EX SITU BIOREMEDIATION ASSESSMENT OF USED ENGINE OIL BY SOIL BACTERIA

By

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Thesis Submitted to the School of Graduate Studies, Universiti Putra Malaysia, in Fulfilment of the Requirements for the Degree of Doctor of Philosophy

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Chairman : Mohd Yusoff bin Ishak, PhD
Faculty : Forestry and Environment

The used engine oil (UEO) contains potentially toxic substances that are harmful, to the ecosystem. Its pollution comprises of dangerous Cpds that requires efficient bioremediation techniques This study investigates the construction of local soil bacteria that can remove petrol-UEO (shell 15-40) by metabolic capability and their bioremediation techniques. The 1st objective of this study was achieved by isolating the UEO degraders from polluted soil of mechanical workshops in Selangor, Malaysia that grows well on UEO as a C-source. These isolates were identified as *Ochrobacterium intermedium* LMG 3301 and *Bacillus paramycoides* MCCC1A04098 strain. The initial results of GC-MS analysis screening results showed that these microbes act on C_9 -C_{29} in 0.74 (%w/v) UEO-EMSM, with the high TPH% removal by *O. intermedium* (0-24d). *O. intermedium* also exhibited increased bacteria numbers than *B. paramycoides* day 24, showing these microbes can utilize UEO.

In the 2nd objective, the RSM optimization and modelling of UEO-HCs biodegradation by *O. intermedium* LMG 3301, allowed the obtaining of the significant model (P <0.001) with R^2 to be 0.9967, and optimal condition (pH7.5, Temperature, 35°C, UEO concentration 5 g/L, and150 rpm), that removed 93% TPH (day 24). The 3rd objective focused on the characterization of Alginate-Attapulgite-Calcium carbonate (AAC) beads, in 20 mL of 1% UEO soil water extract (SWE) for their adsorptive capacities of UEO (in 50-mL capped flasks). The UEO removal efficiency (%RE) of 83 ± 0.32% in SWE associated with the highest number of AAC-beads (No = 20, Ø = 0.45 cm). The studies of SEM and 2, 3, 5-Triphenyl tetrazolium chlroride (TTC) stain of the AAC immobilized cell (IC) systems, showed that the bacteria grows well in this mixed matrix by indicating that this carrier can be applied in the ICs of UEO-pollution adsorptive bioremedies. The AAC matrix stability (4th objective), and its biocatalytic efficiency to remove UEO in a shake flask (150 rpm/24 days) were verified. On day 24, these ICs exhibited the removal of the nC_9 to nC_{17} with 30% increase in TPH obtained with *O. intermedium* and the 18% by *O. intermedium* + *B. paramycoides* (BC) in UEO, as

compared to their counter-free cell systems (FCs). The AAC-ICs of *O. intermedium* and the BC also removed C_9-C_{29} to 93% and 98%, demonstrating adsorptive-bioremediation capacity of these IC-systems.

Additional, studies were conducted *in a* freeze-dryer with the same microbes. The effects of various cryoprotectants carriers on bacteria were verified during this process to cover up part of the 6th objective. The optimum, survival of 98% was observed in the mixed carrier of 1% starch + 10% skimmed milk with the viability of over 12 weeks at the RT. The 5th objective of this study was the scale up of RSM optimal physical-chemical condition, for free cell (*O. intermedium* / BC) in 1.5 L of stirred batch reactor (SBR), to study the bioremediation of UEO under elevated aerobic condition (0-5 days). On day five, the BC was more efficient at biodegrading UEO with 10% (85%) TPH increased removal as compared to *O. intermedium* (77%).

