International Journal of Educational Research and Studies
www.journalofeducation.net
Online ISSN: 2664-6811; Print ISSN: 2664-6803
Received: 05-08-2019; Accepted: 06-09-2019; Published: 13-09-2019
Volume 1; Issue 4; 2019; Page No. 23-29

Crude oil biodegradation using bacterial isolates from river Nile state under aerobic conditions

Nada M Doleib[1, 2], Sulaiman E[3], Hend M Tag [1, 4]
[1] Department of Biology, Faculty of Sciences and Arts, University of Jeddah, Saudi Arabia
[2] Department of Microbiology, Faculty of Applied and Industrial Science, University of Bahri, Khartoum, Sudan
[3] Department of Botany, Faculty of Science and Technology, University of Shendi, Sudan
[4] Department of Zoology, Faculty of Sciences, Suez Canal University, Egypt

Abstract
One-hundred and twenty five strains of petroleum degrading bacteria were isolated from hydrocarbons contaminated soil and wastewater sink at Aljayli refinery in River Nile State, Sudan. Column Gas Chromatography used to detect the residual oil after incubation of isolates in basal medium. The most potential isolates were tentatively identified as: *Pseudomonas aeruginosa, P. putida, Serratia marcences, Cedeca davicae, Enterobacter cloacae, Yersinia enterocolytica and Citrobacter freundi. S. marcences* exhibited the highest emulsification ability to crude oil, nC8 to nC16 was nearly completely disappeared followed by *P. putida* which was active in degradation nC8-nC15 (99%-96%) while nC35-nC37 completely degraded. *P. aeruginosa* represent approximately the same results except nC9 which recorded (84%). *Citrobacter freundii*, was the lowest isolate in degradation of nC8 and nC9 but high for nC10-nC13. It is realized that isolates were able to degrade n-alkane more than total petroleum hydrocarbons.

Keywords: biodegradation, crude oil, gas chromatography, aerobic condition

1. Introduction

Pollution of soil and water environments by crude oil has been, and is still today, an important problem. Crude oil is a complex mixture of thousands of compounds. Among them, alkanes constitute the major fraction. Alkanes are saturated hydrocarbons of different sizes and structures. Although they are chemically very inert, most of them can be efficiently degraded by several microorganisms (Janssen *et al*., 2005). (Rojo, 2009) [1, 2].

The contamination of soil caused by accidental leakage or chronic release of crude oil and refined products to the environment occurs yearly with growing industrialization and demands for energy (Kadafa, 2012) [3]. Contamination by aromatic hydrocarbons has been increasing over the years, due to its use in several industrial segments. Hydrocarbons are described as extremely pollutant, toxic, with carcinogenic and mutagenic potential for humans. The concern with these compounds increases due to the difficulties in removing them from the environment (Calvo *et al*, 2009; Souza *et al*, 2014) [4, 5]

Oil production has a significant impact on the landscape and local environment. Contamination of soil and water is a major common consequence of oil production, particularly in areas with nonexistence or not enforced environmental regulation. For example in Sudan, Hegleg oil field considered of the main oil reserves. Which has a bad impact on the environment directly as well for both surface water and underground water.

Biodegradation is one of the primary mechanisms for elimination of petroleum and other hydrocarbon pollutants from the environment. It is considered an environmentally acceptable way of eliminating oils and fuel because the majority of hydrocarbons In crude oils and refined products are biodegradable. Petroleum hydrocarbon compounds bind to soil components and are difficult to remove and degrade (Calvo *et al*., 2009) [4].

Hydrocarbon-utilizing microorganisms are ubiquitously distributed in the marine environment following oil spills. These microorganisms naturally biodegrade numerous contaminating petroleum hydrocarbons, thereby cleansing the oceans of oil pollutants. Bioremediation, which is accomplished by adding exogenous microbial populations or stimulating indigenous ones, attempts to raise the rates of degradation found naturally to significantly higher rates. Seeding with oil degraders has not been demonstrated to be effective, but addition of nitrogenous fertilizers has been shown to increase rates of petroleum biodegradation (Atlas, 1995) [6]. Oily wastewater, especially from oil field, has posed a great hazard for terrestrial and marine ecosystems.

The single most significant environmental issue for crude oil production facilities in Sudan is the disposal of produce water, especially in Hegleg oil field. Produced water is conventionally treated through different physical, chemical, and biological methods. Microorganisms were given more attention in bioremediation of polluted environments as well as the oil exploitation considering their adaptation to the extreme environment (Das and Chandran, 2011) [7], capabilities to produce biosurfactant (Shekhar *et al*., 2015) [8] and metabolic potential to degrade the hydrocarbons. The objective of this study was to evaluate the biodegradation of crude oil by different strains isolated from different hydrocarbon contaminated soil and form waste produced water.

