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Hydrocarbon degradation potential of some hydrocarbon-utilizing bacterial species associated with Kenaf (*Hibiscus cannabinus* L.) plant

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Abstract

13 Kenaf (Hibiscus cannabinus) plant associated Hydrocarbon-Utilizing Bacterial isolates previously identified based on their morphological, biochemical and molecular characteristics as Providencia vermicola_01, Providencia rettgeriB10_04, Providencia rettgeri D04_10,Pseudomonas aeruginosa D10_10, *Exiguobacterium aurantiacum F03_18, Providencia vermicola F10_16, Klebsiella pneumoniae G10_19, Stenotrophomonas maltophila A04_01, Providencia rustigianii H10_22, Lysinibacillus fusiformis B11_05, Lysinibacillus sphaericus C11_08, Pseudomonas aeruginosa F04_16 and Lysinibacillus sphaericus E11_were investigated for their degradation potential in crude oil, diesel and engine oil. The Kenaf (Hibiscus cannabinus) plant was grown in a Niger Delta soil. Growth of these Kenaf associated bacteria was assessed for 7 days by monitoring the optical density (OD) of the media. OD values were observed to rise majorly between the first and three days of contact between the HUB and the hydrocarbons. The highest OD values measured for crude oil degradation was 1.548nm by Klebsiella pneumoniae G10_19, for engine oil 1.416nm by Klebsiella pneumonia and by Exiguobacterium aurantiacum 1.416nm. Significant difference in o.d. values was observed only for Exiguobacterium aurantiacum F03_18. This study provided information on suitable Kenaf (Hibiscus cannabinus) HUB bacterial species to use for phytoremediation of engine oil, crude oil and diesel impacted soil in Niger Delta Region.*

Keywords: Kenaf, hydrocarbon-utilizing bacteria, crude oil, diesel, engine oil, optical density.

Introduction

Hydrocarbons being organic are made up of majorly carbon and hydrogen with trace quantities of elements like sulphur, nitrogen and oxygen[1]. Examples of hydrocarbons include kerosene, diesel and crude oil. Hydrocarbon utilizing bacteria are heterotrophs which have as their source of energy, carbon and electrons[2]. They are widely distributed in soil, fresh water and marine habitats[3]. The Niger Delta Region occupying over 70,000 square kilometers is made up of nine oil producing states[4,5]. This region is blessed with natural crude oil providing a major source of Nigeria's revenue[6]. Oil exploration and exploitation has exposed this region to pollution and endangerment of the air, land and through spills. These spills occur through corrosion, vandalization, theft and leakage of crude during processing making this oil spill sites a common site in the Niger Delta Region[7]. This has left a deleterious effect on human, aquatic and plant life in the form of hunger, death loss of farm lands, diseases. Phytoremediation is a synergistic process between plants and associated microorganisms in the removing contaminants[8]. Although a slow process, phytoremediation has been largely embraced for the cleanup of contaminants this is due to its environmentally friendly and sustainable effect. This process is enlargely enhanced by processes such as bioaugmentation in which selected strains of microorganisms like bacteria are inoculated to speed up the remediation process. These inoculated bacteria could be sourced from the exterior or isolated from plants with hydrocarbon degrading ability, cultured to increase their numbers and re-inoculated into these plants. These indigenous microbes have been found to be more efficient than allocthonous bacteria because they are better adapted to the physiology of the host plant. Plant associated bacteria may be plant specific and also determined by factors such as climate, soil type therefore an associated bacteria for a plant may be reinoculated into a different plant and may not be as efficient. Isolates of hydrocarbon-Utilizing bacteria (HUB), are still being sought for with the aim of getting stock organisms for bioremediation[9]. Just as nutritional demands vary with humans and animals due to as a result the same may apply to microorganisms such as bacteria and fungi. Microorganism utilize hydrocarbons bases on their chemical nature[10]. Knowledge of this could form a bedrock of information for application in field studies. In this study the degradation ability of 13 HUB associated with Kenaf (*Hibiscus cannabinus*) was tested with three commonly spilled hydrocarbons: Diesel, crude oil and engine oil was tested to know the preferable option for phytoremediation.

Materials and methods

13 Kenaf (*Hibiscus cannabinus*) hydrocarbon-utilizing bacteria isolated by vapour phase transfer method as described were used[11]. These hub species had been identified to the species level by molecular characterization[12].

Hydrocarbon Degradation Test: In this method 9.9mls of sterile mineral salt broth was aseptically dispensed into four test tubes. 0.1ml of crude oil (bonny light), diesel and engine oil (mobil super 1000) was added to the first second and third test tubes respectively. To the fourth test tube, 0.1ml of crude oil (bonny light crude) was added. This process was repeated to meet the number of hydrocarbon utilizing bacterial isolates. 0.1ml of overnight cultures of hydrocarbon-utilizing bacteria (hub) was inoculated into the first, second and third test tubes for all the hub isolates. The fourth test tube was uninoculated and served as a control. Incubation was done at room temperature (28 to 31^{0}C) for 7 days. Constant tilting of the test tubes was done during incubation to enable oil cell phase contact. Optical density of the hub isolates was measured at 610 nanometers using a clincheck plus chemical analyzer (biochemical systems international Sri lanka) at 2 days interval starting from day 1 to day 7.

Statistical analysis: Absorbance values at 610 nanometers using one way analysis of variance (ANOVA) SPSS Version 21.0 were analysed.