In the 6th objective, the bacteria FD-powder and AAC-bead inoculants were tested onto artificial setups of 1.5 kg polluted soil with various UEO concentrations as main C-source; and the urea as partial C-source. The experiments in CRD-FFD$^{3-1}$ showed that the BC was the most effective. The FD-BC, established fast in the soil enabling the removal of UEO, with 12.05 ± 2.26% to 55.12 ± 4.5% of TPH consumed after 84 days whilst 13.54 ± 0.34% to 33.63 ± 1.8% were attained with AAC-bead bioformulates. The research achieved all objectives of the ex-situ bioremedies of soil and water UEO pollution. It charactizes novel methods and substantial formulated agents (i.e., free cell, adsorptive immobilized cell, and freeze-dried cell) that can efficiently decrease UEO contamination. This study has shown the bio effectiveness of the removal of UEO ex-situ exploring two different formulated bacteria isolated from heavily UEO polluted soil of mechanical workshops in Selangor, Malaysia.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Doktor Falsafah

PENILAIAN BIOREMEDIASI EX-SITU MINYAK ENJIN TERPAKAI (UEO) OLEH BAKTERIA TANAH, PENGASINGAN, CIRI-CIRI DAN TEKNIK OPTIMUM

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Minyak enjin terpakai (UEO) mengandungi bahan yang berpotensi toksik yang berbahaya kepada ekosistem dan memerlukan teknik bioremediasi yang cekap. Kajian ini menyelidik pembinaan bakteria tanah tempatan yang boleh mengeluarkan petrol-UEO (Cangkang 15-40) dengan keupayaan metabolik dan teknik bioremediasinya. Objektif pertama kajian ini dicapai dengan mengasingkan pengurai UEO dari tanah yang tercemar di bengkel mekanikal sekitar Selangor, Malaysia yang tumbuh dengan baik pada UEO sebagai sumber C. Pengasingan ini dikenal pasti sebagai *Ochrobacterium intermedium* LMG 3301 dan *Bacillus paramycoides* MCCC1A04098. Keputusan analisis GC-MS menunjukkan bahawa mikrob ini bertindak pada C_9-C_{29} dalam 0.74 (%w/v) UEO-EMSM, dengan penyingkiran TPH% yang tinggi oleh *O. intermedium* (0-24 hari). *O. intermedium* juga menunjukkan peningkatan bilangan bakteria daripada B. paramycoides hari ke-24, dimana mikrob ini boleh menggunakan UEO.

Dalam objektif kedua, pengoptimuman RSM dan pemodelan biodegradasi UEO-HCs oleh *O. intermedium* LMG 3301, menghasilkan model signifikan (P <0.001) dengan R^2 0.9967, dan keadaan optimum (pH 7.5, suhu 35°C, kepekatan UEO 5 g/L, dan 150 rpm), yang mengeluarkan 93% TPH (hari ke-24). Objektif ketiga tertumpu pada pencirian manik Alginate-Attapulgite-Kalsium karbonat (AAC), dalam 20 mL 1% ekstrak air tanah (SWE) UEO untuk mengetahui kapasiti penjerapan terhadap UEO (dalam kelalang bertutup 50 mL). Kecekapan penyingkiran UEO (%RE) sebanyak 83± 0.32% dalam SWE dikaitkan dengan bilangan AAC-manik tertinggi (No = 20, Ø = 0.45 cm). Kajian pewarnaan SEM dan 2, 3, 5-Triphenyl tetrazolium chlroride (TTC) dari sistem sel terimobilisasi AAC (IC), menunjukkan bahawa bakteria tumbuh dengan baik dalam matriks campuran ini dengan pembawa ini boleh digunakan dalam IC bioremedi penjerap pencemaran UEO. Kestabilan matriks AAC (objektif ke-4), dan kecekapan biokatalitiknya untuk mengeluarkan UEO dalam kelalang goncang (150 rpm/24hari) telah disahkan. Pada hari ke-24, IC mempamerkan penyingkiran nC_9 kepada nC_{17} dengan

peningkatan 30% dalam TPH yang diperoleh dengan *O. intermedium* dan 18% oleh *O. intermedium* + *B. paramycoides* (BC) dalam UEO berbanding dengan bebas balasnya system sel. AAC-ICs bagi O. intermedium dan BC juga mengeluarkan C_9-C_{29} kepada 93% dan 98%, menunjukkan kapasiti penjerapan bioremediasi dari sistem IC ini.

