International Journal of Scientific Research in Science, Engineering and Technology (www.ijsrset.com)
© 2020 IJSRSET | Volume 7 | Issue 1 | Print ISSN: 2395-1990 | Online ISSN : 2394-4099
DOI : https://doi.org/10.32628/IJSRSET196658

Biodegradation of used Engine Oil Contaminated Site using Individual and Mixed bacterial isolates

Muh'd A.J., Sa'adatu A.Y., Surayya M. M., Sa'adatu M. I., Nafisa B., Hauwa S. D.
Department of Microbiology and Biotechnology, Faculty of Science, Federal University Dutse, 7156, Dutse-Jigawa State-Nigeria

ABSTRACT

Oil is most widely distributed source of energy in the world and large-scaled environmental pollutant. Oil, oil products, and oil containing industrial waste pollution is ranked second place after radioactive pollution on account of their harmful action to ecosystems. Contamination of soil by organic chemicals (mostly hydrocarbons) is prevalent in oil producing and industrialized countries of the world. Biodegradation, a strategy that uses biological means (i.e microbes) to degrade, stabilize and remove soil contaminants is an alternative green technology remediation of hydrocarbon contaminated soil. This study was carried out to isolate and screen Bacteria capable of degrading used engine oil from oil contaminated site. Soil samples were collected from oil contaminated site. Bacterial species were isolated from the collected soil samples by serial dilution and agar methods. Different bacterial species were isolated but only four were oil degrading isolates. The identity of the various genera of bacterial contaminants were determined by a combination of cultural, morphological as well as preliminary biochemical characteristics of the isolates. The four oil degrading bacteria (AJ1, AJ2, AJ3 and AJ4) were preliminarily identified as Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtillis and Micrococcus spp respectively. The degradation ability of the bacterial isolates was screened and maximum degradation was recorded by AJ 5 (Mixed culture) with 66.9 %, followed by Bacillus cereus (50.3 %), Bacillus subtilis (44 %), Pseudomonas aeruginosa (37.9 %) and the least was seen in Micrococcus spp (35.3 %). These findings revealed that some bacteria species are capable of utilizing the oil and used it as sole source of carbon and energy and the mixed consortia of the bacteria have rapid degradation ability. Biological degradation of hydrocarbon contaminated soil offers a better and more environmentally friendly technique that if properly explored can bring our environment into a better place for both plant and animal.

Keywords: Bacteria, Biodegradation, Hydrocarbon, Oil, Pollution.

I. INTRODUCTION

Rapid industrialization, population growth and complete disregard for environmental health have led to global environmental pollution. Increasing exploration and production activities coupled with improper waste disposal practices has led to widespread contamination of both the aquatic and terrestrial ecological systems (Odokuma and Ikpe, 2003). One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petrochemical industry. From the galaxy of chemicals, heavy metals and polycyclic aromatic hydrocarbons (PAHs) are two of the major environmental pollutants (Dash et al., 2013). Petroleum hydrocarbons pollution (PHP) is becoming increasingly severe with the continuously growing demands for oil and other oil-related products. This form of pollution has attracted

increasing attention owing to its serious harmful effects on both marine and terrestrial ecosystems (Meng et al., 2016).

The release of pollutants into the environment may occur accidentally or due to anthropogenic activities which ultimately results in soil, water and air pollution, leading to many health hazards. From the environment, these chemicals enter into the food chain. The flow of chemicals through the lower constituents of the food chain to different tropic levels imparts risk to the ecosystem, as chemicals tend to bioaccumulate and can be transferred from one food chain to another. Pollutants have been detected in various food chains where the results are usually detrimental to micro-organisms, plants, animals and humans alike (Vinodhini and Narayanan, 2008). The rise in consumption of automotive lubricating oil is a worldwide problem and has increased, ending in large used oil volume and its waste (Ekanem and Ogunjobi, 2017). Contamination of soil by spent lubricating oil is prevalent in oil producing and industrialized countries of the world.

