



## Aerated Static Pile (ASP) Enhanced Bioaugmentation of Oil Sludge Contaminated Soil Using LIBEM Consortium

Nur Zaida Zahari<sup>1\*</sup> and Piakong Mohd Tuah<sup>2</sup>

<sup>1</sup>Faculty of Science & Natural Resources, Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah, Malaysia

\*Corresponding author:

Dr. Nur Zaida Zahari

Faculty of Science & Natural Resources,  
Universiti Malaysia Sabah, 88400 Kota Kinabalu, Sabah,

Tel: +6013-8988754

Fax: +6088-435324

Email: [zaidazahari@ums.edu.my](mailto:zaidazahari@ums.edu.my)

### HISTORY

Received: 21<sup>st</sup> August 2019  
Received in revised form: 22<sup>nd</sup> of October 2019  
Accepted: 6<sup>th</sup> of November 2019

### KEYWORDS

bioremediation  
oil sludge  
microbial consortium  
aerated static pile (ASP)  
LIBEM

### ABSTRACT

This paper focused on efficiency of selective locally isolated beneficial microorganisms (LIBEM) consortia in bioaugmentation of oil sludge contaminated soil on a laboratory scale. Five different selective microbial consortia LIBEM were obtained from Environmental Microbiology Laboratory, Universiti Malaysia Sabah. The arrangement of aerated static pile (ASP)-bioreactor made up of acrylic materials was developed act as continuous aeration to the soil. Six different sets of treatment containing 10 kg of soil mixed with 20% (v/v) oil sludge with five bacterial strains and natural attenuation consisted of indigenous microbial in the soil as a control plot was utilized. The rates of biodegradation of oil sludge were studied for a period of three months and periodic sampling of soil from each bioreactor was carried out at 7 days of interval. Analysis of Total Petroleum Hydrocarbon (TPH) was done along with physiochemical parameters such as pH, moisture content and microbial population (CFUs) in the soil. The results obtained revealed biodegradation of oil sludge contaminated soil in all set treatments were much higher than Natural attenuation by 12.1 fold. The bioreactor augmented with Consortia 4 proved to be a better performance with 94% of TPH degradation followed by Consortia 2 (91.6%), Consortia 5 (90.1%), Consortia 1 (90%), Consortia 3 (87.5%) and Natural attenuation (27.4%) respectively. The variations of physiochemical parameters that influence the reduction of oil sludge in the soil were also discussed in this paper. High degradation of long chain alkanes was observed between consortia treatment as compared to natural attenuation plots suggested that bioaugmentation using consortia LIBEM decrease the level of oil sludge in contaminated soil. It was noted that, the continuous supplied of oxygen from aerated static pile (ASP)-system into the soil showed a good prospect in bioremediation of oil sludge.

### INTRODUCTION

Oil sludge is a complex mixture of oil, water and solids that generate from crude oil exploration, transportation and refining activities from petroleum industries. Oily sludge is categorized as hazardous waste under Environmental Protection Act and Hazardous Waste Handling Rules. It contains a high concentration of petroleum hydrocarbon and other recalcitrant compounds [1]. Due to its hazardous characteristics, the improper disposal of this waste into the environment has attracted widespread attention around the world.

The release of oil sludge from anthropogenic activities in to the environment causes severe damage to ecosystems and accumulation of these pollutants in animal and plant tissues can cause serious genetic mutation [2,3]. Nowadays conventional techniques such as bioremediation are becoming an attractive

alternative for cleaning up hydrocarbon contaminated soil. Due to its cost effectiveness, efficient Technology and environmentally sound treatment, this method has been chosen by researcher in remediation soil hydrocarbon pollutant. One of the best options of bioremediation is bioaugmentation, the addition of selective oil degrading microorganisms that can degrade a wide range of target constituents present in the oil sludge.

This process simply transfers a complex hydrocarbon into a simpler compound that are slightly or non-pollutant into the environment [4]. The process begins with the transformation of complex hydrocarbon into the simple compound (primary degradation) and when the process is complete (mineralization) the hydrocarbon is decomposed into inorganic compound or cellular constituents [5,6].

Numerous researchers have studied that bioaugmentation involve the use of microbial consortium consisting of many hydrocarbon degrading microorganisms showed great performances in removal of recalcitrant toxic substances from the soil. [7] reported that mixed cultures were able to transform chlorinated benzoates and able to degrade chlorocatechols in soil microcosm. Other study by [8], evaluated that capacity of microbial consortium of *Mycobacterium fortuitum*, *Bacillus polyisoprenivorans*, *Microbacteriaceae* bacterium and *Fusarium oxysporum* to degrade and mineralize anthracene, phenanthrene and pyrene in soil. The results showed 96% to 99% degradation rates from (250, 500 and 1000 mgkg<sup>-1</sup>) within 70 days treatment as compared to non-bioaugmented soil.

