HYDROCARBONOCLASTIC POTENTIAL OF BACTERIA ISOLATED FROM HAIR SALON WASTEWATER IMPACTED SOILS

www.akwapolyjournal.org
P-ISSN: 2536-6733 | E-ISSN: 2616-0773

Supported by: tetfund TERTIARY EDUCATION TRUST FUND
TETFund/DESS / POLY / IKOT-OSURUA/ ARJ /1-2)

Christiana Utibe Etuk, Nkeneke E. Akpainyang, Inyang J. Udoh & Rachael S. Umoh
Department of Biological Sciences,
Akwa Ibom State Polytechnic, Ikot Ekpene, Akwa Ibom State

Mayen G. Ben
Departments of Biological Sciences,
Faculty of Science, College of Education, Akwa Ibom State, Nigeria

Emem Ntino
Department of Microbiology,
Faculty of Applied Sciences, University of Uyo,
Uyo, Akwa Ibom State.
chris**etuk2020@gmail.com**

ABSTRACT
The hydrocarbonoclastic potential of bacteria isolated from hair salon wastewater-impacted soils was studied. Isolation of hydrocarbonoclastic bacteria from hair salon wastewater-impacted soil (SWS) and non-salon wastewater-impacted soil (NWS) samples were determined. Total mean bacterial counts of the soil

samples were carried out using standard microbiological techniques. The total bacterial counts were 1.2x106 ±0.5 and 8.0 ±0.2 x105 Cfu g-1 in the SWS and NWS, respectively. Hydrocarbonoclastic bacteria identified included *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Micrococcus*, and *Streptococcus* spp. Utilization of some hydrocarbons tests were carried out on kerosene, petrol, diesel, and waste engine oil on the isolates under aerobic conditions and were determined using Dichlorophenol Indophenol indicator (DCPIP) in hydrocarbon medium and incubated for 48 hours at 30 C. Biosurfactants production potential using the drop collapse test indicated that most of the bacterial isolates which tested positive have good bio-surfactants production potentials. The result of the emulsification index, E24 % of diesel and petrol for *Staphylococcus* sp. was significantly higher (50.70± 1.21 and 51.50± 0.50 respectively) than that obtained for kerosene and waster-engine oil (31.40± 1.05 and 23.47± 0.57 respectively) ($p<0.05$).

In contrast, the amount of waste engine oil was significantly less than that of kerosene. There was no significant difference in emulsification index ($p<0.05$) between diesel (50.70± 1.21) and petrol (51.50± 0.50). Salon wastewater-impacted soils have been shown in this study to harbour bacteria with hydrocarbonoclastic potentials as indicated by the emulsification index E24% of the different isolates. The results suggest that the isolates are potential candidates for hydrocarbon utilization studies and application in bioremediation.

Keywords: Dichlorophenol Indophenol indicator, hydrocarbonoclastic bacteria, polluted soil, hydrocarbon products, salon effluent.

1.0 INTRODUCTION

The ubiquity of microorganisms (an important part of the ecological system) made it easier for them to thrive in different habitats, from which soil impacted by hairdressing salon wastewater is not exempted. Effluent and sludge from municipal wastewater contain a high quantity of hydrocarbon compounds (amongst other components), which may lead to soil contamination, thereby posing a threat to beneficial microorganisms (Havugimana *et al*., 2015**).** In providing its services, the hairdressing salon (a facility used for cosmetic or medicinal preparation for dressing hair) generates wastewater (Onwusah *et al*., 2015), which is discharged into the surrounding soil environment or subsurface disposal systems. The discharged effluents/wastewater, though capable of posing harmful effects as reported by Onwusah *et al*.(2015) is however, reported by (Gielnik *et al*., 2021) to harbor some beneficial microbes (the hydrocarbonoclastic bacteria) most of these microbes are autochthonous, that is indigenous.

In Nigeria, hairdressing salon wastewater is often discharged into surrounding soil environments untreated. However, salon wastewater pollutes soils, and the negative impacts of the discharged salon effluents harbour beneficial bacteria with hydrocarbonoclastic potentials.