Kajian juga telah dijalankan dalam pengering beku dengan mikrob yang sama. Kesan pelbagai pembawa krioprotektan pada bakteria telah disahkan sebagai sabahagian daripada objektif ke-6. Kemandirian optimum 98% diperhatikan dalam pembawa campuran 1% kanji + 10% susu skim dengan daya maju lebih 12 minggu di RT. Objektif ke-5 kajian ini adalah peningkatan skala keadaan fizikal-kimia optimum RSM untuk sel bebas (*O. intermedium* / BC) dalam 1.5L reaktor kelompok kacau (SBR), untuk mengkaji bioremediasi UEO di bawah keadaan aerobic tinggi (0-5 hari). Pada hari kelima, BC lebih cekap dalam membiodegradasi UEO dengan 10% (85%) TPH meningkatkan penyingkiran berbanding *O. intermedium* (77%).

Dalam objektif ke-6, inokulan serbuk FD dan AAC-manik bakteria telah diuji pada tetapan tiruan 1.5kg tanah tercemar dengan pelbagai kepekatan UEO sebagai sumber C utama; dan urea sebagai sebahagian C-sumber. Eksperimen dalam CRD-FFD$^{3-1}$ menunjukkan bahawa BC adalah yang paling berkesan. FD-BC terbentuk dengan cepat di dalam tanah yang membolehkan penyingkiran UEO, dengan 12.05 ± 2.26% hingga 55.12 ± 4.5% daripada TPH yang digunakan selepas 84 hari manakala 13.54 ± 0.34% hingga 33.63 ± 1.8% diperoleh dengan bioformulasi manik AAC. Penyelidikan ini mencapai semua objektif bioremediasi ex-situ pencemaran UEO tanah dan air. Ia mencirikan kaedah baru dan agen yang doformulasikan secara substantial (iaitu sel bebas, sel terimobilisasi penjerap dan sel kering beku) yang boleh mengurangkan pencemaran UEO dengan cekap. Kajian ini telah menunjukkan keberkesanan bio penyingkiran ex-situ UEO yang meneroka dua bakteria yang diformulasikan berbeza yang diasingkan daripada tanah bengkel mekanikal yang tercemar UEO di Selangor, Malaysia.

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LIST OF ABBREVIATIONS

%	Percentage
µM	Micromolar
AAC	Alginate-Attapulgite-Calcium carbonate
AC	Alginate- Calcium carbonate
AC	Alginate-$CaCO_3$
AMSM	acidophilic mineral salt medium
ANOVA	Analysis of variance
A_{xnm}	Optical density at wavelength x nanometer
BC	Bacteria consortium
BOD	Biological oxygen demand
BTEX	Benzene, Toluene, Ethylbenzeneand Xylenes
CCD	Central composite design
CF	Correctional factor
CF	correctional factor
CFU	Colony forming unit
CFU /mL	Colony forming units per milliliters
CFU/g	Colony forming units per g
Cm	Centimeter
CMC	critical micelle concentration
CPD	Chamber of critical point dryer
CPD	critical point dryer
CRD	Completely Randomized Design
CSTR	Continuous Stirred Tank Reactor
DGGE	denaturing gradient gel electrophoresis
DMRT	Duncan multiple range test

DNA	Deoxyribonucleic Acid
DO	Dissolved oxygen
DOE	Design of Experiment
DR	drum reactors
EDTA	Ethylenediamine tetraacetic acid
EMSM	Enrichment mineral salt medium
FCP	fluid cell preparation
FCP	Freeze dryer flued cell preparation
FFD	Fractional Factorial Design
FPA	Freeze drying protective agents
G	Gram
g/L	Gram per liter
GC–MS	Gas chromatography-Mass Spectrometry
GLM	General Linear Model
H/M	High levels of mixed (H/M)
H/S	High levels of individual
ICS	Immobilized Cell System
Kg	Kilogram
L/M	Low levels of mixed
L/S	Low levels of single
LR	Lime requirement
M	Molar
MBS	Molecular Biology Services
MC	Mixed cell
MDC	Microbial desalination cell
mL	Milliliter
MSM	Mineral salt medium

