

The Potential of Some Bacteria for Fuel Residue Removal From Dora Refinery Remnants

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Abstract: The study investigated the effect of two types of microorganisms: *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*, which were diagnosed after being isolated from contaminated soils in Al-Dora refinery. The experiment continued for seven days where tests were implemented on the 1, 3, 5, and 17 days in addition to the control sample to study. The abilities of these bacteria in the treatment of oil compounds and the efficiency of these types of bacteria were studied in the removal and decomposition of these contaminants. *P. aeruginosa* bacteria were efficient in reducing the value of hydrocarbons in the medium with 65.48% and the lowest efficiency of *K. pneumoniae* was 52.41%. *P. aeruginosa* showed their ability to reduce electrical conductivity at 61.10%, reduce biomass at 72.81% and reduce salinity at 62.9%. While *K. pneumoniae* proved a decrease in the value of hydrocarbons at 52.41%, electrical conductivity at 35.5%, biomass at 60.02%, and salinity at 50.05%. The conclusion proved that *P. aeruginosa* bacteria are more efficient than *K. pneumoniae* bacteria in breaking and removing pollutants. This indicates the possibility of using microorganisms of various types to treat and remove much of the waste of laboratories and factories in the future.

Key words: Oil contaminants, microorganisms, removal and decomposition.

Introduction

Oil is located east of Baghdad, next to the Tigris River. Its entire area is 250 h. The Iraqi government constructed the refinery in 1953 with assistance from many international businesses, and it began operations in 1955 (Lim et al., 1999; Nayyef, 2017). This refinery can process crude oil up to 140,000 barrels per day and produces oil derivatives such as diesel fuel, gasoline, and white oil (Toledo et al., 2006). The refinery creates a huge volume of wastewater (ww) due to its considerable water consumption of about 50.000 M³/day for liquidation, which according to Mohammed (2014), can be refined in the treatment facility before being reused or disposed off in the Tigris River. Numerous pollutants that the oil industry may release into the air, soil, and water can have a substantial impact on the physicochemical characteristics of these

ecosystems, which can then have an impact on public health, especially for those workers who are exposed to the environment and their jobs (Bergey's Manual of Determinative Bacteriology, 1994). The purpose of this project is to clean up oil contamination from the refinery using tiny items.

Materials and Methods

Samples

In the soils around the Dora refinery, samples were taken at several points in the vicinity, placed in marked plastic containers thereafter moved to the laboratory.

Microbiology

The microbes *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were isolated and diagnosed on the Nutrient medium and Cetrimide agar medium and then

tested at $(28 \pm 2)^{\circ}\text{C}$ (Bergey's Manual of Determinative Bacteriology, 1994). The experiment continued for seven days, where tests were implemented on the (1,3,5,17) days, in addition to the control sample to study the abilities of these bacteria in the treatment of oil compounds pollution and other pollutants (Nayyef, 2023) (Figure 1).



Figure 1: Preparation of agricultural medium.

Cultivation of Samples

Twenty milliliters of the agricultural medium, with two duplicates for every sample, were added after (0.5) g of the collected soil was suspended in (100) milliliters of distilled water and moved in a small circular motion in all directions to ensure homogeneity of the medium and then left at 30% temperature for 24 hours (Raper & Fennel, 1965).

Separation of Hydrocarbons from Contaminated Water

The compounds associated with industrial waste were extracted using a funnel by mixing 30 ml of industrial water with 99.81 diethyl ether at 1:3 v/v, shaking 10 min. When the organic layer is lifted from the bottom and separated from the aqueous layer. A fresh, dry flask was used to filter and collect the water layer after it had been cleaned once again with 50 ml of diethyl ether (Nadaling et al., 2000).

Physical and Chemical Tests

One of the environmental elements that affect the availability of other nutrients and the bioavailability of pollutants. The biological activity and the impact of soil and water polluted by crude petroleum (AWWA & WEF, 2005). pH and solubility were measured using Multi Metter, while salinity was measured using the calculation method described in Adekunle & Adebambo (2007), and biomass estimation according to Das and Mukherjee (2006).

Statistical Analysis

The SAS (2012) statistical analysis system was employed to determine the impact of various factors

within the limitations of the study. Least significant difference (LSD) test was employed in this study to compare the results statistically (SAS, 2012).