Therefore, it is essential to adopt environmentally friendly, effective measures for dealing with hydrocarbon contamination problems (Mahjoubi *et al*., 2013; Smulek *et al*., 2020), such as utilizing the hydrocarbonoclastic (Hydrocarbon degrading) bacteria, HBC. These bacteria, with their unique ability to degrade and utilize hydrocarbon compounds as their carbon and energy sources, offer a promising solution for environmentally friendly bio-sanitation (Rosenberg *et al*., 1998; Shahid *et al*., 2020).

This study makes a unique contribution to the field of environmental microbiology by focusing on the isolation and characterization of

hydrocarbonoclastic bacteria species from salon wastewater-impacted soils. The assessment of the hydrocarbonoclastic potential of bacterial isolates from salon wastewater-impacted soils further enhances the novelty and significance of this research.

2.0 Methodology

Sample Collection

Three composite salon effluent polluted soil samples (SEs) were collected from where salon wastewater is disposed of, while pristine soil samples (NSs) were collected (10-15 metres away from the salons) with utmost care and precision using a sterile soil auger into sterile polythene bags for microbiological and physicochemical analyses.

2.1 Microbiological Analysis

Soil samples were serially diluted 10-fold down the gradient to 10^{-5}, plated on nutrient agar medium by pour plate method, and incubated at 37oC(± 2) between 24-48 hours**.** Discrete colonies were sub-cultured and preserved in slants at 4oC for further microbiological analysis. Colonial characteristics and cell biochemical reactions of pure cultures were done for characterization according to the methods described by Cheesebrough (2005). They were identified based on the taxonomic schemes of Holt *et al*.(1994).

2.2 Hydrocarbon Utilization Test

Hydrocarbon medium was prepared by dispensing 3g each of glucose and peptone water into 300 ml of distilled water and 1.0 ml of the different hydrocarbon products. 9ml of the medium was dispensed into sterile test tubes. Four drops of Dichlorophenol Indophenol Indicator (DCPIP) were added. The tubes were separated into 4 batches of 6 tubes according to the test hydrocarbons (kerosene, petrol, diesel, and engine oil added 1% (v/v) each) were added to the tubes in batches as appropriately labeled. Each batch had a control tube (containing no hydrocarbon), all tubes were appropriately inoculated with 0.1ml 24 hr old test isolates and incubated for 96 hours at 37o C (±2)and inspected

daily for hydrocarbon utilization indicated by colour changes (from purple to pale yellow) of the medium indicator and the growth patterns were determined by assessing changes in pH (using the Jenway 3310 model pH metre) and optical density (at 600nm using the Spectrumlab spectrophotometer) according to the methods described by Rosenberg (1998).

2.3 Emulsification Index

Distinct isolates were suspended in the mixture of mineral salt medium and 2 ml of each test hydrocarbon in test tubes; they were incubated by placing them at 150 rpm (revolutions per minute) in a shaker at 37o C for 7 days afterward. The cultured tubes were spun using a centrifuge (Model No: 800B) at a speed of 120 rpm for 5 minutes at 25 C and allowed to stand for 24 hours following methods described by El-Gebaly (2020). Calculation of emulsification (E24%):

$$\text{Emulsification } (E_{24\%}) = \frac{\text{Height of emulsification layer}}{\text{Total culture height}} \times \frac{100}{1}$$

2.4 Drop Collapse Test

Overnight culture of isolates (1ml each) was inoculated into tubes containing 9 ml of mineral salt medium (prepared according to methods of Okpokwasili and Okorie, 1990); cultured for 48hours then centrifuged at 150rpm for 15 minutes. And 5µl of each centrifuged supernatants were added unto 2µl of the hydrocarbon product (engine oil) already placed in 96–well micro liter plates and allowed to stand for 1 minute according to the methods described by Youssef *et al*. (2004) and Mahjoubi *et al*. (2013). Observations were made for drop size and for positive and negative drop collapse nature respectively. Distilled water dropped without the hydrocarbons but with supernatants served as negative control while positive control was a mixture of the supernatant without oil.

