

## Bioaugmentation Efficiency of Different LIBEM Strains in ASP Treatment of Oil Sludge-Contaminated Soil

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### Abstract

This study investigates the bioaugmentation potential of five locally isolated beneficial microorganisms (LIBeM), namely *Candida tropicalis*-RETL-Cr1, *Chromobacterium violaceum*-MAB-Cr1, *Pseudomonas aeruginosa*-BAS-Cr1, *Sphingomonas paucimobilis*-RETOS-Cr1, and *Stenotrophomonas maltophilia*-RAS-Cr, for remediating oil sludge. Contaminated soil using an aerated static pile (ASP) system. The experiments were conducted using ASP bioreactor systems that provided continuous aeration to 10 kg soil samples amended with 5%, 10%, 15%, and 20% (v/v) oil sludge, while natural attenuation served as the control treatment. The TPH (total petroleum hydrocarbon) degradation was monitored for three months at 7-day intervals. Rapid TPH reduction occurred within the first 14–28 days, followed by a gradual decline. LIBEM strains *P. aeruginosa*-BAS-Cr1 achieved the highest reductions with 40.0, 85.2, 128.9, and 178.5 g/kg for the respective sludge concentrations, which are 1.0–1.25 fold higher than *C. violaceum*-MAB-Cr1. Other strains demonstrated strong performance, with reductions of 32.5–173.0 g/kg (*C. tropicalis*-RETL-Cr1), 37.2–177.2 g/kg (*S. paucimobilis*-RETOS-Cr1), and 33.4–174.0 g/kg (*S. maltophilia*-RAS-Cr1). All treatments achieved significantly higher TPH removal compared to natural attenuation, which recorded only 27.4% removal. The results indicate that the ASP system's continuous oxygen supply enhances microbial activity, promoting the degradation of long-chain alkanes and overall remediation efficiency. These findings demonstrate that combining LIBeM bioaugmentation with ASP technology offers a scalable, eco-friendly solution for treating hydrocarbon-contaminated soils. The results also provide a basis for optimizing strain combinations and operational parameters in future large-scale applications.

### INTRODUCTION

Bioremediation of hydrocarbon-contaminated soils is most effective in aerobic conditions. Oxygen availability is a critical parameter influencing microbial degradation, yet the role of oxygen mass transfer in solid-phase bioremediation has received relatively limited attention. Active hydrocarbon-degrading microbial populations are often present in the subsurface; however, an insufficient oxygen supply can lead to anoxic conditions, thereby reducing microbial efficiency. Aeration strategies such as daily tillage and soil venting have been employed to improve oxygen diffusion and loosen soil structure. According to Mishra et al. [1] in situ forced aeration enhances biodegradation by supplying oxygen to indigenous microbes, accelerating the breakdown of hydrocarbons into carbon dioxide

and water. The main constraint to rapid biodegradation is often oxygen depletion, which occurs quickly in active microbial communities unless a constant air supply is maintained. In addition to aeration, the use of single and mixed microbial consortia plays a significant role in hydrocarbon degradation.

Numerous microorganisms, including Gram-negative  $\alpha$ -proteobacteria such as *Pseudomonas*, *Acinetobacter*, *Sphingomonas*, *Alcaligenes*, and *Proteus*, have demonstrated the ability to utilize hydrocarbons as their sole source of carbon and energy [2]. Other notable hydrocarbon degraders include *Achromobacter*, *Flavobacterium*, *Mycobacterium*, *Rhodococcus*, *Enterobacter*, and *Micrococcus*. In particular, *Pseudomonas* species have a broad affinity for hydrocarbons, degrading alkanes, alicyclics, thiophenes, and aromatic compounds [3].

Strains such as *Pseudomonas stutzeri* can degrade xylene, toluene, phenol, and polycyclic aromatic hydrocarbons (PAHs), including naphthalene, through key degradation pathways mediated by enzymes like benzoate dioxygenase, toluene 1,2-dioxygenase, and catechol 1,2-dioxygenase. Yeasts also contribute to hydrocarbon degradation, with genera such as *Debaryomyces*, *Saccharomyces*, *Pichia*, *Yarrowia*, and *Candida* isolated from petroleum-contaminated soils [4].

Certain species, including *Candida viswanathii*, have achieved high removal rates for PAHs such as phenanthrene and benzopyrene. Species like *C. tropicalis* and *Trichosporon* can utilize alkanes and partially degrade aromatic hydrocarbons. Different species vary in their susceptibility to and rates of hydrocarbon degradation. Low-molecular-weight hydrocarbons, such as straight and branched-chain alkanes and simple aromatics, are readily degraded, whereas long-chain alkanes and PAHs exhibit lower biodegradability due to their hydrophobicity [5]. Therefore, integrating continuous aeration systems with targeted microbial strains may offer a more efficient strategy for the remediation of oil sludge-contaminated soils.