Results and Discussion

The current study dealt with the use of some microorganisms to remove Doura refinery waste. The experiment continued for seven days. The results indicated the ability of *P. aeruginosa* bacteria to disassemble oil compounds in contaminated areas, and that agrees with the study of Jawetz et al. (1998). *P. aeruginosa* and *K. pneumonia* show good growth. These isolates show that the bacteria were able to adapt to the consumption of oil and the reason may be due to the enzymatic system, which is capable of treating hydrocarbons as indicated by Lawlor et al. (2005). *P. aeruginosa* bacteria are (Figure 2) resistant to many chemical compounds because they have many different mechanisms to help them resist and then disassemble and remove these compounds as well as their ability to compete with other types of bacteria, and this is aided by their production of the pyocyanin which is important in the competition in the same environmental habitat, as indicated by Boratynsky et al. (2005).

The results showed that *K. pneumoniae* has an effective role in the removal of contaminants. This is consistent with the study shown by Levinson & Jawetz (2000), which showed that the effectiveness of these bacteria can integrate with the oil components present in the contaminated soil and its rapid growth (Figure 3), as well as its ability to tolerate high concentrations of pollutants Ko-(Wen et al., 2002).



Figure 2: Removing oil contaminants by *P. aeruginosa* after 7 days.



Figure 3: Removing oil contaminants by *K. pneumoniae* after 7 days.

The results also showed a decrease in the value of hydrocarbon compounds on the seventh day (Figure 4) with (52.41%) causing oil compounds dismantling by *K. pneumoniae*, and its use as a source of carbon and energy and its exploitation in increasing the biomass and building the enzymes necessary for compounds disassembly (Cybulski et al., 2006). The results of biomass showed that the highest percentage on the seventh day of the bacteria *P. aeruginosa* was (72.81%) (Figure 5) then *K. pneumoniae* at (60.02%). As for electrical conductivity, it depends on the removal rate of *P. aeruginosa* on the seventh day is (61.10%) while *K. pneumoniae* showed (35.05) (Figure 6). The decrease in value is due to the dismantling of the organisms using for these compounds and their absorption for ions as a result of dismantling these compounds (Chorom et al., 2010). As for salinity, the highest percentage of *P. aeruginosa* on the seventh day was 62.9% (Figure 7), while *K. pneumoniae* showed 50.05%. The results refer to the existence of a high percentage of dissolved solids and electrical conductivity, which increases the amount of soluble salts in the soil (Tavassoli et al., 2012).

The results agreed with previous studies (Yasin, 2013), which obtained biomass production exceeding 39/1. *P. aeruginosa* is more effective in the biodegradation of oil compounds as compared with other species of bacteria, resulting in this study agreeing with Tavassoli et al. (2012).

The results in this study agree with Ali et al. (2012) who in their study on the efficiency of the old processing plant of North Oil Company indicated that the range of electrical conductivity was 958-1109 Ms/CM and these results were supported by the results of Nwaichi et al. (2017). The EC increased during summer and decreased during the winter and spring. That might be due to the high temperature during summer in Iraq which led to

increased evaporation, increased salt concentration as well and increased many pollutant concentrations. Also, these results are consistent with Okoh (2003) who confirmed that the mixture of bacterial isolates is the most effective in analysing compounds.

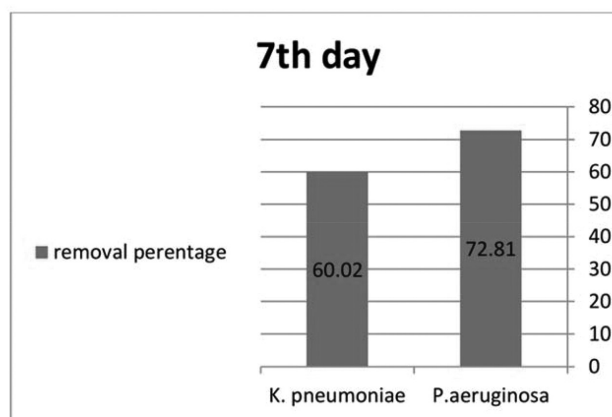


Figure 5: Percentage of biomass for both bacteria on the 7th day.

