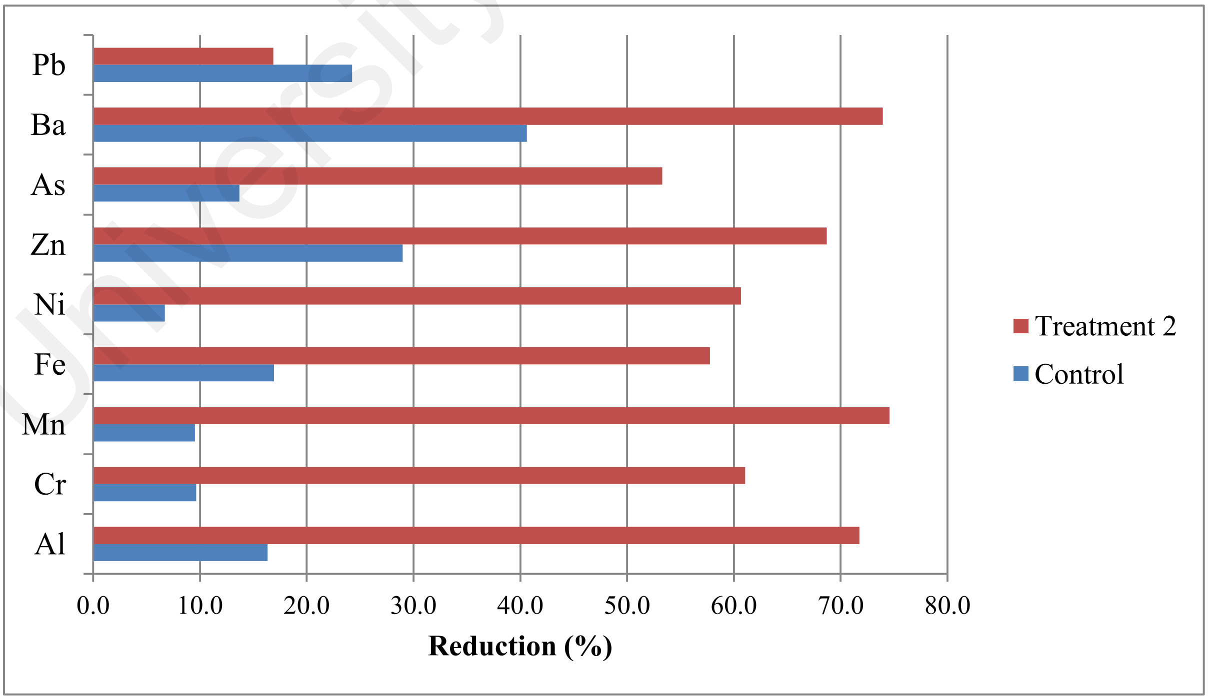

Figure 4.4 Heavy metal analysis of leachate in Treatment 2

The result from the experiment showed a significant removal of heavy metals as opposed to the control experiment with percentage removal of Manganese (75%), Barium (74%), Aluminium (72%), Zinc (69%), Chromium (62%), Nickel (61%), Ferum (58%) and Arsenic (53%). On the other hand, slightly lower removal was observed in Plumbum (18%) compared to that of control experiment. These high removals of heavy metals indicated the potential of mixed culture bacteria in reduction of heavy metals concentration.

The result may reflect the potential of *Bacillus* sp. to readily enhance the uptake of heavy metals and can be supported by Sulaimon *et al.* (2014). Similarly, the reduction of Zinc concentration by 69% may be linked to the presence of *Rhodococcus* sp. in the treatment because it concurs with the degree of Zinc removed by Vásquez *et al.* (2007) using a strain of *Rhodococcus*. Also the overall metal reduction could have been influenced by the presence of *Lysinibacillus* sp. due to the hex-histidine tag (Emenike *et al.*, 2013a).

Mixed culture bacteria consortium enhanced the removal of heavy metals in Treatment 2 by providing additional surface area that significantly increased the heavy metals uptake. Each bacteria or any biological matter have a different functional groups on their surface area thus differs in their interaction with heavy metals in solution (Vásquez *et al.*, 2007). Due to this reason, a single bacterium might effectively accumulate certain type of heavy metals but resistance to others. Similar finding was reported by Emenike *et al.* (2016) that investigated the combined effect of three types of bacteria namely *Basillus* sp., *Lysinibacillus* sp. and *Rhodococcus* sp. in the treatment of leachate polluted soil. The combination of these bacteria created an interaction that yields high removal of Plumbum and Copper with 71% and 86%, respectively.

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4.4 Treatment with bacterial cocktail (Treatment 3)

In Treatment 3 all four bacteria namely *Bacillus salmalaya, Lysinibacillus sphaericus, Bacillus thuringiensis* and *Rhodococcus wratislaviensis* were mixed together to study the potential in treatment of pollutants in the leachate.

4.4.1 Physico-chemical characteristics of leachate in Treatment 3

Table 4.5 summarizes the physico-chemical characteristics of leachate before and after Treatment 3 for 48 hours. From the result of the study, oil and grease content denoted the highest removal of 98.3% that significantly reduced the concentration from 4.4 mg/L to 0.1 mg/L in the treated leachate.

Table 4.5 Physico-chemical characteristics of leachate before and after Treatment 3.

Parameter	Unit	Initial	Final	Reduction percentage (%)
Conductivity	μS/cm	35,830	30,700	14.3
Salinity	ppt	19	17	9.8
TDS	mg/L	20,320	18,450	9.2
Oil and Grease	mg/L	4.4	0.1	98.3
BOD_5	mg/L	1,050	1,230	-18.0
COD	mg/L	11,030	5,390	51.1
Ammoniacal Nitrogen	mg/L	6,400	2,900	54.7

Apart from that, Treatment 3 also showed a remarkable performance in reducing the ammoniacal nitrogen and COD to half of its original value with percentage removal of 54.7% and 51.1%, respectively. The ammoniacal nitrogen content dropped to 2,900 from 6,400 mg/L in the raw leachate. A significant reduction was observed in COD value in treated leachate from 11,030 mg/L to 5,390 mg/L.

A 14.3% reduction was observed in the conductivity value from 35,830 to 30,700 μS/cm. Salinity value showed a minor reduction 9.8% from 19.3 to 17.4 ppt. A slight

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reduction was found in the TDS value from 20,320 to 18,450 mg/L that reflects a percentage reduction of 9.2%. On the other hand, a negative removal (-18%) was observed in BOD_5 value increased to the initial BOD_5 value from 1,050 to 1,230 mg/L.

Figure 4.5 compares the physico-chemical characteristic of treated leachate between Treatment 3 and control experiment. From the observation, Treatment 3 shared the same removal capacity as both Treatment 1 and Treatment 2 for several parameters namely pH, TDS, salinity and conductivity. From the result of this study, no significant difference in these parameters can be observed between Treatment 3 and control experiment.

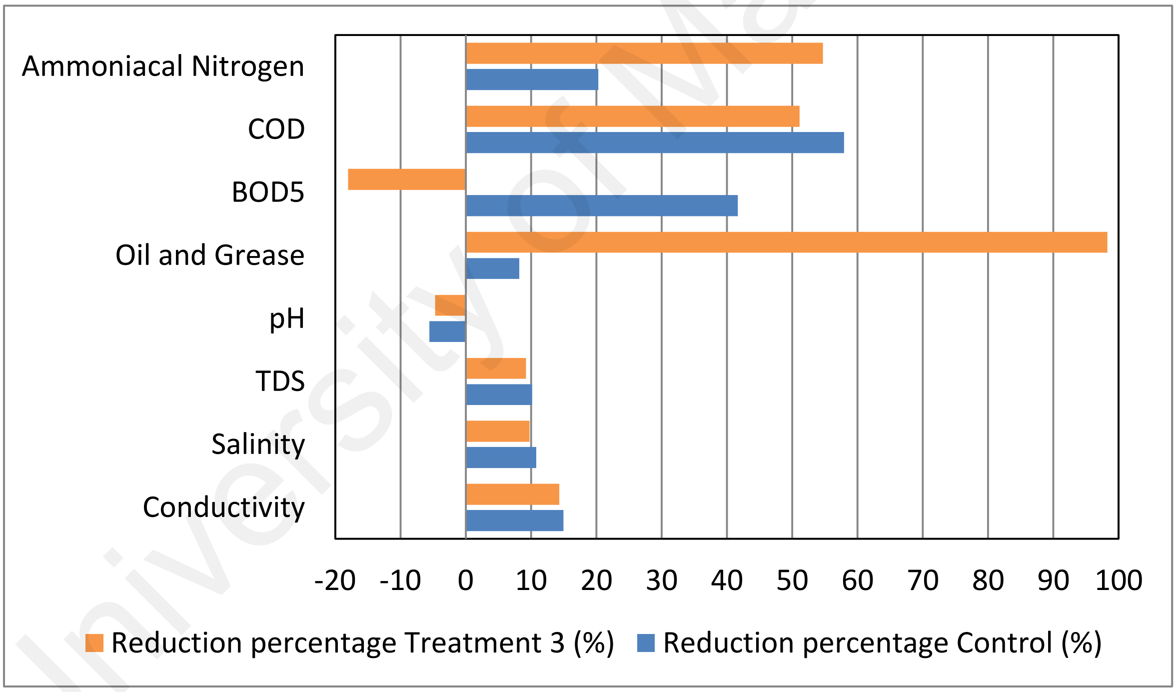

Figure 4.5 Comparison of reduction percentage between Treatment 3 and Control experiments

Treatment 3 presented a remarkable reducing capacity of oil and grease with 98% removal. By contrast only 9% removal was observed in control experiment. This indicates that mixed culture bacteria in Treatment 3 generated a better interaction and synergism in reducing hydrocarbon compound in the leachate. Addition of *Bacillus salmalaya* that is widely known for its hydrocarbon degrading capacity in Treatment 3 significantly improved the degradation of oil and grease compound hence promoting further reduction in their content in the treated leachate (Dadrasnia & Salmah, 2015). On top of that, incorporation of *Lysinibacillus* sp. and *Rhodococcus* sp. into Treatment 3 provide additional agent for microbial degradation and oxidation of hydrocarbon compound(Koshimizu *et al.*, 1997; Pizzul *et al.*, 2007). Apart from that, high removal (57%) of ammoniacal nitrogen was detected in Treatment 3 as to control experiment (20%). It shows the ability of the bacteria consortium to adapt ammoniacal nitrogen as their nitrogen source apart from carbon (Li 2013). On top of that, presence of two types of *Bacillus* sp. that came from ammonia degradation strain significantly improved the reduction percentage (Yu *et al.*, 2012). Similar outcome was reported by (Muthukrishnan *et al.*, 2015) that successfully employed several species of *Bacillus* sp. to remove total ammoniacal nitrogen content in shrimp wastewater.

On the contrary, a 19% increase in the BOD_5 value was observed in Treatment 3 as compared to 41% in control experiment. The result indicated that at the end of the treatment, there were plenty of organic matters present in the solution. This is due to the aforementioned rapid growth and death of the bacteria that resulted in accumulation of biomass in the solution. Higher oxygen is required to degrade the organic matter hence lower down the available dissolved oxygen in the system. Low supply of oxygen retarded the biochemical reaction and chemical oxidation of organic compound thus affected the COD value in Treatment 3. Lower reduction percentage of COD was observed in Treatment 3 with 52% as compared to 58% in control experiment. It is worthy to note that pH value showed no significant change across the treatment, therefore not included in the result.

4.4.2 Heavy metals reduction of leachate in Treatment 3

The potentials of the four bacteria mixture to remedy heavy metals component of raw leachate was evaluated in Treatment 3. Figure 4.6 shows the reduction percentage of heavy metals concentration when mixture of *B. salmalaya, L. sphaericus, B. thuringiensis* and *R. wratislaviensis* was introduced as remediation agent to raw leachate.

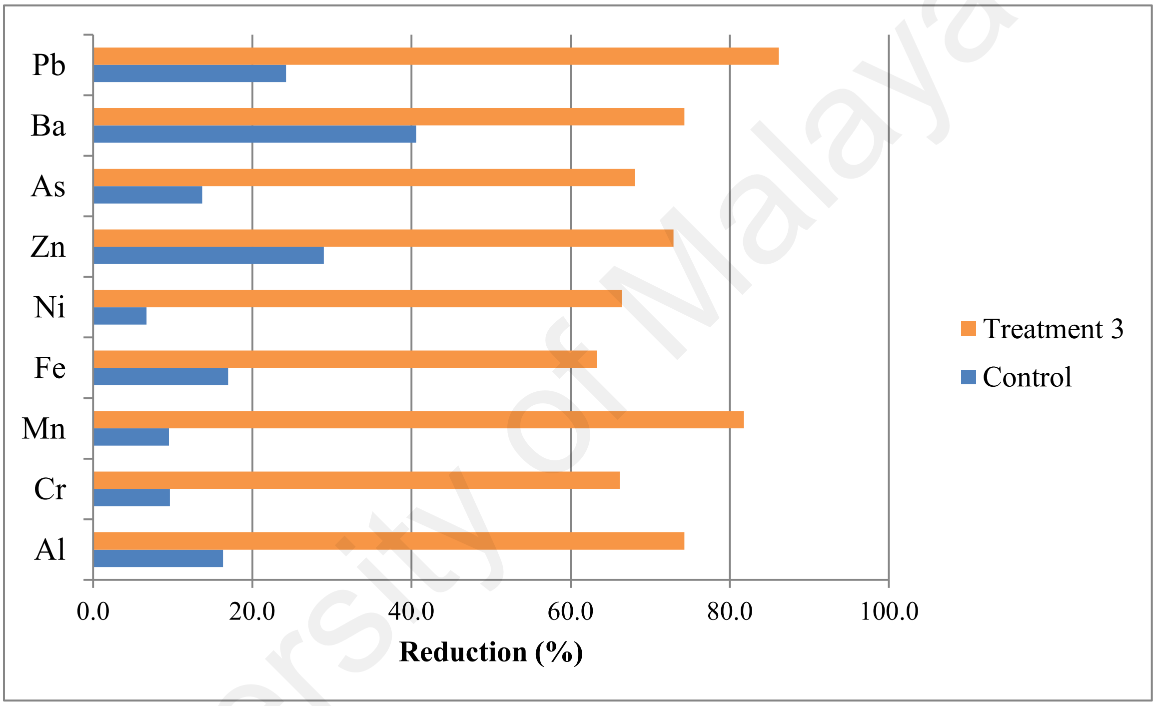

Figure 4.6 Heavy metal analysis of leachate in Treatment 3

Based on Figure 4.6, Treatment 3 showed a great potential in the remediation of heavy metals in leachate. Incorporation of mixed culture bacteria significantly improved the reduction percentage of heavy metals as opposed to the control experiment. It was found that, more than 60% of reduction in all heavy metals component was obtained using Treatment 3. Recorded removal of Lead (86%), Manganese (82%), Barium (74%), Aluminium (74%), Zinc (73%), Arsenic (68%), Nickel (66%), Chromium (66%) and Iron (63%) were obtained. It is interesting to note the control experiment also showed reduction values. There are residential organisms inside the raw leachate as the sample was not autoclaved. This may theoretically have some reduction effects.

