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Bacterial Consortium-Mediated Hydrocarbon Degradation of Waste Engine Oils

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ABSTRACT

The automotive and industrial activities are considered one of the important sources of pollution with the increasing amount of WEO released to the environment. One of the ways to solve this problem is through bioremediation. It is, however, challenging since used engine oil contains complex hydrocarbon compounds and is toxic towards the environment and living organisms. Biodegradation by single species bacterium is limited to a small range of hydrocarbon compounds. Hence, a better strategy is to use a synergistic action of different microbial members in a consortium. Different waste engine oils from the motorcycle workshop (MW), urban vehicle workshop (UVW), and heavy vehicle workshop (HVW) were tested for degradation by a bacterial consortium culture. A two-level factorial design experiment was carried out to evaluate the effect of different WEO and nitrogen concentrations towards the growth of the bacterial culture and hydrocarbon degradation in shake flask fermentation. The bacterial consortium culture grown in mineral salts medium (MSM) supplemented with MW (Viscosity, 82 mPa.s) was shown to exhibit the highest biomass with 0.37 (OD_{600}) with 8% (v/v) WEO and 5 g/L nitrogen from ammonium chloride (NH_4Cl). Pareto chart of biomass with WEO and nitrogen concentration showed a positive effect for MW and UVW, while a negative effect for HVW which was presumed due to the toxicity, higher complexity of hydrocarbon composition and higher viscosity of WEO from heavy vehicles. Subsequently, GC-MS analysis indicated the degradation of various polyaromatic hydrocarbon (PAH) and BTEX compounds such as naphthalene, benzene, and toluene in the three WEO samples. Assessment of cell hydrophobicity of the culture grown on all three WEO exhibited high cell hydrophobicity, with HVW showing the highest cell hydrophobicity of 81%. This suggests that the bacterial culture altered cell hydrophobicity to facilitate hydrocarbon uptake.

Keywords: bacterial consortium, hydrocarbon degradation, waste engine oil, cell hydrophobicity

INTRODUCTION

Over the years, rapid urbanisation and motorised traffic have led to frequent congestion on roads due to the rise in the number of vehicles for daily commuting (Lu & Gao, 2022). This also indirectly increases the waste engine oil (WEO) generation in local automobile workshops reflecting the rise in vehicle maintenance service. Poor waste disposal management by automobile workshops has caused WEO release into the environment, endangering the habitat and the life within. It is estimated that more than 30 billion litres of used oil are accumulated each year, the majority of which is generated by automobile and industrial engines (Ghannam et al., 2021). The presence of polycyclic aromatic hydrocarbons (PAHs) and BTEX (benzene, toluene, ethylbenzene, and xylene) organic compounds in WEO that accumulate during engine operation have long been known as hazardous to human health due to their carcinogenic property (Ali et al., 2023). Chemical treatments are expensive, not environmentally friendly and may lead to incomplete decomposition of contaminants and secondary pollution (Das & Chandran, 2011).

Various approaches to managing the pollutants have been carried out especially through bioremediation due to its environmental-friendly properties and cost-effectiveness (Gaur et al., 2021; Enerijiofi et al., 2020; Varjani & Upasani, 2017). Hydrocarbon bioremediation by naturally occurring microorganisms includes aerobic and anaerobic degradation (Truskewycz et al., 2019). Microorganisms possess several mechanisms for the utilization of hydrocarbons with enzyme-catalyzed ability to break inorganic and organic pollutants (Sakshi et al., 2019). Meanwhile, different species may assist in this process via symbiotic relationships such as the secretion of surfactants to render the oil more bioavailable (Bak et al., 2015). The action of a single species bacterium is limited to the degradation of different hydrocarbon substrates with complex and different chain lengths (Kebede et al., 2021). Hence, a mixed species such as polyculture or consortia with a diverse catabolic pathway provides a much broader arsenal of degradative enzymes to degrade different hydrocarbon components (aliphatic and aromatics) via synergistic metabolism (Yap et al., 2021). In addition, bacterial consortia possess the ability to tolerate more extreme physical parameters of temperature, pH, and salinity and promote higher surface-active biomolecules (biosurfactants) synthesis, to maximize the rate of bioremediation (Hamzah et al., 2017; Moliterni et al., 2012).

This study explores the ability of a bacterial consortium culture previously isolated from a contaminated site from the oil-polluted river in Sg. Pinang, Pulau Pinang, towards degrading several hydrocarbon wastes collected from different types of automobile workshops. Identification of the degraded PAH and BTEX compounds via GCMS analysis was carried out to provide insights into the range of hydrocarbon degraded by the culture.