This study aims to compare the biodegradation potential of five locally isolated beneficial microorganisms (LIBeM) in reducing total petroleum hydrocarbons (TPH) in oil sludge-contaminated soils. In particular, it highlights the biodegradation ratios of two isoprenoid compounds, pristane (C<sub>19</sub>H<sub>40</sub>) and phytane (C<sub>20</sub>H<sub>42</sub>), which are commonly used as biomarkers to assess the degradation progress and efficiency in petroleum-contaminated environments. By focusing on both overall TPH removal and the breakdown of these persistent molecular markers, this work provides a more refined evaluation of each strain's capability. The findings aim to establish a comparative framework for the performance of single LIBeM strains under controlled aerated static pile (ASP) conditions, offering a detailed foundation for optimizing bioaugmentation strategies in oil sludge remediation.

## MATERIALS AND METHODS

### Experimental Design

The five (5) LIBeM strains, namely *Candida tropicalis*-RETL-Cr1, *Chromobacterium violaceum*-MAB-Cr1, *Pseudomonas aeruginosa*-BAS-Cr1, *Sphingomonas paucimobilis*-RETOS-Cr1, and *Stenotrophomonas maltophilia*-RAS-Cr were obtained from the Environmental Microbiology Laboratory, Faculty of Science & Technology, Universiti Malaysia Sabah. The strains were cultured in Ramsay broth, and a standard inoculum was prepared at 30 °C for 24 hours in an orbital shaker at 200 rpm. Soil samples (10 kg each) were sieved through a 0.20 mm mesh to remove stones and debris.

Oil sludge was applied at four concentrations: 5% (0.5 L), 10% (1.0 L), 15% (1.5 L), and 20% (2.0 L) v/v. The sludge was mixed thoroughly into the soil and left for 30 minutes to allow for adsorption [6]. A 10% of inoculation of single LIBeM was conducted every 2 weeks in the ASP bioreactor for the degradation of oil sludge-contaminated soil. Biodegradation experiments were conducted in duplicate using acrylic aerated static pile (ASP) bioreactors (60 × 40 × 20 cm) under open-air, continuously aerated conditions for 84 days.

Treatments consisted of five single-strain LIBeM inoculations: *Candida tropicalis*-RETL-Cr1 (S1), *Chromobacterium violaceum*-MAB-Cr1 (S2), *Pseudomonas aeruginosa*-BAS-Cr1 (S3), *Sphingomonas paucimobilis*-ReTOS-Cr1 (S4), and *Stenotrophomonas maltophilia*-RAS-Cr1 (S5), with natural attenuation serving as the control.

### Analysis of Oil Sludge Contaminated Soil

The total petroleum hydrocarbon (TPH) content was determined using the gravimetric method via Soxhlet extraction (USEPA Method 540C) [7]. A 20 g soil sample was ground, placed in a thimble, and extracted with 175 mL dichloromethane (DCM) in a Soxhlet apparatus. The extraction was conducted for less than 24 hours at cooling and heating temperatures of 4 °C and 170 °C, respectively. The extract was concentrated using a vacuum evaporator at 40–50 °C, followed by drying of the round-bottom flask (RBF) and the extract in an oven at 60 °C for 24 h. The RBF was cooled in a desiccator for 30 min and weighed repeatedly until a constant mass (three readings) was obtained. The percentage of TPH was then calculated using the specified formula below.