The problem is more severe in the developing countries where there are no effective regulatory policies on the environment (Onuoha et al., 2011). The presence of different types of automobile and machinery has resulted in an increase in the use of lubricating oil. Also, oil spills from industries, filling stations, loading and pumping stations, petroleum product depots during transportation and at auto mechanic workshops, all combine to contribute to soil contamination (Onuoha et al., 2011). Hydrocarbon contamination of the air, soil, fresh water especially by polycyclic aromatic hydrocarbon (PAHs) attracts public attention because many PAHs are toxic, mutagenic, and carcinogenic (Clemente et al., 2001). Prolong exposure of high oil concentration may cause the development of liver or kidney diseases, possible damage to the bone marrow and increased risk of cancer (Mishra et al., 2001; Lloyd and Cackette, 2011).

The problems of pollution have led to the exploration of many remedial approaches to affect the cleanup of the polluted soils. Pollution control strategies involving physico-chemical methods have often aggravated the problem rather than eliminate it. Mechanical methods to reduce hydrocarbon pollution are expensive and time consuming. Biodegradation is favored as a good option for the remediation of polluted sites mainly because it uses inexpensive equipment, environmentally friendly and effective. Bioremediation involves the transformation and breakdown of complex organic molecules through bio stimulation and bio augmentation into simpler substances such as fatty acids, carbon-dioxide and water (Atlas, 1985).

Many microorganisms have the ability to utilize hydrocarbons as sole sources of carbon as energy for metabolic activities and these microorganisms are widely distributed in the nature. Hydrocarbons are primarily biodegraded by Bacteria and Fungi (Samuel, 2011). The microbial utilization of hydrocarbons depends on the chemical nature of the compounds within the petroleum mixture and on environmental determinant (Adeline et al., 2009). Microorganisms are known to convert harmful substances to non-toxic substances. Bioremediation is a new and emerging technology that utilizes the capability of microorganisms for the treatment of chemical spills and hazardous waste. Soil contamination by oil leads to loss in its useful properties such as fertility, permeability, water holding capacity and binding capacity. For the treatment of soil and groundwater contamination, bioremediation is emerging as a promising technology. This technology is very effective in the remediation of petroleum hydrocarbon contaminated soil (Samuel, 2011). Thus, the aim of this study was to isolate and screen the Bacteria that is capable of degrading used engine oil from oil contaminated site.

II. METHODS AND MATERIAL

2.1 Sample Collection

Soil samples were collected in a sterile polythene bag from soil contaminated with used engine oil in Mechanic village and Awajil Auto mechanic site, Dutse, Jigawa State, Nigeria. The soil samples were taken to the laboratory immediately for analyses.

Used Engine oil: was collected from Mechanic village and Awajil General Enterprises.

2.2 Media Preparation

All media were prepared according to the manufacturer's instruction.

2.3 Isolation of Bacteria from oil Contaminated Soil

Bacterial species were isolated from the collected soil samples by serial dilution and agar methods wherein the soil sample was diluted from 10^{-1} to 10^{-5} dilutions, and the diluted soil samples were spread on sterile Nutrient agar plates. The inoculated plates were incubated at 37^{o}C for 24 hours (Jahir and Syed, 2011). Mixed cultures obtained after incubation were purified by quadrant streaking on sterile NA plates. The purity of the cultures was cross checked by gram staining procedure and the isolates were named as AJ1, AJ2, AJ3 and AJ4 tentatively.

2.4 Identification of the isolated Bacteria

In order to identify the purified cultures tentatively on the basis of Bergey's manual (Aneja, 2003) various staining and biochemical tests were performed namely Grams staining, spore staining, Vorges Preskers test, Catalase test, Oxidase test, Mixed acid fermentation, Citrate test and Indole test.

2.4 Screening of Bacterial Isolates for Biodegradation of Used Engine Oil

Oil degradation studies of purified cultures was performed against used oil obtained from two sites in Dutse, wherein the components for preparing 100 ml Nutrient broth were dissolved in 75 ml distilled water and 25 ml of used engine oil was added, pH was maintained to 7. Media was autoclaved at 121 ^{0}C for 15 minutes. Cooled media (with oil) was innoculated with 1 ml of 24-hour old grown culture of the respective pure cultures in the first four flasks and the fifth flask was inoculated with mixed culture of the isolated bacteria. The innoculated flasks were incubated at 120 rpm in a shaking incubator at 37 ^{0}C for 10 days. Width of oil and media layer in the flask was recorded on zero day and 10th day. And also the oil degradation was quantified by studying the oil recovery after 10th day of incubation. In this way the oil degradation study was carried out for all the purified cultures (Jahir and Syed, 2011 with slight modification).