Generally, microorganisms develop various resistance mechanisms to heavy metals such as transport across the cell membrane, biosorption to cell walls, entrapment in extracellular capsules, precipitation, complexation and oxidation (Yamina *et al.*, 2014). The application of *B. salmalaya, L. sphaericus, B. thuringiensis* and *R. wratislaviensis* showed a good assimilation of resistance mechanisms that resulted in high removal of heavy metals in leachate.

4.5 Comparison of Treatment

4.5.1 Comparisons of general characteristic of leachate for all treatment

Figure 4.7 shows the comparison on general characteristic of all treatments Treatment 1, Treatment 2, Treatment 3 and control experiments. In general, Treatment 1, Treatment 2 and Treatment 3 recorded a similar pattern of reduction capacity indicating that all four bacteria, either in single or mixed culture have the potential to remediate the leachate that initially have high BOD_5, COD, ammoniacal nitrogen and oil and grease content. They differ only slightly in the reduction and remediation maybe due to the different mechanisms and metabolical activities of the bacteria.

From the observation, all treatments showed similar trend of reduction in conductivity, salinity and TDS with the control experiments. The conductivity value reduced from 35,830 to 15,000 us/cm, salinity value reduced from 19.30 to 2 ppt and TDS value reduced from 20,320 to 2,000 mg/L. In relation to that, the pH values in all set of experiments were in the range of pH 8.3 to pH 8.8. The reductions in these general characteristics are minimal and are of similar values to the control experiments indicating the treatments did not show considerable improvement in the general parameters.

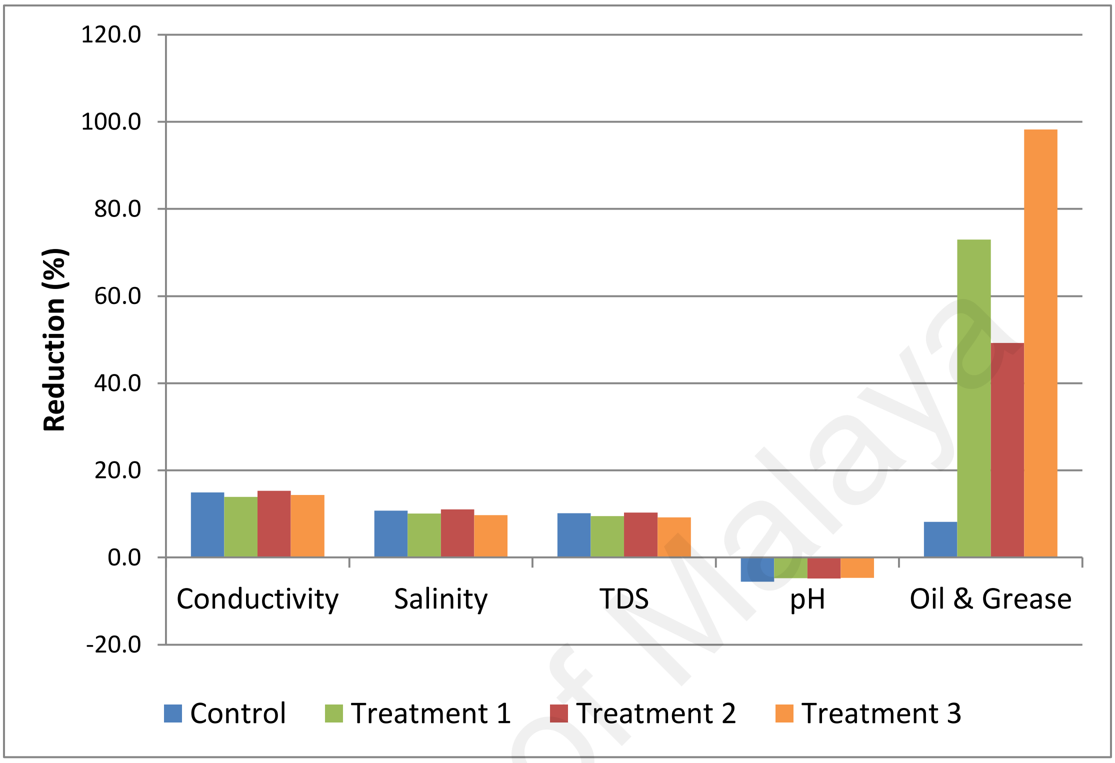

Figure 4.7 Reduction percentages of general characteristics and oil & grease content of leachate for Treatment 1, Treatment 2 and Treatment 3.

On the other hand, high reduction of oil and grease content (> 40%) were observed in all treatments. Treatment 3 recorded the highest reduction at 98.3% followed by Treatment 2 (73%) and Treatment 1 (49.2%). This was in contrast to control experiment which reduced the oil and grease content at only less than 10%. ANOVA analysis (Table 4.6) took into account the level of oil and grease before and after the treatment. The analysis of variance indicated significant differences with $p < 0.05$ for all treatment compared to initial value. Thus, from the result it can be said that the ability of the bacteria to alter and reduce the oil and grease composition in leachate is one of the main highlight of its bioremediation ability.

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Table 4.6 ANOVA analysis of levels oil and grease in the treatment

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.
Initial	control	.36320	.13654	.131
	Treatment 1	3.23613*	.13654	.000
	Treatment 2	2.18387*	.13654	.000
	Treatment 3	4.35853*	.13654	.000
control	Initial	-.36320	.13654	.131
	Treatment 1	2.87293*	.13654	.000
	Treatment 2	1.82067*	.13654	.000
	Treatment 3	3.99533*	.13654	.000
Treatment 1	Initial	-3.23613*	.13654	.000
	control	-2.87293*	.13654	.000
	Treatment 2	-1.05227*	.13654	.000
	Treatment 3	1.12240*	.13654	.000
Treatment 2	Initial	-2.18387*	.13654	.000
	control	-1.82067*	.13654	.000
	Treatment 1	1.05227*	.13654	.000
	Treatment 3	2.17467*	.13654	.000
Treatment 3	Initial	-4.35853*	.13654	.000
	control	-3.99533*	.13654	.000
	Treatment 1	-1.12240*	.13654	.000
	Treatment 2	-2.17467*	.13654	.000

*. The mean difference is significant at the 0.05 level.

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Single species treatment using *B. salmalaya* showed a good performance in the oil and grease biodegradation. This is consistent with previous finding by Dadrasnia and Salmah (2015) that demonstrated 89% oil and grease degradation in soil amended with *B. salmalaya* and organic waste. In addition, RP Singh *et al.* (2010) stated that *Bacillus* sp. strains possess the ability to produce extracellular lipase and cellulose enzymes which stimulates better waste treatment. Hydrolysis of oil by lipase degrades the oil into organic acid and volatile fatty acid which will be further decomposed into carbon dioxide and water (Koshimizu *et al.*, 1997).

However, incorporation of mixed bacteria consortium in Treatment 2 and Treatment 3 tremendously improved the degradation capacity for oil and grease. It was found that, *L. sphaericus* and *R. wratislaviensis* also played a role in the degradation of hydrocarbon compound. Previously, Pizzul *et al.* (2007) reported the efficiency of *Rhodococcus* sp. in the degradation of a mixture of hydrocarbons, gasoline, and diesel oil additives. In another study, two strains of *Rhodococcus* sp. were studied for their ability to degrade a variety of hydrocarbon and fuel additive compounds. It was found that, *Rhodococcus* sp. able to adapt hydrocarbon and fuel as a carbon and energy source and employed co-metabolic process in the degradation mechanism (Auffret *et al.*, 2009).

The result of this study is consistent to previous findings that revealed high degradation activity of mixed culture of organisms than that of single cultures of microorganisms (Benka-Coker & Ekundayo, 1997; Chigusa S *et al.*, 1996; Wakelin & Forster, 1997). It was found that, the synergistic effect of bacteria combination enhanced the performance for effective biodegradation.

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4.5.2 Comparisons of organic pollutants of leachate analysis for all treatment

Figure 4.8 shows the comparison of organic pollutants namely BOD_5 and COD in all treatments Treatment 1, Treatment 2, Treatment 3 and control experiment. From the observation, control experiment showed reduction in BOD_5 at more than 40 percent, indicating that oxygen availability improved tremendously after two days as solid suspended particle started to reside and the indigenous bacteria which is the residential bacteria that exist in the raw leachate performing their natural biodegradation without depleting much oxygen from their environment in the leachate. On the other hand, Treatment 1, Treatment 2 and Treatment 3 showed no reduction in BOD_5. This is may be due to the plenty of organic matters present in the final leachate after 48 hours. Higher organic matters found may be due to the aforementioned rapid growth and death of the bacteria that resulted in accumulation of biomass in the solution.

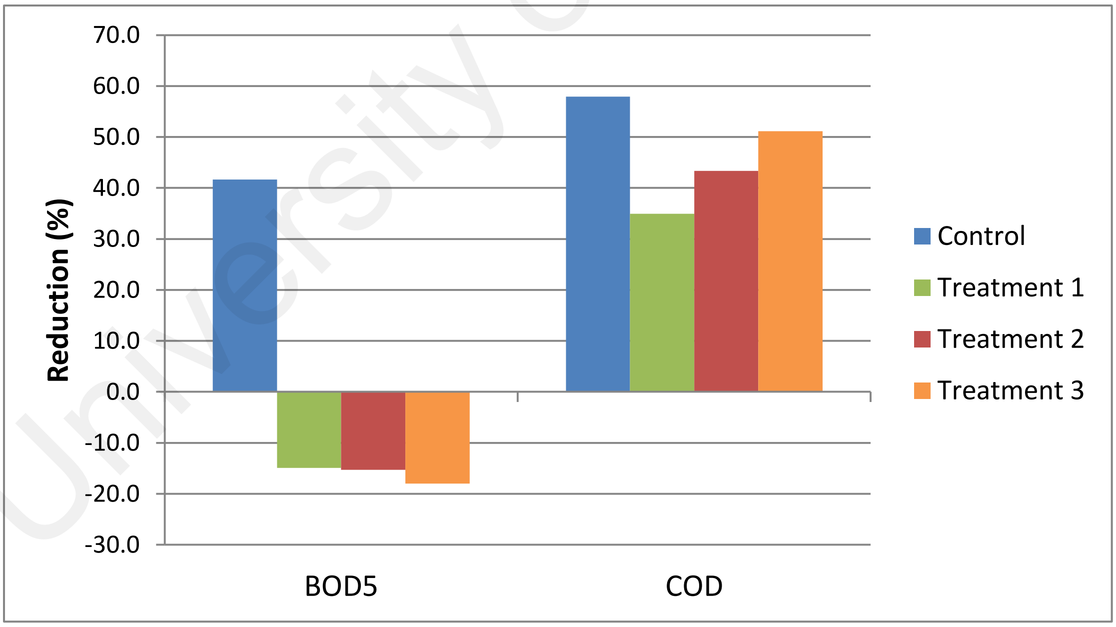

Figure 4.8 Reductions percentage of organic pollutants of leachate analysis of all treatment (Treatment 1, Treatment 2 and Treatment 3).

Furthermore, a reduction in COD was observed in all treatments. Control experiment recorded the highest reduction of COD with 58%, followed by Treatment 3 (50%), Treatment 2 (43%) and Treatment 1 (34%). This indicates that in raw leachate, natural degradation occurred either by indigenous bacteria or chemical transformation of organic materials in the system against time. The existing indigenous bacteria in leachate already adapted and acclimatized to the leachate environment thus increase its efficiency. Natural habitat degradation occurred as showed by reduction of both BOD_5 and COD in control.

The result of this study found that, bacteria consortia in this experiment have showed no apparent BOD_5 reduction ability in the current setting. The dense bacteria population (absorbance reading around 0.6 ABS at inoculation) has proliferated fast and suffocated the available oxygen in the treatment system.

Addition of 'alien' microbes into the system somehow disturbed the natural degradation and the new bacteria require adaptation to the harsh leachate condition thus could not match the natural degrader's reduction efficiency. The toxicity of the leachate could lead to the death of some bacteria adding their organic matter to the wastewater. Adaptation rate might be lower among mixed consortia as different strains competed intra and inter-species for nutrients and optimal metabolic activities. Different strains might also have different optimal growth condition such as salinity and pH whereas 37°C temperature was used in the experiments. This setting was used assuming natural environmental condition in tropical country like Malaysia with average noon temperature of 37°C and ambient salinity and pH of leachate unchanged.

Increase in values of BOD_5 in all treatments might be due to the increase in the total organic content as a result of rapid growth and death of the bacteria. Thus, it resulted in low supply of dissolved oxygen in the solution. In an oxygen-scarce condition, degradation reaction is distressed thus affecting the COD removal as can be seen in Treatment 1, Treatment 2, and Treatment 3. In addition, this may be due to non-optimal

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seeding concentration of bacteria and concentration (v/v) of inoculums for the treatments for the reduction to be visible. There is also possibility of longer treatment duration required as the bacterial just started to enter log exponential phase after 1-2 days of acclimatization.

On the other hand, results showed that mixed cultures of bacteria used in Treatment 2 and Treatment 3 produced higher reduction percentage compared to individual *Bacillus* sp. strain in Treatment 1. The incorporation of mixed culture allows various degradation mechanisms at once thus further reduce the organic matters in the raw leachate. Similar finding was reported in a study by Jameel and Abass Olanrewaju (2011) that achieved 78% COD reduction in mixed consortia application. Sivaprakasam *et al.* (2008) found that the degradation efficiency of single strain or mixed consortia depends on the salinity the solution. Single strain performed well in low salinity (2%) condition while mixed consortia showed high performance at higher salinity (8%) (Sivaprakasam *et al.*, 2008). From the observation, the salinity throughout the incubation period was recorded to be 19.3 ± 0.02 ppt (5 - 8%) which can be considered as high salinity. Higher COD reduction efficiency was observed in Treatment 2 and Treatment 3 (mixed consortia) as compared to single *B. salmalaya* application in Treatment 1. Mixed consortia in this showed better biodegradation ability of organic load.

4.5.3 Comparisons of nitrogenous pollutant of leachate analysis for all treatment

Figure 4.9 shows the comparison of nitrogenous pollutant in treated leachate in Treatment 1, Treatment 2 and Treatment 3 as compared to control experiment. From the observation, the reduction percentage increases from Treatment 1 with 39%, Treatment 2 with 45% and Treatment 3 with 55%. All treatment system were significantly higher than control experiment that gave only 20% reduction.

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The result of the study indicated that presence of single or mixed culture bacteria enhanced the removal of ammoniacal nitrogen in the leachate.