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

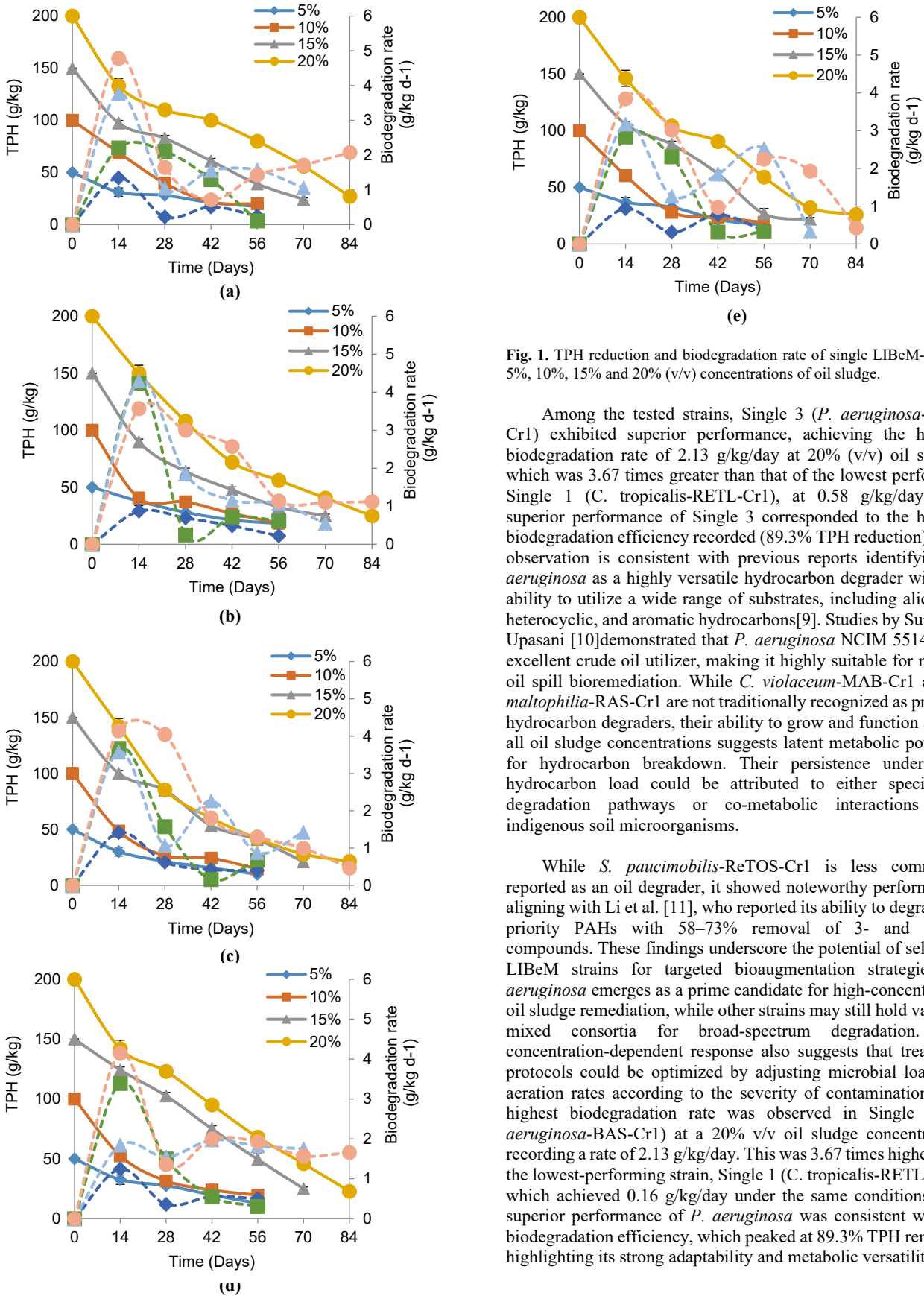
### Biodegradation Ratio of Two Isoprenoids

A Chromatographic analysis using Gas chromatography-mass Spectrometry (GC-MS) focused on the identification of hydrocarbon biomarkers, namely C17 (pristane) and C18 (phytane), as well as the pristane-to-phytane ratio, at 5%, 10%, 15%, and 20% (v/v) contamination levels. Biodegradation efficiency was assessed by calculating the area ratios between readily biodegradable n-alkanes (n-C17 and n-C18) and the more recalcitrant branched alkanes (pristane and phytane), which share similar chromatographic properties [8]

## RESULTS AND DISCUSSIONS

### Reduction of Total Petroleum Hydrocarbon (TPH), Biodegradation Efficiency, and Biodegradation Rate of Oil Sludge Contaminated Soil by Different LIBeM Strains

The variations in Total Petroleum Hydrocarbon (TPH) reduction and biodegradation rates for single LIBeM treatments in the ASP-bioreactor, across different oil sludge concentrations (5%, 10%, 15%, and 20% v/v) over time, are illustrated in Fig. 1 (a–e). For comparison, natural attenuation (control), consisting of oil sludge-contaminated soil with indigenous microorganisms, was also assessed. The findings indicated that indigenous microorganisms achieved only a modest TPH reduction, ranging from 12 to 75 g/kg, across all concentrations tested. The results clearly indicate a positive correlation between oil sludge concentration and biodegradation rate for all single LIBeM treatments in the ASP-bioreactor system, with the order of performance following 20% > 15% > 10% > 5% (v/v). This pattern suggests that higher hydrocarbon availability provided a more substantial carbon and energy source for the microorganisms, thus stimulating enhanced metabolic activity. However, this also implies that the strains maintain tolerance towards higher pollutant loads, which is crucial for field-scale applications in heavily contaminated sites.



**Fig. 1.** TPH reduction and biodegradation rate of single LIBeM-LIQ at 5%, 10%, 15% and 20% (v/v) concentrations of oil sludge.