MATERIALS AND METHODS

Microorganism

The bacterial consortium used in this study was isolated from the oil-polluted river in Sg. Pinang, Pulau Pinang. The isolation of the bacterial consortium culture using a mineral salt medium supplemented with waste engine oil as the sole carbon source showed major bacterial constituents of *Klebsiella* sp., *Enterobacter aerogenes* and *Acinetobacter* sp. (Ismail, 2019). The culture was preserved within 40% glycerol solution and stored at −40°C temperature for further necessary analysis.

Medium Preparation

Mineral salts medium (MSM) supplemented with different waste oils as the sole carbon source was used as the growth medium. The preparation of MSM was adopted from Mohamed Razalli (2020) with a slight modification with composition as follows; KH_2PO_4 (1.2 g/L), K_2HPO_4 (1.8 g/L), $FeSO_4$ (2.5 g/L), $MgSO_4$ (2.8 g/L), NaCl (0.1 g/L), NH_4Cl (4.0 g/L). The pH of the medium was adjusted to 7 before sterilization.

Waste engine oil (WEO) from different types of vehicles was collected from different locations, which were motorcycle workshop (MW) and urban vehicle workshop (UVW), Sg. Dua, and heavy vehicle workshop (HVW), Bayan Baru, Pulau Pinang. All WEO samples were collected from different locations within the same week before they were subjected to experimentation. All waste oils were stored in the laboratory at room temperature. There was a discernible viscosity difference among the three WEOs. The viscosity of each WEO was measured using a viscometer (Model SV-10) and shown in Table 1. Based on GC-MS analysis, the main composition of each oil generally consisted of PAH and BTEX compounds, namely naphthalene, benzene, toluene and xylene.

Table 1 Viscosity of waste engine oils

Waste engine oil	Viscosity (mPa s)
Motorcycle workshop (MW)	82
Urban vehicle workshop (UVW)	113
Heavy vehicle workshop (HVW)	142

Shake Flask Fermentation

The inoculum of the bacterial consortium was propagated in nutrient broth for 24 h before inoculation into MSM. Subsequently, fermentation of the bacterial consortium culture was carried out by inoculating 10% (v/v) inoculum (OD_{600} = 2.5) into each flask containing 50 mL MSM supplemented with respective WEO in triplicates. All flasks were incubated at 30°C at agitation of 200 rpm for 168 h. One mL of sample was taken at every 24 h interval for absorbance measurement at 600 nm until 168 hours. For each flask with different WEO, an uninoculated medium was used as the negative control.

Effect of Different WEO Concentration

Each respective WEO was supplemented as the sole carbon source in the fermentation of the bacterial consortium. Each WEO was added into MSM in shake flasks at concentrations of 3%, 5.5% and 8% (v/v) in triplicates before sterilization by autoclaving. Flasks containing MSM without consortium culture served as the negative control.

Effect of Different Nitrogen Concentration

Ammonium chloride (NH_4Cl) was chosen as a nitrogen source for the fermentation of the bacterial consortium. Different nitrogen concentrations (g/L) were supplied in MSM at 1 g, 3 g, and 5 g of NH_4Cl in triplicates. Flasks containing MSM without consortium culture served as the negative control.

Two-level Factorial Experimental Design

A two-level factorial design was applied to evaluate the effect of two factors, namely, WEO concentration and nitrogen concentration. Table 2 shows a set of five fermentation conditions with one centre point using Design Expert 7.0.0 software. The independent variables were WEO concentration and nitrogen concentration, whereas the dependent variables (response) were biomass concentration.

Table 2 Experimental design of the effect of WEO and nitrogen concentration on biomass of bacterial consortium

Set (Fermentation condition)	Factor A: Concentration of waste engine oil, % (v/v)	Factor B: Concentration of nitrogen (g/L)
1	3	1
2	3	5
3	8	1
4	8	5
5	5.5	3

Residual Oil Determination by Gravimetric Analysis

Bacterial cultures in all flasks were harvested at 168 hours. The culture in each flask was added with n-hexane at a 1 to 1 volume ratio for leftover oil extraction. The flasks were shaken at 200 rpm for 1 hour in an environmental shaker. The flasks were then left overnight to allow phase separation. The topmost layer (hexane layer containing the oil) was transferred into a pre-weighed aluminium weighing dish and left to dry overnight. Water remained in the bottom layer which was not used. The left-over oil concentration (g/L) was expressed as follows, according to a previous study by Jasme et al. (2022):