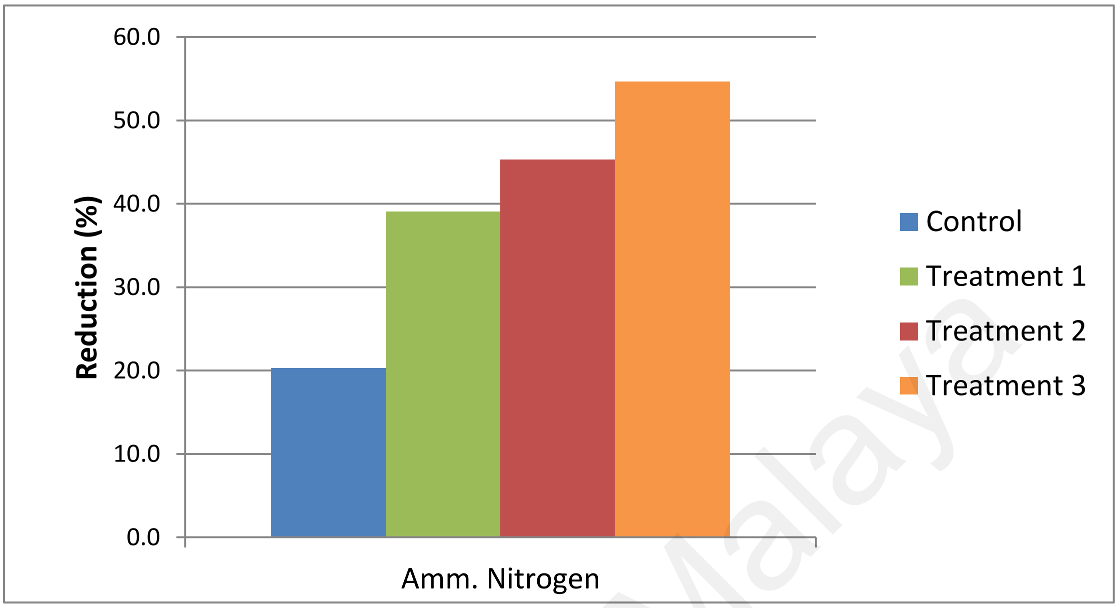

Figure 4.9 Reduction percentages of nitrogenous pollutants of leachate analysis of all treatment (Treatment 1, Treatment 2 and Treatment 3).

In addition, Treatment 3 showed the highest removal of ammoniacal nitrogen from 6,400 to 2,400 mg/L in the treated leachate. It might be the influence of the two *Bacillus* sp. that was introduced in Treatment 3. Previously, a few studies reported on string nitrite removal capacity of some *Bacillus* sp. strain including *B. subtilis* (Chen & Hu, 2011; Rui *et al.*, 2009), *B. lichenformis* (Rui *et al.*, 2009) and *B. cereus* (Lalloo *et al.*, 2007). On top of that, physiological studies on *Bacillus* sp. showed that it capable of utilizing nitrate and nitrite as alternative electron acceptors and nitrogen sources (Hoffmann *et al.*, 1998; Nakano *et al.*, 1998). In addition, *Bacillus* sp. is considered to be the best commercial biological agents for nitrogen removal and water quality enhancement (Hong & Cutting, 2005). Based on ANOVA results (Table 4.7), all three treatments showed statistically significance difference with p value less than 0.05 compared to control experiment. This confirmed that the presence of bacteria improved the removal of ammoniacal nitrogen in raw leachate.

Table 4.7 ANOVA analysis of levels ammoniacal nitrogen in the treatment

(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.
Initial	Control	1300.00000	709.92957	.409
	Treatment 1	2500.00000*	709.92957	.035
	Treatment 2	2900.00000*	709.92957	.015
	Treatment 3	3500.00000*	709.92957	.004
Control	Initial	-1300.00000	709.92957	.409
	Treatment 1	1200.00000	709.92957	.480
	Treatment 2	1600.00000	709.92957	.236
	Treatment 3	2200.00000	709.92957	.067
Treatment 1	Initial	-2500.00000*	709.92957	.035
	Control	-1200.00000	709.92957	.480
	Treatment 2	400.00000	709.92957	.978
	Treatment 3	1000.00000	709.92957	.636
Treatment 2	Initial	-2900.00000*	709.92957	.015
	Control	-1600.00000	709.92957	.236
	Treatment 1	-400.00000	709.92957	.978
	Treatment 3	600.00000	709.92957	.910
Treatment 3	Initial	-3500.00000*	709.92957	.004
	Control	-2200.00000	709.92957	.067
	Treatment 1	-1000.00000	709.92957	.636
	Treatment 2	-600.00000	709.92957	.910

*. The mean difference is significant at the 0.05 level.

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4.5.4 Comparisons of heavy metals analysis for all treatment

Figure 4.10 shows the comparison of heavy metals analysis in all treatment (Treatment 1, Treatment 2 and Treatment 3). The results showed comparatively higher reduction percentages (>50%) in the heavy metals concentration in all treatments. This is with exception of only one heavy metal i.e. Lead in Treatment 2 which showed 17% reduction. Discrete concentrations of the metals across the various 48 hours of biomonitoring showed similar variations.

One-way ANOVA for every single metal took into account concentrations of the heavy metals at both initial and the final monitoring for the 48 hours and the result were significant with $P < 0.05$ except for Barium where there are no significant difference for all the treatment while lead were only significant on Treatment 2 (refer to Appendix G).

The highest degree of reduction recorded in Treatment 3 where all four bacteria were mixed together to remediate the leachate. In Treatment 3, all heavy metals were reduced more than 60% from initial values with two heavy metals reached more than 80% reduction from initial reading. On the other hand, Treatment 1 and Treatment 2 showed comparably similar level of reduction from 46% to 74% for all heavy metals except for Aluminium and Lead. Aluminium reduction rate is higher in Treatment 2 (72%) compared to Treatment 1 (60%) while Lead reduction rate is only 17% in Treatment 2 compared to 46% in Treatment 1. This result indicated that the bacteria have good potentials to remedy heavy metals pollutants either in single application or mixed consortia.

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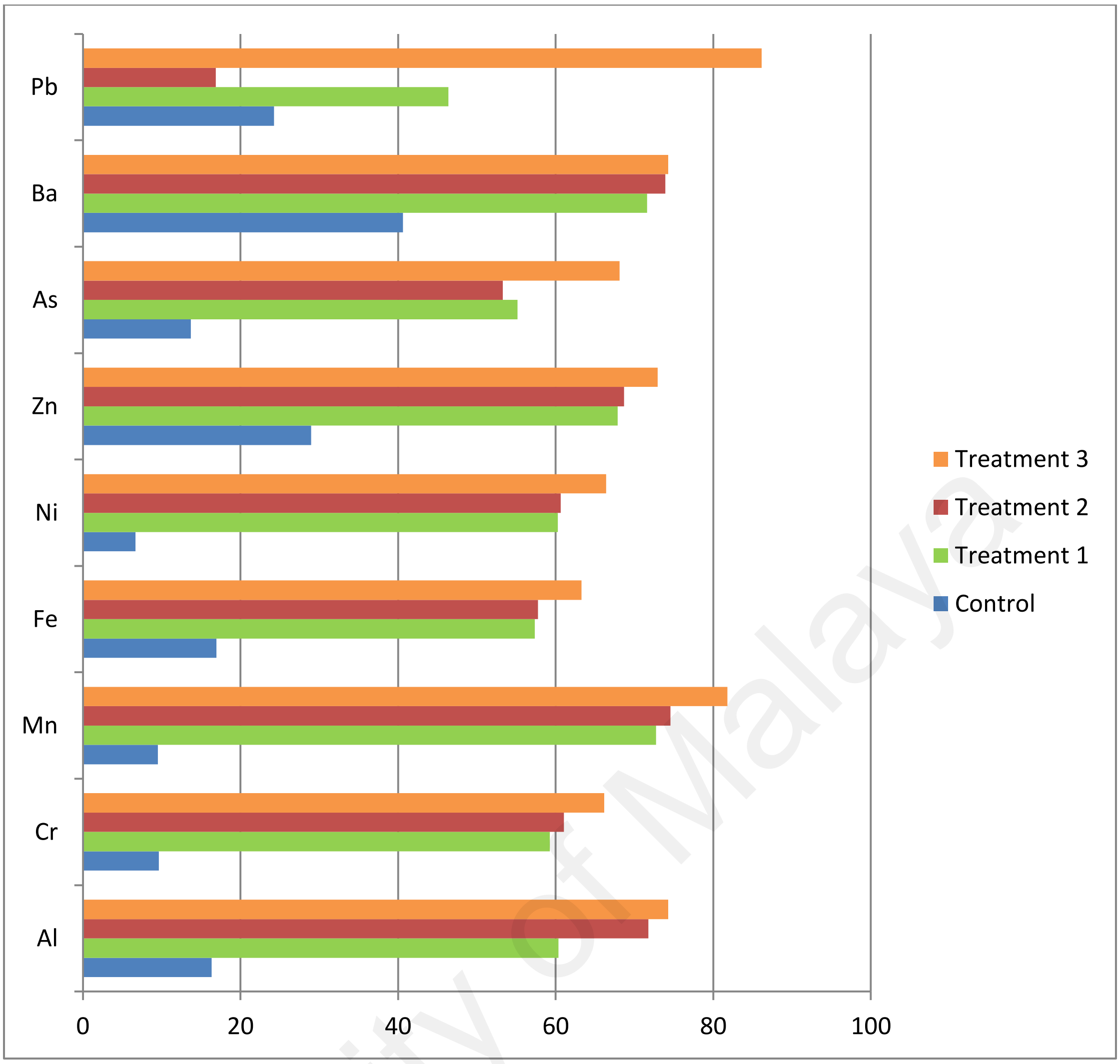

Figure 4.10 Percentage of reduction of heavy metals in leachate analysis of all three treatments (Treatment 1, Treatment 2 and Treatment 3).

B. salmalaya as single specie application in Treatment 1 showed a reduction between 46% to 73% of heavy metals degradation namely Manganese, Barium, Zinc, Aluminium, Nickel, Iron, Arsenic and Lead. To the date, there are no published result was found on the potential of *B. salmalaya* species in heavy metals remediation. This result showed the potential of the *B. salmalaya* as remediating agent for major heavy metals polluter. Previously, several species of *Bacillus* sp. showed similar potential for bioremediation of heavy metal. Sulaimon *et al.* (2014) found that *Bacillus subtilis* was most efficient in the removal of copper with 90.49% and arsenic with 57.7% accumulation under agitated condition. In another study by In a study by Guo *et al.* (2010), an endophytic bacterial strain *Bacillus sp.* EB L14 was profound in the removal

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of divalent heavy metals, especially Pb (II) and Cd (II) with concentration reduction about 80.48% and 75.78% after 24 hours. In addition, *Bacillus sp*. also showed remarkable performance as bioaccumulation medium for zinc ions and had high adsorption yields for the treatment of wastewater containing zinc (Krishna *et al.*, 2013).

In Treatment 2, the incorporation of mixed consortia consisted of *L. sphaericus, B. thuringiensis* and *R. wratislaviensis* in the remediation of heavy metals showed better performance than Treatment 1. The reduction percentages for all heavy metal were between 18% to 75% with no significant difference P>0.05 (refer to Appendix G) except for lead (P=0.01). These bacteria have been showed to have good heavy metal degradation individually in other studies. However, mixed consortia showed even better heavy metals degradation potentials rather than single application. This is mainly due to specific and complex interaction with less antagonistic effect of inter-species. On top of that, synergistic relationship between species in the mixed consortia resulted in better degradation efficiency of heavy metals component in the treated leachate. Also considering that *Lysinibacillus* sp. possessed a hex-histidine tag (His_6- -tag) at the C-terminus of its S-layer protein SbpA, it is possible that the metal binding property of His_6-tag was better expressed when in association with *Bacillus* sp. and *Rhodococcus* sp. hence providing the bioremediation edge for the treatment (Emenike *et al.*, 2013a).

The best results obtained when all four species were combined in Treatment 3. Overall reduction percentages are higher from Treatment 1 and Treatment 2 indicating good synergism in the bacteria growth and metabolism to transform the various heavy metals from ionic form to inactive complexes in their cells. The introduction of *B. salmalaya* in Treatment 3 did not show antagonistic reactions with *L. sphaericus*or and *R. wratislaviensis* owing to its similar catabolic ability as *B. thuringiensis* of the same genus.

Another factor that contributes to the efficient heavy metals removal was pH of the solution. During the incubation period, the pH of the solution was within pH 8-8.5 that is suitable for heavy metal removal. Previously, Sze *et al.* (1996) stated that pH range 5-8 is good for heavy metal removal due to absence of H+ ions. On the other hand, at lower pH, H^+ presents in abundance thus increase the competition with heavy metals that decrease the removal capacity of bacteria.

Cell age is considered as an important microbial factor that affects heavy metals accumulation. Maximum heavy metals uptake by bacterial strains occurred after three days incubation is in conformity with previous findings by (Mondal *et al.*, 2008). This is possibly due to the presence of many highly active enzymes at this growth phase, during which cells are at their most metabolically active stage (Kumar *et al.*, 2010).

On top of that, the formation of biomass in the treatment greatly influenced the heavy metals removal. Heavy metals can be removed via adsorption onto bacterial biomass or can be known as biosorbent (Djefal-Kerrar *et al.*, 2014). The result of this study showed that as time increase, the biomass increased too. Likewise, with increase in biomass, heavy metals bioaccumulation also increased. This is mainly due to the increase in the surface area that improves the adsorptive nature or increases the number of active binding sites on cell surface. The active mode of metal accumulation by living cells is usually designated as bioaccumulation (Krishna *et al.*, 2013). This process is dependent on the metabolic activity of the cell referred to its intrinsic biochemical and structural properties, physiological and/or genetic adaptation, environmental modification of metal specification, availability, and toxicity (Krishna *et al.*, 2013). On the other hand, biosorption using microbial biomass is a passive removal which considered as metabolism-independent process. The efficiency depends on cell surface area and spatial structure of cell wall (Pun *et al.*, 2013). Both living and dead biomass can occur for biosorption because it is independent of cell metabolism (Coelho *et al.,*2015).

4.5.5 General discussion

The death and lysis of the microbial cells may be one of the possibilities for the bacterial strain condition during bioremediation and further studies of other aspects that have not been explored in the study is suggested. This may be another theoretical scenario, in which the leachate should be analyzed in future work on products of the bacteria during the bioremediation process that may help in reducing the recalcitrant contaminants. In summary, treatment of landfill leachate with the bacterial species has showed good results in degrading several components including nitrogenous pollutants and heavy metals, while it showed non-considerable effects to other parameters. There is reduction in chemical organics content but it is the opposite case in the decomposable organics content indicated by increase in the BOD_5 values. This may be due to experimental and bacterial strain optimization that needs to be further refined.

Comparison and ANOVA was done to show statistically difference as parameters were chosen with some limited grounds. The factors for type of bacteria usage and the mix were sufficiently to identify the statistical analysis. Parameters were chosen to sufficiently test the bioremediation capability but not extensively for further research due to other research limitations such as cost and technical constraints. The factors for type of bacteria usage and the mix have been explained in Chapter 3.

There were numbers of studies performed to test the bioremediation potential of locally isolated bacteria in wastewater treatment. Several of the results of recent studies were tabulated in the Table 4.8.