2. Materials and Methods

2.1 Collection of Samples

Soil samples were collected from petroleum hydrocarbons contaminated area in River Nile State, Sudan. For soil sample a scoop was used to remove debris of organic particles from the surface of the soil. One Kg soil sample at a depth of 0-10 cm was collected from sites at random. Samples were kept in separate sterilized plastic bags, stored in an ice-bath and transported immediately to the laboratory.

Water samples were collected from Aljayli refinery particularly from water treatment sinks of produced water in sterile container then transported to the laboratory. Crude oil was obtained from Aljayli refinery.

2.2 Cultivation and isolation of petroleum degrader bacteria:

2.2.1 Isolation of Bacteria strains

One gram of soil samples or 10 ml of produce water was suspended in 250 ml Erlenmeyer flask containing 100ml of basal salt medium which contain g 2.5 g/L NaCl, 4.74 g/L K_2HPO_4, 0.56 g/L KH_2PO_4, 0.5 g/L $MgSO_4.7H_2O$, 0.1 g/L $CaCl.6H_2O$ and 0.5 g/L NH_4NO_3 in dH_2O. 1% v/v of crude oil was added as a sole carbon source. The mixture was shaken at 250 rpm and incubated at room temperature for 10 days. After this period, a volume of one ml of each flask was plated on nutrient agar medium in petri dish and incubated at 37 °C for 48 hours.

2.2.2 Identification of bacterial strain

Identification of bacterial strain was performed according to Olutiola *et al*. (2000) [9]; Fawole and Oso (2004) [10] and Cheesbrough (2006) [11] who describe biochemical characterizations.

2.3 Crude oil degradation

When colonies appear it picked out and then subcultured on agar plate several times until pure strains obtained. The pure colonies tested for their degradation ability by adding a loop full of purified strains into 250 ml shake flask containing 100ml of mineral salt medium and one ml crude oil as a carbon sours the flasks were shaken at a room temperature of 25 ± 2 on a rotary shaker at 250 rpm for 10 days. Growth of bacteria indicates the capability of degradation. The degree of degradation estimated visually. The visual observation was based on the change in the color of crude oil, clearance of the medium, microbial growth and the disappearance of the oily surface. The results were recorded in the form of pluses (1-4 pluses). The best crude oil degrader bacteria were selected and tested for residual oil in flask using gas chromatography.

2.4 Extraction and analysis of oil:

Method of extraction of residual oil was modified from those described by Primandari *et al*. (2013) [12]. Oil was extracted from cultures by liquid – liquid extraction as follows:

Sixty ml of normal hexane was added to fermentation flask and shaken well until the oil dissolved. The mixture was pure in separating funnel. Bacterial cells were removed by centrifugation at 30.000 for 15 min. The normal hexane was evaporated with heat at 70^o C. The samples to be analyzed were injected into a gas chromatograph in Central Petroleum Laboratories, Khartoum. Di- Chloromethylen was added to the samples to be lighter.

2.5 Gas chromatography

The samples to be analyzed were injected into a gas chromatograph in Central Petroleum Laboratories, Khartoum. Di- Chloromethylen was added to the samples to be lighter. Analyses were performed on a Varian 3800 GC. (Beaconsfield, Buckinghamshire, UK) with the FID detector at 250°C. Nitrogen was used as the carrier gas at inlet pressure of 80.4 kPa. The Pyro-probe® interface was directly connected to the split/splitless injector (1/60 split ratio) and heated at 300°C. Chromatographic separation was performed on a Supelco (Bellefonte, PA) SPBTM OCTYL fused, silica capillary column 60 m x 0.25 mm x 1.0 tm. GC oven temperature was programmed at 40°C with a ramp rate of 4°C/min up to 250°C, holding for 76.50 min. Data recording was carried out using Millennium32® software supplied by Waters (Milford, MA).

Petroleum samples to be analyzed (0.5 tl to 1.0 tl) were intro-duced on the surface of the adsorbent with a microsyringe (Zeng *et al*., 2012) [13].