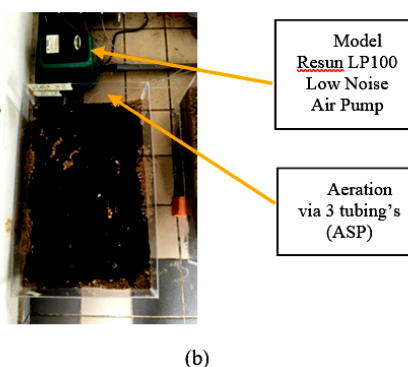
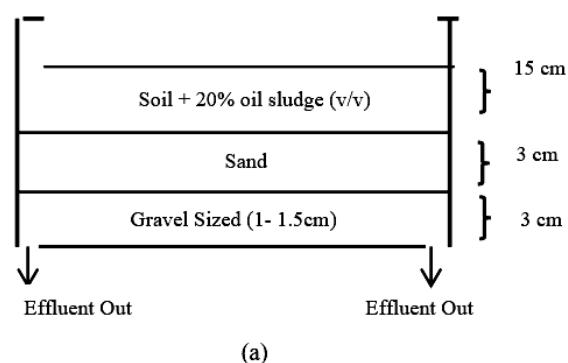
The key observation was that microbial consortium was more effective in comparison with bacterial and fungal isolates inoculated separately to the soil. Similarly, the high efficacy of PAH degradation in artificially contaminated sediments by consortium has been reported [9]. They studied the biodegradation of a mixture of fluorene, phenanthrene and pyrene by a bacterial consortium made up of three strains of *Rhodococcus* sp., *Acinetobacter* sp. and *Pseudomonas* sp. The results showed that the addition of this consortium into soil significantly enhanced the efficiency of fluorene and phenanthrene biodegradation. However, few studied focused on supplement of aeration into the soil to enhance the biodegradation rates.

A common method like regularly tillage has been applied as aeration in to the soil. In this paper, we focused on effectiveness of five different consortium of LIBeM namely *Candida tropicalis*-RETL-Cr1, *Chromobacterium violaceum*-MAB-Cr1, *Pseudomonas aeruginosa*-BAS-Cr1, *Sphingomonas paucimobilis*-ReTOS-Cr1 and *Stenotrophomonas maltophilia*-RAS-Cr1 in bioaugmentation of oil sludge contaminated soil. This study was enhanced with aerated static pile (ASP) as a continuous aeration into the soil.

## MATERIAL AND METHODS

### Bioreactor Design

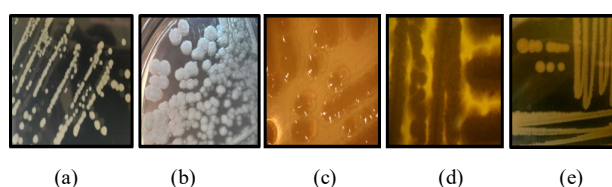
An aerated static pile ASP- bioreactor system made up of acrylic material with 3 silicone tubing's at the side of the reactor connected to air pump (Model RESUN LP100 Low Noise Air Pump) (Fig. 1b). Aerated static piles (ASP) was used by forming composting into piles and aerated with blowers or vacuum pumps. The bioreactor divided into 3 parts where the base is filled with gravel-sized (1-1.5 cm) followed by sand and soil on the surface (Fig. 1a). The soil used in the study was mixed thoroughly with oil sludge after being put in the treatment plot. The effluent collected in the universal bottle located on the bottom of the reactor.



**Fig. 1.** Bioreactor supplied with aerated static pile for bioaugmentation of oil sludge contaminated soil, (a) front view, (b) top view.

### Sources of Microorganisms

LIBeM strains were obtained from the Environmental Microbiology Laboratory, Faculty of Sci & Natural Resources, Universiti Malaysia Sabah. The cultures were maintained on Nutrient agar (NA) and slant at 4°C for prior used. These strains have capability to degrade oil and phenol based on the previous research done by [10,11]. The colony of these microorganisms is shown in Fig. 2.



**Fig. 2.** Colony morphology of LIBeM (a) *Candida tropicalis*-RETL-Cr1, (b) *Chromobacterium violaceum* -MAB-Cr1, (c) *Pseudomonas aeruginosa* -BAS-Cr1, (d) *Sphingomonas paucimobilis* -ReTOS-Cr1 (e) *Stenotrophomonas maltophilia* -RAS-Cr1 observed under stereo microscope at magnification x20.

## Culture Medium

Ramsay broth was used as the culture medium to supply nutrients for the microbial growth. All the components and constituents listed in **Table 1** were mixed with 1.0 L of sterile distilled water. The liquid medium was transferred to Scott bottle and autoclaved for 15 minutes at 121°C.