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Table 4.8 Various examples of microorganisms having biodegradation potentials comparing with this study

Parameters Studied	Removal percentage (%)	Bacteria (Single/Mono or Mixed Application)	Source	References
BOD	42.86	*Bacillus licheniformis* NW16 + *Aeromonas hydrophilia* NS17	Municipal wastewater	Sonune & Garode (2015)
TDS	81.4	*Paenibacillus sp.* NW9		
COD	82.76	*Bacillus licheniformis* NW16		
	81.61	*Paenibacillus sp.* NW9		
BOD	41.9	*Rhodopseudomonas palustris* + *E.coli*	Untreated river wastewater	Shrivastava *et al.* (2013)
COD	92.64			
BOD	93.55	*Bacillus subtilis*		
COD	73.9			
Oil and grease	79	*Bacillus salmalaya* 139SI	Water contaminated with crude oil waste	Salmah & Dadrasnia (2015)
Copper	90.49	*Bacillus subtilis*	Dumpsite leachate	Sulaimon *et al.* (2014)
Arsenic	57.7			
Zinc	54	*Bacillus thuringiensis*	Industrial wastewater	Kumar *et al.* (2015)
Lead	43	*Bacillus subtilis*		
Ammoniacal Nitrogen	93	*Bacillus amyloliquefaciens*	Industrial wastewater	Yu *et al.* (2012)
Oil and grease	73	*Bacillus salmalaya*	Jeram sanitary landfill leachate	This report
Oil and grease	98.3	*Bacillus salmalaya* + *Lysinibacillus sphaericus* + *Bacillus thuringiensis* + *Rhodococcus wratislaviensis*		
Ammoniacal Nitrogen	54.7			
Lead	86			
Zinc	73			
Arsenic	68			
BOD	-18			
COD	51.1			
TDS	9.2			

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Sonune and Garode (2015) screened and isolated 44 bacteria from municipal wastewater and sludges which 8 specie were successfully grown in wastewater environment. The bacteria were used in treatment as single isolate or monoculture. The highest percentage of 42.86% removal of BOD after 72 hours of treatment was observed by *Bacillus licheniformis NW16* and *Aeromonas hydrophilia*. The COD removal of more than 80% was observed by *Bacillus licheniformis NW16* and *Paenibacillus sp. NW9*. The specie *Paenibacillus sp. NW9* also showed high TDS reduction of 81.4% after 72 hours treatment in municipal wastewater. In this study, it is interesting to note that although wastewater samples were pre-sterilized there were 10-30% reduction of BOD_5, TDS and COD recorded.

Another study by Shrivastava *et al.* (2013) tested 31 isolated bacteria with untreated polluted river water and found that 8 isolates showed degrading capacity of waste water pollutants. *Rhodopseudomonas palustris* and *E.coli* recorded 41.9% BOD removal and 92.64% COD removal when used in combination to degrade waste water. Other combinations also showed similar removal potential. Single isolate or monoculture of *Bacillus subtilis* recorded highest reduction in BOD and COD at 93.55% and 73.9%. This study indicated that bacteria can be used in both single and combination to degrade waste water.

In bioremediation study of water contaminated with crude oil waste by Salmah and Dadrasnia (2015) a novel specie *Bacillus salmaya* 139SI showed good oil and grease reduction potential of 79%. This is the same specie used by author in this study which recorded nearly similar reduction percentage of 73%.

The specie *Bacillus subtilis* also showed potential in reduction of Copper and Arsenic metals level in dumpsite leachate (Sulaimon *et al*., 2 014). The Copper content reduced by 90.49% and arsenic level reduced to 57.7% after treatment of leachate with the

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bacteria for duration of 10 to 15 days. Another study by Kumar *et al.* (2015) isolated bacteria from heavy metal contaminated soil and tested in bioaccumulation assay. After 3 days, the average Zinc reduction of 54% achieved by *Bacillus thuringiensis* and lead reduction of 43% recorded by *Bacillus subtilis* in single culture. The microorganisms' metal bio-accumulation capacity showed the potential role in the bioremediation of heavy metals in contaminated aquatic environment by heavy metal containing leachate.

In general, the genus *Bacillus* bacteria have shown highly potent activity in degradation of pollutants in wastewater across various studies including author's work in this report. Different species for example *Bacillus licheniformis, Bacillus subtilis, Bacillus thuringiensis* and *Bacillus salmalaya* may have different specific mechanism of metabolism of organic and inorganic pollutants but however in overall showed reduction of parameters of BOD_5, COD, ammoniacal nitrogen and several metals such as Arsenic, Zinc and Lead. It is also interesting to note that the new novel specie locally identified *Bacillus salmalaya* have also showed good remediation potential indicating that there is possibility of other wild strain or local soil bacteria which could perform the same degradation process. That is also the possible reason of high reduction percentage observed in control experiments, apart from the fact that the samples were not pre-sterilized to mimicked real application.

In situation where single bacteria application is not favorable, the mixed bacteria species treatment of wastewater could also be carried out. This report showed that this setting improved the reduction percentage and achieved best results. *Bacillus salmalaya* have been tested with *Lysinibacillus sphaericus, Bacillus thuringiensis and Rhodococcus wratislaviensis* isolated from previous work of Emenike (2011) and showed good potential of bioremediation. This may due to the synergistic mechanism of all bacteria in the concoction and highly adaptation ability of the specie in the harsh leachate environment.

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CHAPTER 5: CONCLUSION

The study concludes that the landfill leachate is indeed contaminated with toxic components such as dissolved organics matter, ammonia and heavy metals. Comparison with previous studies also proved that the characteristics of the JSL landfill leachate are more or less within the same range and contains toxic compounds (organics matter and ammoniacal nitrogen) that exceed the discharged limits. The leachate also contains high content of oil and grease, and traces of heavy metals but still within maximum limits permitted. Proper landfill leachate treatment is still needed to remedy this wastewater before it is discharged.

Bioremediation of the leachate has been successfully carried out, using several strains of bacteria previously isolated either in single specie or mixed consortia application. In general, all treatments setups have not shown any observable reduction in general characteristics of the leachate (conductivity, salinity and pH) but significant reduction in oil and grease content. There is also noticeable reduction in COD although the opposite case is showed for the BOD_5 as the BOD_5 increase for all treatments after 48 hours. Ammoniacal nitrogen content has been reduced to approximately 50% of initial value in all treatments setup. Highlight of the remediation is the significant reduction in heavy metals content which ranging from minimum 40% to 89% reduction. Comparing between the species, *B. salmalaya* (Treatment 1) showed a good bioremediation potential followed by the mixture of *L. sphaericus, B. thuringiensis* and *R. wratislaviensis* in Treatment 2. The best results were obtained when all four strains were combined in Treatment 3 which resulted in highest reductions were recorded in all parameters such as oil and grease (98.3%), ammoniacal nitrogen (57%), Lead (86%),

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Manganese (82%), Barium (74%), Aluminium (74%), Zinc (73%), Arsenic (68%), Nickel (66%), Chromium (66%) and Iron (63%).

In conclusion, the microbial mixture have showed potential in remediating highly heterogeneous and polluted wastewater such as JSL landfill leachate. It is worthy to note that the bacterial growth in such environment is highly unfavorable but the strains managed to adapt, metabolize and somehow degrade the pollutants in the system. There are some positive results for the metal reduction study although not across all metals analysed and further analysis in future work could be done to elucidate the scenario. The implication of this result is that it could be tested on more highly polluted leachate or wastewater in future works to study the bioremediation ability of the bacteria. The findings fit the purpose of the study planned at the minimum to partially and at best scenario completely fulfilling the requirement of all of the objectives.

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REFERENCES

Abbas, A. A., Jingsong, G., Ping, L. Z., Pan, Y. Y., & Al-Rekabi, W. S. (2009). Review on Landfill Leachate Treatments. *American Journal of Applied Sciences, 6*(4), 672.

Abdullah , N., Shuhaimi, S., Toh, Y., Shafee, A., & Maznorizan, M. (2011). The Study of Seasonal Variation of PM10 Concentration in Peninsula, Sabah and Sarawak. *Malaysian Meteorological Department*(9).

Abou-Shanab, R., Van Berkum, P., & Angle, J. (2007). Heavy metal resistance and genotypic analysis of metal resistance genes in gram-positive and gram-negative bacteria present in Ni-rich serpentine soil and in the rhizosphere of Alyssum murale. *Chemosphere, 68*(2), 360-367.

Aderemi, A. O., Oriaku, A. V., Adewumi, G. A., & Otitoloju, A. A. (2011). Assessment of groundwater contamination by leachate near a municipal solid waste landfill. *African Journal of Environmental Science and Technology, 5*(11), 933-940.

Adhikari, B., Dahal, R., & Khanal, N. (2014). A Review of Factors Affecting the Composition of Municipal Solid Waste Landfill Leachate. *International Journal of Engineering Science and Innovative Technology (IJESIT), 3*(5), 273-281.

Adhikari, B., Manandhar, D., & Khanal, S. (2013). Study Of Leachate And Waste Composition At Different Landfill Sites Of Nepal. *Journal of Science, Engineering and Technology, 9*(2), 15-21.

Agamuthu, P. (2001). *Solid waste: principles and management; with Malaysian case studies*: Inst. of Biological Sciences, University of Malaya.

Agamuthu, P., & Fauziah, S. (2011). Challenges and issues in moving towards sustainable landfilling in a transitory country-Malaysia. *Waste Management & Research, 29*(1), 13-19.

Agamuthu , P., Fauziah, S., Khidzir, K., & Aiza, A. N. (2007). *Sustainable waste management-Asian perspectives.* Paper presented at the Proceedings of the International Conference on Sustainable Solid Waste Management.

Agamuthu, P., & Fauziah, S. H. (2011). Challenges and issues in moving towards sustainable landfilling in a transitory country - Malaysia. *Waste Manag Res, 29*(1), 13-19. doi:10.1177/0734242X10383080

Agarwal, S. K. (1998). *Environmental biotechnology*: APH Publishing.

Agbozu, I., Oghama, O., & Odhikori, J. (2015). Physico-Chemical Characterization and Pollution Index Determination of Leachates from Warri Waste Dumpsite, Southern Nigeria. *Journal of Applied Sciences and Environmental Management, 19*(3), 361-372.

Ahmed, F. N., & Lan, C. Q. (2012). Treatment of landfill leachate using membrane bioreactors: A review. *Desalination, 287*, 41-54.

Akinbile, C. O., & Yusoff, M. S. (2011). Environmental impact of leachate pollution on groundwater supplies in Akure, Nigeria. *International Journal of Environmental Science and Development, 2*(1), 81-89.

Al-Yaqout, A., & Hamoda, M. (2003). Evaluation of landfill leachate in arid climate—a case study. *Environment international, 29*(5), 593-600.

Alkassasbeh, J. Y., Heng, L. Y., & Surif, S. (2009). Toxicity Testing and the Effect of Landfill Leachate in Malaysia on Behavior of Common Carp (Cyprinus carpio L., 1758; Pisces, Cyprinidae) *American Journal of Environmental Sciences, 5*(3), 209.

Alvarez-Vazquez, H., Jefferson, B., & Judd, S. J. (2004). Membrane bioreactors vs conventional biological treatment of landfill leachate: a brief review. *Journal of chemical technology and biotechnology, 79*(10), 1043-1049.

Alvarez, P. J., & Illman, W. A. (2005). *Bioremediation and natural attenuation: process fundamentals and mathematical models* (Vol. 27): John Wiley & Sons.

Amokrane, A., Comel, C., & Veron, J. (1997). Landfill leachates pretreatment by coagulation-flocculation. *Water research, 31*(11), 2775-2782.

Anand, A., & Singh, S. S. (2014). Membrane Technique for Leachate Treatment-A Literature Review. *International Journal of Environmental Research and Development, 4*(1), 33-36.

Ansari, M. I., & Malik, A. (2007). Biosorption of nickel and cadmium by metal resistant bacterial isolates from agricultural soil irrigated with industrial wastewater. *Bioresource Technology, 98*(16), 3149-3153.

Antonello, S. D. (2007). *Frontiers in Ecology Research*: Nova Science.

APHA, A. W.(2012). *Standard Methods for the Examination of Water and Wastewater. American Public Health Association, American Water Works Association & Water Environment Federation.(EW Rice, RB Baird, AD Eaton, & LS Clesceri, Ed.)(22nd ed.). Washington*.

Ariyanto, E., Ang, H. M., & Sen, T. (2011). *Effect of initial solution pH on solubility and morphology of struvite crystals.* Paper presented at the CHEMECA Conference, Sydney.

ASTM D5673-16 (2010) Standard Test Method for Elements in Water by Inductively Coupled Plasma—Mass Spectrometry, ASTM International, West Conshohocken, PA

Aucott, M. (2008). The Tidal Exchange. *The fate of heavy metals in landfills: A Review,* (21).

Auffret, M., Labbé, D., Thouand, G., Greer, C. W., & Fayolle-Guichard, F. (2009). Degradation of a mixture of hydrocarbons, gasoline, and diesel oil additives by Rhodococcus aetherivorans and Rhodococcus wratislaviensis. *Applied and environmental microbiology, 75*(24), 7774-7782.

University of Malaya

Aziz, Aziz, H. A., Yusoff, M. S., Bashir, M. J., & Umar, M. (2010). Leachate characterization in semi-aerobic and anaerobic sanitary landfills: A comparative study. *Journal of environmental management, 91*(12), 2608-2614.

Aziz, H. A., Adlan, M. N., Zahari, M. S. M., & Alias, S. (2004). Removal of ammoniacal nitrogen (N-NH3) from municipal solid waste leachate by using activated carbon and limestone. *Waste Management & Research, 22*(5), 371-375.

Aziz , H. A., Daud, Z., Adlan, M. N., & Hung, Y.-T. (2009). The use of polyaluminium chloride for removing colour, COD and ammonia from semi-aerobic leachate. *International Journal of Environmental Engineering, 1*(1), 20-35.

Baig, S., Coulomb, I., Courant, P., & Liechti, P. (1999). Treatment of landfill leachates: Lapeyrouse and Satrod case studies. *Ozone: Sciences & Engineering, 21*(1), 1-22.

Bala, J. D., Lalung, J., & Ismail, N. (2015). Studies on the reduction of organic load from palm oil mill effluent (POME) by bacterial strains. *International Journal of Recycling of Organic Waste in Agriculture*, 1-10.

Ball, J. (2005). *Landfill site selection.* Paper presented at the Tenth International Waste Management Symposium S. Margherita di Pula, Cagliari, Italy.

Barnes, K. K., Christenson, S. C., Kolpin, D. W., Focazio, M. J., Furlong, E. T., Zaugg, S. D., . . . Barber, L. B. (2004). Pharmaceuticals and other organic waste water contaminants within a leachate plume downgradient of a municipal landfill. *Groundwater Monitoring & Remediation, 24*(2), 119-126.

Baun, D. L., & Christensen, T. H. (2004). Speciation of heavy metals in landfill leachate: a review. *Waste Management & Research, 22*(1), 3-23.

Benka-Coker, M., & Ekundayo, J. (1997). Applicability of evaluating the ability of microbes isolated from an oil spill site to degrade oil. *Environmental monitoring and assessment, 45*(3), 259-272.