3. Results & Discussion

Degradation percentage of oil

The best eleven isolates were selected to test their degree of degradation they attained in an oil-mineral medium (table 1). There was a variation in degradation depending on the isolate, isolate N27.122 showed the greatest percentage of degradation it gave 72%, while isolate N27.120 recorded 62%. Other more isolates had percentage of degradation range from 58% to 31%. The aromatic hydrocarbons are complex pollutants and therefore less easily degraded. In many ecosystems, there is already an adequate indigenous microbial community capable of extensive oil biodegradation, as these environmental conditions are favorable for oil-degrading metabolic activity. The ability to utilize hydrocarbons is distributed among a diverse microbial population. This population occurs in natural ecosystems and either independently or in combination metabolizes various hydrocarbons (Olajire and Essien, 2014) [14]

Isolation and characterization of crude oil degrading strains

Morphological and Biochemical characterization of isolates were identified in table 2. All isolated strains were gram negative and single short rod. They were tentatively identified as follows: two isolates (S20.94 and S20.95) as *Pseudomonas aeruginosa*, two strain (S2096, and N27.120 as *Pseudomonas putida*, other strain as (N27.122) *Serratia marcences*, strain (D1.2 and D2.10) as *Cedeca davicae*, (11.54 and D9.49) as *Enterobacter cloacae*, (D11.55) as *Yersinia enterocolytica* and (W23.103) as *Citrobacter freundii*.

Analysis of residual hydrocarbons in liquid culture by gas chromatography GC

Gas chromatography was used to detect residual oil in fermentation flasks for the best performing three isolates and one isolate that had weak performance. The analysis profile of crude oil before fermentation was taken as the standard to compare fermented oil (Figure 1) with two hundred and twelve beaks obtained.

N27.122 (*Serratia marcences*)

Isolate exhibited the highest emulsification ability and though the strongest adherence to the oil from all investigated

microorganisms in this study (Figure 2). It was not only the better microorganisms in degradation but also the better alkane degrader. Its average percentages of degradation was 57.89%. The results showed that nC8 to nC16 (Figure 3) was nearly completely degraded as these are small molecules then degradation percentage decreased gradually from nC17- nC38, until 5%, while nC40 recorded 27%. Comparing with the control there is decrease in the peaks of a wide range of compounds from nC8-nC28, then it decrease. There are limited reports describing the involvement of Serratia in biodegradation of hydrocarbons (Rojas-Avelizapa *et al*. (2002) [15], and they are mostly degraders of aromatic compounds. It was observed that this strain not only utilized aromatic compounds but also long-chain alkanes (Wongsa *et al*; 2004) [16].

Isolate N27.120 (*Pseudomonas putida*)

Is more active than N27.122 in the range nC8-nC15and the range of nC41-nC44 (Figure 4). The average percentage for this isolate was 62.38%. The degradation percentage of nC8-nC15 was 99%-96%. There was decreased in degradation from nC16-nC34, however, degradation increased again until 86% for nC42. In addition, nC35-nC37 completely disappears.

As shown in Figure 5; the average percentage of degradation was 70.47% for isolate S20.94 (*Pseudomonas aeruginosa)*. There was high degradation percentage of rang form nC8-nC17 (99%-91%) except nC9 which recorded (84%). Percentage decreased for nC18-nC38, then increased till (91%) for nC40.

Pseudomonas is a common bacterium capable of degrading hydrocarbons (Das & Brooijmans *et al*., 2009) [17]. Therefore, it is not uncommon for a number of Pseudomonas strains capable of degrading petroleum hydrocarbon should be isolated from areas receiving petroleum waste discharges. This bacteria have the ability to grow on both aromatic and aliphatic fractions of petroleum (Brzeszcz and Kaszycki, 2018) [18], which demonstrated the broad substrate spectra of this genus not only on hydrocarbons but also on diverse range of xenobiotic compounds (Amund and Adebiyi, 1991; Wackett and Hershberger, 2001; Parales *et al*., 2002) [19, 20, 21]. Similarly, Christopher and Christopher (2004) have demonstrated the importance of Pseudomonas species in the early stage of petroleum land treatment unit.

Pseudomonas putida strains were isolated from crude oil contaminated soil by many researchers it showed high ability to degrade oil (Vinothini *et al*; 2015) [22]. It is able to degrade hydrocarbons such as benzene (Meliani and Bensoltane, 2014) [23], toluene, p-xylene (Otenio *et al*. 2005) [24], and phenol (Hasan and Jabeen, 2015) [25]. Also this strain isolated from different soil locations contaminated with fuel spills, it demonstrated its ability to degrade diesel fuel (Xu *et al*., 2018) [26].

Pseudomonas aeruginosa was found in a consortium of bacteria from sandy and loamy soils that can degrade hydrocarbons in light fuel oil (Hawle-Ambrosch *et al*. 2007) [27]. It was also shown that *P. aeruginosa*, can grow on hydrocarbon as sole carbon source and is a good degrader of oil (Wu *et al*., 2018) [28]. Prevalence of members of the genus *Pseudomonas* in tested soil samples and waste water confirms previous reports about the widespread distribution of such bacteria in hydrocarbon-polluted soils and reflects their potential in utilizing these hydrocarbon contaminants for growth and thus able to eliminate oil particle for waste water and soils.