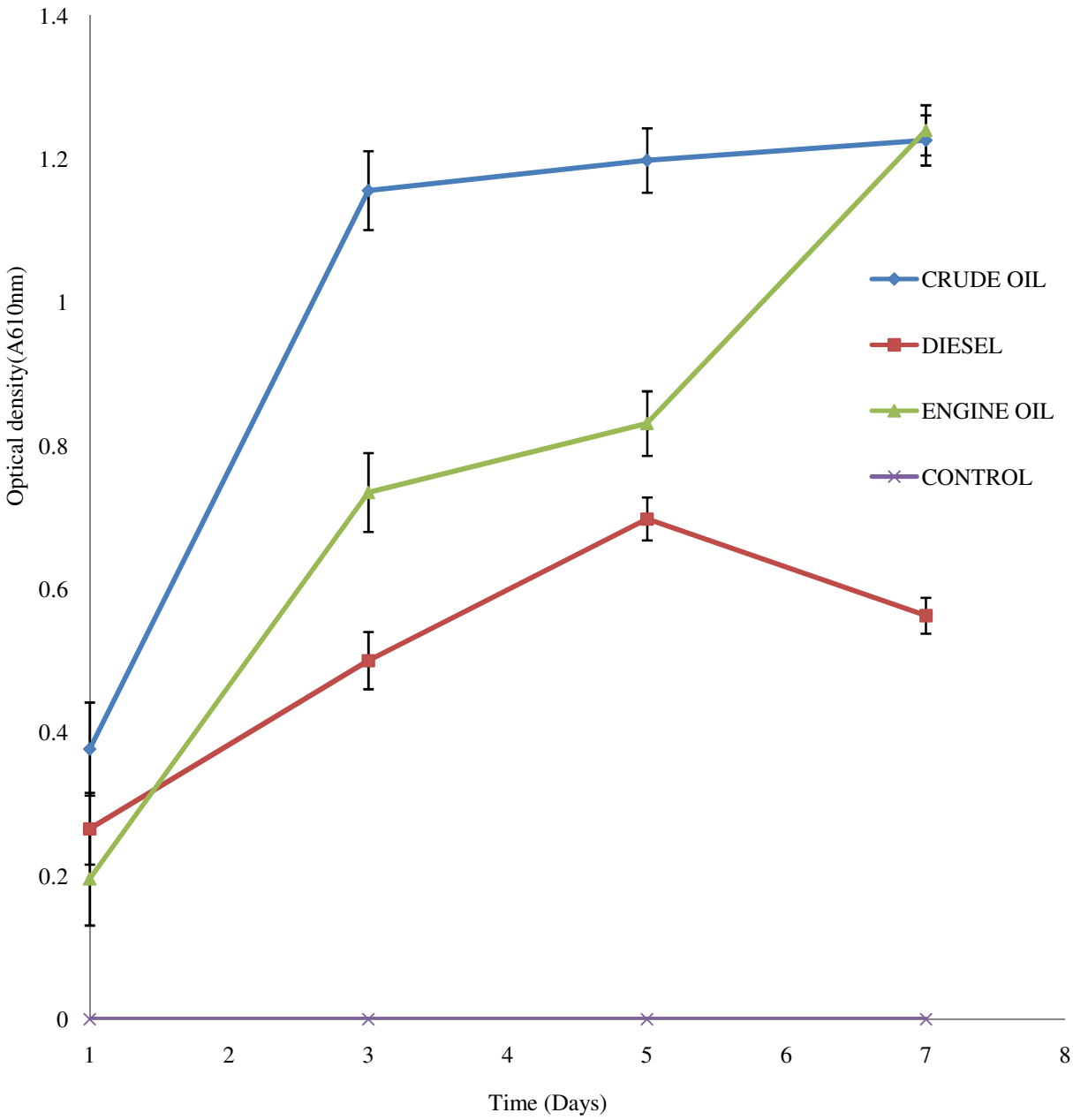

Figure-1: Optical density of *Providencia vermicola* A10_01 grown in mineral salt broth containing crude oil, diesel and engine oil as sole carbon and energy source.

Results and discussion

The results of this study reveal that these 13 *Hibiscus cannabinus* associated bacteria have the ability to degrade diesel, engine oil and crude oil. When hydrocarbons are broken down it brings about release of water, carbon iv oxide and energy which hydrocarbon–utilizing microbes use for growth. This growth or increase in biomass is indicated by turbidity in the growth medium. Optical density readings were observed to increase generally for all 13 isolates[13]. This may have been due to probably to the age of the spill. *Providencia* spp isolates were observed to have their peak growth for diesel at day 5 and drop by day 7 with the exception of *Providencia rettgeri* D04_10 that showed a slight increase on day 7 (Figure-3). This may suggest that optimum phytodegradation by Kenaf (*Hibiscus cannabinus*) of diesel using *Providencia* spp is probably around the 5th day of contact with either of the hydrocarbons: Crude oil, diesel or engine oil. Crude oil utilization by the *Providencia* spp was observed to rise generally from day 1 to day 7 with the exception of *Providencia rettgeri* D04_10 that experienced a decline phase after day 1 and grow exponentially afterwards (Figure-3). This decline may have been due to acclimatization, competition among species for nutrients, space thereby exhausting some of the species present. The observation was similar for engine oil breakdown with the exception of *Providencia rustigianii* H10-22that declined by day 7 (Figure-9). For *Lysinibacillus fusiformis* B11_05 a decline phase was observed by day 7 for diesel only (Figure-10). *Lysinibacillus sphaericus* C11_08 had an exponential growth in all the three hydrocarbon types had and gradual increase in optical density for all hydrocarbon sources (Figure-11). This result suggests that this strain, could be the best choice for bioremediation in the event of contamination of any media with the three hydrocarbon types used for the test in the event of phytoremediation of hydrocarbons with *Hibiscus cannabinus*. *Pseudomonas spp, Stenotrophomonas maltophila* and *Lysinibacillus sphaericus* E11_14 (Figures-4, 12, 8 and 13 showed best preference for crude oil degradation. *Exiguobacterium aurantiacum* F03_18 showed best preference for diesel degradation giving the highest O.D. reading amongst the other HUB isolates (Figure-5). *Exiguobacterium aurantiacum* has been reported to be a preferential degrader of diesel in the midst of crude oil and engine oil. The highest optical density measured for crude oil was 1.548nm and this was by *Klebsiella pneumoniae* G10_19, suggesting that this strain among other isolates may be the best utilizer of crude oil when compared to other HUB isolates. For engine oil the optical density reading was highest on the 7th day by *Klebsiella pneumoniae* G10_19 (Figure-7). Similar study, reported a greater utilization of engine oil by *Klebsiella* sp than utilization of diesel. The control being uninoculated did not show any growth in microbial populations[16].