**Table 1.** Ingredients for the preparation of Ramsay broth [12].

Ingredients	Composition (g/L)
Ammonium nitrate (NH <sub>4</sub> NO <sub>3</sub> )	2.0
Monopotassium phosphate (KH <sub>2</sub> PO <sub>4</sub> )	0.5
Dipotassium phosphate (K <sub>2</sub> HPO <sub>4</sub> )	1.0
Potassium chloride (KCl)	0.1
Calcium chloride dihydrate (CaCl <sub>2</sub> ·2H <sub>2</sub> O)	0.01
Yeast extraction	0.06
Glucose	20
Magnesium sulfate heptahydrate MgSO <sub>4</sub> ·7H <sub>2</sub> O	0.5

## Standard Inoculum Preparation

One loop of each strain of LIBeM was harvested from the overnight sub-cultured using streak plate method [13]. The inoculation of LIBeM into the oil sludge contaminated soil was conducted in liquid form. Microbial consortia LIBeM was inoculated into Ramsay broth at 30°C for 24 hours in an orbital shaker at 200 rpm. A total of 10% of the cultured (1x10<sup>7</sup>) with OD 0.5 and above at 600 nm was used as inoculum. Then 10% (v/v) inoculants of microbial consortia LIBeM were added into the contaminated soil with 20% (v/v) of oil sludge for bioaugmentation studies. The inoculation of microbial consortia LIBeM in the ASP- bioreactor was conducted for every 2 weeks [14].

## Soil Preparation

10 kg soil was sieved through 0.20 mm sieve size to remove stone and dust particle. The physical analysis of soil (pH, & moisture content) was determined according to [15]. 2 liters (20% v/v) oil sludge was sprinkled over the soil and allowed to get adsorbed for 30 minutes for further bioremediation studies [16].

## Experiments Set-up

The bioaugmentation studied of oil sludge in contaminated soil was evaluated by setting different plots in the bioreactors as shown in **Table 1**. The experimental set-up includes one controlled plot and five different microbial consortia treatments. Experiments for each ASP-bioreactor will be carried out for 90 days at open and air-ventilated environment. The soil samples were treated with microbial consortia LIBeM, which are denoted as Consortia 1, 2, 3, 4, 5 and Natural attenuation (control plot) by following treatment combination:

**Table 1.** Bioaugmentation study set-up of oil sludge in contaminated soil.

Treatment	Content
Consortia 1 :	Soil + oil sludge + (ReTOS-Cr1+ BAS-Cr + RAS-Cr1)
Consortia 2 :	Soil + oil sludge + (RETL-Cr1+ MAB-Cr1+RAS-Cr1)
Consortia 3 :	Soil + oil sludge + (RETL-Cr1+ RAS-Cr1+BAS-Cr1)
Consortia 4 :	Soil + oil sludge + (RETL-Cr1+ MAB-Cr1+BAS-Cr1)
Consortia 5 :	Soil + oil sludge + (ReTOS-Cr1+ BAS-Cr + RAS-Cr1+ RETL-Cr1+ MAB-Cr1)
NA (Control plot):	Soil + oil sludge + non-augmented

## Technical Procedures Protocol

For each of the experimental unit the soils were tillage daily and watering with distilled water at an interval 2 days to maintain the water holding capacity of soil. The inoculation of microbial consortia LIBeM into each plot was done for every 2 weeks during 60 days treatment periods. Initial readings were taken immediately after the inoculation of the bacterial culture and further. The soil samples were drawn after every 7<sup>th</sup> day from the respective experimental unit. The experiment was lasted 90 days under the ambient temperature. Oily sludge in natural attenuation plot was not subjected to any treatment.

## Physiochemical Analysis of Soil

### pH

Analysis pH was carried out as according to [17]. 20 g of soil was weighed and mixed in a beaker containing 40 ml of distilled water. Analysis was done by taking three reading and average for accuracy using the pH meter.

### Soil Moisture Content

The soil moisture was analyzed by using gravimetric method as mentioned by [18]. An empty crucible was weighed and record as (M1) before putting the soil sample. Then, 10 g of soil sample was grained with mortar and pestle and recorded as (M2). The soil sample was dried at 110 °C for 24 hours in an oven. The soil was cooled in desiccator and weighed the crucible as (M3). The soil moisture content was measured by using the formula:

$$\% \text{ Moisture content} = \frac{(M2 - M3)}{(M2 - M1)} \times 100$$

Where;

M1 = Weight of empty crucible (g)

M2 = Wet of crucible + wet soil in (g)

M3 = Weight of crucible + dry soil in (g)

### Soil Microbial Population

Biological microbial population was determined by the serial dilution method [14]. 10 g of soil sample was mixed with 100 ml sterile distilled water in conical flask. 1 ml of soil sample mixture was diluted with 9 ml of sterile distilled water. The dilution series (10<sup>-3</sup> to 10<sup>-5</sup>) were used to calculate the microbial population. 0.1 ml of three dilutions (10<sup>-3</sup> to 10<sup>-5</sup>) was spreader on a nutrient agar plate and incubated under 30 °C for 24 hours. The bacterial colonies in each plate were counted as the number of colony-forming units (CFU) per gram of dry soil by using the formula below:

$$CFU/ml = \frac{CFU \text{ per plate} \times \text{dilution factor}}{\text{volume of sample taken (ml)}}$$

### Total Petroleum Hydrocarbon (TPH) Analysis

Total petroleum hydrocarbon was carried out based on gravimetric method (Soxhlet extraction) (USEPA 3540C) [19]. 20 g of soil sample was grained and placed in thimble and extracted with dichloromethane (DCM). The thimble was placed in soxhlet extractor. 175 ml of dichloromethane was added into round bottomed flask (RBC). The cooling temperature and mantel heater were set at 17 °C and 4 °C respectively. The extraction process took place for less than 24 hours.