This study demonstrated that isolate W23.103 (*Citrobacter freundii*), was the lower isolate in degradation of nC8 and nC9, but high degradation of nC10-nC13 as shown in Figure 6. The degradation decreased gradually from nC23-nC34, but nC35 gave 16%. Besides the result displayed a high percentage for nC41 and nC42. The average percentage of degradation is for crude oil by *Citrobacter freundii* was 59.89%.

As mentioned previously, many microorganisms (Rojo, 2009; Wang and Shao, 2013) [2, 29] contain several sets of alkane degradation systems, each one being active on a particular kind of alkane or being expressed under specific physiological conditions. Varjani (2017) [30] evaluated the biodegradation potential of ten species of hydrocarbonoclastic bacteria, almost all isolates showed 50% or more degradation of kerosene except F.S19a (*C. freundii*) which showed only 14.13% degradation. Also *C. freundii* exhibited lowest engine oil degradation capacity. In contrast the work done by Singh and Lin (2008) [31] under standard degradation conditions showed that *Citrobacter freundii* (MRC3) achieved 86.94% of diesel degradation in 2 weeks.

Various microorganisms, including bacteria, filamentous fungi and yeasts, can degrade alkanes (Rojo, 2009; Palanisamy *et al*., 2014) [2, 32]. Notably, some recently characterized bacterial species are highly specialized for hydrocarbon degradation. These species are called hydrocarbonoclastic bacteria (HCB), and they play a key role in the removal of hydrocarbons from polluted and non-polluted environments (Teramoto *et al*., 2013; Berry and Gutierrez, 2017, Thompson *et al*., 2017) [33, 34, 35].

In agreement with Ibrahim (2016) [36] the present study demonstrated the potential of bacterial strain *C. freundii* for degradation of crude oil with percent equal 59.89. The author displayed that the ability of *C. freundii* in biodegradation of used engine oil (UEO) where the bacterial strains started utilization of UEO as sole energy and carbon sources.

4. Conclusions

In conclusion, identified isolates present in this study displayed as potent hydrocarbon degradation. The pattern of degradation showed that the microorganisms first attacked the lower and higher hydrocarbon chains and those of middle length were attacked at a lesser extent.

Table 1: Amount of Crude oil degraded and Percentage Degradation for the best strains.

Isolates code	Weight after degradation	Percentage degradation (%)
D.11.55	0.67	31%
D.1.2	0.49	33%
W.23.103	0.60	36%
D.9.49	0.49	40%
D.2.10	0.69	51%
D.11.54	0.42	51%
S.20.96	0.44	52%
S.20.95	0.48	56%
S.20.94	0.64	58%
N.27.120	0.38	62%
N.27.122	0.28	72%

Weight of crude oil before degradation was 1 gm.

Table 2: Morphological and Biochemical characterization of isolates

Tests	**N27.122**	**N27.120**	**W23.103**	**S20.94**
Shape	Rod	Rod	Rod	Rod
Gram reaction	-ve	-ve	-ve	-ve
Motility	+	+	+	+
Growth on air	+	+	+	+
Catalase	+	+	+	+
Oxidase	-	+	-	+
Fermentation:				
Glucose	+	+	+	+
Lactose	-	-	+	-
Maltose	+	-	+	-
Mannitol	+	-	+	+
Fructose	+	+	0	+
Indole	-	-	-	-
Lysine	+	-	-	-
Xylose	-	+	-	+
Citrate	+	-	+	-
Urease	-	-	+	-
Starch hydrolysis		-	-	-
Nitrate to nitrite	+	-	+	+
Voges Proskauer	+	-	+	-
Pigment	+	-	+	+
Growth at 42°C		-		+
Growth at 5°C		-		-
Utilization of citrate	+	+		+
Tentative genus	*Serratia marcences*	*Pseudomonas. putida*	*Citrobacter freundii*	*Pseudomonas aeruginosa*