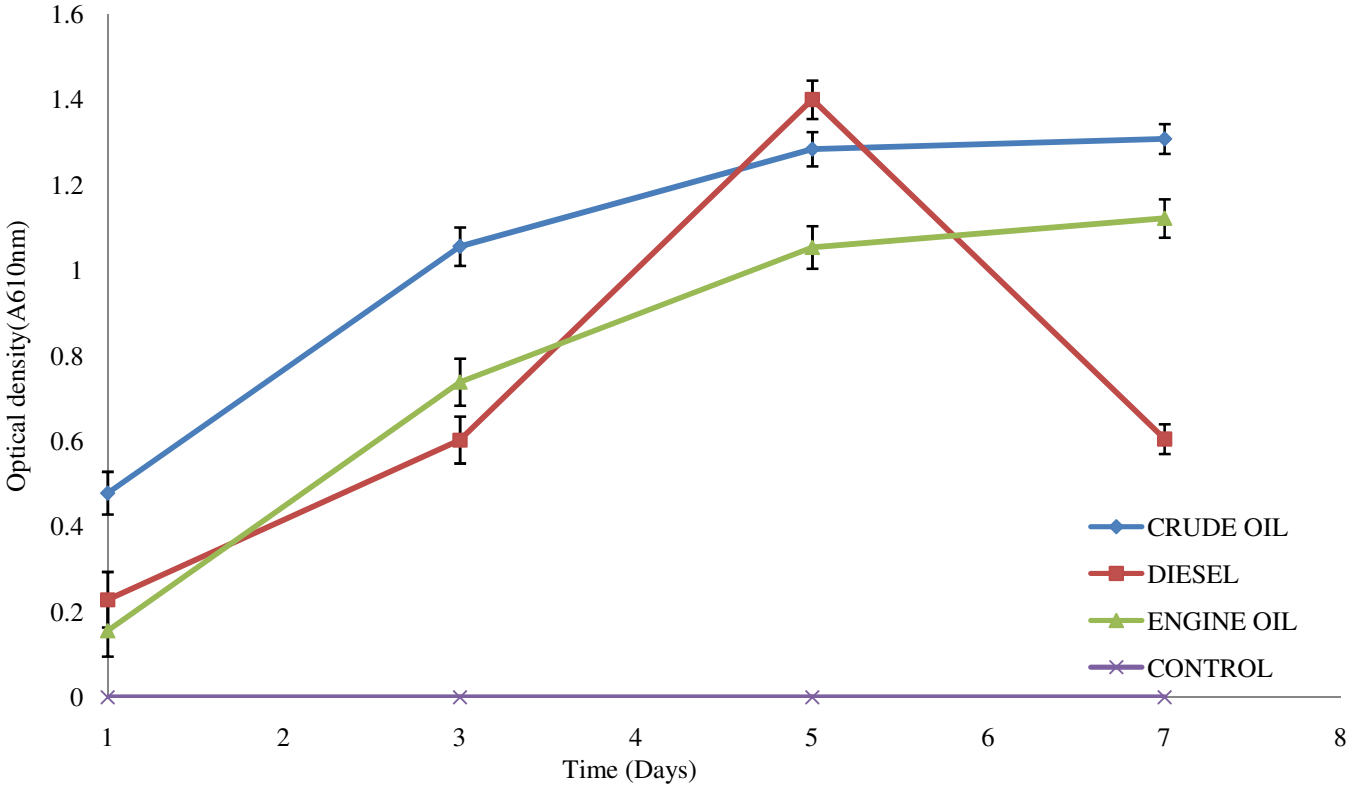

Figure-2: Optical density of Providencia rettgeri B10_04 grown in mineral salt broth containing crude oil, engine oil and diesel as sole carbon and energy source.

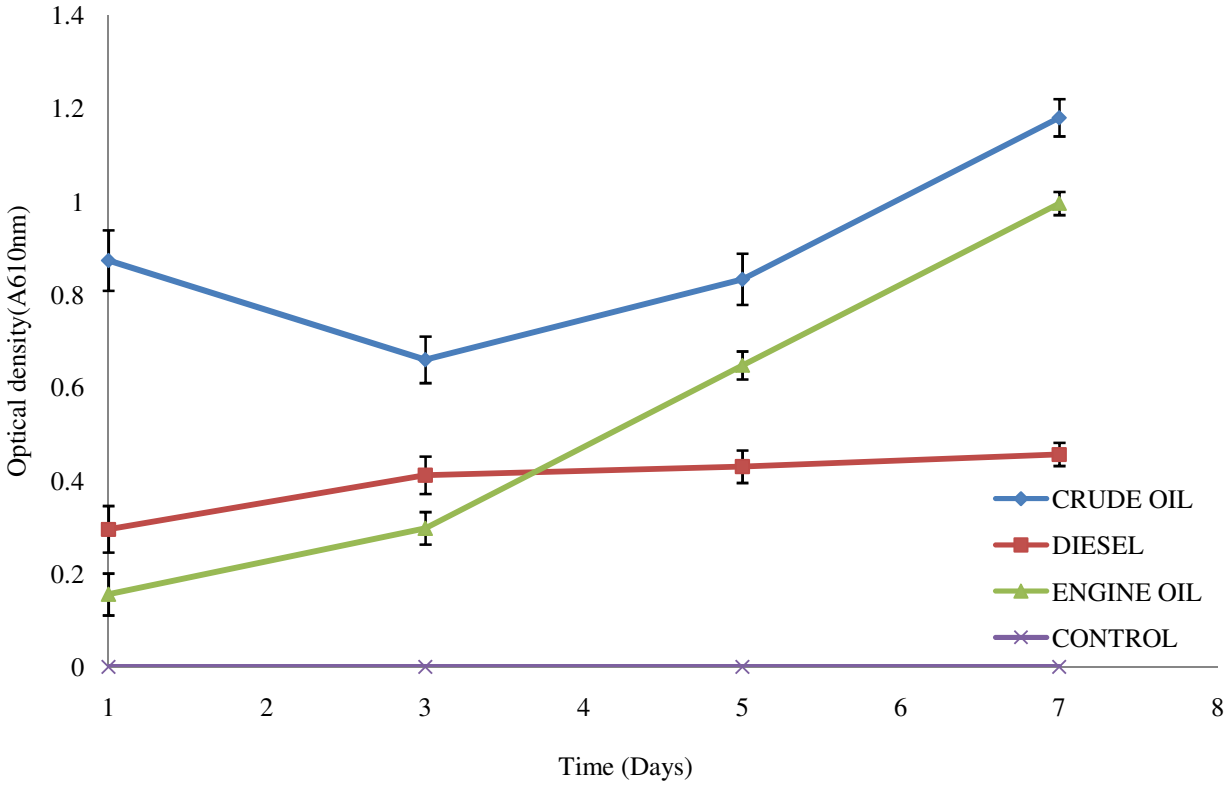

Figure-3: Optical density of Providencia rettgeri D04_10 grown in mineral salt broth containing crude oil, engine oil and diesel as sole carbon and energy source.