NA	Nutrient agar
NB	Nutrient broth
NEO	New engine oil
Ng	Nano gram
ng/µL	Nanograms per micro liter
OD	Optical density
PAHs	polycyclic aromatic hydrocarbons
PW	Peptone Water
RBR	Risk-based remediation
RPM	Rotations per minute
RSM	Response surface methodology
SBR	Stirred batch reactor
SC	Single cell
SEM	Scanning electron microscope
SF	Shake flask
SM	Skim milk
SSCP	Single-strand conformational polymorphism
STR's	Stirred tank bioreactors
SVE	Soil vapor extraction
SW 305	Spent lubricating oil
TAE	Tris-acetate-ethylene diamine tetra acetate
TBA	Tributyrin Base Agar
TC	Total concentration of single/mixed bacteria viable counts
TF	Formazan
TPH	Total Petroleum Hydrocarbons
TTC	2,3,5-Triphenyltetrazolium chloride
TVC	Total viable counts

UEO	Used engine oil
USCS	Unified Soil Classification System
v/v	Volume/Volume
VBNC	Visible but non-culturable
VOCs	Volatile organic compounds
w/v	Weight per volume
w/w	Weight per weight
WHC	Water holding capacity
YE	Yeast extract

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Pollution of soil by engine oil is a global problem caused by used engine oil (UEO) (Pongsilp & Nimnoi, 2022). The UEO leakages, indiscriminate discharge and careless handling of UEO from and in the mechanic, workshops have been a significant source of this pollution problem (Bulai et al., 2021). That has not been addressed equally as to those of crude oil, diesel and petrol spills occuring in large areas (Ghribi et al., 2019). This has been a very common over growing problem that has not been tackled for many years in many countries. Because, of the environmental dangers of this waste (Wolak et al., 2020), the dumping of this waste in the soil (Bulai et al., 2021), has become an environmental serious pollution trouble. The increasing and accumulation of this waste type in the urban soil areas, and left untreated for several years, it represents an environmental fear (Ghribi et al., 2019). Due to the fact, that it can lead to transitory/permanent damage to the soil properties, plants, microorganisms (Adedokun et al., 2007; Hossain et al., 2022); and the pollution of surface and/or ground water resources (Hossain et al., 2022). The UEO contaminations are dangerous to human health as well as to the ecosystem. They may contain the toxic hydrocarbons, cyclic alkanes, heavy metals etc. (Koma et al., 2003; Jain, et al., 2010; Kim et al., 2013; Adeyinka et al., 2020), that are dangerous to the soil, water resources, food chain, human health etc. (Adedokun et al., 2007; Bulai et al., 2021; Hossain et al., 2022). Of those Cpds some are carcinogenic, mutagenic and immunotoxicants to which it is a direct potential danger to the human (De Silva et al., 2021; Hambali et al., 2021; Ibe et al., 2021; Gaur et al., 2022). The UEO-waste Cpds may also have a great impact on the local system, by imbalancing the soil C-N-P ratio (Ławniczak et al., 2020), due to the increasing of C% (Okolo & Odu, 2005). They damage the soil physical (e.g., deccrease soil porosity, permeability etc) and/or harm biological characteristics (e.g., fertility, microbial diversity etc.), by preventing oxygen transfers between the soil/water surface and atmosphere (Bulai et al., 2021; Ambaye et al., 2022; Gaur et al., 2022).

However, because the soil oil contaminated areas are rich with indegeneous microorganisms that can contribute in bioremediation processes (Hossain et al., 2022). Many researchers isolated various soil bacteria genus that can degrade UEO in pure cultures or in consortia (Ibrahim, 2016; Hossain et al., 2022.; O Jesubunmi et al., 2022; Javed et al., 2022; Muhammad & Adamu, 2022). The biodegradation of hydrocarbon pollutant by the same native microorganisms isolated from the same contaminated site is substantially faster than exotic microorganisms (Parach et al. 2017). Therefore, the uses of native biopotential in bioremediation are favoured.

The quality of the environment requires safe and efficient biotreatments of contaminants to sustain healthy environment (Bulai, et al., 2021). An appropriate remediation method to deal with the UEO-contamination is required.