Among the tested strains, Single 3 (*P. aeruginosa*-BAS-Cr1) exhibited superior performance, achieving the highest biodegradation rate of 2.13 g/kg/day at 20% (v/v) oil sludge, which was 3.67 times greater than that of the lowest performer, Single 1 (*C. tropicalis*-RETL-Cr1), at 0.58 g/kg/day. The superior performance of Single 3 corresponded to the highest biodegradation efficiency recorded (89.3% TPH reduction). This observation is consistent with previous reports identifying *P. aeruginosa* as a highly versatile hydrocarbon degrader with the ability to utilize a wide range of substrates, including alicyclic, heterocyclic, and aromatic hydrocarbons[9]. Studies by Sunita & Upasani [10] demonstrated that *P. aeruginosa* NCIM 5514 is an excellent crude oil utilizer, making it highly suitable for marine oil spill bioremediation. While *C. violaceum*-MAB-Cr1 and *S. maltophilia*-RAS-Cr1 are not traditionally recognized as primary hydrocarbon degraders, their ability to grow and function across all oil sludge concentrations suggests latent metabolic potential for hydrocarbon breakdown. Their persistence under high hydrocarbon load could be attributed to either specialized degradation pathways or co-metabolic interactions with indigenous soil microorganisms.

While *S. paucimobilis*-ReTOS-Cr1 is less commonly reported as an oil degrader, it showed noteworthy performance, aligning with Li et al. [11], who reported its ability to degrade 16 priority PAHs with 58–73% removal of 3- and 4-ring compounds. These findings underscore the potential of selective LIBeM strains for targeted bioaugmentation strategies. *P. aeruginosa* emerges as a prime candidate for high-concentration oil sludge remediation, while other strains may still hold value in mixed consortia for broad-spectrum degradation. The concentration-dependent response also suggests that treatment protocols could be optimized by adjusting microbial load and aeration rates according to the severity of contamination. The highest biodegradation rate was observed in Single 3 (*P. aeruginosa*-BAS-Cr1) at a 20% v/v oil sludge concentration, recording a rate of 2.13 g/kg/day. This was 3.67 times higher than the lowest-performing strain, Single 1 (*C. tropicalis*-RETL-Cr1), which achieved 0.16 g/kg/day under the same conditions. The superior performance of *P. aeruginosa* was consistent with its biodegradation efficiency, which peaked at 89.3% TPH removal, highlighting its strong adaptability and metabolic versatility.

Among all single LIBeM tested, *P. aeruginosa* demonstrated the most consistent and robust degradation capacity across all concentrations. Literature supports this finding, as *P. aeruginosa* is widely recognized for its broad substrate affinity, degrading diverse hydrocarbon classes, including alicyclics, heterocyclics, and aromatics [12]. Its well-documented ability to produce biosurfactants likely enhanced hydrocarbon bioavailability, facilitating rapid degradation.

Conversely, yeast strain *C. tropicalis* showed the lowest biodegradation rate, possibly due to slower enzymatic adaptation to complex hydrocarbons or competition with indigenous microorganisms. Despite this, *C. tropicalis* still demonstrated measurable TPH removal, indicating potential synergy if used in a consortium. The ASP system provided continuous aeration, ensuring sufficient oxygen supply to sustain aerobic degradation. Aeration is critical in hydrocarbon biodegradation, as oxygen acts as the terminal electron acceptor for many hydrocarbon-degrading pathways. The high performance at 20% oil sludge suggests that the ASP effectively mitigated oxygen limitation, even at higher contamination loads, preventing anaerobic zones that could reduce microbial efficiency.

#### Biodegradation Ratios of n-C17: Pristane, n-C18: Phytane and Pristane: Phytane

The early effect of microbial degradation can be monitored by the ratios of biodegradable to the less degradable compounds. The rate of degradation of these isoprenoid compounds compared to corresponding length n-alkanes provides useful insights into the extent of biodegradation and microbial activity in hydrocarbon-rich samples [13]. The values of n-C17/Pr, n-C18/Ph, and Pr/Ph for the initial and post-biodegradation of oil sludge by the single were calculated based on the data obtained in **Table 1**.

The analysis of the isoprenoids n-C17:Pr and n-C18:Ph ratio index shows that single LIBeM achieved a substantial decrease by the end of the incubation periods. Based on the results obtained, the n-C17:Pr and n-C18:Ph ratios range from 0.1 to 8.5 and 0.1 to 7.0, respectively, while the Pr/Ph ratios range from 0.1 to 8.8, respectively. The highest ratio of n-C17:Pr and n-C18:Ph in 5% (v/v) decreased to 5.8 and 0.3 in treatment Single 5, 3.9 and 3.2 at 10% (v/v) for treatment Single 5. The decreasing ratio of n-C17/Pr and n-C18/Ph in this present work shows that biodegradation was in progress. Both C17 and C18 are easily subjected to biodegradation compared to pristane and phytane, which are very resistant to degradation except in the presence of biosurfactants. A study by Milton et al. [14] supported this finding, stating that as easily degraded hydrocarbons (C17 and C18) are lost, the more degradation-resistant isoprenoids (pristane and phytane) remain. In this study, the Pristane/Phytane remained constant and only experienced slight decreases.