2.5 Biodegradation of Contaminated Soil using Isolated Bacterial Specie

According to Oyeleke et al., (2008), four separate flasks of 250ml set up were mounted for each identified isolate and one separate flask was mounted with the mixture of the four isolated bacterial samples. One is to examine the action of individual and mixed bacterial isolates. The pH was adjusted to 7 using sodium hydroxide and hydrochloric acid solution. Then the flasks were sterilized at 121^{0}C for 15 minutes. Then the flasks were inoculated under aseptic conditions with 3ml suspension of isolated bacterial specie into 250ml conical flasks containing 200ml of sterile soil sample. The flasks were incubated in a shaking incubator at 200rpm for 10 days at 37^{0}C. Samples were drawn at the interval of 48 hours for observation. Three millilitre of each sample were filtered and centrifuged at 5000rpm for 20 minutes. Biodegradation of the samples were determined by monitoring the decrease in absorbance at the maximum wavelength of soil samples (λmax. 600nm) by using a UV- visible spectrophotometer (Spectrumlab 755s). The rate of biodegradation was expressed in percentage.

$$\% \text{ Biodegradation} = \frac{\text{Initial absorbance} - \text{Final absorbance}}{\text{Initial absorbance}} \times 100$$

III. RESULTS AND DISCUSSION

3.1 Morphology and Gram Reaction of the Bacteria Isolated from Oil Contaminated Soil

Different bacterial isolates were isolated from used engine oil contaminated soil samples. The bacterial isolates were identified using morphology and gram reaction. Table 1 shows the morphology and gram reaction of the bacterial isolates.

Table 1 : Morphology and Gram Reaction of the isolated bacteria

Bacterial Isolates	Shape	Colour	Grams reaction
AJ 1	Rod	White	+
AJ2	Rod	Cream	-
AJ3	Rod	Cream	+
AJ4	Cocci	White	+

Keywords: + Positive - Negative

3.2 Biochemical Characteristics of Bacterial Isolates from Oil Contaminated Soil

Table 2 shows the biochemical tests conducted on the degrading isolates. Various biochemical tests and staining techniques were carried out so as to identify the bacterial species. The four oil degrading bacteria AJ1, AJ2, AJ3 and AJ4 were identified as Bacillus cereus, Pseudomonas aeruginosa, Bacillus subtillis and Micrococcus spp respectively.

Table 2 : Biochemical Characteristics of bacterial isolates from oil contaminated samples

Isolates	Spore	Indole	Cat	Oxi	VP	Cit	MAF	Suspected org.
AJI	+	-	+	+	-	+	-	Bacillus cereus
AJ2	-	+	+	+	-	+	-	P. aeruginosa
AJ3	+	-	+	-	+	+	-	Bacillus subtillis
AJ4	-	-	+	+	-	-	+	Micrococcus spp

Key words:

+ Positive
- Negative
Ind – Indole
MAF – Mixed Acid Fermentation
VP – Vorges Preskers
Cit – Citrate test
Oxi – Oxidase test
Cat – Catalase test
Spore – Spore staining

3.3 Screening of the Degradation ability of the individual and mixed Bacterial Isolates on Used Engine Oil

The degradation ability of the bacterial isolates was screened by measuring the volume and the width of the used engine oil in conical flasks after the 10th day.

Table 3 and 4 below shows the quantification oil degradation by two methods used in this study. From the tables below the isolate AJ4 shows the minimum degradation and AJ5 (mixed culture) shows the maximum oil degradation in both the two parameters followed by AJ3 which is an individual isolate.