However, although the remediation of oil contaminated environments are challenging due to their low solubility, none polarity, hydrophobic nature, co-contamination with metals etc (Vidali, 2001; Singh et al., 2009; Liu et al., 2011; Adeyinka et al., 2020). But there are several remediation methods for oil-soil contamination, including physical (burning or thermal), chemical, and biological (bioremediation) (Gallegos Martínez et al., 2000). The conventional chemical and thermal or burning methods used to banish oil from soil are expensive, none environmentally friendly, and sometimes inefficient (Bulai, et al., 2021; Ambaye et al., 2022). The biological methods are the most environmentally acceptable and economically feasible remedies to remove oil soil pollution (Varjani, 2017; Ławniczak et al., 2020). Amongst the methods, microbial bioremediation has been considered as the most promising (Karigar & Rao, 2011).

Consequently, bioremediation has been applied to remove petrol oil spill (Liu et al., 2022, Zahed et al., 2022), used motor oil (Hossain et al., 2022), and diesel fuel (Nkem et al., 2022) from the soil/water environment. The bioremedies of various bacteria strains has been reported as effective and efficient treatment to cleanup UEO in the contaminated environments (Hossain et al., 2022; Kumar et al., 2022; Pongsilp & Nimnoi, 2022).

Nevertheless, since the removal efficiency by biological methods can be some times variable, low and/or slow (Angehrn and Zeyer, 1998), depending on nutrient imbalances (e.g., high/low (C:N:P) ratio, etc.), availability (e.g., solubility, etc) (Okolo& Odu, 2005; Ambaye et al., 2022), and spent oil composition (Bhattacharya et al., 2015; Wu et al., 2017; Rațiu et al., 2020). The efficiency by biological methods can also be subjected to the oil bioremediation techniques (Partovinia et al., 2018).

To anticipate the maximum impact of any microbial bio-treatment, it is of a significant importance to assess its liquid culture free cell systems (FCs) requirements that can lead to enhanced biodegradation process before any other carrier/support material systems. The "one factor at time" optimization approach to assess the optimal nutritional requirement of a defined culture medium is a time consuming and unrealistic procedure. The use of a statistical approach that provides profound insight on the interactive outcome of inputs that controls a bioprocess can be robust and realistic. The approach of response surface methodology (RSM) has been applied in various controlled physical-chemical bioremediation studies (Concepta Goveas, et al., 2022; Umar et al., 2022; Shi et al., 2022) as an effective tool to resolve the interactions, modelling and determine optimal process conditions.

The oil degradation processes are mostly influenced by physical-chemical limiting factors (Bhattacharya et al., 2015; Olawale et al., 2015; Ibrahim, 2016). To anticipate the maximum impact of this process for UEO in basal UEO-MSM culture, the RSM optimization and modelling of physical-chemical factors is crucial to this process.

However, apart from nutritional physical-chemical requirements to enhance bioremediation rates in various environments, the bioremediation processes can also be enhanced by the techniques of microorganism transfer to polluted water/soil media that can be applied in-situ / ex-situ (Mrozik and Piotrowska-Seget, 2010; Partovinia et al., 2018). The most common procedure is the direct use of the liquid culture stage or free cell (FC) system (Partovinia et al., 2018). However, appropriate distribution of microorganisms, especially in soil profile, their activity, and viability cannot be completely assured using FC systems. In order to increase bioremediation efficacy, various techniques of microorganism transfer to polluted area have been investigated (Mrozik and Piotrowska-Seget, 2010). One of the more promising techniques in this field is to use different carrier or support materials for pure culture/consortia cell wet coarse formulates (e.g., beads)/freeze dryer protective agents in fine dry powder formulates (e.g., freeze dried bacteria powder) to maintain sufficient microbial activity for a long period of time, slow release protection, even distribution etc. (Banerjee & Ghoshal, 2013; Bayat et al., 2015; Partovinia et al., 2018; Li et al., 2021) thereby enabling higher bioremediation efficiency (Cassidy et al., 1996). Several bioremediation techniques of novel characteristics and eco-friendly mixed carrier matrices that support bacteria viability and enhance UEO bioremediation has been reported elsewhere (Zhen-Yu et al., 2012; Hou et al., 2013; Partovinia & Rasekh, 2018; Li et al., 2021; Zhou, et al., 2021).