Then the content was cooled, and the DCM was flown to the round bottomed flask from the extractor. The total solvent was cleared completely with the vacuum evaporator at 40°C to 50°C. By using the rotary pump, DCM was left as the extracted sample. The RBC together with the extract was dried in oven at 60°C for 24 hours. The RBC then was cooled in desiccator for 30 minutes. The RBC was measured until constant weights were obtained for 3 readings. The percentage of total petroleum hydrocarbon (TPH) was calculated using the formula below:

$$\% \text{TPH} = \frac{\text{Min extract weight in RBC}}{\text{Weight of sample}} \times 100$$

## RESULTS & DISCUSSION

### Biodegradation of Oil Sludge

The effect of microbial consortia of LIBeM in preparation for bioaugmentation of oil sludge at 20% (v/v) contaminated soil is shown in Fig. 3. The total petroleum hydrocarbon (TPH) decreased by 31% to 94% in soil treated with microbial consortia LIBeM respectively. At the same time activation of natural attenuation (non-augmented soil) with indigenous microbial decreased the TPH by 6.5%-27.4% within 90 days incubation periods. The fact that the living indigenous microorganism also can degrade oily sludge in the soil can be observed with small reduction of TPH degraded in the natural attenuation plot.

The oil sludge degradation increased almost 12-fold higher than the control plot when augmented consortia LIBeM were applied. Therefore, the obtained data are consistent with a previous comparative study [20], which demonstrated that bioaugmentation was more effective than natural attenuation on the degradation of the TPH. The result also showed that significant degradation of TPH occurs most rapid during first month of the experiment and the rate declined with time.

It is reported that the initial attack is generally occur when microbial consortia LIBeM have been introduced into the oil sludge contaminated soil. This process achieved through various mechanism such as the attachment of microbial cells to the substrates, the production of biosurfactant and bioemulsifier and production of gases and acids [21]. At this stage, the sufficient of aeration supplied with aerated static pile was most helpful in order to complete the process. Bioreactor augmented with Consortia 4 denoted by strains *C. tropicalis*-RETL-Cr1, *C. violaceum* -MAB-Cr1 and *P. aeruginosa* -BAS-Cr has showed great potential with 94% TPH degradation as compared to other treatment. At the 3 months observation, the highest degradation of TPH can be shown in Consortia 4 (94%) followed by Consortia 2 (91.6%), Consortia 5 (90.1%), Consortia 1 (90%), Consortia 3 (87.5%) and Natural attenuation (27.4%) respectively.

The results also demonstrated that after 56 days treatment, there was no significant difference in TPH reduction in all augmented reactor. The physical changes can be seen through the light color of the soil indicated that TPH degradation almost complete to mineralization process [22].

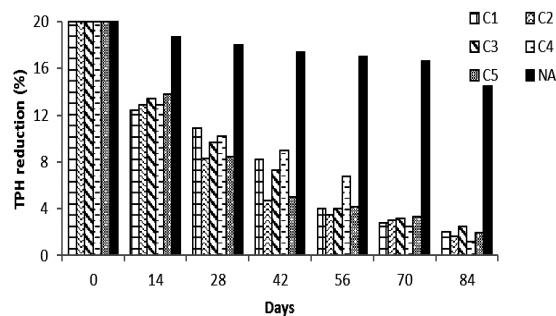


Fig. 3. Degradation of TPH by bioaugmentation of oil sludge contaminated soil during three months experiment (C1= ReTOS-Cr1+ BAS-Cr + RAS-Cr1, C2= RETL-Cr1+ MAB-Cr1+RAS-Cr1, C3 = RETL-Cr1+ RAS-Cr1+BAS-Cr1, C4= RETL-Cr1+ MAB-Cr1+BAS-Cr1, C5= ReTOS-Cr1+ BAS-Cr + RAS-Cr1+ RETL-Cr1+ MAB-Cr1 and NA = Control plot)

It can be hypothesized that the increase in TPH removal when soil inoculated was performed due to different species of hydrocarbon degrading ability present in the soil. The combination of Consortia 4 consists of strains *P. aeruginosa*, *C. tropicalis* and *C. violaceum* showed a great performance in TPH degradation as these three strains play an important role in petroleum degradation [23]. As well-known on their individual performance, the combination of these strains attributed the synergistic effect through the reduction of TPH in the soil. This fact suggested that intermediates of a catabolic pathway of one strain may be further degraded by other strains possessing suitable catabolic pathway [24]. Thus our finding suggested that bioaugmentation with microbial consortia (*C. tropicalis*-RETL-Cr1, *C. violaceum* -MAB-Cr1, *P. aeruginosa* -BAS-Cr1, *S. paucimobilis* -ReTOS-Cr1, *S. maltophilia* -RAS-Cr1) assisted by aerated static pile (ASP) was much better as compared to [25] and [26] in terms of the duration (short time) needed to degrade the hydrocarbon in contaminated soil.