According to Haven et al. [15], a higher ratio of Pr/Ph (>3.0) indicates a terrigenous input under oxic conditions, while a low Pr/Ph (<0.8) indicates anoxic/hypersaline or carbonate environments. Groune et al. [16] also stated that the low values Pr/Ph (<2.0) indicate the sample mainly comes from aquatic depositional environments including marine, fresh and brackish water while the intermediate value (2-4) indicates the fluviomarine and coastal swamp environments, whereas high values (>10) are related to peat swamp depositional environments (oxidizing conditions). In comparison to the obtained results, the ratio of Pr/Ph is mainly <0.8, which confirms that this oil originated from anoxic/hypersaline environments. As shown in **Table 1**, the reduction of the intensity of these biomarker peaks in the final chromatogram confirms the

excellent biodegradation abilities of single LIBeM in the biodegradation of oil sludge.

**Table 1.** Comparison of the n-C17: Pristane, n-C18: Phytane, and Pristane: Phytane peak area ratios of LIBeM single from gas chromatograms of saturated hydrocarbons.

Concentration of Oil Sludge	Week	Treatment	C <sub>17</sub> : Pristane	C <sub>18</sub> : Phytane	Pristane: Phytane
5%	0	S1	1.7	1.0	1.0
	8		0.1	0.4	0.1
	0	S2	0.9	5.2	2.6
	8		0.5	0.6	1.2
	0	S3	2.4	2.4	0.8
	8		2.1	0.1	0.4
	0	S4	1.1	0.9	2.4
	8		0.7	0.7	0.8
	0	S5	7.3	0.6	0.9
	8		1.5	0.3	0.3
10%	0	S1	5.0	1.0	1.5
	8		0.5	0.7	1.3
	0	S2	1.2	0.8	1.0
	8		0.8	0.3	0.9
	0	S3	1.6	3.1	0.7
	8		0.3	0.8	0.6
	0	S4	1.0	2.5	2.0
	8		0.7	1.9	0.7
	0	S5	4.4	4.3	2.6
	8		0.6	1.1	1.6
15%	0	S1	2.0	0.4	0.4
	10		0.2	0.3	0.2
	0	S2	3.6	1.1	2.5
	10		2.1	0.6	0.1
	0	S3	4.2	0.9	0.8
	10		2.5	0.3	0.5
	0	S4	1.2	3.5	7.1
	10		0.6	0.5	2.1
	0	S5	1.8	7.0	0.2
	10		0.9	1.1	0.1
20%	0	S1	4.1	3.0	2.7
	12		3.4	1.0	0.8
	0	S2	0.8	3.7	1.4
	12		0.6	2.2	0.8
	0	S3	0.8	1.0	1.8
	12		0.5	0.1	0.2
	0	S4	7.1	6.1	1.5
	12		3.9	5.2	1.3
	0	S5	8.5	1.6	0.8
	12		1.9	1.2	0.6

The data obtained illustrated that n-alkanes degrade faster than isoprenoids (branched alkanes), leading to a decrease in the ratio values of C17:Pr and C18:Ph, especially when they are highly degraded. According to Paudyn et al., [17], due to their branched nature, isoprenoids are relatively resistant to biodegradation and are more slowly degraded when compared to linear ones. Waples [18] also mentioned that by increasing maturity, n-alkanes are generated faster than isoprenoids, in contrast to biodegradation. It is difficult to compare the gravimetric data with previous research done by other authors. Biodegradation of oil sludge has been studied extensively; however, there is significant variability in the initial concentration of oil sludge used, the type of oil sludge, the incubation period, and the methodologies used to quantify degradation. Therefore, from the comparison with these studies,

the decreasing potential of oil sludge degrader from high to moderate ranked is in the order of 5% (v/v);

S5>S2>CS3>S1>S4, 10%(v/v); S5>S1>S3>S2>S4; 15%(v/v); S5 >S4>S3>S2>S1 and 20% (v/v); S5>S4>S1>S2>S3 respectively.

### Degradation of Aliphatic Hydrocarbon (n-alkanes)

To further understand the biodegradative effectiveness of oil sludge by single LIBeM, the composition of both the initial and final samples across all treatment systems was analyzed by GC-MS. The chromatogram obtained from the GC-MS analysis illustrated the changes in the composition of the selected aliphatic hydrocarbon (n-alkanes; C17-C24) presented in the oil sludge studied. Based on the results obtained from Fig. 2, there was a significant change in the saturate fraction in different concentrations of oil sludge across the incubation period.