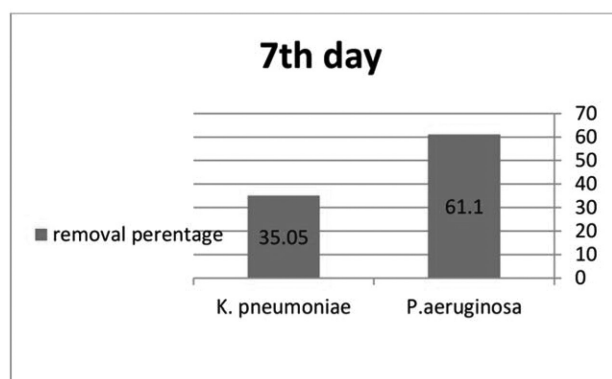


Figure 6: The percentage of electrical conductivity in Al-Dora Refinery water.

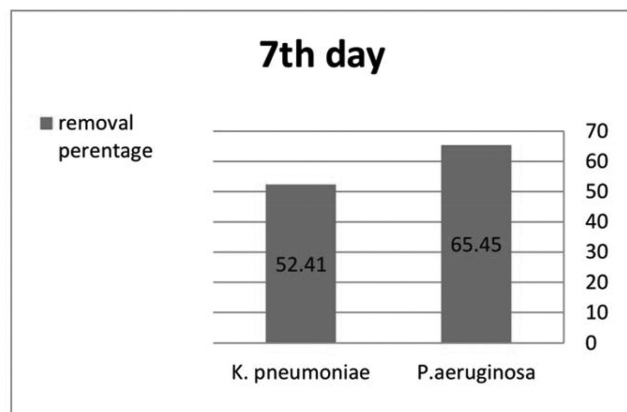


Figure 4: Percentage of hydrocarbon removal by both bacteria on the 7th day.

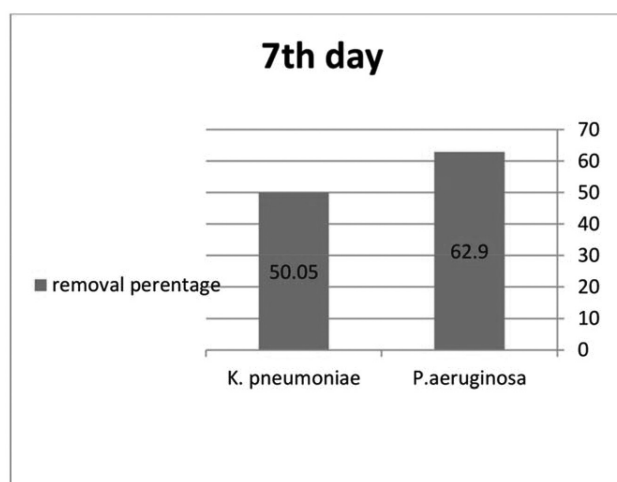


Figure 7: The percentage of salinity removal.

Conclusion

It is evident from the data that *K. pneumoniae* is more effective than *P. aeruginosa* bacteria at cleaning up oil pollution.