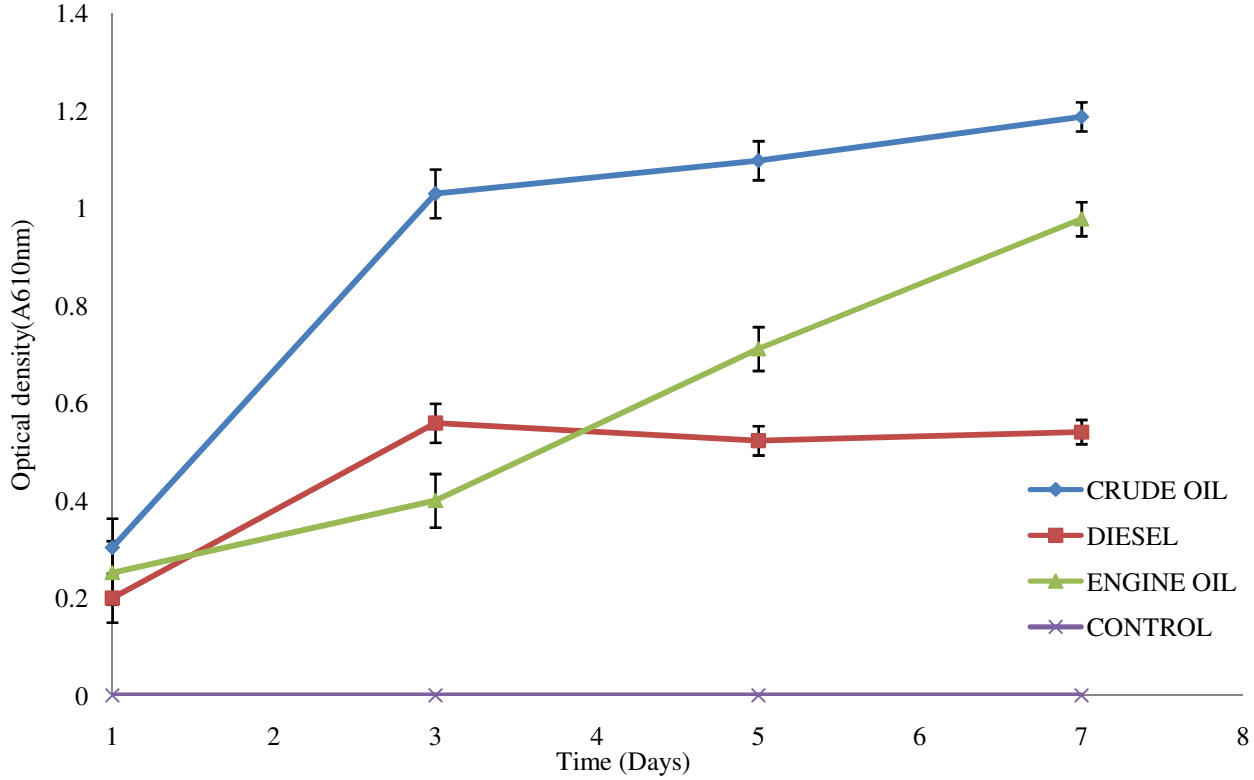

Figure-4: Optical density of Pseudomonas aeruginosa D10_10 grown in mineral salt broth containing crude oil, diesel and engine oil as sole carbon and energy source.

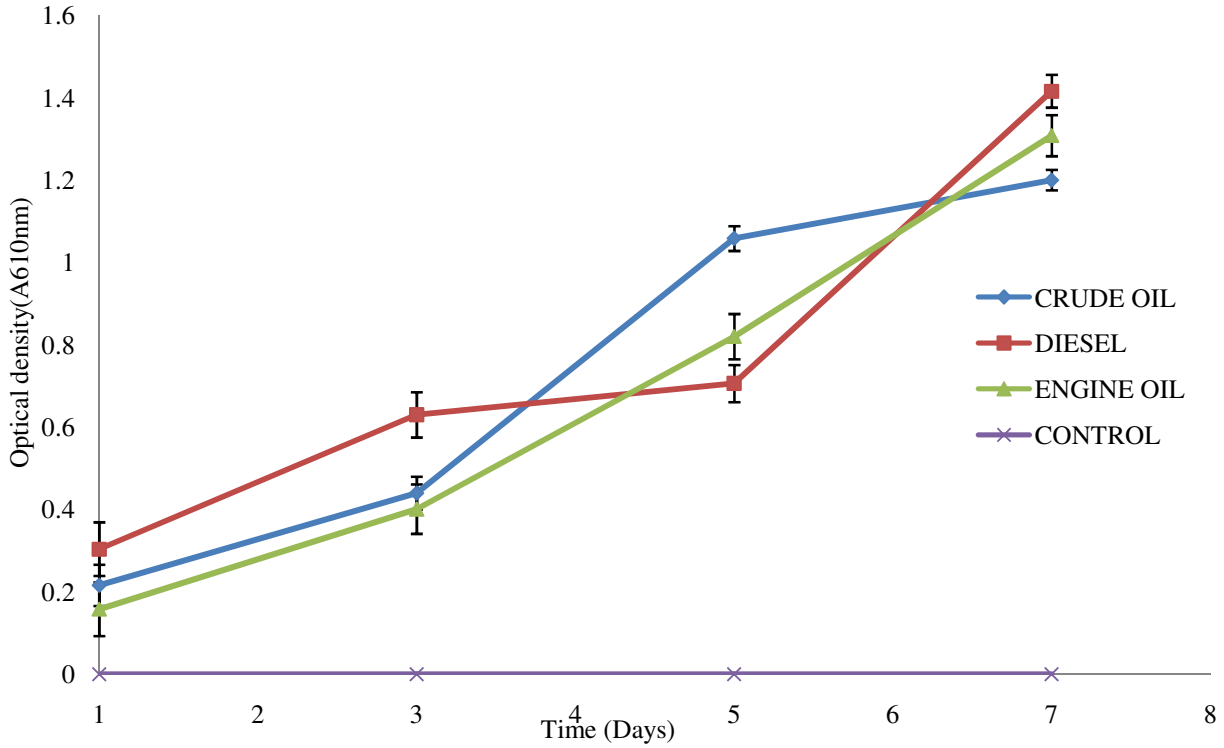

Figure-5: Optical density of Exiguobacterium aurantiacum F03_18 grown in mineral salt broth containing crude oil, diesel and engine oil as sole carbon and energy source.