### Degradation of Hydrocarbon Content in Oil Sludge by GCMS

The eight types of hydrocarbon aliphatic components, namely heptadecane (C<sub>17</sub>H<sub>36</sub>), octadecane (C<sub>18</sub>H<sub>38</sub>), nonadecane (C<sub>19</sub>H<sub>40</sub>), eicosane (C<sub>20</sub>H<sub>42</sub>), heneicosane (C<sub>21</sub>H<sub>44</sub>), docosane (C<sub>22</sub>H<sub>46</sub>), tricosane (C<sub>23</sub>H<sub>48</sub>) and tetracosane (C<sub>24</sub>H<sub>50</sub>) are identified through the GC-MS analysis. It was clearly observed that the percentage reduction of each individual hydrocarbon compounds by consortia LIBeM was nearly 100% except for compounds nonadecane, heneicosane, tricosane and tetracosane. The results demonstrated that both microbial consortia LIBeM C3 and C4 exhibited good performance in degrading n-alkanes hydrocarbons with more than 95% reduction in all compounds (Fig. 4).

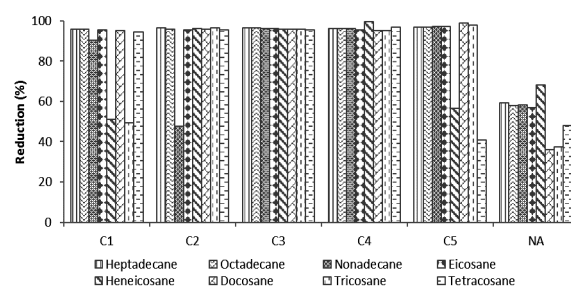


Fig. 4. Reduction of selected hydrocarbons compounds presented in the contaminated oil sludge studied

The decrease in the peak heights of hydrocarbons and areas implies that selected LIBeM used to degrade oil sludge in this study have the ability to exploit and degrade the hydrocarbon as sole carbon source [27]. As the height and area of the peaks decrease, the potency of consortia LIBeM to act as a petroleum hydrocarbon degrader is greater. By comparing the reduction percentage between consortia LIBeM and natural attenuation plots, there was small reduction of hydrocarbon compounds recorded with range of 36-68% in all compounds. Heneicosane experienced highest degradation (99.53%) in C4 while docosane was degraded the least with (36.0%) in natural attenuation plot. This can be explained that docosane composed of long chain aliphatic hydrocarbon as compared to heneicosane. Thus, from the results obtained, it was recognized that consortia LIBeM C4 (RETL-Cr1+ MAB-Cr1+BAS-Cr1) has greater degradation capability as compared to others.

**Enumeration of Microorganism in Soil**

The data on soil microbial growth in the oil sludge contaminated soil during bioremediation process are shown in **Table 2**. At the initial time, the total viable cells in all set treatment were range from  $1.0 \times 10^4$  to  $5.4 \times 10^5$  CFU/g. The viable cell counts increase approximately 10 to 100 times for the first 2 weeks at all treatments except in natural attenuation plot. Treatment with Consortia 4 recorded the highest cell counts with  $4.6 \times 10^9$  CFU/g during 14 days of experiment. Then the viable cells decreased slightly and remained almost unchanged after 56 days of experiment.

The addition of microbial consortia LIBeM and nutrient medium (Ramsay broth) in the soil increased the number of indigenous microorganism and inoculant augmented in the treatment plot. Amendment of nutrients every 2 weeks was needed for stimulating the growth of microbial consortia LIBeM and assisting the degradation of residual hydrocarbon in the soil. Treatment natural attenuation however was used as biotic control in which the loss of residual hydrocarbon was not significant and could be neglected. Thus, these findings indicated that the viable cell counts in the augmented soil with microbial consortia LIBeM are higher than natural attenuation plot.