3.0 RESULTS

3.1. Total Bacterial Count of Soil Samples

Table 1 shows the total bacterial counts of the salon effluent impacted soil and control soil (unpolluted) samples. The morphological and biochemical identification of the bacterial isolates revealed *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Streptococcus*, and *Micrococcus* spp. (Table not shown here).

Table 2 shows the pH value of the different soil samples which ranged between 5.02 ±0.01to 5.10±0.2& 6.11±0.1 to 6.56±0.1 in SWS and NWS respectively. The pH value of the SEs were more acidic compared to the control.

Table 3 shows the results of the drop collapse assay of the different isolates. It revealed that *Staphylococcus*, *Bacillus* and *Pseudomonas* spp. were positive while *Streptococcus* and *Micrococcus* spp. were negative (Table 3).

Table 4 shows the emulsification ability of the isolates. It revealed that all the isolates have good emulsification ability on the hydrocarbons exception of waste engine oil (Table 4).

Figure 1 shows the reactions of the isolates after 24hrs incubation in mineral salts medium in which waste keroosene and the DCPIP indicator were added. Figure 2 shows the determination of engine oil utilization by the bacterial isolates.

Table 1: Total bacterial counts of soil samples

Sample location	Total bacterial counts (CFU g^{-1})	
	SWS	NWS
A	$1.1 \pm 0.2 \times 10^6$	$6.0 \pm 0.3 \times 10^5$
B	$9.0 \pm 0.1 \times 10^5$	$3.0 \pm 0.2 \times 10^5$
C	$1.2 \pm 0.5 \times 10^6$	$8.0 \pm 0.2 \times 10^5$

Keys: SWS =Salon wasrewater soil, NWS = (non-polluted soil/control), A–C=locations of sample collection.

Table 2: pH of Soil Samples

Sample locations	pH	
	SWS	NWS
A	5.10±0.2	6.11±0.1
B	5.02±0.01	6.48± 0.2
C	5.12±0.2	6.56±0.1

Keys: SWS = Salon wastewater soil, NWS =(non-polluted soil), A – C = Different locations of sample collection, values are means of triplicates data ±standard deviation

Table 3: Drop Collapse Assay

Probable organism	Drop collapse assay
Staphylococcus sp.	+
Bacillus sp.	+
Pseudomonas sp.	+
Streptococcus sp.	-
Micrococcus sp.	-

Key: + =positive, - =negative reaction

Table 4: Emulsification index , E_{24} (%)

	Hydrocarbon			
Isolates	Kerosene	Diesel	Petrol	Waste-engine oil
Staphylococcus sp.	*31.40±1.05* *b*	50.70±1.21 c	51.50±0.50 c	23.47±0.57 a
Bacillus sp.	35.20±0.75 b	46.77±1.80 c	61.20±0.60 d	28.90±0.17 a

Pseudomonas sp.	30.37±0.21 b	46.23±0.40 d	41.80±1.25 c	21.00±1.73 a
Streptococcus sp.	24.53±1.46 b	33.10±0.17 c	51.83±1.07 c	15.43±1.10 a
Micrococcus sp.	25.67±0.25 b	41.63±0.55 c	45.03±1.00 d	18.03±0.93 a

Similar superscript letter means not significantly different (p>.05) while different superscript letters means significantly different.