Residual oil concentration (g/L) =

$$\frac{\text{Weight of leftover oil (g)}}{\text{Total volume of sample taken (mL)}} \times \rho \times 1000 \text{ mL/L}$$

Where is ρ the constant of the density of oil; waste engine oil = 0.86

Gas Chromatography-Mass Spectrometry (GC-MS) analysis of waste oils

The GCMS analysis of hydrocarbon compounds in WEO was carried out using GCMS-QP2010 Ultra (Shimadzu, Japan) with a BPX 70 (0.25 mm × 60 m × 0.25 µm) capillary column using helium as carrier gas at a flow rate of 3.0 mL/min. The column temperature was maintained at 100°C for 2 minutes and thereafter increased at 2°C/min to 230°C (Ichihara & Fukubayashi, 2010). The GCMS was equipped with an auto-injector which has an injection volume of 1 µL. The GC-MS Spectral Database and NIST 2008 mass spectral library were used for the identification of the compounds represented by different peaks in the resulting chromatogram.

The untreated oil samples were added to n-hexane at a volume ratio of 1:4 and mixed well. The mixture was filtered through a 0.22 µm nylon filter membrane into a glass vial. For treated oil samples, solvent extraction with n-hexane was performed at a 1:1 volume ratio and left to stand for 10 minutes. The topmost layer was transferred onto a glass Petri dish and left to dry in the fume hood. The left-over oil was then transferred into a universal bottle and mixed with n-hexane at a ratio of 1:4. The solvent mixture was then pipetted by syringe and filtered using a 0.22 µm nylon filter membrane into a vial. Degradation of hydrocarbon was measured for each WEO and compared to the untreated WEO.

Screening for Biosurfactant Production

Oil Spreading Test

An oil spreading test was performed to observe the presence of biosurfactants by oil displacement activity. A volume of 20 µL waste oil sample was added onto the surface of 50 mL distilled water in a 125 mm diameter Petri dish to form a thin oil layer at the water surface. Then, 10 µL bacterial consortia culture was gently placed on the centre of the oil layer. Displacement of oil forming a clearing zone in the oil layer was an indication of the presence of a biosurfactant. The diameter of the clearing zone was measured, which correlated with the surfactant concentration.

Emulsification Index (E_{24})

The emulsifying activity of the cell-free supernatant from the culture broth of bacterial consortium using different waste oils was determined by adding 2 mL of kerosene to the same volume of the supernatant in a test tube. The tubes were vortexed vigorously for 2 minutes using a vortex (Vortex Mixer, Vision Scientific) and left to stand for 24 hours. Tween® 80, a chemical surfactant, was used as a positive control while uninoculated MSM served as a negative control. The emulsification index (E_{24}) was determined as the percentage of the height of the emulsified layer (mm) divided by the total height of the liquid column (mm) as follows (Zulkhifli et al., 2023):

$$E_{24}(\%) = \frac{\text{Height of emulsified layer (mm)}}{\text{Height of total liquid mixture (mm)}} \times 100$$

Bacterial Adhesion to Hydrocarbon (BATH) Assay

The bacterial consortium culture was sampled at 168 hours and centrifuged at 8,000 rpm for 8 minutes at 4°C. Subsequently, it was washed twice to remove any interfering solutes, particularly oil. The supernatant was discarded, and the cell pellet was re-suspended in PBS accordingly to achieve an optical density (OD) of 1.0 at 600 nm wavelength. A volume of 4.0 mL cell suspension and 1.0 mL of oil were mixed in a test tube, and vortexed at 2,000 rpm for 1 min. The turbidity of the aqueous phase was measured at 600 nm using a spectrophotometer (Genesys 20, model 4001-04, Thermo Scientific, USA). Cell-free PBS served as a blank. Hydrophobicity is expressed as the percentage of cells bound to the hydrophobic phase (Zupančič et al., 2019):

$$\text{Cell hydrophobicity}(\%) = \frac{(1 - \text{OD of aqueous phase mixed with hydrophobic phase})}{\text{OD of initial cell suspension without hydrophobic phase}} \times 100$$