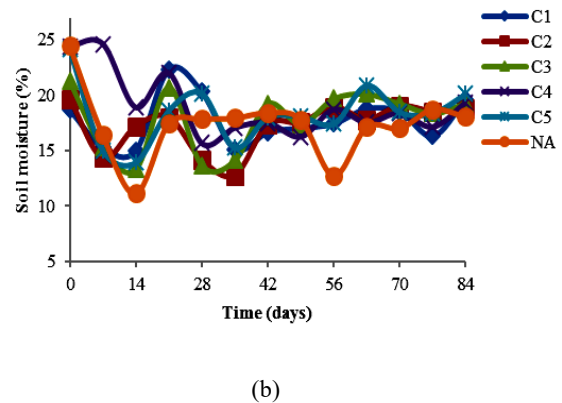
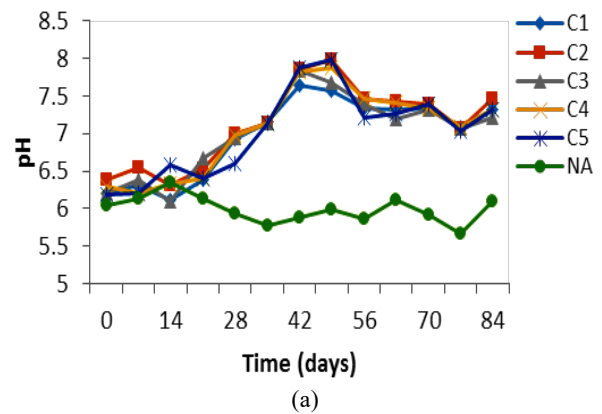
**Table 2.** Microbial activity monitoring in the soil and control plot during bioremediation study.

Treat ment	Monitoring (days) (CFU g <sup>-1</sup> soil)						
	Initial	14	28	42	56	70	84
C1	$1.0 \times 10^4$	$2.0 \times 10^6$	$7.5 \times 10^6$	$3.7 \times 10^6$	$8.1 \times 10^6$	$1.9 \times 10^6$	$8.5 \times 10^6$
C2	$5.4 \times 10^5$	$2.4 \times 10^6$	$1.7 \times 10^7$	$5.6 \times 10^7$	$7.9 \times 10^7$	$1.0 \times 10^7$	$1.3 \times 10^6$
C3	$1.0 \times 10^5$	$3.0 \times 10^6$	$3.0 \times 10^6$	$1.0 \times 10^6$	$3.5 \times 10^6$	$1.6 \times 10^6$	$4.5 \times 10^6$
C4	$6.6 \times 10^4$	$4.6 \times 10^6$	$7.7 \times 10^6$	$2.1 \times 10^7$	$5.8 \times 10^6$	$2.6 \times 10^6$	$1.5 \times 10^6$
C5	$3.3 \times 10^5$	$2.3 \times 10^6$	$4.7 \times 10^6$	$2.8 \times 10^6$	$6.6 \times 10^6$	$1.1 \times 10^6$	$2.7 \times 10^6$
NA	$5.0 \times 10^4$	$1.6 \times 10^5$	$9.7 \times 10^5$	$4.7 \times 10^5$	$3.3 \times 10^5$	$3.8 \times 10^5$	$2.4 \times 10^5$

**Environmental Parameters Monitoring During Bioaugmentation of Oil Sludge**

Soil pH is one of the most important factors in biodegradation of oil sludge. The relative acidity or alkalinity of soil will influence the metabolism activity of microorganisms and the availability of essential nutrients in the soil. **Fig. 5(a)** showed the results of pH monitoring within 3 months the experimental studies. During the first two weeks of treatment period, the pH of soil was recorded in between 6.1 – 6.6. However, after 40 days treatment, pH in bioreactor augmented with microbial consortia C1- C5 showed slightly increased to range 7.0 - 8.0. This is possible that after prolonged time of soil contamination, the microbial activity has changed the ideal ratio of inorganic to

organic essential nutrients into the soil [28]. In this study, the growth of microbial consortia LIBeM augmented in the soil was observed highly in the first two weeks of the experiment in which the range of pH 6.1-6.6 respectively. This data suggested that our microbial consortia LIBeM are capable in tolerate with acidic condition in the presence of oily sludge. This finding was supported by [29], reported that the optimal pH range for the degradation of hydrocarbons in soil is between 6.0 - 8.0 [30]. On the contrary, the pH value in natural attenuation plots was remained slightly acidic in range 5.8 – 6.3 respectively. In attempt to accelerate the biodegradation process, the range of pH in the soil must be added with lime for not pronounce the acidic value. As pH is one of the key factors for microbial metabolism, the pH value must be monitored weekly in order to avoid the degradation drop evidently. The results also showed that the fluctuation of pH on weekly basis was synchronized with the time of the microbe’s preparation addition into the soil. This suggested that the inoculation of microbial consortia LIBeM into the soil had an effect on decontamination of oily sludge.