The highest reduction in the saturation fraction indicated that the hydrocarbon compound was degraded gradually throughout the incubation period, and a biodegradation process had occurred in the present study. Among all single LIBeM studied, soil augmented with Single 3 (*P. aeruginosa*-BAS-Cr1) and Single 4 (*S. maltophilia*-RAS-Cr1) showed comparable performances in degrading oil sludge at all concentrations studied, with the highest reduction observed in 5% v/v oil sludge, ranging from 88.5% to 99.8%. By referring to selected hydrocarbon compounds, heptadecane (C17H36), and octadecane (C18H38) are the most preferable compounds degraded by single LIBeM at all concentration studied. Otherwise, tetracosane (C24H50) was the most difficult compound to degrade in this present work.

It is essential to note that most hydrocarbon-degrading microorganisms are more efficient at degrading the smallest group of hydrocarbons compared to long-chain alkanes. As aliphatic hydrocarbons are divided into four molecular weight groups: the gaseous alkanes, lower molecular weight (C8–C16), medium molecular weight (C17–C28), and high molecular weight (>C28), the lower the molecular weight, the greater the advantages in bioremediation of oil sludge. Low molecular weight alkanes are sparingly soluble in water, ensuring a sufficient mass transfer to the bacterial cell. In contrast, high molecular weight alkanes (medium and long chain n-alkanes) are accessible to the cell either through adherence or a surfactant-mediated process.

In this study, mid-length molecular weight (C17–C24) is non-polar, virtually water-insoluble hydrocarbons with increasing melting and boiling points as the carbon number increases within the molecule. Typically, these alkanes have low aqueous solubilities, and hydrocarbon degradation is subjected to several physical, chemical, and biological processes in soil, with microbial degradation representing the major loss process of hydrocarbons[19]. Therefore, it was revealed that the uptake mechanism of n-alkanes in this experiment is subjected to Single LIBeM, along with suitable physicochemical environment conditions in the ASP-bioreactor systems. The molecular mechanism of n-alkanes was investigated through aerobic degradation with the augmentation of single LIBeM using O<sub>2</sub> as a reactant.

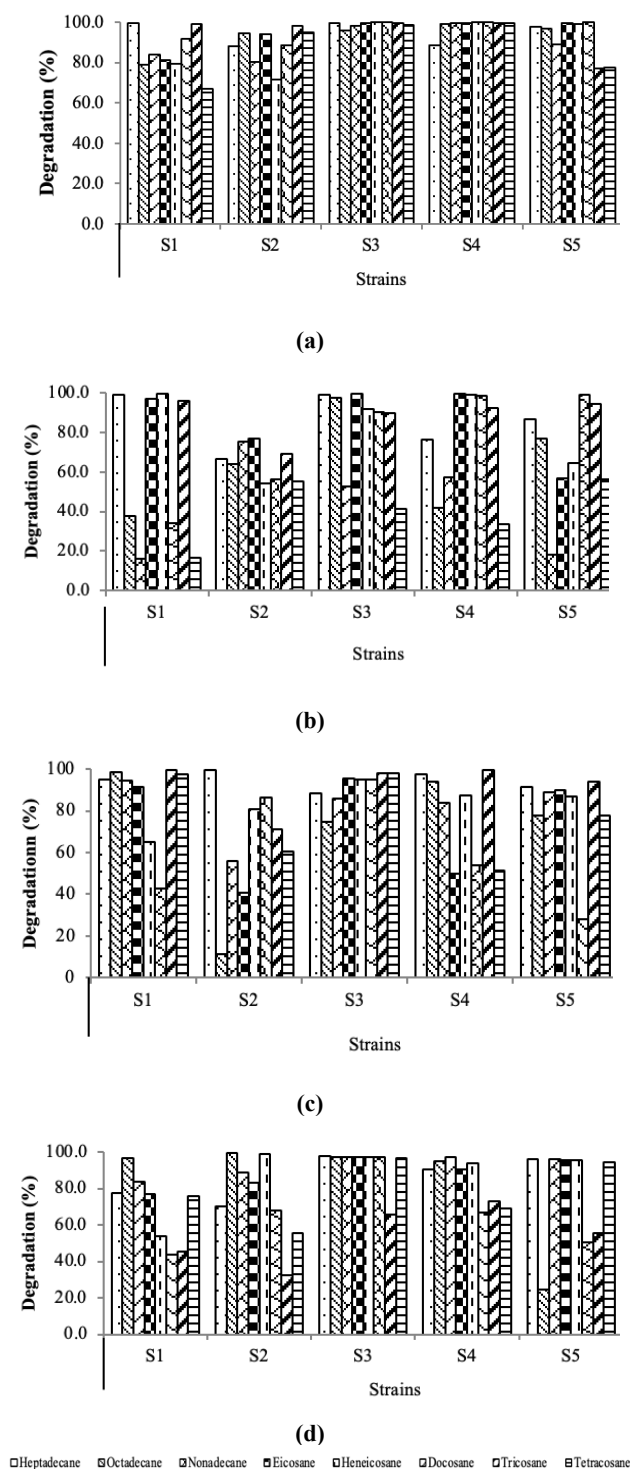


Fig. 2. Degradation of aliphatic hydrocarbons (n-alkane) by single strains LIBeM in (a) 5%, (b) 10%, (c) 15% and (d) 20%(v/v) of oil sludge contaminated soil after 70 and 84 days of bioremediation period.