Białowiec, A. (2011). Hazardous Emissions from Municipal Solid Waste Landfills *Contemporary Problems of Management and Environmental Protection* (Vol. 9, pp. 7-28).

Bodzek, M., Łobos-Moysa, E., & Zamorowska, M. (2006). Removal of organic compounds from municipal landfill leachate in a membrane bioreactor. *Desalination, 198*(1), 16-23.

Bohdziewicz, J., Neczaj, E., & Kwarciak, A. (2008). Landfill leachate treatment by means of anaerobic membrane bioreactor. *Desalination, 221*(1), 559-565.

Bruins, M. R., Kapil, S., & Oehme, F. W. (2000). Microbial resistance to metals in the environment. *Ecotoxicology and environmental safety, 45*(3), 198-207.

University of Malaya

Butkovskyi, A. (2009). *Leachate Treatment at Filborna Landfill with Focus on Nitrogen Removal.* Masters Thesis, Department of Chemical Engineering, Lund University, Sweden.[Links].

Cecen, F., & Gursoy, G. (2000). Characterization of landfill leachates and studies on heavy metal removal. *Journal of Environmental Monitoring, 2*(5), 436-442. doi:10.1039/B004729P

Çelen, I., & Türker, M. (2001). Recovery of ammonia as struvite from anaerobic digester effluents. *Environmental technology, 22*(11), 1263-1272.

Chemtrade. (2014). Struvite Control. Retrieved from http://www.generalchemical.com/assets/pdf/Struvite_Control_in_Wastewater.pdf

Chen , J.-Z., Tao, X.-C., Xu, J., Zhang, T., & Liu, Z.-L. (2005). Biosorption of lead, cadmium and mercury by immobilized Microcystis aeruginosa in a column. *Process Biochemistry, 40*(12), 3675-3679.

Chen, S.-z., & Hu, Y.-y. (2011). Use of Bacillus subtilis in purification of slightly-polluted water. *Acta Scienctiae Circumstantiae, 31*(8), 1594-1601.

Chen , X. C., Wang, Y. P., Lin, Q., Shi, J. Y., Wu, W. X., & Chen, Y. X. (2005). Biosorption of copper (II) and zinc (II) from aqueous solution by Pseudomonas putida CZ1. *Colloids and Surfaces B: Biointerfaces, 46*(2), 101-107.

Chian, E. S., & DeWalle, F. B. (1975). *Compilation of methodology for measuring pollution parameters of landfill leachate*: US Environmental Protection Agency, Office of Air, Land, and Water Use, Municipal Environmental Research Laboratory.

Chigusa S, Hasegawa T, Yamamoto N, & Y, W. (1996). Treatment of waste-Chappe water from oil manufacturing plant by yeasts. . *Wat Sci Tech, 34*(11), 51-58.

Christensen, T. H., Kjeldsen, P., Bjerg, P. L., Jensen, D. L., Christensen, J. B., Baun, A., . . . Heron, G. (2001). Biogeochemistry of landfill leachate plumes. *Applied geochemistry, 16*(7), 659-718.

Chu, L., Cheung, K., & Wong, M. (1994). Variations in the chemical properties of landfill leachate. *Environmental Management, 18*(1), 105-117.

Coelho, L. M., Rezende, H. C., Coelho, L. M., de Sousa, P. A., Melo, D. F., & Coelho, N. M. (2015). Bioremediation of Polluted Waters Using Microorganisms. *Agricultural and Biological Sciences»" Advances in Bioremediation of Wastewater and Polluted Soil", book edited by Naofumi Shiomi*.

Colberg, P. J., & Young, L. Y. (1995). Anaerobic degradation of nonhalogenated homocyclic aromatic compounds coupled with nitrate, iron, or sulfate reduction. *Microbial transformation and degradation of toxic organic chemicals, 307330*.

University of Malaya

Crutcher, A. J., & Yardley, J. R. (1991). Implications of Changing Refuse Quantities and Characteristics on Future Landfill Design and Operations. *Municipal solid waste management: making decisions in the face of uncertainty*, 171.

Cybulski, Z., Dziurla, E., Kaczorek, E., & Olszanowski, A. (2003). The influence of emulsifiers on hydrocarbon biodegradation by Pseudomonadacea and Bacillacea strains. *Spill Science & Technology Bulletin, 8*(5), 503-507.

Dadrasnia, A., Chuan Wei, K. S., Shahsavari, N., Azirun, M. S., & Ismail, S. (2015). Biosorption Potential of Bacillus salmalaya Strain 139SI for Removal of Cr (VI) from Aqueous Solution. *International journal of environmental research and public health, 12*(12), 15321-15338.

Dadrasnia, A., & Salmah, I. (2015). Bio-enrichment of Waste Crude Oil Polluted Soil: Amended with Bacillus 139SI and Organic Waste. *International Journal of Environmental Science and Development, 6*(4), 241-245.

Dadrasnia, A., Usman, M. M., Wei, K. S. C., Velappan, R. D., Jamali, H., Mohebali, N., & Ismail, S. (2016). Native soil bacterial isolate in Malaysia exhibit promising supplements on degrading organic pollutants. *Process Safety and Environmental Protection, 100*, 264-271.

Dandautiya, R. (2012). *Comparative Study Of Existing Leachate Treatment Methods*. Paper presented at the International Conference on Recent Trends in Engineering & Technology (ICRTETreatment 2012), India.

Das, S. (2014). *Microbial Biodegradation and Bioremediation* (S. Das Ed. 1 ed.): Elsevier Science.

Davis, E. B. (2006). *Frontiers in Environmental Research*: Nova Science Publishers.

Department of Statistics Malaysia. (2010). *Population Distribution and Basic Demographic Characteristics*.

Dhillon, R. (2014, Nov 15, 2014). Govt to make it mandatory for households to separate solid waste from Sept next year. *The Rakyat Post*.

Djefal-Kerrar, A., Abdoun-Ouallouche, K., Khadraoui, L., & Belounis, A. (2014). Bioaccumulation of Heavy Metals by Non-living Rhodococcus Erythropolis B4.

Donlon, D., & Bauder, J. (2006). A General Essay on Bioremediation of Contaminated Soil. *Department of land Resources an Environmental Sciences. Montana State University. waterquality. montana. edu/docs/methane/Donlan. shtml*.

DWLC. (1998a). *Window on Water*. NSW.

Edi, M., & Fellner, J. (2013). *Guidelines for Design and Operation of Municipal Solid Waste Landfills in Tropical Climates*. Retrieved from

Ejlertsson, J., Meyerson, U., & Svensson, B. (1996). Anaerobic degradation of phthalic acid esters during digestion of municipal solid waste under landfilling conditions. *Biodegradation, 7*(4), 345-352.

University of Malaya

El-Fadel, M., Bou-Zeid, E., Chahine, W., & Alayli, B. (2002). Temporal variation of leachate quality from pre-sorted and baled municipal solid waste with high organic and moisture content. *Waste management, 22*(3), 269-282.

Emenike, C., Agamuthu, P., & Fauziah, S. (2016). Blending Bacillus sp., Lysinibacillus sp. and Rhodococcus sp. for optimal reduction of heavy metals in leachate contaminated soil. *Environmental Earth Sciences, 75*(1), 1-8.

Emenike, C. U., Fauziah, S. H., & Agamuthu, P. (2011). Characterization of active landfill leachate and associated impacts on edible fish (Orechromis mossambicus). *Malaysian Journal of Science*, *30*(2), 99-104.

Emenike, C., Fauziah, S., & Agamuthu, P. (2012). Characterization and toxicological evaluation of leachate from closed sanitary landfill. *Waste Management & Research, 30*(9), 888-897.

Emenike, C., Fauziah, S., & Agamuthu, P. (2013a). *Bioaugmenting Leachate Polluted Soil for Optimal Removal of Heavy Metals.* Paper presented at the International Conference on Agriculture Science and Environment Engineering, ICASEE, Beijing, China.

Emenike, C., Fauziah, S., & Agamuthu, P. (2013b). Whole Effluent Toxicity from Active and Inactive Landfills: Variation of Leachate Impact on Fish (Pangasius sutchi). *Advanced Science Letters, 19*(9), 2681-2689.

Erdogan, E. E., & Karaca, A. (2011). Bioremediation of crude oil polluted soils. *Asian J. Biotechnol, 3*, 206-213.

Errasquın, E. L., & Vazquez, C. (2003). Tolerance and uptake of heavy metals by Trichoderma atroviride isolated from sludge. *Chemosphere, 50*(1), 137-143.

Fauziah, S., & Agamuthu, P. (2012). Trends in sustainable landfilling in Malaysia, a developing country. *Waste Manag Res, 30*(7), 656-663. doi:10.1177/0734242X12437564

Foul, A. A., Aziz, H. A., Isa, M. H., & Hung, Y.-T. (2009). Primary treatment of anaerobic landfill leachate using activated carbon and limestone: batch and column studies. *International Journal of Environment and Waste Management, 4*(3), 282-298.

Fried, S., Mackie, B., & Nothwehr, E. (2012). Nitrate and phosphate levels positively affect the growth of algae species found in Perry Pond. *Tillers, 4*, 21-24.

Geenens, D., Bixio, B., & Thoeye, C. (2001). Combined ozone-activated sludge treatment of landfill leachate. *Water Science & Technology, 44*(2-3), 359-365.

Giller, K. E., Witter, E., & McGrath, S. P. (1999). Assessing risks of heavy metal toxicity in agricultural soils: do microbes matter? *Human and Ecological Risk Assessment: An International Journal, 5*(4), 683-689.

Goltapeh, E. M., Danesh, Y. R., & Varma, A. (Eds.). (2013). *Fungi as Bioremediators* (Vol. 32). Springer Science & Business Media.

University of Malaya

Grisey, E., Belle, E., Dat, J., Mudry, J., & Aleya, L. (2010). Survival of pathogenic and indicator organisms in groundwater and landfill leachate through coupling bacterial enumeration with tracer tests. *Desalination, 261*(1), 162-168.

Guiné, V., Martins, J., Causse, B., Durand, A., Gaudet, J.-P., & Spadini, L. (2007). Effect of cultivation and experimental conditions on the surface reactivity of the metal-resistant bacteria Cupriavidus metallidurans CH34 to protons, cadmium and zinc. *Chemical geology, 236*(3), 266-280.

Guo, H., Luo, S., Chen, L., Xiao, X., Xi, Q., Wei, W., Zeng, G., Liu, C., Wan, Y., Chen, J. & He, Y. (2010). Bioremediation of heavy metals by growing hyperaccumulator endophytic bacterium Bacillus sp. L14. *Bioresource Technology, 101*(22), 8599-8605.

Halim, C. E., Scott, J. A., Natawardaya, H., Amal, R., Beydoun, D., & Low, G. (2004). Comparison between acetic acid and landfill leachates for the leaching of Pb (II), Cd (II), As (V), and Cr (VI) from cementitious wastes. *Environmental science & technology, 38*(14), 3977-3983.

Hamidi, A. A. (2015). *Control and Treatment of Landfill Leachate for Sanitary Waste Disposal*.

Harmsen, J. (1983). Identification of organic compounds in leachate from a waste tip. *Water research, 17*(6), 699-705.

Hess, A., Zarda, B., Hahn, D., Häner, A., Stax, D., Höhener, P., & Zeyer, J. (1997). In situ analysis of denitrifying toluene-and m-xylene-degrading bacteria in a diesel fuel-contaminated laboratory aquifer column. *Applied and environmental microbiology, 63*(6), 2136-2141.

Hetzer, A., Daughney, C. J., & Morgan, H. W. (2006). Cadmium ion biosorption by the thermophilic bacteria Geobacillus stearothermophilus and G. thermocatenulatus. *Applied and environmental microbiology, 72*(6), 4020-4027.

Hoffmann, T., Frankenberg, N., Marino, M., & Jahn, D. (1998). Ammonification in Bacillus subtilisUtilizing Dissimilatory Nitrite Reductase Is Dependent onresDE. *Journal of bacteriology, 180*(1), 186-189.

Hong, H. A., & Cutting, S. M. (2005). The use of bacterial spore formers as probiotics. *FEMS microbiology reviews, 29*(4), 813-835.

Husni, B. (2008). Bioremediation of oil contaminated waste water using mixed culture. *Bachelor of Chemical Engineering (Biotechnology) Thesis, Universiti Malaysia Pahang, pp1-4*.

Ikram, I. (2014). Malaysians producing more solid waste than before [Press release]. Retrieved from http://www.themalaymailonline.com/malaysia/article/malaysians-producing-more-solid-waste-than-before

University of Malaya

Inanc, B., Calli, B., & Saatci, A. (2000). Characterization and anaerobic treatment of the sanitary landfill leachate in Istanbul. *Water science and technology, 41*(3), 223-230.

Jameel, A. T., & Abass Olanrewaju, A. (2011). Aerobic biodegradation of oil and grease in palm oil mill effluent using consortium of microorganisms. In M. Z. Alam, A. T. Jameel, & A. Amid (Eds.), *Current Research and Development in Biotechnology Engineering at IIUM* (1 ed., Vol. III, pp. 43-52). International Islamic University Malaysia: IIUM Press.

Jensen, D. L., & Christensen, T. H. (1999). Colloidal and dissolved metals in leachates from four Danish landfills. *Water research, 33*(9), 2139-2147.

Johannessen, L. M. (1999). *Guidance note on leachate management for municipal solid waste landfills*: World Bank.

Johari, A., Alkali, H., Hashim, H., Ahmed, S. I., & Mat, R. (2014). Municipal Solid Waste Management and Potential Revenue from Recycling in Malaysia. *Modern Applied Science, 8*(4), p37.

Jones, D., Williamson, K., & Owen, A. (2006). Phytoremediation of landfill leachate. *Waste management, 26*(8), 825-837.

Kabdasli, I., Tnay, O., Yilmaz, S., & Arikan, O. (2000). Ammonia removal from young landfill leachate by magnesium ammonium phosphate precipitation and air stripping. *Water science and technology, 41*(1), 237-240.

Kadlec, R. H., & Wallace, S. (2008). *Treatment wetlands*: CRC press.

Kang, K.-H., Shin, H. S., & Park, H. (2002). Characterization of humic substances present in landfill leachates with different landfill ages and its implications. *Water research, 36*(16), 4023-4032.

Kapley, A., Purohit, H. J., Chhatre, S., Shanker, R., Chakrabarti, T., & Khanna, P. (1999). Osmotolerance and hydrocarbon degradation by a genetically engineered microbial consortium. *Bioresource Technology, 67*(3), 241-245.

Kargi, F., & Yunus Pamukoglu, M. (2003). Simultaneous adsorption and biological treatment of pre-treated landfill leachate by fed-batch operation. *Process Biochemistry, 38*(10), 1413-1420.

Kjeldsen, P., Barlaz, M. A., Rooker, A. P., Baun, A., Ledin, A., & Christensen, T. H. (2002). Present and long-term composition of MSW landfill leachate: a review. *Critical reviews in environmental science and technology, 32*(4), 297-336.