1.2 Problem Statement

Used engine oil (UEO) pollution on soil is a worldwide problem. The indiscriminate discharge and improper handling of UEO from and in the mechanic workshops have been a significant source of this issue. The accumulation of UEO in the urban soil areas, left untreated for years, has turn out to be a potential environmental concern, as this contamination can inflict transitory and permanent damages to the ecosystem and human health.

The UEO contaminations are dangerous as they contain the toxic compounds that can pollute the water and/or food chain. They also imbalances the soil C-N-P ratio, while damaging the soil physical (e.g., deccrease soil porosity, permeability etc) and/or harm biological characteristics (e.g., fertility, microbial diversity etc.), by preventing oxygen transfers between the soil/water surface and atmosphere.

The UEO comprises mainly the toxic n-Alkane hydrocarbons and heavy metals that makes it an EPA very significant waste problem. Due to UEO assorted compounds, the inorganic compounds (i.e., heavy metals) are expected to complicate the remediation of oil contaminated environments. Generally, the remediations of oil contaminated environments are complex, owing to their low solubility, non polarity, hydrophobic nature, and co-contamination. Those environmental pollution attributes, requires bioremediation techniques of combined efficacies of biological and non biological method, with the former as the core to the overall bioremediation process, and the latter complementary.

However, the soil oil contaminated areas are rich with indegeneous microorganisms that can contribute effectively in bioremediation processes allowing researchers to isolate various soil bacteria UEO degraders in pure cultures or in consortia with many biodegradative capabilities. The biodegradation of HCs pollutant by native microorganisms isolated from the same contaminated site is substantially faster than exotic microorganisms. While the uses of native microbial biopotential in remediation studies are favoured; the activity and viability of those microbes as transfered in culture suspensions to the soil/water UEO polluted sites are some times not assured. In order to increase their bioremediation efficacy, numerous techniques of microorganism transfer to polluted area have been investigated. One of the promising techniques in this field is to use different carrier or support materials to protect the innoculum, maintain longer viability and improve the toleration to environmental loadings (e.g., high pH, high UEO concentration etc), thereby enabling higher bioremediation efficiency and rates. Several bioremediation techniques of novel characteristics and eco-friendly mixed carrier matrices that support excellent bacteria viability and enhance UEO bioremediation have been established.

1.3 Significance of the Study

The used engine oil mostly comprises of miscellaneous toxic compounds (Jain, et al., 2010; Kim et al., 2013; Adeyinka et al., 2020), with the n-Alkane HC-compounds forming the highest percentage. Therefore, a study on used engine oil is of important, as it comprises of a range of simple to complex n-Alkane HC-Compounds of various characteristics, heavy metals etc. (Adeyinka et al., 2020) that are dangerous to the soil, water resources, food chain etc. (Adedokun et al., 2007; Bulai et al., 2021; Hossain et al., 2022). They can be considered as useful "arbitrary model" to hydrocarbons and cyclic alkanes (Koma et al., 2003) for pollution further studies.

Besides that, the used engine oil is often co-contaminated with inorganic compounds e.g., heavy metals (Adeyinka et al., 2020). This pollution characteristics complicates the conventional bioremediation of free cell systems (FCs) to oil compounds, and leads to more harmful effects (Wang et al., 2020), that demands adsorptive-biosorptive efficient systems to enhance adsorptive bioremediation process e,g., composite matrix of immobilized cell techniques to entrap the cells and protect them from toxicity (Wang et al., 2014; Partovinia & Rasekh, 2018; Elhamrouni et al., 2023), pH and temperature changes.