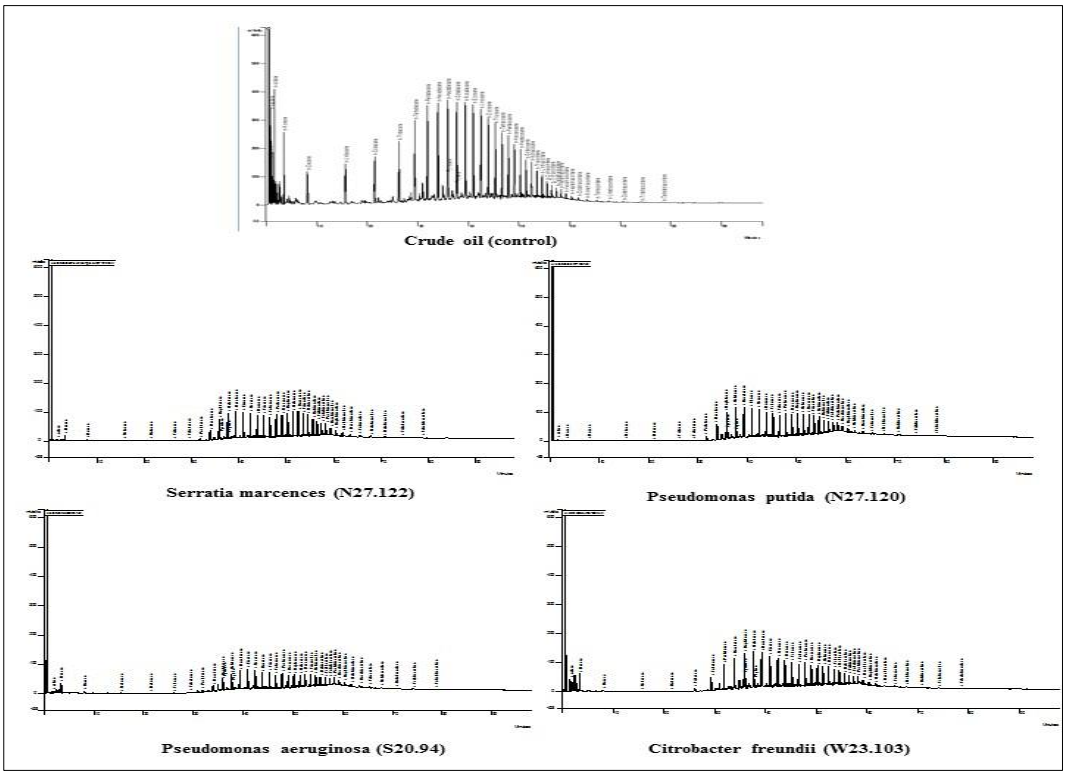

Fig 1: chromatographic analysis for crude oil degradation by different bacterial strains isolated from different contaminated soil and from produced wastewater collected.

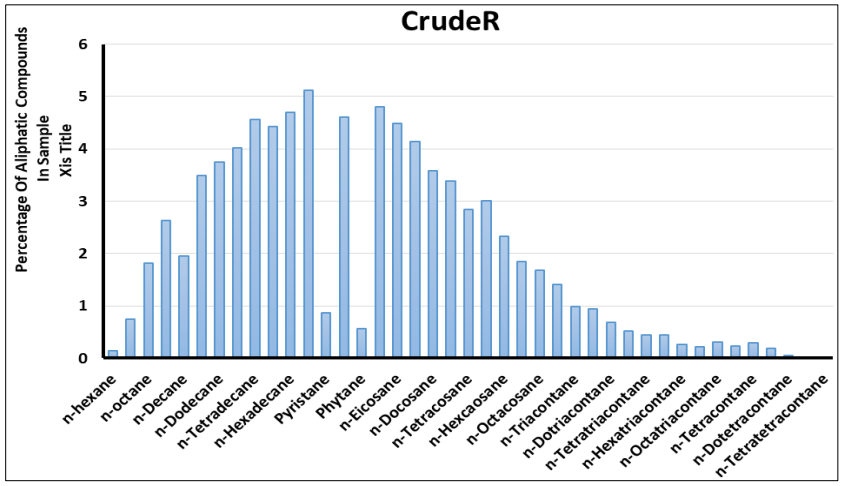

Fig 2: Mass spectra crude oil before biodegradation.