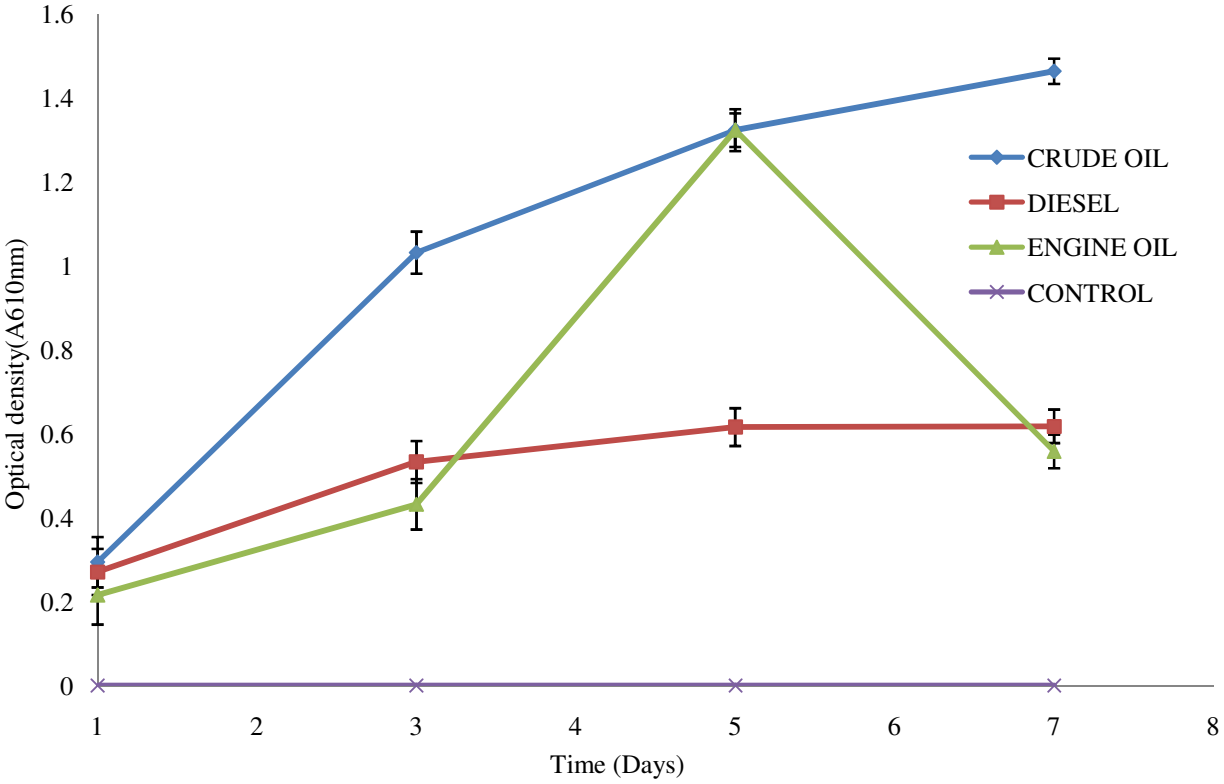

Figure-6: Optical density of Providencia vermicola F10_16 grown in mineral salt broth containing crude oil, engine oil and diesel as sole carbon and energy source.

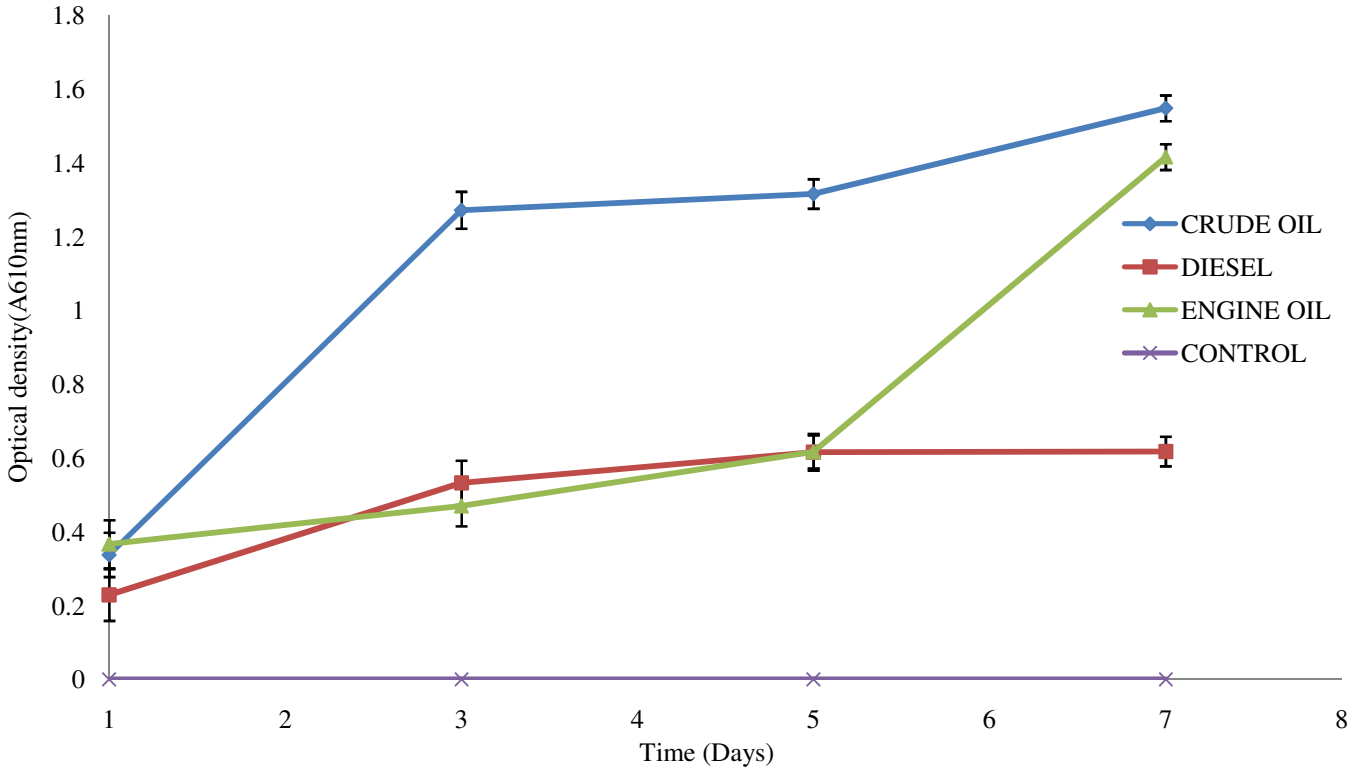

Figure-7: Optical density of Klebsiella pneumonia*e* G10_19 grown in mineral salt broth containing crude oil, engine oil and diesel as carbon and energy source.