Table 3 : Oil degradation studies (oil recovery)

Bacterial isolates	Volume of oil on zero day (ml)	Volume of oil on 10thday (ml)
AJ1	25	13.5
AJ2	25	15.0
AJ3	25	11.0
AJ4	25	18.5
AJ5 (Mixed isolates)	25	5.5

Table 4 : Oil degradation studies (width)

Bacterial isolates	Width of oil on zero day (mm)	Width of oil on 10thday (mm)
AJ1	6	4.0
AJ2	6	4.5
AJ3	6	3.5
AJ4	6	5.0
AJ5 (Mixed isolates)	6	2.5

3.4 Determination of the Biodegradation of the Bacterial Isolates using Spectrophotometer

Fig. 3.1 shows the biodegradation ability of the individual and mixed culture of the microbial isolates. Maximum degradation was recorded by AJ 5 (Mixed culture) with 66.9 %, followed by Bacillus cereus (50.3 %), Bacillus subtilis (44 %), Pseudomonas aeruginosa (37.9 %) and the least was seen in Micrococcus spp (35.3 %).

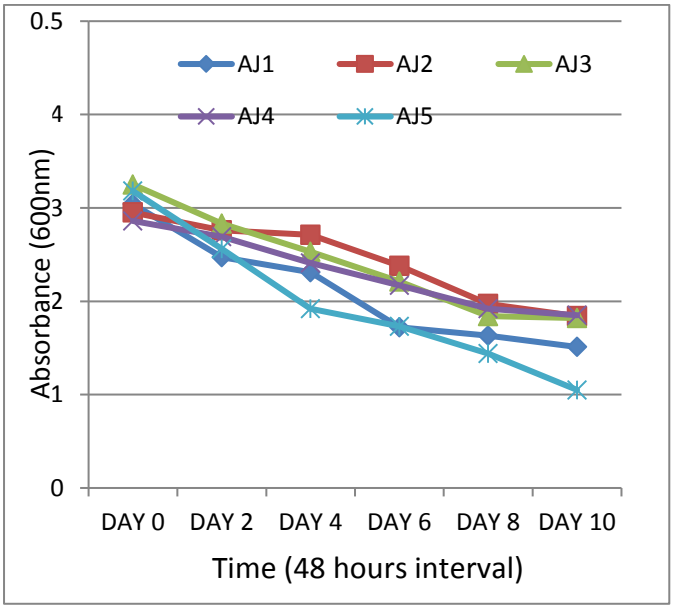

Fig. 4.1 Graph of Absorbance against Time

From the figure 4.1 above, it was noticed that there was a slow rate of degradation in the samples AJ1, AJ2, AJ3 and AJ4 from day 8 to day 10. The slow rate of degradation occurs because the isolated bacteria is exposed to the pollutant (used engine oil) for a prolonged period of time and hence results in limited metabolic activity. To revive the rate of biodegradation activity in the samples, growth-stimulating nutrients such as nitrogen and phosphorus should be added, a process called Biostimulation or through the addition of specialized microbial culture into the samples in a process called Bioaugmentation. These processes ultimately promotes the growth of the microbes and subsequently the rate of biodegradation is increased. Biostimulation is generally used concomitantly with bioaugmentation to improve survival of the added cells and/or to optimize their metabolic capabilities, thus resulting in robust long term biodegradation efficiency. Fig. 4.2 below shows the percentage degradation of the individual and mixed bacterial isolates.