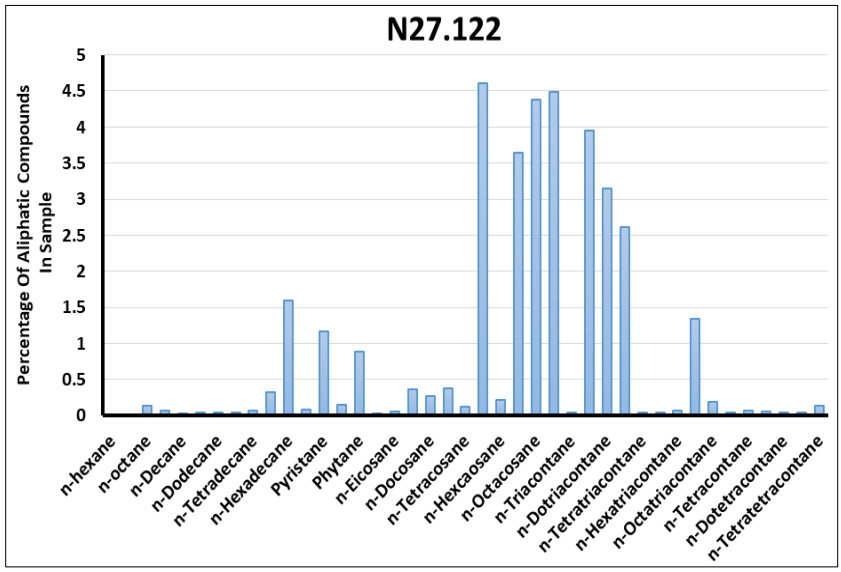

Fig 3: Mass spectra crude oil after biodegradation by isolate N27.122 (*Serratia marcences*)

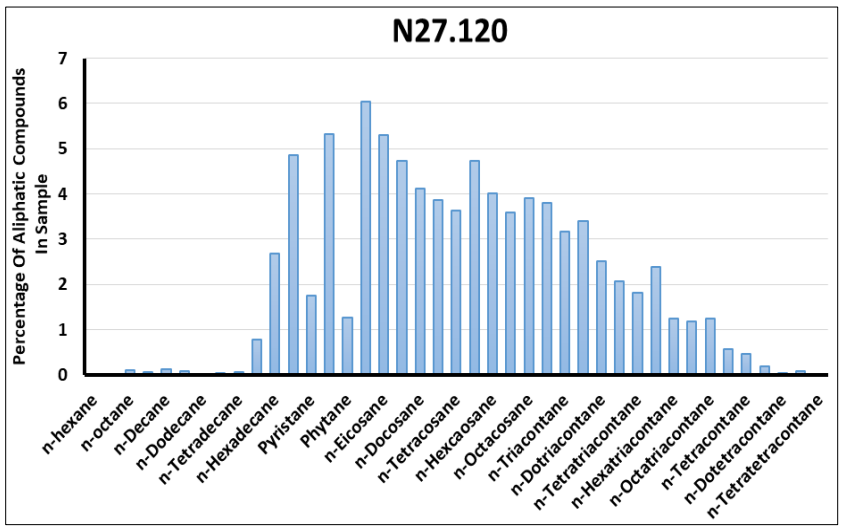

Fig 4: Mass spectra crude oil after biodegradation by isolate N27.120 (*Pseudomonas putida***)**

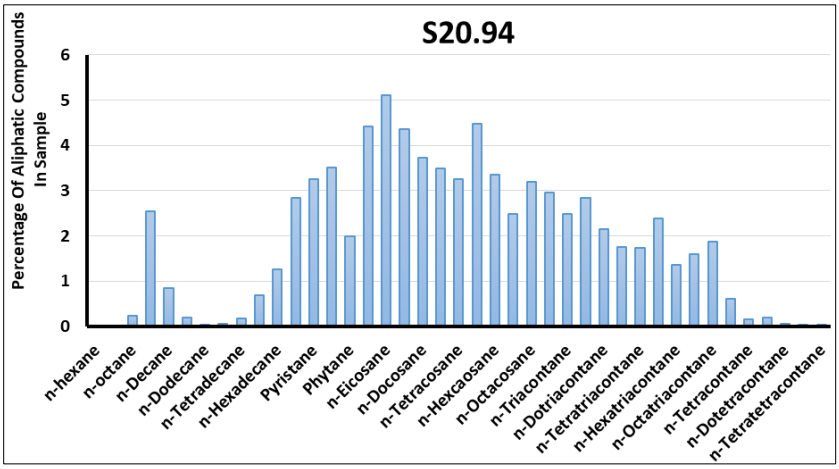

Fig 5: Mass spectra crude oil after biodegradation by isolate S20.94 (*Pseudomonas aeruginosa)*