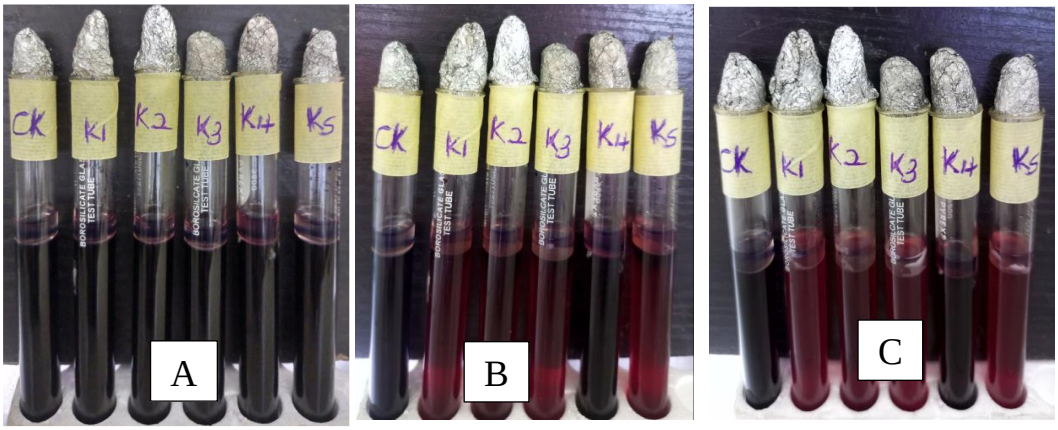

Fig 1: Determination of kerosene utilization by bacterial isolates
Keys: A= Before incubation, B= After 24hrs of incubation, C= After 48hrs of incubation, CK=Control & K_1= Isolate 1,2,3,4 &5 (*Staphylococcus*, *Bacillus*, *Pseudomonas*, *Streptococcus* & *Micrococccus* spp. respectively)

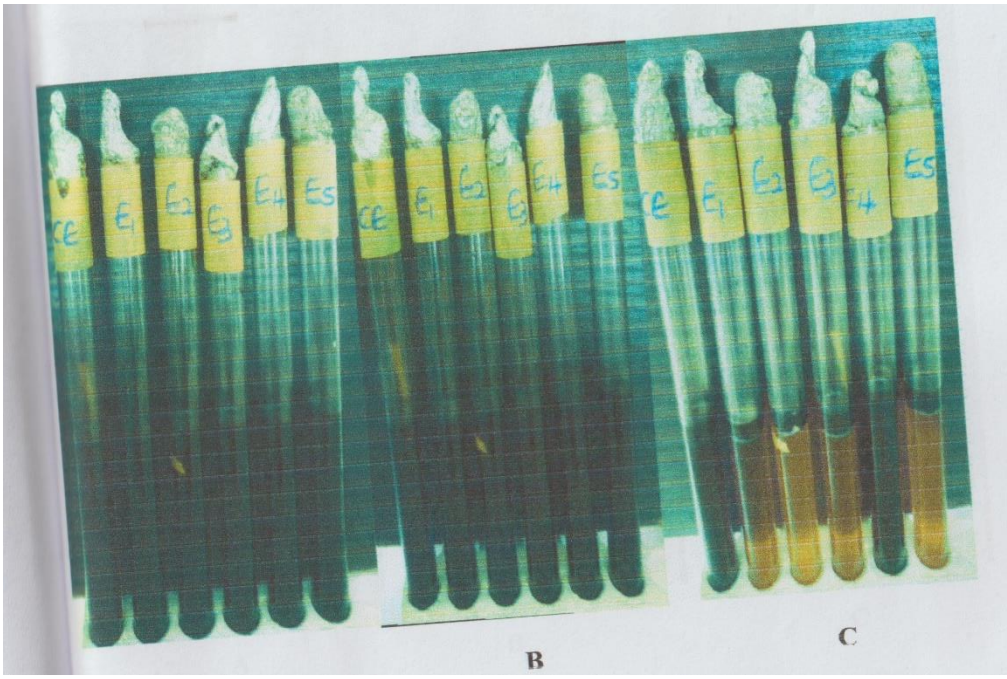

Fig 2: Determination of engine oil utilization by bacterial isolates
Keys: A= Before incubation, B= After 24hrs of incubation, C= After 48hrs of incubation, CK=Control & E1, 2, 3, 4 & 5 (*Staphylococcus*, *Bacillus*, *Pseudomonas*, *Streptococcus* & *Micrococcus* spp. respectively).