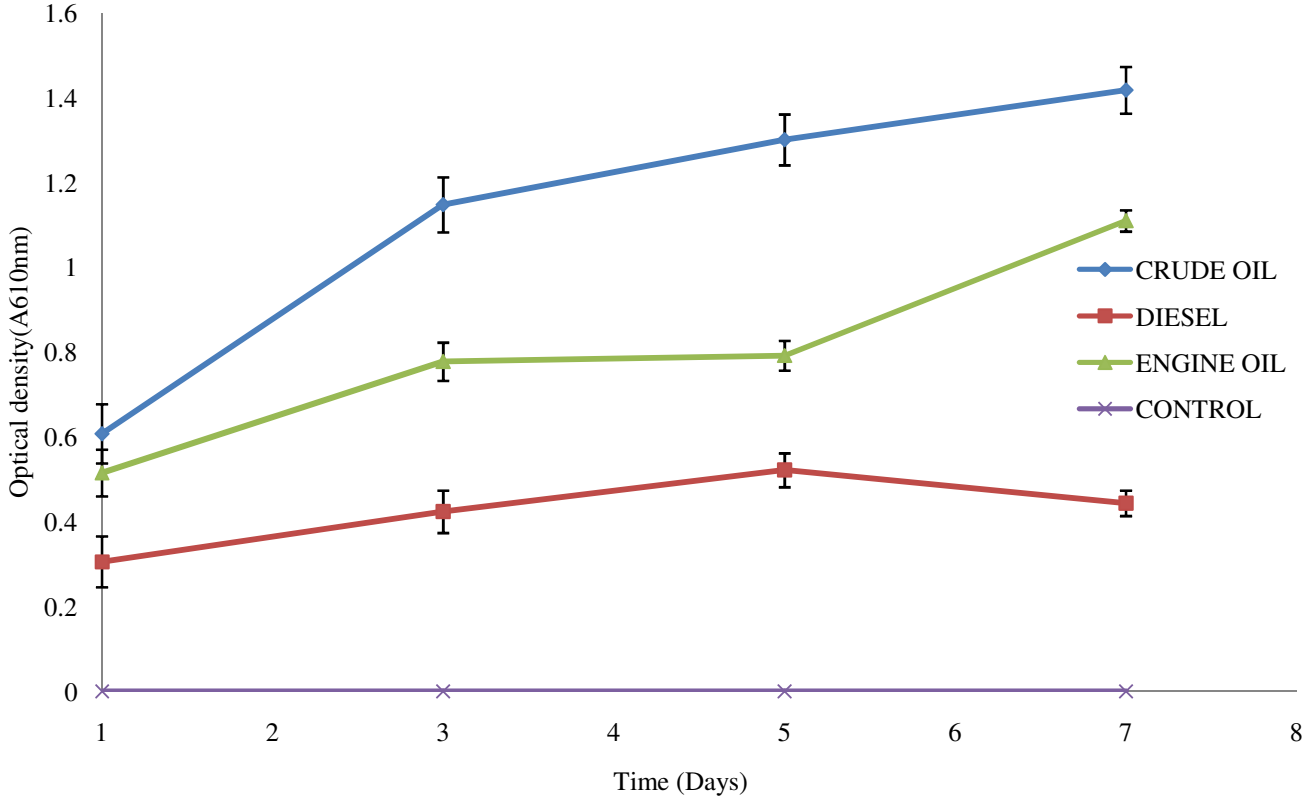

Figure-8: Optical density of Stenotrophomonas maltophila A04_01 grown in mineral salt broth containing crude oil, diesel and engine oil as sole carbon and energy source.

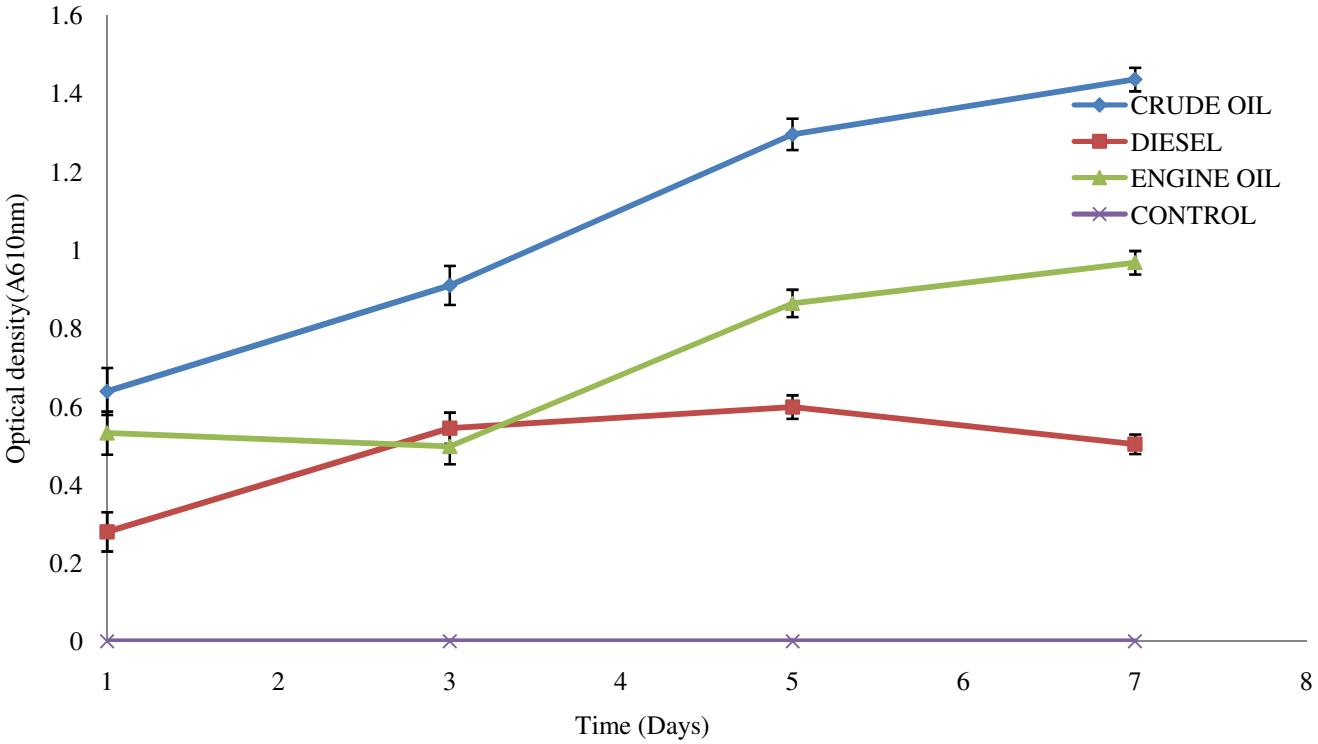

Figure-9: Optical density of Providencia rustigianii H10_22 grown in mineral salt broth containing crude oil, engine oil and diesel as sole carbon and energy source