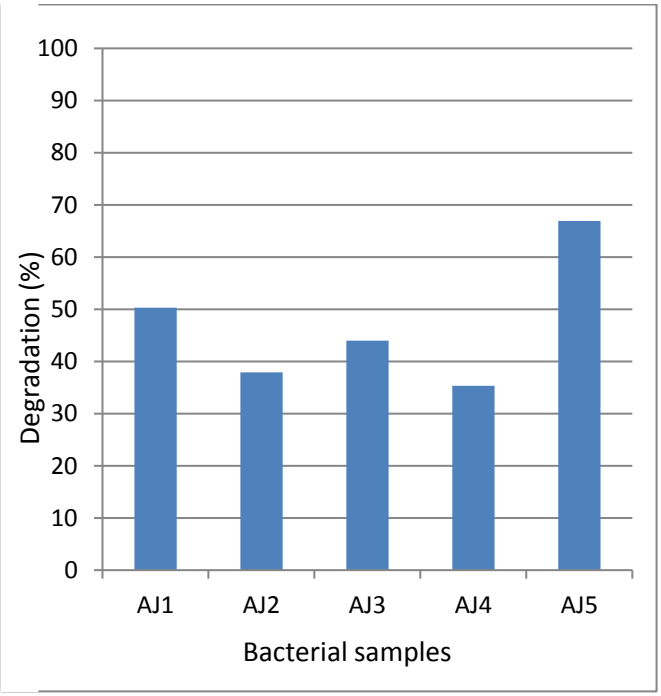

Fig. 4.2 Percentage degradation of the bacterial isolates

Discussion

Soil samples were collected from two oil contaminated sites i.e Mechanic village and Awajil Auto mechanic site as done earlier by (Ojo, 2006; Okoh, 2003; Emtiazi, et al., 2005). In motor mechanic shop there is constant change in microorganism of oil contaminated soil as the colour and texture changes. Further, Bacteria was isolated by serial dilution method and agar plating method as done previously by (Udeani et al., 2009). Cultures were purified by quadrant streaking technique and the purity was cross checked by Grams staining procedure. Purified cultures were characterized for the various staining and biochemical activities and was compared with Bergey's manual which serves as a guide in identifying the isolated microbes as done earlier by (Udeani et al., 2009).

The individual and mixed isolates were screened for their biodegradation ability on the used engine oil by measuring the volume and the width of the used engine oil after incubation for 10 days. The isolate showing maximum oil degradation abilities was the mixed microbial culture containing all the four isolated microbial species with a massive reduction in quantity from 25 ml to 5.5 ml within the period of 10 days. Many studies have confirmed that a consortium of microorganisms degrades used engine oil rapidly within a short period of time through synergistic activity as no single microorganism is capable of degrading used engine oil completely. The mixed consortia of microorganisms were able to degrade the used engine oil by utilizing the oil as their sole carbon source through the use of some strategies which are; use of constitutive enzymes, production of biosurfactants, co-metabolism, enzyme induction etc.
The individual isolates that shows maximum biodegradation was Bacillus subtilis. Few studies (Annweiller et al., 2000); (Ijah and Antai, 2003); (Rahman et al., 2002; Sepahi et al., 2008) have been reported on the roles of Bacillus spp. in hydrocarbon biodegradation; although there are several reports on biodegradation of pollutants by the action of Bacillus spp. occurring in extreme environments. (Ijah and Antai, 2003) reported Bacillus spp. as being the predominant isolate of all the crude oil utilizing bacteria characterized from highly polluted soil samples (30 and 40 % crude oil). It has been postulated that Bacillus subtilis are more tolerant to high levels of hydrocarbons in soil due to their resistant endospores, a feature common to all Bacillus spp. There is growing evidence that isolates belonging to the Bacillus spp. could be effective in clearing oil spills (Ghazali et al., 2004).

Also, the contaminated soil samples were dissolved in dislilled water and the microbial isolates were inoculated in the solution, then the decrease in absorbance for each solution was monitored using spectrophotometer (Spectrumlab 755s, made in China) at 48 hours interval for the period of 10 days. The decrease in absorbance of the mixed culture sample (AJ5) appeared to be more than that of the remaining samples with Biodegradation ability of 66.9 % within the period of 10 days. This is also unprecedented as it was mentioned earlier that the mixed consortia microorganisms have rapid degradation ability. For the individual isolates, Bacillus cereus appeared to have the maximum degradation with percentage degradation of 50.3 % which also has a resistant endospore.

IV.CONCLUSION

Four different species capable of degrading used engine oil were isolated from oil contaminated site. The experimental study conducted confirmed that mixed microbial consortium as a result of their diverse metabolic activities and individual microbial isolates are potential degraders of hydrocarbon contaminants (used engine oil) in soil. Employing the activities of these microbes in a conical flask and incubating in a shaking incubator to remove or degrade hydrocarbon contaminants (oil) thus proved

to be an efficient technique in dealing with hydrocarbon contaminants in a soil.