RESULTS AND DISCUSSION

Growth of Bacterial Consortium with Waste Engine Oil as the Sole Carbon Source

In this study, the bacterial consortium culture was able to grow on all types of WEO substrates at different concentrations of waste oil and nitrogen sources as shown in Figure 1. Figure 1 (a) shows that the highest biomass density for culture grown on MW was produced in the fermentation condition of Set 4, with 8% (v/v) WEO and 5 g/L nitrogen. The biomass density was 0.370 (OD_{600}) with 49 g/L of leftover oil. In contrast, for UVW and HVW, the highest biomass and the lowest left-over oil were obtained from

the fermentation condition of Set 1 with 3% (v/v) UEO and 1 g/L nitrogen (Figure 1 (b) and Figure 1(c)). Among the three waste oils tested, UVW exhibited the lowest growth with 0.219 (OD_{600}) biomass and 19 g/L leftover oil.

Figure 1 and Figure 2 depict an experimental design of a two-level factorial design for 5 sets of fermentation conditions and one centre point constructed using statistical software (Design Expert 7.0.0), with the results of biomass of bacterial consortium using different waste engine oils. Factors that influence the bacterial consortium growth were evaluated by the Pareto chart and the surface plot.

Figures 2(a), 2(b) and 2(c) show the Pareto charts of biomass densities with the factors of substrate concentration (A) and nitrogen sources (B) for each respective waste engine oil. It was observed that both factor A and B values did not reach above the Bonferroni limit of significance. Figure 2(a) shows that both concentrations of used engine oil and nitrogen sources have a positive effect towards biomass production with the latter giving a higher positive effect. Similarly, Figure 2(b) shows that the concentration of used engine oil has a positive effect towards biomass production. However, the positive effect of nitrogen concentration was not significant. On the other hand, Figure 2(c) depicts that both factors of waste engine oil and nitrogen concentrations had a negative effect towards biomass production.

The interaction of variables was determined by plotting three-dimensional (3D) response surface curves. The effect of the three types of waste engine oil on biomass production of the bacterial consortium was assessed using ANOVA. The results of the p-value were greater than 0.05, indicating that the differences were not significant.

Figures 3(a), 3(b) and 3(c) represent the interaction between the substrate concentration (waste engine oil) and nitrogen concentration, which showed that the maximum biomass production was independent of the type of waste oil and nitrogen supplied. The shape of the response surface slopes revealed a positive interaction between these tested variables. The interaction between substrate concentration and nitrogen source was not significant, presumably due to the viscosity and toxicity of waste engine oil that might inhibit the bacterial consortium growth, thus affecting hydrocarbon degradation.

Effect of Different Carbon Source Concentrations

It was observed that waste oil concentration affected cell growth differently depending on the type of WEO. At low concentrations, bacterial consortium supplemented with UVW and HVW demonstrated higher growth and lower left-over oil compared to the one grown in MW, regardless of their relatively higher viscosity.

This was suspected to be affected by the different chemical compositions and complexity of each waste oil (Ghannam et al., 2021). The UVW and HVW oils are formulated to withstand high pressure and temperature from combustion within the vehicle engine which is larger in size compared to the motorcycle engine. Furthermore, automobile engine oil contains friction modifiers to reduce friction between moving parts to provide good fuel economy and efficiency. These friction modifiers and

other additives in UVW and HVW were suspected to aid growth as nutrients such as phosphorus and sodium. These are absent in motorcycle oils (Pimda & Bunnag, 2015; Ratoi et al., 2014; Tang & Li, 2014). Moreover, the presence of detergent additives within the larger vehicle engine oil may provide better accessibility for the culture to take up the nutrients within the oils. Motorcycle oils of MW, on the other hand, are formulated with fewer additives to function for both smaller engines and gearboxes by protecting engine components and gears. From the result, lower bacterial growth was observed when the consortium was grown in this oil.

At higher concentrations, a lower viscosity of MW (82 mPa s) allows better dispersion in the form of oil droplets as shown in Figure 4(a), providing more surface contact for cell uptake, thereby resulting in higher growth. In comparison, bacterial growth for UVW and HVW was lower. Water immiscible nature of higher WEO volume with higher viscosity from UVW and HVW (113 mPa s and 142 mPa s) caused the formation of water-oil emulsion, which resulted in the clumping of oil substrate, reducing surface contact area for cell uptake. This was suspected due to the high viscosity of the oils that tend to resist motion, thus reducing oil dispersion within the cultures (Figure 4(b)). This would suggest that cell growth was independent of oil concentration as increasing oil did not contribute to higher growth, due to immiscibility and higher viscosity.