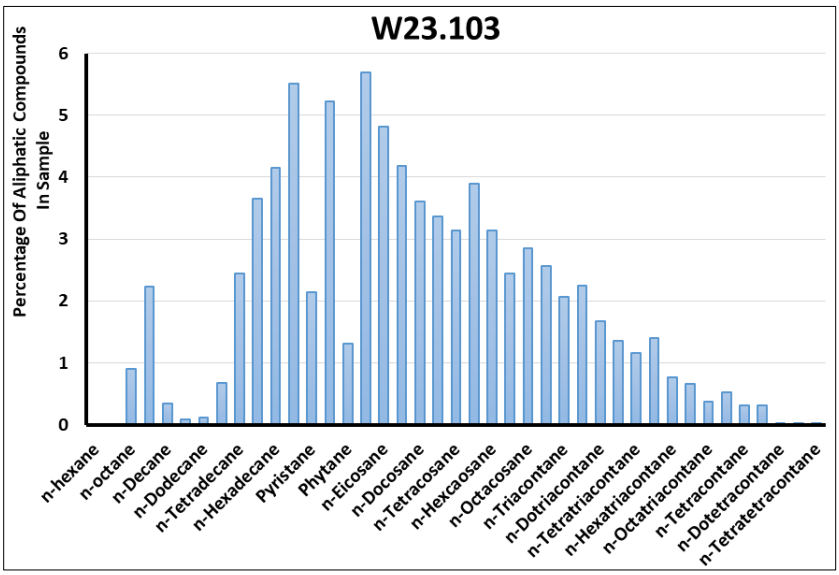

Fig 6: Mass spectra crude oil after biodegradation by isolate W23.103 (*Citrobacter freundii*)

6. References

1. Janssen DB, Dinkla IJ, Poelarends GJ, Terpstra P. Bacterial degradation of xenobiotic compounds: evolution and distribution of novel enzyme activities. Environmental microbiology. 2005; 7(12):1868-1882.
2. Rojo F. Degradation of alkanes by bacteria. Environmental microbiology. 2009; 11(10):2477-2490.
3. Kadafa AA. Environmental impacts of oil exploration and exploitation in the Niger Delta of Nigeria. Global Journal of Science Frontier Research Environment & Earth Sciences. 2012; 12(3):19-28.
4. Calvo C, Manzanera M, Silva-Castro GA, Uad I, González-López J. Application of bioemulsifiers in soil oil bioremediation processes. Future prospects. Science of the total environment. 2009; 407(12):3634-3640.
5. Souza EC, Vessoni-Penna Oliveira RPSO. Biosurfactant-enhanced hydrocarbon bioremediation: An overview. International Biodeterioration & Biodegradation. 2014; (89):88-94.
6. Atlas RM. Petroleum biodegradation and oil spill bioremediation. Marine Pollution Bulletin. 1995; 31(4-12):178-182.
7. Das N, Chandran P. Microbial degradation of petroleum hydrocarbon contaminants: an overview. Biotechnology research international, 2011.
8. Shekhar S, Sundaramanickam A, Balasubramanian T. Biosurfactant producing microbes and their potential applications: a review. Critical Reviews in Environmental Science and Technology. 2015; 45(14):1522-1554. Brooijmans RJ, Pastink MI, Siezen RJ. Hydrocarbon degrading bacteria: the oil- spill clean- up crew. Microbial biotechnology. 2009; 2(6):587-594.
9. Olutiola PO, Famurewa O, Sonntag HG. Introduction to General Microbiology: A Practical Approach. 2nd Edn., Bolabay Publications, Ikeja, Nigeria, 2000.
10. Fawole MO, Oso BA. Characterization of Bacteria: Laboratory Manual of Microbiology. 4th Edn., Spectrum Book Ltd., Ibadan, Nigeria, 2004, pp: 24-33.