Klinck, B., & Stuart, M. (1999). Human health risk in relation to landfill leachate quality. *British Geological Survey, Technical report WC/99/17*.

Koshimizu, S., Ohtake, I., Yoshioka, H., Saito, Y., & Taki, H. (1997). Development of kitchen wastewater treatment system using fats and oils degrading microorganisms. *Kuuki-chouwa Eiseikougaku, 71*, 999-1009.

University of Malaya

Koshy, L., Jones, T., & BéruBé, K. (2008). Bioreactivity of municipal solid waste landfill leachates—Hormesis and DNA damage. *Water research, 42*(8), 2177-2183.

Krishna, M., Varghese, R., Babu, V. A., Jyothy, S., & Hatha, A. M. (2013). Bioremediation of Zinc Using Bacillus sp. Isolated from Metal-Contaminated Industrial Zone *Prospects in Bioscience: Addressing the Issues* (pp. 11-18): Springer.

Kulikowska, D., & Klimiuk, E. (2008). The effect of landfill age on municipal leachate composition. *Bioresource Technology, 99*(13), 5981-5985.

Kulshreshtha, A., Agrawal, R., Barar, M., & Saxena, S. (2014). A Review on Bioremediation of Heavy Metals in Contaminated Water. *IOSR Journal of Environmental Science, Toxicology and Food Technology (IOSR-JESTFT), 8*(7), 44-50.

Kumar , A., Bisht, B., Joshi, V., & Dhewa, T. (2011). Review on Bioremediation of Polluted Environment:: A Management Tool. *International journal of environmental sciences, 1*(6), 1079.

Kumar, A., Bisht, B. S., & Joshi, V. D. (2010). Biosorption of heavy metals by four acclimated microbial species, Bacillus spp., Pseudomonas spp., Staphylococcus spp. and Aspergillus niger. *J Biol Environ Sci, 4*(12), 97-108.

Kumar, V., Singh, S., Kashyap, N., Singla, S., Bhadrecha, P., Kaur, P., Datta, S., Kalia, A., & Singh, J. (2015). Bioremediation of heavy metals by employing resistant microbial isolates from agricultural soil irrigated with industrial waste water. *Oriental Journal of Chemistry, 31*(1), 357–361.

Lalloo, R., Ramchuran, S., Ramduth, D., Görgens, J., & Gardiner, N. (2007). Isolation and selection of Bacillus spp. as potential biological agents for enhancement of water quality in culture of ornamental fish. *Journal of Applied Microbiology, 103*(5), 1471-1479.

Latifah, A. M., Mohd Armi, A. S., & Nur Ilyana, M. Z. (2009). Municipal solid waste management in Malaysia: Practices and challenges. *Waste management, 29*(11), 2902-2906.

Le, A. (2013). Bioremediation Nature's Way to a Cleaner Environment.

Lee, G., & Jones, R. (1993). *Groundwater pollution by municipal landfills: Leachate composition, detection and water quality significance.* Paper presented at the Proc. Sardinia.

Lee , S.-H., Lee, S., Kim, D.-Y., & Kim, J.-g. (2007). Degradation characteristics of waste lubricants under different nutrient conditions. *Journal of hazardous materials, 143*(1), 65-72.

Lefebvre, Olivier, & Moletta, R. (2006). Treatment of organic pollution in industrial saline wastewater: a literature review. *Water research, 40*(20), 3671-3682.

University of Malaya

Lema, J., Mendez, R., & Blazquez, R. (1988). Characteristics of landfill leachates and alternatives for their treatment: a review. *Water, Air, and Soil Pollution, 40*(3-4), 223-250.

Levlin, E. (2010). *Conductivity measurements for controlling municipal waste-water treatment.* Paper presented at the Proceedings of a Polish-Swedish-Ukrainian Seminar Utron.

Li , Zhao, Y., Shi, L., & Gu, Y. (2009). Three-stage aged refuse biofilter for the treatment of landfill leachate. *Journal of Environmental Sciences, 21*(1), 70-75.

Li , W. (2013). Study on Characteristics in the Removal Process of Ammonia Nitrogen and Nitrate Nitrogen by an Isolated Heterotrophic Nitrification-Aerobic Denitrification Strain Rhodococcus sp. *Journal of Environmental Protection, 4*(01), 74.

Li , W., Zhou, Q., & Hua, T. (2010). Removal of organic matter from landfill leachate by advanced oxidation processes: a review. *International Journal of Chemical Engineering, 2010*.

Li, X., & Zhao, Q. (2001). Efficiency of biological treatment affected by high strength of ammonium-nitrogen in leachate and chemical precipitation of ammonium-nitrogen as pretreatment. *Chemosphere, 44*(1), 37-43.

Li, Y., Low, G. K.-C., Scott, J. A., & Amal, R. (2007). Microbial reduction of hexavalent chromium by landfill leachate. *Journal of hazardous materials, 142*(1), 153-159.

Lian, L. W. (Producer). (2011, 1 January 2016). Malaysia's population hits 28m amid slowing growth. Retrieved from http://www.themalaysianinsider.com/malaysia/article/malaysias-population-hits-28m-amid-slowing-growth

Liu, S. (2013). *Landfill leachate treatment methods and evaluation of Hedeskoga and Måsalycke landfills.* Master thesis.

Lloyd, J. R., & Lovley, D. R. (2001). Microbial detoxification of metals and radionuclides. *Current Opinion in Biotechnology, 12*(3), 248-253.

Lu, J., Eichenberger, B., & Stearns, R. J. (1985). *Leachate from municipal landfills; production and management* (Vol. 119): Noyes Publications.

Ludwig, C., Hellweg, S., & Stucki, S. (2012). *Municipal solid waste management: strategies and technologies for sustainable solutions*: Springer Science & Business Media.

Lutz, W., Sanderson, W., & Scherbov, S. (2001). The end of world population growth. *Nature, 412*(6846), 543-545. doi:http://www.nature.com/nature/journal/v412/n6846/suppinfo/412543a0_S1.html

Malik, A. (2004). Metal bioremediation through growing cells. *Environment international, 30*(2), 261-278.

University of Malaya

Mansor, H., Mat, J., & Tahir, W. P. A. W. M. (2011). Treatment Of Leachate Using Cultured Spirulina Platensis. *International Journal of Engineering and Technology, 8*(2), 57-60.

Maqbool, F., Bhatti, Z., Malik, A., Pervez, A., & Mahmood, Q. (2011). Effect of landfill leachate on the stream water quality. *International Journal of Environmental Research, 5*(2), 491-500.

Marco, A., Esplugas, S., & Saum, G. (1997). How and why combine chemical and biological processes for wastewater treatment. *Water science and technology, 35*(4), 321-327.

Marina, B., Noor Azlina, I., & Aweng Eh, R. (2013). Biodegradation of oily wastewater by pure culture of Bacillus cereus. *ARPN Journal of Agricultural and Biological Sciences, 8*(2), 1-8.

Marttinen, S., Kettunen, R., Sormunen, K., Soimasuo, R., & Rintala, J. (2002). Screening of physical–chemical methods for removal of organic material, nitrogen and toxicity from low strength landfill leachates. *Chemosphere, 46*(6), 851-858.

Masirin, M., Idrus, M., Ridzuan, M. B., & Mustapha, S. (2008). *An overview of landfill management and technologies: a Malaysian case study at Ampar Tenang*. Paper presented at the 1st National Seminar on Environment, Development and Sustainability (PSISenviro2008), Selangor.

McEldowney, S., Hardman, D. J., & Waite, S. (1993). *Pollution: ecology and biotreatment*: Longman Scientific & Technical.

Megharaj, M., Ramakrishnan, B., Venkateswarlu, K., Sethunathan, N., & Naidu, R. (2011). Bioremediation approaches for organic pollutants: a critical perspective. *Environment international, 37*(8), 1362-1375.

Meng R, H. L., Xi BD, Hu X, Li YY. (2009). Experimental study on purifying aquaculture wastewater between Bacillus and nitrifying bacteria. *Environ Sci Technol, 32*(11), 28-31.

Mersiowsky, I. (1999). *Long-term behaviour of pvc products under soil-buried and landfill conditions: final report of the research project*: Techn. Univ.

Moh, Y. C., & Manaf, L. A. (2014). Overview of household solid waste recycling policy status and challenges in Malaysia. *Resources, Conservation and Recycling, 82*, 50-61.

Mohd Armi, A. S., Manaf, L. A., Ahsan, A., Sulaiman, W. N. A., Agamuthu, P., & D'Silva, J. L. (2013). Household solid waste composition in Balakong City, Malaysia: trend and management. *Polish Journal of Environmental Studies, 22*(6), 1807-1816.

Mohd Harun, A. (2012). *Principles in Water Analysis for Environmental Science.*: Penerbit Universiti Malaysia Sabah.

Mojiri, A., Aziz, H. A., & Aziz, S. Q. (2013). Trends in physical-chemical methods for landfill leachate treatment. *Int. J. Sci. Res. Environ. Sci, 1*(2), 16-25.

University of Malaya

Mondal, P., Majumder, C., & Mohanty, B. (2008). Treatment of arsenic contaminated water in a laboratory scale up-flow bio-column reactor. *Journal of hazardous materials, 153*(1), 136-145.

Muhammad, U., Hamidi, A., & Mohd Suffian, Y. (2010). Variability of parameters involved in leachate pollution index and determination of LPI from four landfills in Malaysia. *International Journal of Chemical Engineering, 2010*.

Mukherjee, S., Mukhopadhyay, S., Hashim, M. A., & Sen Gupta, B. (2015). Contemporary environmental issues of landfill leachate: assessment and remedies. *Critical reviews in environmental science and technology, 45*(5), 472-590.

Muthukrishnan, S., Sabaratnam, V., Tan, G. Y. A., & Chong, V. C. (2015). Identification of indegenous bacteria isolated from shrimp wastewater with bioremediation application: Total ammonial nitrogen (TAN) and nitrite removal. *Sains Malaysiana, 44*(8), 1103-1110.

Nadiah, M., Noor Suraya, R., Fatimah, S., & Osmi, C. (2012). *Bse-504: Leachate Treatment Using Limestone Filled-Tank and Constructed Wetland*. Paper presented at the Malaysian International Conference on Trends in Bioprocess Engineering (MICOTriBE), Meritus Pelangi Beach Resort & Spa, Langkawi, Kedah.

Nagarajan, R., Thirumalaisamy, S., & Lakshumanan, E. (2012). Impact of leachate on groundwater pollution due to non-engineered municipal solid waste landfill sites of erode city, Tamil Nadu, India. *Iranian Journal of Environmental Health Science & Engineering, 9*(1), 35. doi:http://doi.org/10.1186/1735-2746-9-35

NAHRIM. (2009). *Desktop study on groundwater contamination at landfill sites in Selangor*. Retrieved from

Naik, M. M. G., & Duraphe, M. M. D. (2012). Review Paper On–Parameters Affecting Bioremediation. *Life, 50*, 77.

Nakano, M. M., Hoffmann, T., Zhu, Y., & Jahn, D. (1998). Nitrogen and oxygen regulation of Bacillus subtilis nasDEF encoding NADH-dependent nitrite reductase by TnrA and ResDE. *Journal of bacteriology, 180*(20), 5344-5350.

Ntampou, X., Zouboulis, A., & Samaras, P. (2006). Appropriate combination of physico-chemical methods (coagulation/flocculation and ozonation) for the efficient treatment of landfill leachates. *Chemosphere, 62*(5), 722-730.

Öman, C. B., & Junestedt, C. (2008). Chemical characterization of landfill leachates–400 parameters and compounds. *Waste management, 28*(10), 1876-1891.

Osman, K. T. (2013). *Soil Degradation, Conservation and Remediation*: Springer Netherlands.

Paisio, C. E., Quevedo, M. R., Talano, M. A., González, P. S., & Agostini, E. (2014). Application of two bacterial strains for wastewater bioremediation and assessment of phenolics biodegradation. *Environmental technology, 35*(14), 1802-1810.

University of Malaya

Panasiuk, O. (2010). Phosphorus removal and recovery from wastewater using magnetite.

Pandhare, G. G., Trivedi, N., & Dawande, S. (2013). Adsorption Of Color From A Stock Solution Using Neem Leaves Powder As A Low-Cost Adsorbent. . *International Journal of Engineering Sciences & Emerging Technologies, 5*(2), 97-103.

Patel, P. C., Goulhen, F., Boothman, C., Gault, A. G., Charnock, J. M., Kalia, K., & Lloyd, J. R. (2007). Arsenate detoxification in a Pseudomonad hypertolerant to arsenic. *Archives of microbiology, 187*(3), 171-183.

Pathak, H. (2011). Alcaligenes-the 4T engine oil degrader. *Journal of Bioremediation and Biodegradation*.

Paxus, N. (2000). Organic compounds in municipal landfill leachates. *Water Science & Technology, 42*(7-8), 323-333.

Pires, C. (2010). *Bacteria in heavy metal contaminated soil: diversity, tolerance and use in remediation.* (PhD), Cranfield University.

Pivato, A., & Gaspari, L. (2006). Acute toxicity test of leachates from traditional and sustainable landfills using luminescent bacteria. *Waste management, 26*(10), 1148-1155.

Pizzul, L., del Pilar Castillo, M., & Stenström, J. (2007). Effect of rapeseed oil on the degradation of polycyclic aromatic hydrocarbons in soil by Rhodococcus wratislaviensis. *International biodeterioration & biodegradation, 59*(2), 111-118.

PRB. (2015). 2015 World Population Data Sheet Retrieved from http://www.prb.org/pdf14/2014-world-population-data-sheet_eng.pdf

Pun, R., Raut, P., & Pant, B. R. (2013). Removal of Chromium (VI) From Leachate Using Bacterial Biomass. *Scientific World, 11*(11), 63-65.

Rajendran, P., Muthukrishnan, J., & Gunasekaran, P. (2003). Microbes in heavy metal remediation. *Indian journal of experimental biology, 41*(9), 935-944.

Rathoure, A. K. (2015). *Toxicity and Waste Management Using Bioremediation*.

Renou, S., Givaudan, J., Poulain, S., Dirassouyan, F., & Moulin, P. (2008). Landfill leachate treatment: Review and opportunity. *Journal of hazardous materials, 150*(3), 468-493.

Robinson, H. (2007). The composition of leachates from very large landfills: an international review. *Communications in waste and resource management, 8*(1), 19-32.

Rowe, R. K., Quigley, R. M., Brachman, R. W., Booker, J. R., & Brachman, R. (2004). *Barrier systems for waste disposal facilities*: Spon Press.

University of Malaya

Rui, M., LianSheng, H., BeiDou, X., Xiang, H., & YueYan, L. (2009). Experimental study on purifying aquaculture wastewater between Bacillus and nitrifying bacteria. *Environmental Science & Technology (China), 32*(11), 28-31.

Sakawi, Z. (2011). Municipal solid waste management in Malaysia: solution for sustainable waste management. *Journal of Applied Sciences in Environmental Sanitation, 6*(1), 29-38.

Salmah, I., & Dadrasnia, A. (2015). Biotechnological potential of Bacillus salmalaya 139SI: A novel strain for remediating water polluted with crude oil waste. *PloS one, 10*(4), e0120931.