4.0 Discussion

4.1 Total bacterial counts of soil samples

The highest mean bacterial counts revealed 1.2 ±0.5 x 106 and 8.0 ±0.2 x 105 Cfu g-1 for salon wastewater-impacted soils (SWS) and controlled soil or non-wastewater-impacted soils (NSS), respectively. Higher counts of bacterial isolates in salon wastewater or effluent polluted soil samples than in the control soil suggest the ability of salon effluent soil to support the growth of a wide diversity of bacteria, which affirmed the ubiquity of microorganisms (Willey *et al*., 2008; Al-Saleh *et al*., 2009).

4.2 Morphological and Biochemical Identification of Bacterial Isolates

The morphological and biochemical identification of the bacterial isolates revealed Staphylococcus, *Bacillus*, *Pseudomonas*, *Streptococcus*, and *Micrococcus* spp. (Table not shown here). These bacterial species obtained were similar to those reported by Al-Saleh *et al*. (2009) as hydrocarbon utilizers. They also opined that *Bacillus* and *Pseudomonas species* have great potential for improving oil recovery from specific locations they studied.

4.3 pH of Soil Sample

Table 2 shows the pH values of the different soil samples, which ranged between 5.02 ±0.01to 5.10±0.2 and 6.11±0.1 to 6.56±0.1 in SWS and NWS, respectively. The pH value of the SWS was more acidic than the control. This could be attributed to the presence of chemicals in hair relaxers and dyes, as also previously suggested by Diaz *et al*. (2001).

4.4 Drop Collapse Assay

The drop collapse assay of the different isolates revealed that *Staphylococcus*, *Bacillus*, and *Pseudomonas sp*. were positive, while *Streptococcus* and Micrococcus spp. were negative (Table 3). The drop collapse test (a rapid method used for screening bio-surfactant production) determines a reduction in surface tension by testing the stability of a droplet on an oil-covered surface. This assay relies on surfactants to destabilize lipid droplets. This work corroborates with the findings of Saminathan and Rajendran (2014), who reported that strongly positive isolates for drop collapse test were an indication of good bio-surfactant production potential, and Kubicki *et al*.(2019) reported that microorganisms recording negative drop-collapse test (round shape of broth surface) were unable to produce bio-surfactant.

4.5 Determination of Hydrocarbon Utilization by Bacterial Isolates using Redox Indicator (DCPIP) and Emulsification Index Assay.

The hydrocarbonoclastic potential of the bacterial isolates was determined by including the Dichlorophenol indophenols indicator

(DCPIP) in the hydrocarbon medium as a lipophilic mediator and electron acceptor capable of providing microbial metabolism; thus, by incorporating this electron acceptor, DCPIP, it was possible to ascertain the ability of the bacterial isolates to utilize the hydrocarbon substrate by simply observing the colour change in the hydrocarbon-indicator medium as the carbon and energy source. The Calculation of the emulsification index(E24%) of the isolates is shown in Table 4.

NOTES: E24 (above 30%) was recorded for all the isolates for all the hydrocarbon products except waste engine, especially for *Staphylococcus, Bacillus*, and *Pseudomonas*.

However, higher values for engine oil (66.8 & 81%) have been reported by Sidkey *et al*. (2016); Saminathan and Rajendran (2014), respectively. Kubicki*et al*. (2019) opined that an emulsification index (E24) of ≥30.0% is considered to correlate with the positive drop collapse nature and, hence, possesses a high ability to degrade hydrocarbons. Thus, this study's E24(above 30%) of all the isolates for all the hydrocarbon products except waste engine oil correlate with their positive drop collapse, especially for *Staphylococcus, Bacillus*, and *Pseudomonas*. The result of the emulsification ability of the isolate revealed that all the isolates have good emulsification ability on the hydrocarbons, except for waste engine oil (Table 4).