**Fig. 5.** Variation of (a) pH and (b) soil moisture content during 90 days bioaugmentation of oil sludge by microbial consortia of LIBeM

Soil moisture is one of the most required factors for all biological processes in order to transport nutrients, foods and waste product in and out of the microorganisms. The optimum ratio of moisture content will depend on the climate and soil type used. For biodegradation to be successful, the optimum range of soil moisture is required in between 20%-60% [31]. Failure to achieve this fact would lead to maintenance of excessive or moderate water levels in soil and concomitant reduction in oxygen availability [32]. **Fig. 5(b)** showed the variation of soil moisture content (%) in soil augmented with microbial consortia LIBeM and natural attenuation plot. In the first two weeks of

experiments, the soil moisture content was ranged between 15-25%. The soil moisture content continued to remain unchanged in between 13-20% along the bioremediation period. During the experimental studies, the soil was tillage daily and watering in interval 2 days to maintain 20%-60% of water holding capacity and to avoid dehydration in the soil. Tillage can redistribute carbon, nitrogen, water and also reduces spatial distribution within the soil [33]. According to [34], soil moisture content lower than 10% (v/v) of the holding capacity will affect the microbial activity in the soil. The loss of moisture content in the soil may due to several reasons such as evaporation due to hot weather, uptake of water for microbial activity and insufficient frequency of watering into the soil [35]. However, if water content in soil is oversaturated, oxygen transfer to the microorganisms will be resisted by the soil and the rate of degradation of hydrocarbon will decrease. Our finding suggested that treated soil need to be watering every day in order to achieve the optimum soil moisture content for favorable in hydrocarbon degradation process.

## CONCLUSION

This study revealed that bioaugmentation of oil sludge using selective microbial consortia LIBeM achieved a great performance with more than 87% degradation of TPH within 90 days experimental set up. The assistance of aerated static pile (ASP) as continuous aeration into the soil system much helps in accelerating the microbial activity in the soil. The experiments proved that the microbial consortia C4 (*C. tropicalis*-RETL-Cr1, *C. violaceum* -MAB-Cr1 and *P. aeruginosa* -BAS-Cr1) was the most effective consortia in treated oil sludge contaminated soil. The study demonstrated the potential of this consortia in hydrocarbon degradation with further research in different delivery technique such powder or capsule form. Their effectiveness can be improved by monitoring the bioremediation parameters such as pH, soil moisture content, microbial population and substrate concentration.

## ACKNOWLEDGEMENT

This study was financially supported by a grant (GUG0035-SG-P-1/2016) from the Center of Research & Innovation, Universiti Malaysia Sabah. We are indebted to Department of Environment (DOE) Malaysia and Labuan Shipyard & Engineering Sdn. Bhd. for granting us permission to use and treat the oil sludge for this research.