Sang, N., Li, G., & Xin, X. (2006). Municipal landfill leachate induces cytogenetic damage in root tips of Hordeum vulgare. *Ecotoxicology and environmental safety, 63*(3), 469-473.

Sanphoti, N., Towprayoon, S., Chaiprasert, P., & Nopharatana, A. (2006). The effects of leachate recirculation with supplemental water addition on methane production and waste decomposition in a simulated tropical landfill. *Journal of environmental management, 81*(1), 27-35.

Sar , P., & D'Souza, S. F. (2001). Biosorptive uranium uptake by a Pseudomonas strain: characterization and equilibrium studies. *Journal of chemical technology and biotechnology, 76*(12), 1286-1294.

Sar, P., Kazy, S., Asthana, R., & Singh, S. (1999). Metal adsorption and desorption by lyophilized Pseudomonas aeruginosa. *International biodeterioration & biodegradation, 44*(2), 101-110.

Sarret, G., Avoscan, L., Carriere, M., Collins, R., Geoffroy, N., Carrot, F., . . . Gouget, B. (2005). Chemical forms of selenium in the metal-resistant bacterium Ralstonia metallidurans CH34 exposed to selenite and selenate. *Applied and environmental microbiology, 71*(5), 2331-2337.

Sasikumar, C. S., & Papinazath, T. (2003). *Environmental management: bioremediation of polluted environment.* Paper presented at the Proceedings of the third international conference on environment and health, Chennai, India.

Savage, G. M., Diaz, L., Golueke, C., Martone, C., & Ham, R. (1998). *Guidance for landfilling waste in economically developing countries*: EPA, CalRecovery.

Schiopu, A. M., & Gavrilescu, M. (2010). Options for the treatment and management of municipal landfill leachate: common and specific issues. *CLEAN–Soil, Air, Water, 38*(12), 1101-1110.

Schrab, G., Brown, K., & Donnelly, K. (1993). Acute and genetic toxicity of municipal landfill leachate. *Water, Air, and Soil Pollution, 69*(1-2), 99-112.

Schwarzbauer, J., Heim, S., Brinker, S., & Littke, R. (2002). Occurrence and alteration of organic contaminants in seepage and leakage water from a waste deposit landfill. *Water research, 36*(9), 2275-2287.

University of Malaya

Sharifah Norkhadijah, S. I., & Latifah, A. (2013). The challenge of future landfill: A case study of Malaysia. *Journal of Toxicology and Environmental Health Sciences, 5*(6), 86-96.

Sharma, S. (2012). Bioremediation: features, strategies and applications. *Asian Journal of Pharmacy and Life Science ISSN, 2231*, 4423.

Shrivastava, J. N., Verma, S., & Kumar, V. (2013). Bioremediation of Yamuna water by mono and dual bacterial isolates. *Ind. J. Sci. Res. and Tech*, *1*(1), 56-60.

Sierra-Alvarez, R., Field, J. A., Cortinas, I., Feijoo, G., Teresa Moreira, M., Kopplin, M., & Jay Gandolfi, A. (2005). Anaerobic microbial mobilization and biotransformation of arsenate adsorbed onto activated alumina. *Water research, 39*(1), 199-209.

Singh, R. (2014). Microorganism as a tool of bioremediation technology for cleaning environment: A review. *Proceedings of the International Academy of Ecology and Environmental Sciences, 4*(1), 1.

Singh, R., Ibrahim, M. H., Esa, N., & Iliyana, M. (2010). Composting of waste from palm oil mill: a sustainable waste management practice. *Reviews in Environmental Science and Bio/Technology, 9*(4), 331-344.

Sivaprakasam, S., Mahadevan, S., Sekar, S., & Rajakumar, S. (2008). Biological treatment of tannery wastewater by using salt-tolerant bacterial strains. *Microbial Cell Factories, 7*(1), 15.

Smith, V. H., Graham, D. W., & Cleland, D. D. (1998). Application of resource-ratio theory to hydrocarbon biodegradation. *Environmental science & technology, 32*(21), 3386-3395.

Soni, S. K. (2007). *Microbes: A Source of Energy for 21st Century*: New India Publishing Agency.

Sonune, N. A., & Garode, A. M. (2015). Bioremediation Potential of Bacterial Isolates for Municipal Wastewater Treatment. *Current World Environment, 10*(2).

Stegmann, R., Heyer, K., & Cossu, R. (2005). *Leachate treatment.* Paper presented at the Proceedings Sardinia.

Strong, P. J., & Burgess, J. E. (2008). Treatment methods for wine-related and distillery wastewaters: a review. *Bioremediation Journal, 12*(2), 70-87.

Sulaimon, A. M., Odeyemi, A. T., Ogunjobi, A. A., & Olasunkanmi, I. (2014). Bioaccumulation of Heavy Metals using Selected Heavy Metal Tolerant organisms Isolated from Dumpsite Leachate. *Nature & Science, 12*(10).

Sundaravadivel, M., & Vigneswaran, S. (2001). Constructed wetlands for wastewater treatment. *Critical reviews in environmental science and technology, 31*(4), 351-409.

Sze, K., Lu, Y., & Wong, P. (1996). Removal and recovery of copper ion (Cu 2+) from electroplating effluent by a bioreactor containing magnetite-immobilized cells of Pseudomonas putida 5X. *Resources, Conservation and Recycling, 18*(1), 175-193.

Tang, C. Y., Fu, Q. S., Criddle, C. S., & Leckie, J. O. (2007). Effect of flux (transmembrane pressure) and membrane properties on fouling and rejection of reverse osmosis and nanofiltration membranes treating perfluorooctane sulfonate containing wastewater. *Environmental science & technology, 41*(6), 2008-2014.

Tao, W., Hall, K. J., & Duff, S. J. (2007). Microbial biomass and heterotrophic production of surface flow mesocosm wetlands treating woodwaste leachate: Responses to hydraulic and organic loading and relations with mass reduction. *ecological engineering, 31*(2), 132-139.

Tatsi, A., & Zouboulis, A. (2002). A field investigation of the quantity and quality of leachate from a municipal solid waste landfill in a Mediterranean climate (Thessaloniki, Greece). *Advances in Environmental Research, 6*(3), 207-219.

Tatsi, A., Zouboulis, A., Matis, K., & Samaras, P. (2003). Coagulation–flocculation pretreatment of sanitary landfill leachates. *Chemosphere, 53*(7), 737-744.

Tchobanoglous, G., Theisen, H., & Vigil, S. (1993). *Integrated solid waste management: engineering principles and management issues*: McGraw-Hill, Inc.

Tengrui, L., Al-Harbawi, A. F., Bo, L. M., Jun, Z., & Long, X. Y. (2007). Characteristics of nitrogen removal from old landfill leachate by sequencing batch biofilm reactor. *American Journal of Applied Sciences, 4*(4), 211.

Thuku, G. K., Gachanja, P. M., & Obere, A. (2013). The impact of population change on Economic growth in Kenya. *International Journal of Economics and Management Sciences, 2*(6), 43-60.

Tränkler, J., Visvanathan, C., Kuruparan, P., & Tubtimthai, O. (2005). Influence of tropical seasonal variations on landfill leachate characteristics—Results from lysimeter studies. *Waste management, 25*(10), 1013-1020.

Tyre, B. W., & Dennis, M. C. (1997). On-Site Sewage Disposal for a Subdivision in a High Groundwater Area. *ASTM SPECIAL TECHNICAL PUBLICATION, 1324*, 283-293.

UNEP. (2009). *Waste Management Partnership* Retrieved from Geneva: http://www.unep.or.jp/ietc/SPC/news-nov09/DratfReport_ConsultationWorkshopOnWaste_Geneva09.pdf

Usman, M. M., Dadrasnia, A., Lim, K. T., & Fahim, A. (2016). Application of biosurfactants in environmental biotechnology; remediation of oil and heavy metal. *AIMS Bioengineering, 3*(3), 289-304. doi:10.3934/bioeng.2016.3.289

Vaidya, R. D. (2002). *Solid waste degradation, compaction and water holding capacity.* (Master of Science), Blacksburg, Virginia.

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Valls, M., & De Lorenzo, V. (2002). Exploiting the genetic and biochemical capacities of bacteria for the remediation of heavy metal pollution. *FEMS microbiology reviews, 26*(4), 327-338.

Vásquez, T. G. P., Botero, A. E. C., De Mesquita, L. M. S., & Torem, M. L. (2007). Biosorptive removal of Cd and Zn from liquid streams with a Rhodococcus opacus strain. *Minerals engineering, 20*(9), 939-944.

Vidali, M. (2001). Bioremediation. An overview. *Pure and Applied Chemistry, 73*(7), 1163-1172.

Wakelin, N., & Forster, C. (1997). An investigation into microbial removal of fats, oils and greases. *Bioresource Technology, 59*(1), 37-43.

Wan Razarinah, W., Noor Zalina, M., & Abdullah, N. (2011). *Screening method for selecting the potential fungi for use in the bioremediation of leachate*. Paper presented at the 2nd International Conference on Environmental Science and Technology IPCBEE Singapore

Wang, Q., Matsufuji, Y., Dong, L., Huang, Q., Hirano, F., & Tanaka, A. (2006). Research on leachate recirculation from different types of landfills. *Waste management, 26*(8), 815-824.

Wang , Z.-p., Zhang, Z., Lin, Y.-j., Deng, N.-s., Tao, T., & Zhuo, K. (2002). Landfill leachate treatment by a coagulation–photooxidation process. *Journal of hazardous materials, 95*(1), 153-159.

Wiszniowski, J., Robert, D., Surmacz-Gorska, J., Miksch, K., & Weber, J. (2006). Landfill leachate treatment methods: A review. *Environmental Chemistry Letters, 4*(1), 51-61.

World Health Organization. (2006). *Guidelines for drinking-water quality: First addendum to volume 1, Recommendations* (Vol. 1): World Health Organization.

Worldwide Environment. (2015). Jeram Sanitary Landfill. *Our projects.*

Wu, H., Zhang, J., Ngo, H. H., Guo, W., Hu, Z., Liang, S., . . . Liu, H. (2015). A review on the sustainability of constructed wetlands for wastewater treatment: Design and operation. *Bioresource Technology, 175*, 594-601.

Xie, F., Zhu, T., Zhang, F., Zhou, K., Zhao, Y., & Li, Z. (2013). Using Bacillus amyloliquefaciens for remediation of aquaculture water. . *SpringerPlus,, 2*, 119. doi:http://doi.org/10.1186/2193-1801-2-119

Yamina, B., Tahar, B., Lila, M., Hocine, H., & Laure, F. M. (2014). Study on Cadmium Resistant-Bacteria Isolated from Hospital Wastewaters. *Advances in Bioscience and Biotechnology, 2014*.

Yilmaz, D. D., & Akbulut, H. (2011). Effect of circulation on wastewater treatment by Lemna gibba and Lemna minor (floating aquatic macrophytes). *International journal of phytoremediation, 13*(10), 970-984.

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Yimer, S., & Sahu, O. (2013). Management of Liquid Solid Waste. *Journal of Applied & Environmental Microbiology, 1*(1), 12-17.

Yu, C.-H., Wang, Y., Guo, T., Shen, W.-X., & Gu, M.-X. (2012). Isolation and Identification of Ammonia Nitrogen Degradation Strains from Industrial Wastewater. *Engineering, 4*, 790-793. doi:http://dx.doi.org/10.4236/eng.2012.411101

Zainab H, M., Mohamed R, O., & Abdullaha, M. P. (2015). Electrochemical Oxidation of Landfill Leachate: Investigation of Operational Parameters and Kinetics Using Graphite-PVC Composite Electrode as Anode. *J. Braz. Chem. Soc, 26*(5), 939-948.

Zainab, H. M., Mohamed Rozali, O., Md Puazi, A., & Norazzizi, N. (2013). Decolorization Of Landfill Leachate Using Electrochemical Technique. *Int. J. Chem. Sci, 11*(4), 1636-1646.

Zouboulis, A. I., Chai, X.-L., & Katsoyiannis, I. A. (2004). The application of bioflocculant for the removal of humic acids from stabilized landfill leachates. *Journal of environmental management, 70*(1), 35-41.

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APPENDICES

APPENDIX A: Characteristics of Raw Leachate (Initial Reading)

Characteristics	Average Value
Apparent colour	Deep black
Odour	Slightly ammoniac
Conductivity (µS/cm)	35,829.67 ± 293.29
pH	8.38 ± 0.08
Salinity (ppt)	19.27 ± 0.02
Total Dissolved Solid (mg/L)	20,321.17 ± 9.93
Biological Oxygen Demand (mg/L)	1,046 ± 154.50
Chemical Oxygen Demand (mg/L)	11,031.67 ± 153.65
BOD_5 / COD	0.09
Ammoniacal Nitrogen (mg/L)	6,400 ± 624.50
Oil and Grease (mg/L)	4.43 ± 0.03

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APPENDIX B: Physicochemical analysis of leachate after 48 hours (control)

Characteristics	Average Value
Apparent colour	Deep black
Odour	Slightly ammoniac
Conductivity (µS/cm)	30466.33 ± 162.03
pH	8.85 ± 0.09
Salinity (ppt)	17.19 ± 0.13
Total Dissolved Solid (mg/L)	18260.67 ± 120.97
Biological Oxygen Demand (mg/L)	610 ± 206.11
Chemical Oxygen Demand (mg/L)	6771.67 ± 328.65
BOD_5 / COD	0.09
Ammoniacal Nitrogen (mg/L)	5100 ± 1587.45
Oil and Grease (mg/L)	4.07 ± 0.03

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APPENDIX C: Physicochemical analysis of leachate after Treatment 1

Characteristics	Average Value
Apparent colour	Deep black
Odour	Slightly ammoniac
Conductivity (µS/cm)	30844 ± 175.58
pH	8.78 ± 0.09
Salinity (ppt)	17.33 ± 0.07
Total Dissolved Solid (mg/L)	18395 ± 67.55
Biological Oxygen Demand (mg/L)	1202 ± 155.03
Chemical Oxygen Demand (mg/L)	7176.67 ± 421.58
BOD_5 / COD	0.17
Ammoniacal Nitrogen (mg/L)	3900 ± 519.62
Oil and Grease (mg/L)	1.20 ± 0.11

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APPENDIX D: Physicochemical analysis of leachate after Treatment 2

Characteristics	Average Value
Apparent colour	Deep black
Odour	Slightly ammoniac
Conductivity (µS/cm)	30347.67 ± 893.65
pH	8.79 ± 0.07
Salinity (ppt)	17.14 ± 0.55
Total Dissolved Solid (mg/L)	18230.33 ± 478.28
Biological Oxygen Demand (mg/L)	1206 ± 83.19
Chemical Oxygen Demand (mg/L)	6251.67 ± 1692.60
BOD_5 / COD	0.19
Ammoniacal Nitrogen (mg/L)	3500 ± 754.98
Oil and Grease (mg/L)	2.25 ± 0.35

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APPENDIX E: Physicochemical analysis of leachate after Treatment 3