11. Cheesbrough M. District Laboratory Practice in Tropical Countries. 2nd Edn., Cambridge University Press, Cambridge, UK, 2006. ISBN-13: 9781139449298.
12. Primandari SP, Yaakob Z, Mohammad M, Mohamad AB. Characteristics of residual oil extracted from palm oil mill effluent (POME). World Applied Sciences Journal. 2013 27(11):1482-1484.
13. Zeng H, Zou F, Lehne E, Zuo JY, Zhang D. Gas chromatograph applications in petroleum hydrocarbon fluids. Advanced gas chromatography—progress in agricultural, biomedical and industrial applications, In Tech, 2012, 363-388.
14. Olajire AA, Essien JP. Aerobic degradation of petroleum components by microbial consortia. Journal of Petroleum & Environmental Biotechnology. 2014; 5(5):1.
15. Rojas-Avelizapa NG, Cervantes-Gonzalez E, Cruz-Camarillo R, Rojas-Avelizapa LI. Degradation of aromatic andasphaltenic fractions by Serratia lique fasciens and Bacillussp. Bull Environ Contam Toxicol. 2002; 69:835-842.
16. Wongsa P, Tanaka M, Ueno A, Hasanuzzaman M, Yumoto I, Okuyama H. *et al*. Isolation and characterization of novel strains of Pseudomonas aeruginosa and Serratia marcescens possessing high efficiency to degrade gasoline, kerosene, diesel oil, and lubricating oil. Current microbiology. 2004; 49(6):415-422.
17. Brooijmans RJ, Pastink MI, Siezen RJ. Hydrocarbon degrading bacteria: the oil spill clean up crew. Microbial biotechnology. 2009; 2(6):587-594.
18. Brzeszcz J, Kaszycki P. Aerobic bacteria degrading both n-alkanes and aromatic hydrocarbons: an undervalued strategy for metabolic diversity and flexibility. Biodegradation. 2018; 29(4):359-407.
19. Amund OO, Adebiyi AG. Effect of viscosity on the biodegradability of automotive lubricating oils. Tribology International. 1991; 24(4):235-237.
20. Wackett LP, Hershberger CD. Biocatalysis and biodegradation: microbial transformation of organic compounds (No. QP517. B5 W33). Washington, DC: ASM press, 2001.
21. Parales RE, Bruce NC, Schmid A, Wackett LP. Biodegradation, biotransformation, and biocatalysis (B3). Appl. Environ. Microbiol. 2002; 68(10), 4699-4709.
22. Vinothini C, Sudhakar S, Ravikumar R. Biodegradation of petroleum and crude oil by Pseudomonas putida and Bacillus cereus. International Journal of Current Microbiology and Applied Sciences. 2015; 4(1):318-329.
23. Meliani A, Bensoltane A. Enhancement of hydrocarbons degradation by use of Pseudomonas biosurfactants and biofilms. Journal of Petroleum & Environmental Biotechnology. 2014; 5(1):1.
24. Otenio MH, Silva MTLD, Marques MLO, Roseiro JC, Bidoia ED. Benzene, toluene and xylene biodegradation by Pseudomonas putida CCMI 852. Brazilian Journal of Microbiology. 2005; 36(3):258-261.
25. Hasan SA, Jabeen S. Degradation kinetics and pathway of phenol by Pseudomonas and Bacillus species. Biotechnology & Biotechnological Equipment. 2015; 29(1):45-53.
26. Xu X, Liu W, Tian S, Wang W, Qi Q, Jiang P. *et al*. Petroleum Hydrocarbon-Degrading Bacteria for the Remediation of Oil Pollution Under Aerobic Conditions: A Perspective Analysis. Frontiers in microbiology, 2018, 9:2885.
27. Hawle- Ambrosch E, Riepe W, Dornmayr- Pfaffenhuemer M, Radax C, Holzinger A, Stan- Lotter H. Biodegradation of fuel oil hydrocarbons by a mixed bacterial consortium in sandy and loamy soils. Biotechnology Journal: Healthcare Nutrition Technology. 2007; 2(12):1564-1568.
28. Wu T, Xu J, Xie W, Yao Z, Yang H, Sun C. *et al*. Pseudomonas aeruginosa L10: a hydrocarbon-degrading, biosurfactant-producing, and plant-growth-promoting endophytic bacterium isolated from a reed (Phragmites australis). Frontiers in microbiology, 2018; 9:1087.
29. Wang W, Shao Z. Enzymes and genes involved in aerobic alkane degradation. Frontiers in microbiology, 2013; 4:116. doi:10.3389/fmicb.2013.00116
30. Varjani SJ. Microbial degradation of petroleum hydrocarbons. Bioresource technology. 2017; 223:277-286.
31. Singh C, Lin J. Isolation and characterization of diesel oil degrading indigenous microrganisms in Kwazulu-Natal, South Africa. African Journal of Biotechnology, 2008; 7(12).
32. Palanisamy N, Ramya J, Kumar S, Vasanthi NS, Chandran P, Khan S. *et al*. Diesel biodegradation capacities of indigenous bacterial species isolated from diesel contaminated soil. Journal of environmental health science and engineering. 2014; 12(1):142.
33. Teramoto M, Queck SY, Ohnishi K. Specialized hydrocarbonoclastic bacteria prevailing in seawater around a port in the Strait of Malacca. PloS one. 2013; 8(6):e66594.
34. Berry D, Gutierrez T. Evaluating the detection of hydrocarbon-degrading bacteria in 16S rRNA gene sequencing surveys. Frontiers in microbiology, 2017; 8:896.
35. Thompson H, Angelova A, Bowler B, Jones M, Gutierrez T. Enhanced crude oil biodegradative potential of natural phytoplankton- associated hydrocarbonoclastic bacteria. Environmental microbiology. 2017; 19(7):2843-2861.
36. Ibrahim HM. Biodegradation of used engine oil by novel strains of Ochrobactrum anthropi HM-1 and Citrobacter freundii HM-2 isolated from oil-contaminated soil. 3 Biotech. 2016; 6(2):226.