## REFERENCES

- Yang X, Tan W, Bu Y. Demulsification of Asphaltenes and Resins Stabilized Emulsions via the Freeze/Thaw Method. *Energy and Fuels*. 2009;23(1):481-6.
- Collins LD, Daugulis AJ. Benzene/toluene/ p -xylene degradation. Part II. Effect of substrate interactions and feeding strategies in toluene/benzene and toluene/ p -xylene fermentations in a partitioning bioreactor. *Applied Microbiology and BioTechnology*. 1999;52(3):360-5.
- Taiwo AM. Composting as a sustainable waste management technique in developing countries, *J Environ Sci Technol*. 2011;4:93- 102.
- Megharaj M, Ramakrishnan B, Venkateswarlu K, Sethunathan N, Naidu R. Bioremediation approaches for organic pollutants: A critical perspective. *Environ Int*. 2011;37(8):1362-75.
- Kamada F, Tanaka H, Abe S, Wariishi H, Hiratsuka N. Mineralization of aromatic compounds by brown-rot basidiomycetes – mechanisms involved in initial attack on the aromatic ring. *Microbiology*. 2002;148(6):1939-46.
- Madigan M, Martinko J, Parker J (2003) *Brock Biology of microorganisms*. Pearson.
- Havel J, Reineke W. Degradation of Aroclor 1221 and survival of strains in soil microcosms. *Appl Microbiol Biotechnol* 1992;38:129-34.
- Jacques RJS, Okeke BC, Bento FM, Teixeira AS, Peralba MCR, Comargo FAO. Microbial consortium bioaugmentation of a polycyclic aromatic hydrocarbons contaminated soil. *Bioresour Technol*, 2008;99:2637-43
- Yu SH, Ke L, Wong YS, Tam NFY. Degradation of polycyclic aromatic (PAHs) by a bacterial consortium enriched from mangrove sediments. *Environ Int*, 2005;32: 149-54.
- Piakong MTH, Jamaluddin S, Hasila R, Haron A Yahya, M. Md. Salleh, Noor Aini AR. Biodegradation of phenol by locally isolated strains from petrochemical wastewater treatment plants. *Wat Environ Manage Series*. 2004:109-114.
- Nurulhuda, Z, Piakong, MT. Isolation, characterization and screening of hydrocarbon-degrading bacteria from environmental samples for treatment of oil-sludge. *Proceeding Int Conference on Environmental Research and Technology*. Universiti Sains Malaysia. 2008:521- 525
- Ramsay BA, Cooper DG, Margaritis A, Zajic JE. *Rhodochorou*s bacteria: Biosurfactant production and demulsifying ability. *Microbial Enhanced Oil Recove*. 1983:61-65.
- USEPA. 1978. Bioassay procedures for the ocean disposal permit program. Environmental Research Laboratory, Gulf Breeze, Florida. EPA-600/9-78-010.
- Zhang X, Wang Z, Liu X, Hu X, Liang X, Hu Y. Degradation of diesel pollutants in Huangpu-Yangtze River estuary wetland using plant-microbe systems. *Int Biodeterior Biodegrad*. 2013;76: 71-75.
- Alef K, Nannipieri P (ed). *Methods in applied soil microbiology and biochemistry*. London, Academic Press, 1995. 576p.
- Sharma A, Rehman MB. Laboratory scale bioremediation of diesel hydrocarbon in soil by indigenous bacterial consortium. *Indian J Exp Biol*. 2009;47:766-769.
- American Society of Testing and Materials (ASTM) (1995) *Annual Book of ASTM Standards*.
- Gardner, C.M.K., D.A. Robinson, K. Blyth and J.D. Cooper, 2001: Soil water content. In: K.A. Smith, and C.E. Mullins, *Soil and Environmental Analysis: Physical Methods*, Marcel Dekker, New York, pp. 1-64.
- U.S. EPA, 1996. United States Environmental Protection Agency, Method 8270C. Semi- volatile Organism Compounds by Gas Chromatography/Mass Spectrometry, Report SW-846, pp. 1-72.
- Agnello AC, Bagard M, van Hullebusch ED, Esposito G, Huguenot D. Comparative bioremediation of heavy metals and petroleum hydrocarbons co-contaminated soil by natural attenuation, phytoremediation, bioaugmentation and bioaugmentation-assisted phytoremediation. *Sci Total Environ* 2016;563-564:693-703.
- Varjani SJ, Rana DP, Jain AK, Bateja S, Upasani VN. Synergistic exsitu biodegradation of crude oil by halotolerant bacterial consortium of indigenous strains isolated from on shore sites of Gujarat, India. *Int Biodeterior Biodegrad*, 2015;103: 116-124
- Kamada F, Abe S, Hiratsuka N, Wariishi H, Tanaka H Mineralization of aromatic compounds by brown-rot basidiomycetes - mechanisms involved in initial attack on the aromatic ring. *Microbiology* 2002;148: 1939-1946.
- Bartha R, Atlas RM. *The microbiology of aquatic oil spills*. *Adv Appl Microbiol*. 1977;22:225-266
- Heinaru E, Merimaa M, Viggor S, Lehist M, Leito I, Truu J, et al. Biodegradation efficiency of functionally important populations selected for bioaugmentation in phenol- and oil-polluted area. *FEMS Microbiol Ecol*. 2005;51:363-73.
- Ouyang W, Liu H, Murygina V, Yu Y, Xiu Z, Kalyuzhni S. Comparison of bio-augmentation and composting for remediation of oily sludge: A field-scale study in China. *Process Biochem*. 2005;40:3763-3768.
- Mishra S, Jyot J, Kuhad RC, Lal B. Evaluation of inoculum addition to stimulate in situ bioremediation of oily-sludge-contaminated soil. *Appl Environ Microbiol*. 2001;67(4):1675-1681.
- Vinas M, Grifoll M, Sabate J, Solanas AM. Biodegradation of a crude oil by three microbial consortia of different origins and metabolic capabilities. *J Ind Microbiol BioTechnol*. 2002;28(5): 252-260.

28. Thomas JM, Ward CH, Raymon RL, Wilson JT, Loehr RC. *Bioremediat Encyclo Microbio*. 1992;1:369-385.
29. Chaineau CH, Rougeux G, Yepremian C, Oudot J. Effects of nutrient concentration on the biodegradation of crude oil and associated microbial populations in the soil. *Soil Biol Biochem*. 2005;37:1490–1497.
30. Morgan P, Watkinson RJ. Hydrocarbon degradation in soils and methods for soil biotreatment. *CRC Crit Rev BioTechnol*, 1989;4: 305-333.
31. Rosenberg E . Exploiting microbial growth on hydrocarbons: new markets. *Trends Biotechnol*. 1993;11:419-424.
32. Baker KH . Bioremediation of surface and subsurface soils, in: Baker KH, Herson DS (eds), *Bioremediation*. McGraw-Hill, Inc., 1994:209-259.
33. Rhykerd RL, Crews B, McInnes KJ, Weaver RW . Impact of bulking agents, forced aeration and tillage on remediation of oil contaminated soil. *Bioresour Technol*. 1999;67:279-285.
34. Testa SM, Winegardner DL. *Restoration of Petroleum-contaminated Aquifers*. Lewis Publishers. London.1991.
35. Sihag S, Pathak H, Jaroli DP. Factors Affecting the Rate of Biodegradation of Polyaromatic Hydrocarbons. *Int J Pure Appl BioSci*. 2014;2(3):185-202.