Characteristics	Average Value
Apparent colour	Deep black
Odour	Slightly ammoniac
Conductivity (µS/cm)	30696.67 ± 105.51
pH	8.78 ± 0.02
Salinity (ppt)	17.39 ± 0.10
Total Dissolved Solid (mg/L)	18449.17 ± 91.08
Biological Oxygen Demand (mg/L)	1234 ± 18.16
Chemical Oxygen Demand (mg/L)	5393.33 ± 1257.02
BOD_5 / COD	0.23
Ammoniacal Nitrogen (mg/L)	2900 ± 173.21
Oil and Grease (mg/L)	0.08 ± 0.01

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APPENDIX F: Heavy Metals analysis of leachate after Treatment 1, 2, & 3

Heavy metal	Initial			Average	SD
	Run1	Run2	Run3		
Mg		11.3200	11.7400	11.5300	0.2970
Al	0.5398	0.5937	0.4800	0.5378	0.0569
Ca	0.6506	0.7950	0.8339	0.7598	0.0966
Cr	0.0659	0.0748	0.0771	0.0726	0.0059
Mn	0.0157	0.0183	0.0191	0.0177	0.0017
Fe	0.5744	0.6593	0.7745	0.6694	0.1004
Ni	0.0255	0.0294	0.0295	0.0281	0.0023
Zn	0.1085	0.0649	0.0557	0.0764	0.0282
As	0.0099	0.0119	0.0140	0.0119	0.0021
Ba	0.2711	0.1344	0.2030	0.2028	0.0684
Pb	0.0039	0.0023	0.0088	0.0050	0.0033

Heavy metal	Control			Average	SD
	Run1	Run2	Run3		
Mg	10.3142	9.7563	9.4495	9.8400	0.4384
Al	0.5261	0.3650	0.4590	0.4500	0.0809
Ca	0.5160	0.3990	0.6510	0.5220	0.1261
Cr	0.0711	0.0485	0.0772	0.0656	0.0151
Mn	0.0140	0.0150	0.0190	0.0160	0.0026
Fe	0.5610	0.4820	0.6250	0.5560	0.0716
Ni	0.0320	0.0284	0.0183	0.0262	0.0071
Zn	0.0602	0.0544	0.0481	0.0542	0.0060
As	0.0087	0.0082	0.0140	0.0103	0.0032
Ba	0.0683	0.1574	0.1355	0.1204	0.0464
Pb	0.0011	0.0045	0.0058	0.0038	0.0024

Heavy metal	Treatment 1			Average	SD
	Run1	Run2	Run3		
Mg	4.5340	4.5650	4.2630	4.4540	0.1661
Al	0.3645	0.1549	0.1203	0.2132	0.1321
Ca	0.3277	0.3181	0.3052	0.3170	0.0113
Cr	0.0322	0.0283	0.0282	0.0296	0.0023
Mn	0.0055	0.0047	0.0043	0.0048	0.0006
Fe	0.2855	0.3132	0.2575	0.2854	0.0279
Ni	0.0118	0.0111	0.0106	0.0112	0.0006
Zn	0.0244	0.0263	0.0227	0.0245	0.0018
As	0.0058	0.0055	0.0047	0.0054	0.0006
Ba	0.0955	0.0498	0.0275	0.0576	0.0347
Pb	0.0028	0.0034	0.0020	0.0027	0.0007

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Heavy metal	Treatment 2			Average	SD
	Run1	Run2	Run3		
Mg	4.5369	4.6520	4.4220	4.5370	0.1150
Al	0.1520	0.1776	0.1262	0.1519	0.0257
Ca	0.3102	0.3092	0.3109	0.3101	0.0009
Cr	0.0285	0.0291	0.0275	0.0283	0.0008
Mn	0.0045	0.0046	0.0044	0.0045	0.0001
Fe	0.2829	0.3019	0.2638	0.2829	0.0191
Ni	0.0113	0.0118	0.0103	0.0111	0.0008
Zn	0.0240	0.0253	0.0225	0.0239	0.0014
As	0.0055	0.0056	0.0055	0.0056	0.0001
Ba	0.0813	0.0488	0.0284	0.0529	0.0267
Pb	0.0071	0.0073	0.0073	0.0072	0.0001

Heavy metal	Treatment 3			Average	SD
	Run1	Run2	Run3		
Mg	4.1890	4.0360	3.7510	3.9920	0.2223
Al	0.1600	0.1205	0.1340	0.1382	0.0201
Ca	0.2981	0.2763	0.2686	0.2810	0.0153
Cr	0.0260	0.0249	0.0228	0.0245	0.0016
Mn	0.0036	0.0033	0.0028	0.0032	0.0004
Fe	0.2689	0.2346	0.2335	0.2457	0.0201
Ni	0.0098	0.0095	0.0090	0.0094	0.0004
Zn	0.0207	0.0207	0.0208	0.0207	0.0001
As	0.0043	0.0038	0.0033	0.0038	0.0005
Ba	0.0259	0.0559	0.0745	0.0521	0.0245
Pb	0.0005	0.0009	0.0008	0.0007	0.0002

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APPENDIX G : ANOVA analysis of heavy metal for Treatment 1, 2, 3 & Control

Multiple Comparisons

Tukey HSD

Dependent Variable	(I) sample	(J) sample	Mean Difference (I-J)	Std. Error	Sig.	95% Confidence Interval Lower Bound	95% Confidence Interval Upper Bound
Al	Control	Treatment 1	.2367967*	.0646425	.026	.029789	.443805
		Treatment 2	.2980967*	.0646425	.007	.091089	.505105
		Treatment 3	.3118633*	.0646425	.006	.104855	.518871
	Treatment 1	Control	-.2367967*	.0646425	.026	-.443805	-.029789
		Treatment 2	.0613000	.0646425	.781	-.145708	.268308
		Treatment 3	.0750667	.0646425	.665	-.131941	.282075
	Treatment 2	Control	-.2980967*	.0646425	.007	-.505105	-.091089
		Treatment 1	-.0613000	.0646425	.781	-.268308	.145708
		Treatment 3	.0137667	.0646425	.996	-.193241	.220775
	Treatment 3	Control	-.3118633*	.0646425	.006	-.518871	-.104855
		Treatment 1	-.0750667	.0646425	.665	-.282075	.131941
		Treatment 2	-.0137667	.0646425	.996	-.220775	.193241

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Cr	Control	Treatment 1	.0360433*	.0062855	.002	.015915	.056172
		Treatment 2	.0372533*	.0062855	.002	.017125	.057382
		Treatment 3	.0410533*	.0062855	.001	.020925	.061182
	Treatment 1	Control	-.0360433*	.0062855	.002	-.056172	-.015915
		Treatment 2	.0012100	.0062855	.997	-.018918	.021338
		Treatment 3	.0050100	.0062855	.854	-.015118	.025138
	Treatment 2	Control	-.0372533*	.0062855	.002	-.057382	-.017125
		Treatment 1	-.0012100	.0062855	.997	-.021338	.018918
		Treatment 3	.0038000	.0062855	.928	-.016328	.023928
	Treatment 3	Control	-.0410533*	.0062855	.001	-.061182	-.020925
		Treatment 1	-.0050100	.0062855	.854	-.025138	.015118
		Treatment 2	-.0038000	.0062855	.928	-.023928	.016328

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Mn	Control	Treatment 1	.0111833*	.0011233	.000	.007586	.014781
		Treatment 2	.0115033*	.0011233	.000	.007906	.015101
		Treatment 3	.0127800*	.0011233	.000	.009183	.016377
	Treatment 1	Control	-.0111833*	.0011233	.000	-.014781	-.007586
		Treatment 2	.0003200	.0011233	.991	-.003277	.003917
		Treatment 3	.0015967	.0011233	.521	-.002001	.005194
	Treatment 2	Control	-.0115033*	.0011233	.000	-.015101	-.007906
		Treatment 1	-.0003200	.0011233	.991	-.003917	.003277
		Treatment 3	.0012767	.0011233	.679	-.002321	.004874
	Treatment 3	Control	-.0127800*	.0011233	.000	-.016377	-.009183
		Treatment 1	-.0015967	.0011233	.521	-.005194	.002001
		Treatment 2	-.0012767	.0011233	.679	-.004874	.002321

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Fe	Control	Treatment 1	.2706000*	.0333534	.000	.163791	.377409
		Treatment 2	.2731333*	.0333534	.000	.166324	.379943
		Treatment 3	.3103333*	.0333534	.000	.203524	.417143
	Treatment 1	Control	-.2706000*	.0333534	.000	-.377409	-.163791
		Treatment 2	.0025333	.0333534	1.000	-.104276	.109343
		Treatment 3	.0397333	.0333534	.649	-.067076	.146543
	Treatment 2	Control	-.2731333*	.0333534	.000	-.379943	-.166324
		Treatment 1	-.0025333	.0333534	1.000	-.109343	.104276
		Treatment 3	.0372000	.0333534	.691	-.069609	.144009
	Treatment 3	Control	-.3103333*	.0333534	.000	-.417143	-.203524
		Treatment 1	-.0397333	.0333534	.649	-.146543	.067076
		Treatment 2	-.0372000	.0333534	.691	-.144009	.069609

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Ni	Control	Treatment 1	.0150667*	.0029352	.004	.005667	.024466
		Treatment 2	.0150967*	.0029352	.004	.005697	.024496
		Treatment 3	.0168000*	.0029352	.002	.007400	.026200
	Treatment 1	Control	-.0150667*	.0029352	.004	-.024466	-.005667
		Treatment 2	.0000300	.0029352	1.000	-.009370	.009430
		Treatment 3	.0017333	.0029352	.932	-.007666	.011133
	Treatment 2	Control	-.0150967*	.0029352	.004	-.024496	-.005697
		Treatment 1	-.0000300	.0029352	1.000	-.009430	.009370
		Treatment 3	.0017033	.0029352	.935	-.007696	.011103
	Treatment 3	Control	-.0168000*	.0029352	.002	-.026200	-.007400
		Treatment 1	-.0017333	.0029352	.932	-.011133	.007666
		Treatment 2	-.0017033	.0029352	.935	-.011103	.007696

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Zn	Control	Treatment 1	.0297500*	.0026383	.000	.021301	.038199
		Treatment 2	.0302867*	.0026383	.000	.021838	.038735
		Treatment 3	.0335167*	.0026383	.000	.025068	.041965
	Treatment 1	Control	-.0297500*	.0026383	.000	-.038199	-.021301
		Treatment 2	.0005367	.0026383	.997	-.007912	.008985
		Treatment 3	.0037667	.0026383	.518	-.004682	.012215
	Treatment 2	Control	-.0302867*	.0026383	.000	-.038735	-.021838
		Treatment 1	-.0005367	.0026383	.997	-.008985	.007912
		Treatment 3	.0032300	.0026383	.630	-.005219	.011679
	Treatment 3	Control	-.0335167*	.0026383	.000	-.041965	-.025068
		Treatment 1	-.0037667	.0026383	.518	-.012215	.004682
		Treatment 2	-.0032300	.0026383	.630	-.011679	.005219

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As	Control	Treatment 1	.0049500*	.0013521	.026	.000620	.009280
		Treatment 2	.0047500*	.0013521	.032	.000420	.009080
		Treatment 3	.0064933*	.0013521	.006	.002163	.010823
	Treatment 1	Control	-.0049500*	.0013521	.026	-.009280	-.000620
		Treatment 2	-.0002000	.0013521	.999	-.004530	.004130
		Treatment 3	.0015433	.0013521	.676	-.002787	.005873
	Treatment 2	Control	-.0047500*	.0013521	.032	-.009080	-.000420
		Treatment 1	.0002000	.0013521	.999	-.004130	.004530
		Treatment 3	.0017433	.0013521	.594	-.002587	.006073
	Treatment 3	Control	-.0064933*	.0013521	.006	-.010823	-.002163
		Treatment 1	-.0015433	.0013521	.676	-.005873	.002787
		Treatment 2	-.0017433	.0013521	.594	-.006073	.002587

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Ba	Control	Treatment 1	.0628433	.0279012	.189	-.026506	.152193
		Treatment 2	.0675400	.0279012	.150	-.021809	.156889
		Treatment 3	.0683067	.0279012	.144	-.021043	.157656
	Treatment 1	Control	-.0628433	.0279012	.189	-.152193	.026506
		Treatment 2	.0046967	.0279012	.998	-.084653	.094046
		Treatment 3	.0054633	.0279012	.997	-.083886	.094813
	Treatment 2	Control	-.0675400	.0279012	.150	-.156889	.021809
		Treatment 1	-.0046967	.0279012	.998	-.094046	.084653
		Treatment 3	.0007667	.0279012	1.000	-.088583	.090116
	Treatment 3	Control	-.0683067	.0279012	.144	-.157656	.021043
		Treatment 1	-.0054633	.0279012	.997	-.094813	.083886
		Treatment 2	-.0007667	.0279012	1.000	-.090116	.088583

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Pb	Control	Treatment 1	.0010733	.0010388	.736	-.002253	.004400
		Treatment 2	-.0034133*	.0010388	.044	-.006740	-.000087
		Treatment 3	.0030700	.0010388	.071	-.000256	.006396
	Treatment 1	Control	-.0010733	.0010388	.736	-.004400	.002253
		Treatment 2	-.0044867*	.0010388	.011	-.007813	-.001160
		Treatment 3	.0019967	.0010388	.292	-.001330	.005323
	Treatment 2	Control	.0034133*	.0010388	.044	.000087	.006740
		Treatment 1	.0044867*	.0010388	.011	.001160	.007813
		Treatment 3	.0064833*	.0010388	.001	.003157	.009810
	Treatment 3	Control	-.0030700	.0010388	.071	-.006396	.000256
		Treatment 1	-.0019967	.0010388	.292	-.005323	.001330
		Treatment 2	-.0064833*	.0010388	.001	-.009810	-.003157

*. The mean difference is significant at the 0.05 level.

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APPENDIX H: Specification for Nutrient Broth E

Nutrient Broth "E"

LAB 68

Description
An inexpensive broth for the growth of nutritionally non-demanding organisms. Ideal for teaching purposes.

Typical Formula	g/litre
Beef Extract	1.0
Yeast Extract	2.0
Peptone	5.0
Sodium chloride	5.0

Method for reconstitution
Weigh 13 grams of powder, add to1 litre of deionised water. Heat to dissolve then dispense into bottles or tubes. Sterilise by autoclaving at 121°C for 15 minutes.

Appearance: Straw coloured, clear.

pH: 7.4 ± 0.2

Minimum Q.C. organisms: *S. aureus* WDCM 00034
E. coli WDCM 00031

Storage of Prepared Medium: Capped containers – up to 3 months at 15-20°C in the dark.

Inoculation and incubation: To suit chosen organism.

Growth indicator: Turbidity.

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LIST OF PRESENTATION

Seminar:

1. Poster presentation in "Evaluation And Prediction Of Nutrients Availability From Biowaste Using Sensor And Cloud Technology To Meet Crop Demands In Malaysia Workshop 2016", 19 February 2016, University Malaya, Malaysia

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