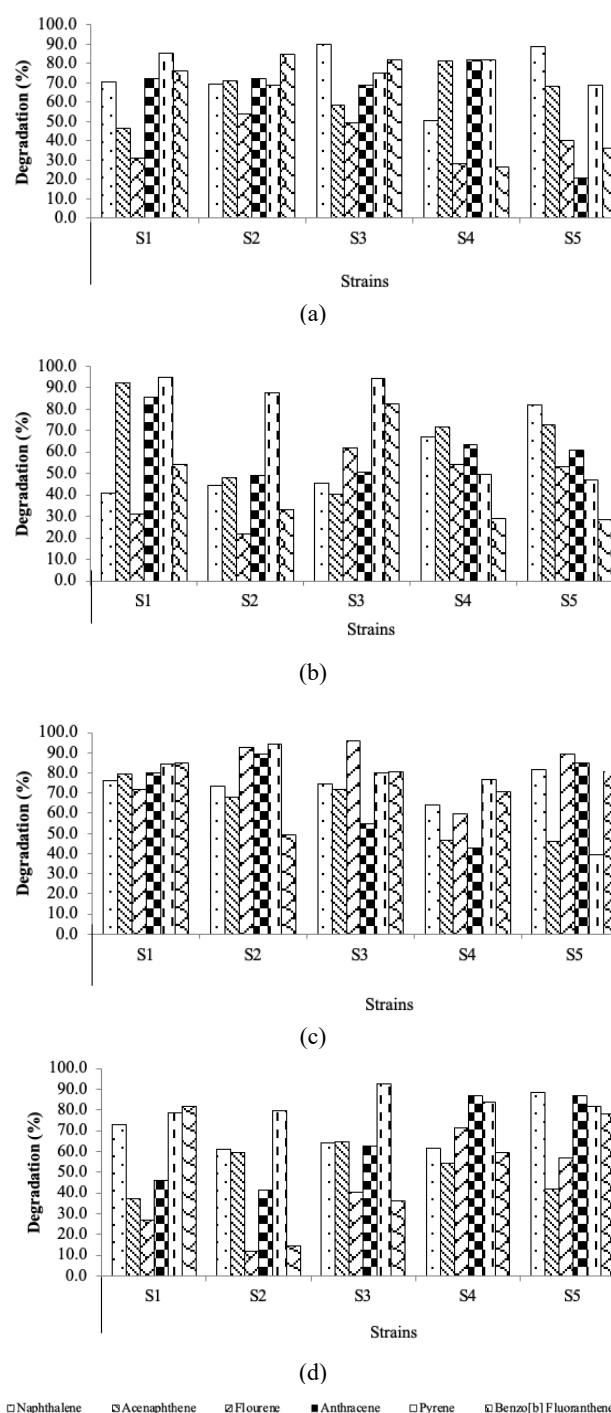
The alkane-activating monooxygenase overcomes the low reactivity of the hydrocarbon by producing reactive oxygen species. Oxidation of methane leads to the formation of methanol, which is subsequently transformed into formaldehyde and then into formic acid. This compound is either converted to CO<sub>2</sub> or assimilated for the biosynthesis of other organic compounds, either through the ribulose monophosphate pathway or the serine pathway, depending on the organism [20].

### Degradation of Polycyclic Aromatic Hydrocarbons (PAHs)

Polycyclic aromatic hydrocarbons (PAHs) are organic compounds containing only carbon and hydrogen that are composed of multiple aromatic rings. This compound is a hydrophobic substance that has low solubility in water. These natural characteristics make them unfit for natural degradation. However, it has been reported that several microorganisms have the capability to degrade soil with PAH compounds efficiently.

In this present work, the ability of single LIBeM to degrade polycyclic aromatic hydrocarbon compounds (PAHs) was assessed by GC-MS analysis. The chromatogram obtained from the GC-MS analysis illustrated the changes in the composition of selected polycyclic aromatic hydrocarbon (PAHs) compounds (C<sub>10</sub>-C<sub>20</sub>) that were present in the oil sludge studied. Six types of polycyclic aromatic hydrocarbons (PAHs) compounds, namely naphthalene (C<sub>10</sub>H<sub>8</sub>), acenaphthalene (C<sub>12</sub>H<sub>8</sub>), fluorene (C<sub>13</sub>H<sub>10</sub>), anthracene (C<sub>14</sub>H<sub>10</sub>), pyrene (C<sub>16</sub>H<sub>10</sub>), and benzo [b] fluoranthene (C<sub>20</sub>H<sub>12</sub>), are identified through the GC-MS analysis.