It is well known that oil viscosity plays a very important role in the availability of surface area needed for biodegradation (Ke et al., 2019). The increase in oil viscosity interferes with the adherence and mass transfer within the culture. It was observed that oils with high viscosities tend to show three types of physical morphology during fermentation, namely clumps and emulsified form or both. As the oil concentration increased, the oil substrate tended to clump within the aqueous broth causing very low dispersion of oil resulting in low contact surface area, thus decreasing the adherence and accessibility of bacterial cells to the hydrophobic substrates. Furthermore, high viscosity also caused the formation of a stable oil-in-water emulsion that led to non-Newtonian behaviour, thus impeding substrate uptake (Du et al., 2022). The occurrence of emulsion was more frequent in oil with higher viscosity, as observed on HVW.

Effect of Different Nitrogen Concentrations

In addition to scouting for different WEO as the carbon source, the effect of nitrogen on the bacterial consortium culture was also evaluated. It was observed that the maximum biomass densities for the cultures grown on different WEOs were achieved at different nitrogen concentrations. For MW, notable differences were observed in growth with increased nitrogen concentration, indicating the limiting effect of nitrogen concentration towards bacterial consortium growth. However, the opposite outcome was observed for UVW (Set 2), HVW (Set 2), and HVW (Set 4), where an increase in nitrogen concentration caused a decrease in biomass growth. The higher concentration of nitrogen was suspected to increase osmotic pressure, causing the medium to be slightly hypertonic for cell growth (Breznak & Costilow, 2007). A previous study by Costa et al. (2002) reported similar results when the organic nitrogen source became

the limiting factor for the Gram-negative bacterium *Pantoea agglomerans* growth. In this case, the amount of nitrogen availability was more significant than the nitrogen source since a previous study had proven that there was no significant influence on degradation-related activity (Wrenn et al., 2006). This study found that nitrogen concentration was limiting for both MW and UVW.

Hydrocarbon Degradation by GC-MS Analysis

The hydrocarbon degradation of different waste oils by bacterial consortium was observed by comparing the untreated and treated oil samples as shown in Figure 5 (a to c). Based on Figure 5, the presence of PAH and BTEX compounds such as naphthalene, benzene, toluene and xylene were detected at early retention time (between 23 minutes to 40 minutes). However, the hydrocarbon compounds were detected at a lower percentage in the treated samples indicating degradation activity has occurred. The degraded hydrocarbons amongst the waste oil were slightly different in which MW waste oil GCMS analysis shows the degradation of xylene, benzene, naphthalene and phenanthrene, UVW waste oil GCMS analysis shows the degradation of benzene, tetradecane, naphthalene, toluene and pentadecane and HVW waste oil GCMS analysis shows the degradation of toluene, naphthalene, and benzene.

At a later retention time (above 40 minutes), a curve was detected on all three waste oils chromatograms and was not able to be identified by the library equipped with the GCMS software. This finding was like various previous reports that work on waste oil and were assumed as an unresolved complex mixture (UCM) that is recalcitrant towards biodegradation due to its high molecular weight (Isaacman-VanWertz et al., 2020; Jasmine & Mukherji, 2019; Ramadass et al., 2021). A higher percentage area of unresolved complex mixture was observed in the treated sample in Figure 5(a) compared to its untreated sample. This new emergence of peaks was suspected to be caused by the transformation of long hydrocarbon chains to shorter or different numbers of hydrocarbon chains. Similar findings were observed by Li et al. (2022) who observed the ability of bacterial consortium to convert medium and long-chain hydrocarbon into short-chain hydrocarbons and then further degrade.

The chromatograms also showed that the degradation peak was not just observed for one hydrocarbon compound but for multiple compounds. This indicated the ability of the bacterial consortium to degrade a wide range of hydrocarbons. This supports the previous studies by Kim et al. (2009) and Tzintzun-Camacho et al. (2012), about the cooperative metabolic activities among consortium members, where the process facilitated the exchange of metabolic products during hydrocarbon degradation. Their studies have shown that mixing a degrading species broadens the number of hydrocarbon compounds utilised without inhibiting other consortium members' growth.