The result shows that the emulsification index of diesel and petrol for *Staphylococcus* sp. was significantly higher than that obtained for kerosene and waster-engine oil ($p<.05$). In contrast, that of waste engine oil was significantly less than that of the kerosene but between diesel and petrol, there was no significant difference in emulsification index($p<.05$). The result also shows that the emulsification index was significantly lower for all isolates when waste-engine oil was used compared with another hydrocarbon ($p<.05$). Chaerum*et al*. (2004) reported that most bacteria could only effectively degrade or utilize specific hydrocarbon components while some bacteria are completely incapable.

After 24hrs incubation, there was incomplete decolorization of the colour of the DCPIP indicator in all the tubes containing isolates within a medium incorporated with kerosene) which did not record a colour change (Figure 1).

After 48hrs of incubation, there was complete decolorization of the colour of the DCPIP indicator in the tubes, except tubes K4 and E4 (*Streptococcus* sp. in medium incorporated with kerosene and engine oil, respectively), which did not record a colour change (Figure 2). It could, therefore, be said that *Streptococcus* sp. was not able to utilize the hydrocarbon substrate in the medium, as similarly reported by Olayide *et al*. (2010), who were able to show that some soil bacteria were capable of being used in the bioremediation of some hydrocarbon polluted soil.

However, Pathak and Jaroli (2014) revealed that factors such as temperature, pH, nutrients, oxygen, and microbial consortium can influence hydrocarbon degradation. The result shows that *Streptococcus*, obtained in both diesel and petrol, was significantly higher than that of another hydrocarbon ($p<.05$). In contrast, between diesel and petrol, there was no significant difference in their emulsification index ($p>.05$). Rambeloarisoa *et al*. (1984) states that compounds such as saturated, aromatic and polar compounds present in different hydrocarbons are degraded at different degrees by the same organism**.**

5.0 Conclusion

The production of emulsifiers has proven bacteria's hydrocarbonoclastic potential or hydrocarbon degradation ability. The results show *Staphylococcus*, *Bacillus*, *Pseudomonas*, *Streptococcus*, and Micrococcus spp. isolated from hair salon effluent-impacted soils can utilize some hydrocarbons effectively and could be relevant in the bioremediation of ecosystems that may be contaminated with hydrocarbons.

6.0 Recommendations

From this research, it is recommended that,

1. Biodegradation should be considered a key component in the clean-up strategy and should be adopted for the treatment of contaminated sites as it could be a significant sustainable tool in the Bioeconomy.
2. Further investigation is needed to identify more strains of bacterial isolates that can utilize other hydrocarbons.

REFERENCES

Al-Saleh, H. J. ,and Drobiova, C. O. (2009).Predominant culturable crude oil degrading bacteria in the coast of Kuwait. *International Biodetorioration and Biodegradation*, **63**(4): 400-406.

Antia, S. P. (1990). Biodegradation of Bony light crude oil by *Bacillus* sp. and *Pseudomonas* species. *Waste Management*, **60**(10): 61-64.

Chaerum, S. K. , Tazaki, U. K ., Asada, R. R. and Kogure, O. K. (2004). Bioremediation of coastal areas 5 years after the Nakhodkaoil spill in the sea of Japan: isolation and characterization of hydrocarbon degradation.*Bacteria Environ. Internationa*l, **30**: 911-922.

Cheesebrough, M. (2005).*District laboratory practice in tropical countries* (2nd part).Cambridge University press, Pp 23-39,372.

Diaz, E., Ferrandez, J. L. (2001). Biodegradation of aromatic compounds by *E. coli*. *Microbiology, Molecular Biology Review*, **65**: 523-569.

El-Gebaly, E. O. (2020).Screening of biosurfactant production by bacterial strains isolated from oil contaminated sites near gas stations in Egypt .*Az. Journal of Pharmaceutical Science*, **61**: 134-145 .

Holt, J. G., Kreig, N. R., Sneath, P. H. A., Staley, J. T. ,and Williams, S. T. (1994). *Bergey's Manual of Determinative Bacteriology* (9th ed.). Lippincott; Williams and Wilkins, 894p.

Kubicki, S., Bollinger, A., Katzke, N., Jaeger, K., Loeschcke, A., and Thies S. (2019). Marine biosurfactants: Biosynthesis, structural diversity and biotechnological applications. *Marine Drugs*, **17**(7): 408-420.