The research on remediation in the past was mostly focused on crude oil, and diesel oil pollutions of large areas (Ghribi et al., 2019; Adeyinka et al., 2020), mostly using conventional physical and/or chemical approaches. The emphasis on those approaches in the past, often did not pay attention to the merits of sustainable UEO microbial remediation techniques for contaminated sites (e.g., garage) to remove organic and/or inorganic compounds. Adsorptive-biosorptive microbial based remediation techniques are the best method for the ecological recovery of complex mineral lubricating oil contaminated sites. The bacteria are well known to be safe and the core of those whole process in the soil and water (Okino-Delgado et al. 2019; Ye et al. 2019), and in those bioremediation techniques. The bacteria solubilize, absorb, biosorp, transform, or degrade HC-Compounds, and can reduce the mobility and bio-availability of the contaminants in the environment (Wu et al. 2010). However, although the methods of crude/fuel oil remediation are not a new method, they have been known for consuming a long time and requires local biopotential efficient techniques that can enhance the qualitative over all process.

The physical and chemical approaches to remediate complex contaminants are expensive; and sometimes unsafe, and partial (Bulai, et al., 2021; Ambaye et al., 2022) unless properly managed, and/or supplemented with bioagents to sustain and enhance process efficiency. Great deals of literature have reported the entrapment alginate and skimmed milk-based carrier matrix techniques that efficiently enhanced the removal of oil pollutants (Zhen-Yu et al., 2012; Hou et al., 2013; Partovinia & Rasekh, 2018; Li et al., 2021; Zhou, et al., 2021). Therefore, the investigation of those techniques that allows the combined attributes of physical, chemical and biological to enhance bioremediation process has flexible potential to be used for ex-situ bioremediation of pollutants associated with mineral oil wastes (e.g., UEO) in the soil and/or water environments of urban cities in Malaysia.

1.4 Research Objectives

1.4.1 The general objectives of the study:

1. Identify and characterize indigenous bacteria capable of degrading used engine oil from mechanic workshops in Selangor, Malaysia;

1.4.2 The specific objectives of the study:

1. Analyse, modelling of growth and degradation conditions of used engine oil (UEO) rate in mineral salt medium (MSM) shake flask system, using the most potent bacterial isolate strain, and the statistical approach of one-factor-at-a-time (OFAT) and response surface methodology (RSM);
2. Characterize of adsorptive properties of a composite carrier (sodium alginate-attapulgite-calcium carbonate), for the bioaugmentation of risk-based remediation (RBR) approach of used engine oil in the soil and aqueous system;
3. Optimize kinetic studies and development of free and entrapped pure/mixed consortia bacteria techniques in the remediation studies of used engine oil (UEO) in shake flask;
4. Scaling up and the characterization of growth, physiological activity of UEO potential degrading bacterium and mixed bacteria (consortium), in the stirred batch bioreactor (SBR);
5. The formulate of freeze dried and entrapped pure/mixed consortia bacteria techniques for the applications in the remediation studies of artificially UEO contaminated soil microcosm.

1.5 Research scope

This research aims to investigate the factors and the best method for removing used engine oil (UEO) pollution. Soil samples were collected from three different mechanic workshops in Selangor, Malaysia. Analytical remediation processes were used, including locally isolated and mixed microorganisms in shake flask systems and an artificially UEO contaminated soil microcosm. Potential UEO bioremediation isolates were chosen and identified. UEO degradation bioremediation processes were tested in aqueous and soil medium biological-chemical-physical conditions. In shake flask experiments, analytical bioremediation studies of UEO were introduced using the mineral salt medium (Zajic & Supplisson, 1972). The most potent locally isolated bacterium strain was used to optimise and model the UEO bioremediation conditions.

UEO and yeast extract was added to the basal mineral salt medium (MSM), and growth was monitored along with pH and UEO-residues throughout the bioremediation process. The optimised bioremediation conditions obtained from shake flask experiments were scaled up in aerobic stirred batch reactor to further investigate the physiological responses of bioremediation under elevated oxygen conditions. As a high priority of the systems investigated in MSM-shake flask and artificially UEO-contaminated soil microcosm, the characterization of adsorptive/sorptive properties of a composite carrier (sodium alginate-attapulgite-calcium carbonate) and the corresponding mixed bacteria culture biocatalytic potential were undertaken. Specific studies were conducted to compare the performance of different bioremediation processes, growth, enzyme activities, and bioremediation kinetics.

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