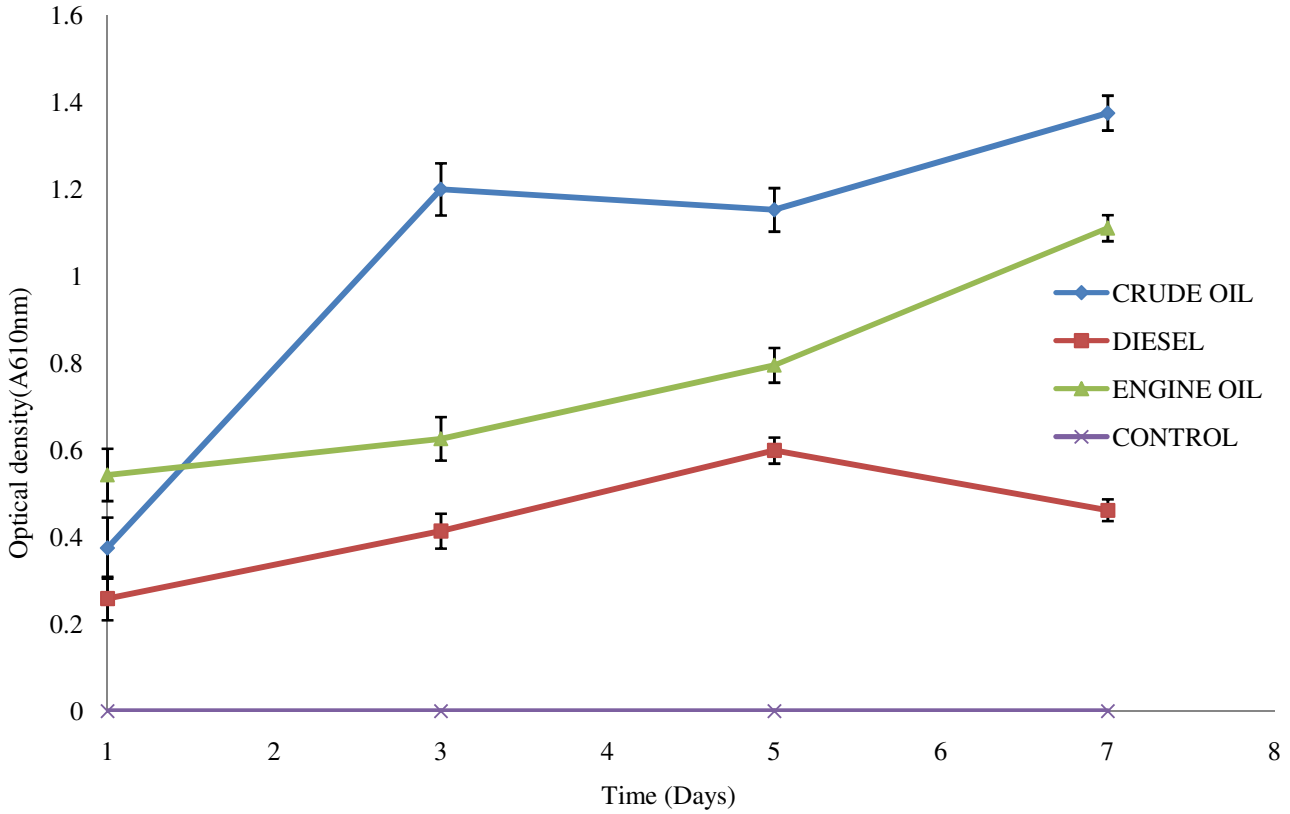

Figure-10: Optical density of Lysinibacillus fusiformis B11_05 grown in mineral salt broth containing crude oil, engine oil and diesel as sole carbon energy source.

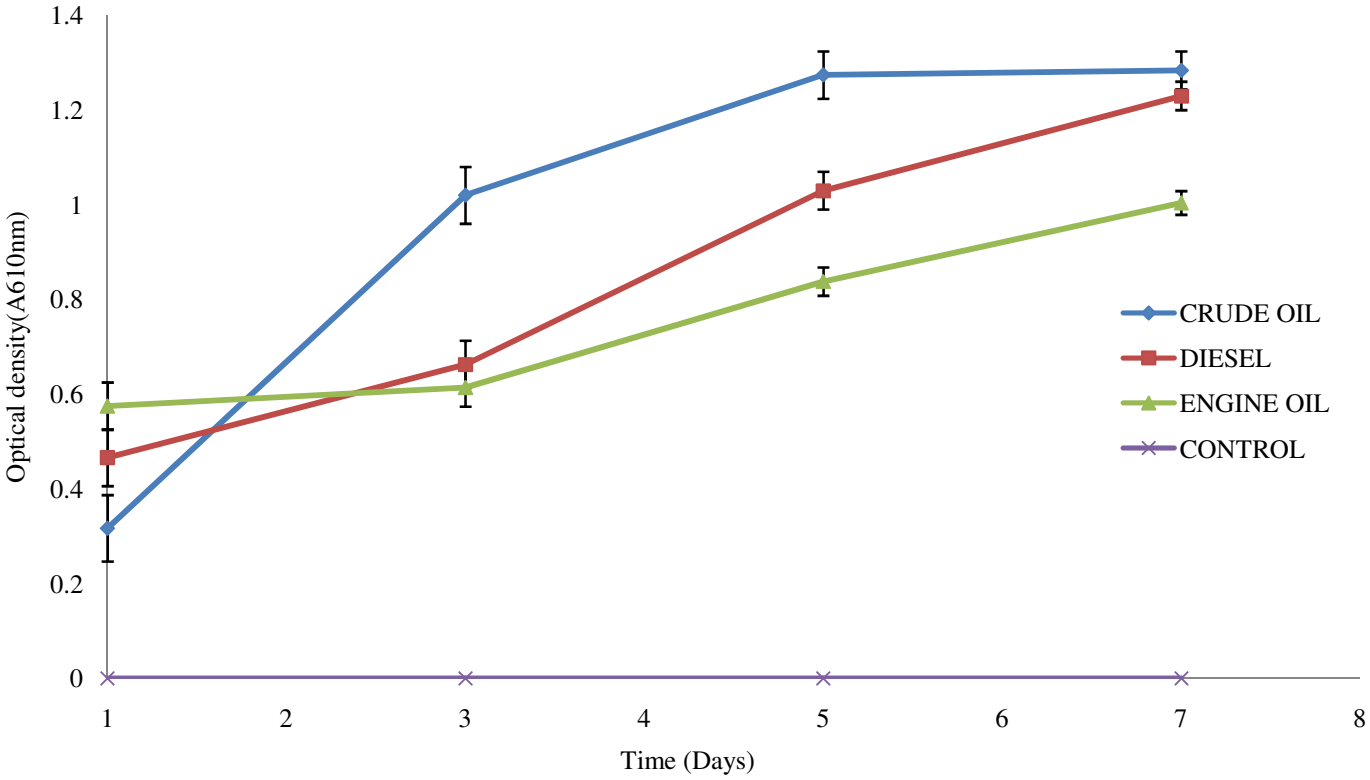

Figure-11: Optical density of Lysinibacillus sphaericus C11_08 grown in mineral salt broth containing crude oil, diesel and engine oil as sole carbon and energy source.