Degradation of hydrocarbon contaminated soil is necessary in order to have a safe and healthy environment that will in turn result in healthy lifestyle across the world. Biological degradation of hydrocarbon contaminated soil offers a better and more environmentally friendly technique that if properly explored can bring our environment into a better place for both plant and animal and animal well-being due to its enormous advantages over other treatment methods. However, despite these enormous advantages of biological treatment methods, its potential is yet to be fully utilized in restoration of oil contaminated soil. This is due to the fact that it takes a long period of time for the complete restoration of the environment. This limitation can be overcome through nutrient addition and introduction of microbes with degradative capability to degrade hydrocarbon.

Conflict of Interest

The Authors declare that there is no existence of conflicting interest in this research financially or otherwise.

V. REFERENCES

[1]. Odokuma, L. O and Ikpe, M. D. 2003. Role of composition on the degradability and toxicity of drillingmuds. Afri. J. Appl. Zool. Environ. Biol. 5: 6-13.

[2]. Vindhoni R., and Narayanan M., 2008. Bioaccumulation of heavy metals in organs of fresh water fish. Int. J Environ. Sci. Tech. 5(2) 179-182.

[3]. Atlas, R.M., (1981). "Biological Degradation of Petroleum Hydrocarbons: An Environmental Perspective". Microbiol. Rev. 45, pp.180-209.

[4]. Samuel A. A. (2011). "Biodegradation of used lubricating engine oil contaminated water using indigenous hydrocarbon degrading microbes in a fixed bed bioreactor system."

[5]. Nilanjana D., & Preethy C., (2010). "Microbial Degradation of Petroleum Hydrocarbon Contaminants: An Overview," Biotechnology Research International, vol. 2011, Article ID 941810, 13 pages, 2011. doi:10.4061/2011/941810.

[6]. Jahir A.K. and Syed H.A., (2011). "Isolation amd characterization of micro organisms from oil contaminated sites." Advances in Applied Sciences Research, 2011, 2(3):455-460

[7]. Aneja, K. R., (2003), " Identification of Bacteria isolated from highly contaminated soil samples using Biochemical tests"

[8]. Oyeleke E. O., and Manga K. I., (2008) "Isolation and Characterization of Bacterial collected from spent oil contaminated sites."

[9]. Udeani CKT, Obroh A A, Okwuosa NC, Achukwu U P, and Azubike N, 2009, African J.

[10]. Annweiller E, Richnow HH, Antranikian G, Hebenbrock S, Garms C, Franke S, Francke W, Michaelis W, Appl. Environ. Microbiol, 2000, 66:518-523.

[11]. Ijah UJJ and Antai SP, Biodegradation, (2003), 51: 93-99.

[12]. Rahman KSM, Lakshmanaperumalsamy P, Tahira RJ, Banat IM, 2002, Bioresour. Technol, 85: 257-261.

[13]. Sepahi AA, Golpasha DI, Emami M and Nakhoda MA, 2008, Iran. J. Environ. Health. Sci. Eng, 5(3): 149-154.

[14]. Ghazali FM., Rahman., RNZA., Salleh, A.B., Basri, M. (2004). Int. Biodeterior. Biodegradation, 54: 61-67.

[15]. Okoh IA, African J. Biotechnol, 2003, 2(5):104-108. Emtiazi G, Shakarami H, Nahvi I and Mirdamadian SH, African J. Biotechnol, 2005, 4(2): 172-176.

[16]. Ojo AO, African J. Biotechnol , 2006, 5 (4):333-337.

[17]. Dash H.R., Mngwani N.,Chakraborty J., Kumari S., Das S., 2013 Marine bacteria: potential

candidates for enhanced bioremediation Appl Microbiol, Biotechnol. 97(2), 561-571.

Cite this article as :

Muh'd A. J., Sa'adatu A.Y., Surayya M. M., Sa'adatu M. I., Nafisa B., Hauwa S. D., "Biodegradation of used Engine Oil Contaminated Site using Individual and Mixed bacterial isolates", International Journal of Scientific Research in Science, Engineering and Technology (IJSRSET), Online ISSN : 2394-4099, Print ISSN : 2395-1990, Volume 7 Issue 7, pp. 38-45, January-February 2020. Available at doi : https://doi.org/10.32628/IJSRSET196658
Journal URL : http://ijsrset.com/IJSRSET196658