Screening for Biosurfactant Production

Attempts were made to investigate the possible mechanism of hydrocarbon degradation by the bacterial consortium, either via biosurfactant production or changing cell hydrophobicity to facilitate hydrocarbon uptake as reported in Table 3. Cell-free supernatant obtained from cultures supplemented with respective oils were subjected to an oil spreading test (OST) and emulsification index assay (E_{24}). The highest clearing zone observed was from HVW with 16 mm followed by that of UVW and MW with 11 mm. Similarly, for the E_{24} assay, the E_{24} index was only 11% for HVW and below 10% for MW and UVW. Low values of OST and E_{24} indicate the low presence of biosurfactants in the samples. The cell pellets collected upon centrifugation of each fermentation broth were tested for cell surface hydrophobicity via bacterial adherent to hydrocarbon (BATH) assay.

Table 3 Screening of biosurfactant production and cell hydrophobicity measurement

Waste engine oil	Oil spreading test (mm)	Emulsification index, E_{24}	BATH assay (%)
Motorcycle workshop (MW)	11	6	70
Urban vehicle workshop (UVW)	11	2	81
Heavy vehicle workshop (HVW)	16	11	69

Based on Table 3, it was observed that the bacterial consortium grown on all three WEOs exhibited cell hydrophobicity. Bacterial consortium supplemented with UVW showed the highest cell hydrophobicity of 81%, followed by similar values of MW at 70% and HVW at 69% of cell hydrophobicity. This signifies the hydrocarbon degradation was most probably performed through the cell surface hydrophobicity mechanism. Previous studies towards cell surface hydrophobicity suggest that bacteria may use the outer part of the surface such as thin fimbriae to adhere to hydrocarbon (Zadeh et al., 2021). A previous study also suggested that some microorganisms modify their cell surface to increase their affinity towards hydrophobic substrates, thereby facilitating substrate absorption (Kaczorek & Olszanowski, 2011). On the other hand, the oil spreading test and emulsification index (E_{24}) indicated the absence of biosurfactants.

The main challenge and limitation in this study was the inconsistency in WEO analysis measurement, which stemmed from the rheology and complex composition of the waste oils. As the fermentation progressed, the oil dispersion within the aqueous broth caused the formation of a stable oil-in-water emulsion that led to non-Newtonian behaviour. In addition, the behaviour of oil emulsions in waste oils is complex. Due to the complex composition of hydrocarbon in the oils, investigation of the synergistic activity within the bacterial consortium members could be carried out in future studies to identify the range of hydrocarbon compounds that can be degraded by respective bacterial species.

CONCLUSION

This study documented the ability of a bacterial consortium isolated from a contaminated site in Sg. Pinang, Pulau Pinang to degrade a wide range of hydrocarbon compounds in different types of waste engine oils. Among all WEOs, MW exhibited the highest biomass density, followed by HVW and UVW. The higher concentration of UVW and HVW could have given rise to a toxic effect toward bacterial growth, manifesting in lower biomass densities with increasing oil concentrations. Response surface analysis exhibited a positive effect of carbon and nitrogen concentrations from MW and UVW, but a negative effect from HVW waste engine oil. Nitrogen concentration was proven to be limiting for cultures supplemented with MW, where it helped the bacterial consortium assimilate the carbon source within the waste oil. However, increasing both nitrogen and oil concentrations of HVW and UVW did not result in high biomass. At low WEO concentration, the composition of engine oil, whether it was used for small engines such as motorcycles, or larger vehicles, was observed to play a crucial role in the growth of bacterial consortium. At higher WEO concentrations, the viscosity of oil starts to affect the oil uptake by the cells. This consequentially led to emulsion formation that caused the oil to clump together. Moreover, the toxicity of WEO such as UVW and HVW, seems to be inhibiting the growth of the bacterial consortium. The GC-MS analysis supported the event of biodegradation when the untreated and treated oil by bacterial consortium were compared. The presence of PAH and BTEX compounds such as naphthalene, benzene and toluene were detected in the three WEO samples and were degraded. Assessment of degradation mechanism by bacterial consortium grown on all three WEO exhibited high cell hydrophobicity, suggesting that hydrocarbon degradation most probably occurred by changing cell surface hydrophobicity to facilitate the uptake of hydrophobic substrates.

This study explores the use of waste engine oil as a carbon source for bacterial growth which is significant for environmental remediation. The synergistic metabolism among the different bacterial species in the consortium offers a better strategy than that of a single culture. Nevertheless, natural environmental conditions pose continuous fluctuations and inconsistencies influencing hydrocarbon degradation. Therefore, the optimization of relevant parameters should be considered for developing microbial consortia cultures for contaminated environmental clean-up.

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