Naphthalene and acenaphthene are known as the simplest compounds of PAHs, consisting of 2 benzene rings that are mostly able to degrade by microorganisms. In fact, fluorene and anthracene consist of 3 benzene rings, while pyrene has four benzene rings, and benzo [b] fluoranthene consists of 5 benzene rings. Based on the results obtained (Fig. 3), at 5% (v/v) of oil sludge, the degradation of PAH compounds can be observed in the range of 11%-89%. The highest degradation compound can be found in the Single three treatment with *P. aeruginosa*-BAS-Cr1, which achieved 89% degradation of naphthalene in soil. However, treatment with Single 5 *S. maltophilia*-RAS-Cr1 was found to have moderate tolerance levels to PAHs. It was clearly shown that benzo[b]fluoranthene was the most difficult compound to degrade, with a low percentage of degradation of less than 50% in all treatment sets. The degradation of PAH compounds at 10% (v/v) oil sludge showed that the range of PAHs degraded was from 24 to 94%. It can be observed that treatment with Single 1, *C. tropicalis*-RETL-Cr1, accelerated pyrene degradation by 94% in soil, which is 3-fold higher than that of the other selected compound studied. When 15% (v/v) oil sludge was introduced into the soil, the performance of single LIBeM varied in its utilization of PAH compounds. Augmenting soil with Single 2 *C. violeceum*-MAB-Cr1 and Single 3 *P. aeruginosa*-BAS-Cr1 into oil sludge-contaminated soil enabled the maximum degradation of the fluorene compound, achieving 92% and 95% degradation, respectively. At 20% (v/v) oil sludge, the degradation of PAH compounds ranged from 26% to 93%, respectively.



**Fig. 3.** Degradation of polycyclic aromatic hydrocarbon (PAHs) by single strains LIBeM in (a) 5%, (b) 10%, (c) 15% and (d) 20%(v/v) of oil sludge contaminated soil after 70 and 84 days of bioremediation period.

Meanwhile, compound benzo[b]fluoranthene was the hardest compound to degrade in this study. Juhasz et al. [21] have reported that the biodegradation of 4-ring PAHs and PAHs with a higher number of rings is difficult but possible to degrade. Taking into account the results mentioned above, the addition of single LIBeM, together with native microorganisms, exhibited high degradation levels of PAHs, thus demonstrating that augmenting LIBeM in the soil improved the degradation. Bioaugmentation of LIBeM leads to different levels of degradation rate of selected PAH compounds. This selective degradation was observed to be higher in naphthalene, pyrene, and anthracene at all concentrations of oil sludge studied. Pyrene is known for its high molecular weight as a PAH compound, and the environmental persistence of this compound has a deleterious effect on human health. Conversely, most bacterial species show limited ability to degrade these hydrophobic compounds [22].

However, in this present work, *P. aeruginosa*-BAS-Cr1 was found to have a high tendency to degrade most PAH compounds, especially pyrene. This data has been supported by previous research by Nie et al. [23], which asserted that *P. aeruginosa* NY3 has efficacy in degrading a mixture containing amounts of fluorene, anthracene, phenanthrene, pyrene, and fluoranthene. Studies by Maliszewska [24] have reported that the properties of PAHs, such as solubility in water, determine the leaching of PAHs into the depth of the soil profile and its susceptibility to biological and physical degradation. Oleszczuk and Baran [25] mentioned that a 50% decrease was observed in 3-ring PAHs within 25 days of the experiment, followed by fluorene and pyrene (4 rings). However, he observed that Benz[a]anthracene with lower solubility had markedly lower degradation after the final days of the experiment. It can be concluded that the use of LIBeM in this study provides the most effective degradation of PAHs, with more than 30% to 95% compared to the indigenous population in the natural attenuation plot.

## CONCLUSION

In conclusion, this study demonstrated *P. aeruginosa*-BAS-Cr1 achieved the highest biodegradation rate (2.13 g/kg/day) and removal efficiency (89.3%), confirming its adaptability and strong hydrocarbon-degrading capacity. It was observed that *P. aeruginosa*-BAS-Cr1 was able to achieve maximum degradation of fluorene compound with 95% and a biodegradation ratio of n-C17:Pr, n-C18:Ph, and Pr/Ph decreased of n-C17:Pr, n-C18:Ph with 3.9 and 5.2 at 20% (v/v) of oil sludge. These findings support the use of adaptable, high-performance strains, particularly *P. aeruginosa*, for targeted microbial formulations to improve ex-situ oil sludge bioremediation efficiency and reduce environmental impact.

## CONFLICT OF INTEREST

The authors declare no conflict of interest.

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