References

- APHA, AWWA and WEF (2005). Standard methods for the examination of water and wastewater; APHA, AWWA and WEF, 21ST Edition, 2005.
- Adekunle, A.A. and O.A. Adebambo (2007). Petroleum hydrocarbon utilization by fungi isolated from *Detarium senegaleuse* (J. F. Gmelin) seeds. *Journal of American Sciences*, **3(1)**: 69-76.
- Ali, H.R., El-Gendy, N.Sh., Moustafa, Y.M., Roushdy, M.I. and A.I. Hashem (2012). Degradation of asphaltenic fraction by locally isolated halotolerant bacterial strains. International Scholarly Research Network (ISRN). <https://doi.org/10.5402/2012/435485>.
- Bergey's Manual of Determinative Bacteriology (1994). 9th ed. Williams and Wilkins, USA.
- Boratynsky, J., Syper, D., Weber-Dabrowska, B., Iusiak-Szelachowska, L., Pozniak, G. and A. Gorski (2004). Preparation of endotoxin-free bacteriophage. *Cellular and Molecular Biology Letters*, **9**: 253-259.
- Chorom, M., Hosseini S.S. and H. Motamedi (2010). Bioremediation of crude oil polluted soil as affected by sewage – sludge. In: 19th World Congress of Soil Science, Soil Solutions for a Changing World. pp. 4-7.
- Cybulski, Z., Dzuirla, E., Kaczorek, E. & Olszauowski, A. (2003). The influence of emulsifiers on hydrocarbon biodegradation by Pseudomonadacea and Bacillacea strains. *Spill Science and Technology Bulletin*, **8**: 503-507.
- Das, K. and A.K. Mukherjee (2006). Crude petroleum-oil biodegradation efficiency of Bacillus subtilis and Pseudomonas aeruginosa strains isolated from a petroleum-oil contaminated soil from North-East India. *Bioresour Technol*, **98(7)**: 1339-1345.
- Jawetz, M.D., Melnick, J.L. and E.A. Adelberg (1998). Pseudomonas. In: Medical Microbiology Review, 21st ed., USA: Appleton and Lange, pp. 231-233.
- Ko-Wen, C., Paterson, D.L., Sagnimeni, A.J., Hansen, D.S., Von Gottberg, A., Mohapatra, S., Casellase, J.M., Gossens, H., Mulazimoglu, L., Treuhme, G., Klugman, K.P., McCormack, J.G. and V.L. Yu (2002). Community-acquired *Klebsiella pneumoniae* bacteremia: Global differences in clinical patterns. *Emerging Infectious Diseases*, **8**: 160-166.
- Lawlor, M.S., Hsu, J., Rick, P.D. and V.L. Miller (2005). Identification of *Klebsiella pneumoniae* virulence determinants using an intranasal infection model. *Mol. Microbiol.*, **58(4)**: 1054-1073. doi: 10.1111/j.1365-2958.2005.04918.x.
- Levinson, W. and E. Jawetz (2000). Medical Microbiology & Immunology (examination & board review). 6th ed. Singapore, New Delhi: 582 pp.
- Lim, L.H., Harrison, R.M. and S. Harrad (1999). The contribution of traffic to atmospheric concentration of polycyclic aromatic hydrocarbons. *Environ. Sci. Technol.*, **33**: 3538- 3542.
- Mohammed, M.Kh. (2014). The use of certain bacterial species to treat polluted water with some hydrocarbon compound. Doctoral Dissertation. College of Science, University of Baghdad.
- Nadaling, T.N., Raymond, Gilewicz, M. and H. Budziniski (2000). Development of a protocol to study aerobic bacterial degradation of PAHs. *Ploy Cyclic Aro. Comp.*, **18**: 177-192.
- Nayyef, R.A. (2017). Testing efficiency of *Pseudomonas aurigios* and *Staphylococcus aureus* bacteria in reducing the concentration of glyphosphin and toluene used as fuel and fertilizer. *ARPJ Journal of Agriculture and Biological Science*, **12(9)**: 269-272.
- Nayyef, R.A. (2023). Inhibition effect of water and alcohol extracts of *Cinnamomun zeylanicam* (cinnamomun plants) and *Zingiber officinale* (ginger plant) in growth and efficiency of some microorganism. *Asian Journal of Water, Environment and Pollution*, **20(5)**: 95-98.
- Nwaichi, E.O., Essien, E.B. and E. Vabayide (2013). characterization of Warri Refinery effluent and its recipient medium. *Int. J. Pure App. Biosci.*, **1(4)**: 22-27.
- Okoh, A.L. (2003). Biodegradation of bonny light crude oil in soil microcosm by some bacterial strain isolated from crude oil flow strains Saver pits in Nigeria. *African. J. of Biotech.*, **2**: 104-108.
- Raper, B.K. and D.I. Fennel (1965). The Genus. Baltimore, USA: Williams and Winkins Co.
- Statistical Analysis System (SAS) (2012) . User's Guide. Statistical. Version 91th ed. SAS. Inst. Inc. Cary. N.C. USA.
- Tavassoli, T., Mousari, S.M., Shojaosadati, S.A. and M.H. Salehizedeh (2012). Asphaltene biodegradation using microorganisms isolated from oil samples. *Fuel*, **93**: 142-148.
- Toledo, F.L., Calro, C., Rodelas, B. and L.G. Lopez (2006). Selection and identification of bacteria isolated from waste crude oil with polycyclic aromatic hydrocarbons removal capacities. *Syst. Appl. Microbiol.*, **29**: 244-252.
- Yasin, A.R. (2013). Biodegradation of polluted water with crude oil discharged from South Baghdad power plant. MSc. Thesis College of Science for University of Baghdad.