Lal, V. P. and Khama, T. C. (1996).Effect of hydrocarbon pollution on the microbial properties of a sandy and clay soil.*Chemosphere*, **66**:1863-1871.

Mahjoubi, M. , Jaouani, A., Guesmi, A., Ben Amor, S., Jouini, A., Cherif, H. A., Boudabous, A., Koubaa, N., and Cherif, A. (2013). "Hydrocarbonoclastic bacteria isolated from petroleum contaminated sites in Tunisia: Isolation, identification and characterization of the biotechnological potential". *New Biotechnology*, **30**(6): 723-733.

Nakuleshivas, D. J., Richa, S. S., and Subhash, C. Q. (2018). Isolation and identification of microorganism from playhouse agriculture soil of Rajasthan. *Afri. J. Mocrobiol. Research*, **7**(4):4886-4891.

Okpokwasili,G. C. and Okorie, B. B.(1990).Degradation of aldrin by bacterial isolates. Nigerian *J. of Technological Research*, **2**: 1-6

Olayide, F. O., Angus, C. O., and Simon, C. N. (2010). Bioremediation of crude petroleum polluted stagnant water. *Journal of Botany* and *Microbiology*, **2(**11): 81-86.

Onwusah, B. N., Amafina, I. M., and Obire, O. (2015). Microflora of different wastewater from some hair dressing salons in Port Harcourt in Nigeria. Current Studies in Comparative Education, Science and Tech, **2**(2):303-311.

Pathak, S. H., and Jaroli, D. P. (2014).Factors affecting the rate of biodegradation of polycyclic aromatic hydrocarbons(PAHs). *Journal of Bioremed. Biodegradation*, **1**(3):124-133.

Rahman, K. S. (2002). Bioremediation of gasoline contaminated soil by a bacterial consortium amended with poultry litter,coir pith and rhamnolipid biosurfactant. *Bioresource Technology*, **81**(3): 25-32

Rosenberg, E. (1998). The hydrocarbon-oxidizing bacteria. In E. Rosenberg,E. DeLong and P. Thompson, S. Lore and E. Stackebrandt (Eds.),*The prokaryotes*(4thEd.,Chapter 5).Springer.

Saminathan, P., and Rajendran, P. (2014). Screening and identification of potential biosurfactant producing microorganisms isolated from oil contaminated soil. *International Journal of Pharma and Biosciences*, **5**(1): 1096-1106.

Shahid, M , Al-surhanee, A., Kouadri, F., Ali, S., Nawaz, N., Afzal, M., Rizwan, M., Ali, B. and Spoliman, M. (2020).Role of microorganisms in bioremediation nof wastewater in floating wetlands: Review.*Sustainability*, **12**: 55-59. Doi-10.3390/su12145559

Sidkey, N. M., Mohamed, H. F. , and Elkhouly, H. I. (2016). Evaluation of different screening methods for biosurfactant producers isolated from contaminated Egyptian samples grown on industrial olive oil processing waste.*British Microbiology Research Journal*, **17**(4):1-9.

Smulek, W., Zdarta, A., Grzwaczyk, A., Guzik, U., Siwińska-Ciesielczyk, K., Ciesielczyk, F., Strzemiecka, B., Jesionowski, T., Voelkel, A., and Kaczorek, E. (2020). Evaluation of the physico-chemical properties of hydrocarbons-exposed bacterial biomass. *Journal of Biointerfaces*, **196**: 111-116.

Willey, J. M., Sherwood, L. M., Woolverton, C. J., Presscott, B., Harley, R. and Klein, A. (2008).*Microbiology*(seventh edition),McGrawHill, pp.643-742.

Youssef, N.H., Duncan, K. E., Nagle, D. P., Savage, K. N., and Knapp, R. M. (2004).Comparison of methods to detect biosurfactant production by diverse microorganisms. *Journal of Microbial Methods*, **56**: 339-347.