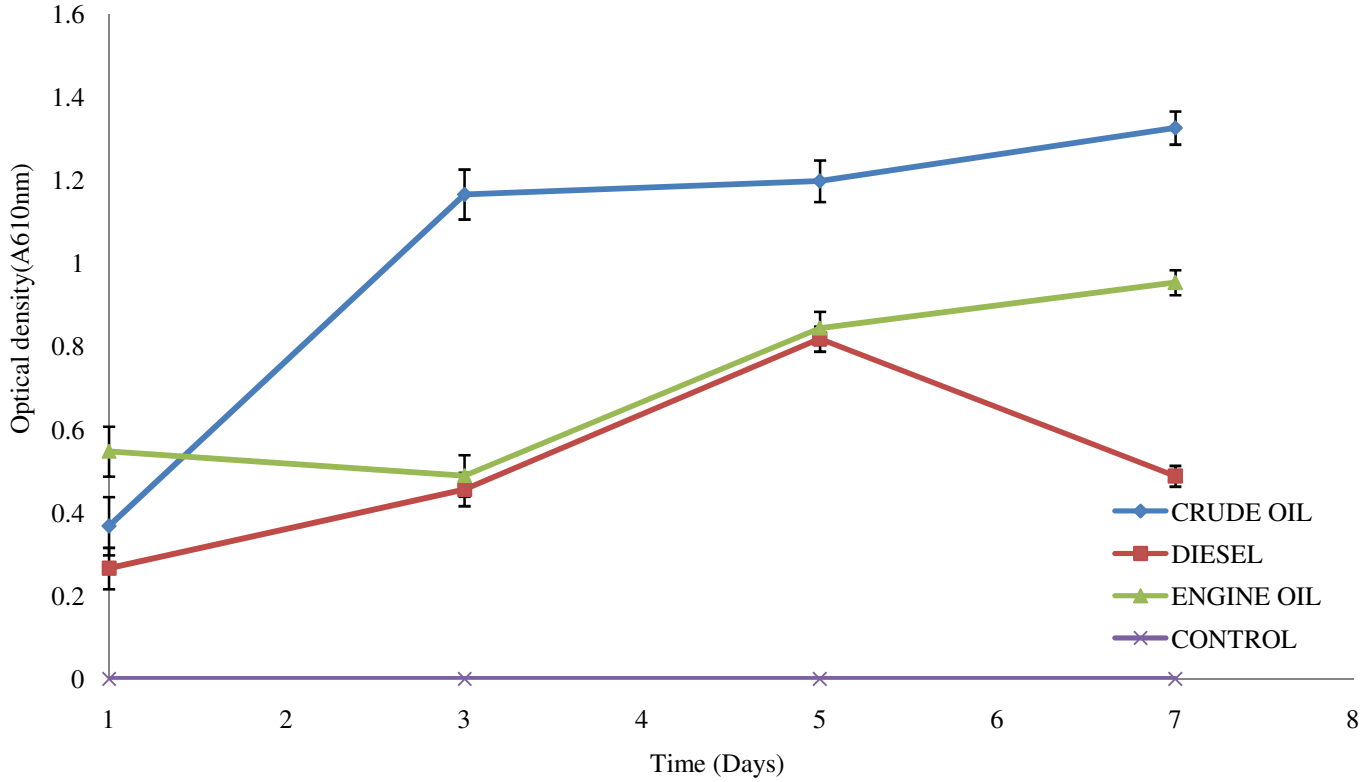

Figure-12: Optical density of Pseudomonas aeruginosa F04_16 in mineral salt broth containing crude oil, diesel and engine oil as sole carbon and energy source.

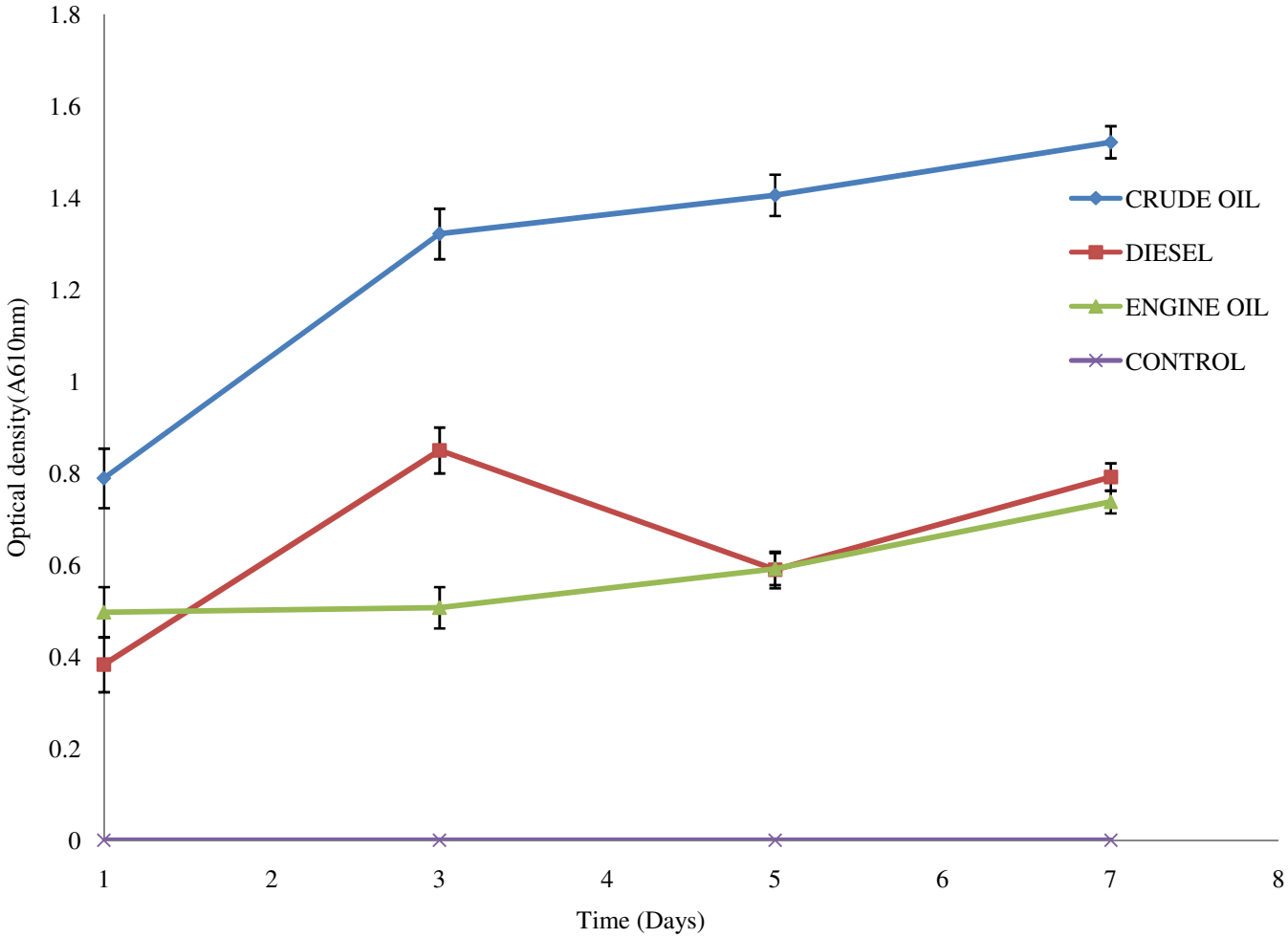

Figure-13: Optical density of *Lysinibacillus sphaericus* E11_14 grown in mineral salt broth containing crude oil, engine oil and diesel as sole carbon and energy source

Conclusion

Based on the results obtained the *Hibiscus cannabinus* associated 13 HUB are efficient utilizers of engine oil, crude oil and diesel, crude oil being unrefined is a better preferred carbon and energy source. Time is a determining factor in the utilization of these hydrocarbons. Isolation of large concentrations degrading bacterial these species for stocking should be within 1-3 days of contact with these hydrocarbons since optimum growth was measured within this time interval.

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