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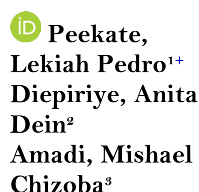
Effect of glucose on biodegradation of hydrocarbons in crude-oil polluted soil undergoing bioremediation



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ABSTRACT

Bioremediation of crude oil polluted soil can be enhanced by addition of easily assimilated carbon sources such as glucose. The aim of this study was to determine the effect of increasing concentration of glucose on biodegradation of hydrocarbons in crude-oil polluted soil undergoing bioremediation. Some hydrocarbon utilizing bacteria and fungi were used as bioremediation agents in the study. Soil artificially polluted with crude-oil was divided into 5 setups: CT (control), S1, S2, S4, and S8. Suspension of the bioremediation agents and nutrients were added to the setups. About 5, 10, 20, and 40 g glucose were added to S1, S2, S4, and S8 respectively: equivalent to 1, 2, 4, and 8 % glucose concentration. Soil moisture in the setups was maintained at 10-20 % during the experimental period. Total hydrocarbon concentration (THC), and population of total heterotrophic bacteria (THB), hydrocarbon utilizing bacteria (HUB), total fungi (TF), & hydrocarbon utilizing fungi (HUF) were determined on day 1 and 21. Results obtained showed that increase in HUB occurred only in setup S1, and setup S1 had the highest extent of hydrocarbon degradation (18.93 %). Setup S8 had the lowest extent of hydrocarbon degradation (0.79 %). It is concluded that glucose concentration of 1 % and below will enhance biodegradation of hydrocarbons in crude-oil polluted soils, whereas high glucose concentration will retard hydrocarbon biodegradation. Also, the study indicates the possibility of fungi not partaking in hydrocarbon degradation when glucose is made available.

Contribution/Originality: This study is different from other previous studies in that the effect of increasing concentration of glucose on the extent of total-hydrocarbon biodegradation was investigated. Also, the microbial group (bacteria or fungi) participating in hydrocarbon degradation in the presence of glucose was elucidated.

1. INTRODUCTION

Bioremediation of crude oil polluted soil can be enhanced by addition of easily assimilated carbon sources. A small amount of glucose, an easily assimilated carbon source, has been observed to enhance the biodegradation of the hydrocarbon naphthalene [1]. This enhancement can be explained in terms of co-metabolism. Co-metabolism have been investigated by some researchers [2-6] in the biodegradation of some environmental pollutants such as methyl-tert-butyl ether and chlorinated solvents, with some level of success. These pollutants were degraded due to the catalytic activities of enzymes that were produced for the degradation of easier utilizable substrates as source of

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energy. However, the effect of increasing or reducing concentrations of added easier utilizable substrates on pollutant biodegradation has not been determined.

Glucose is the most important source of energy for many organisms; it is a simple sugar and the most abundant monosaccharide [7]. It is metabolized in microorganisms through glycolysis, the pentose phosphate pathway, and the Entner-Doudoroff pathway [8, 9]. It is further metabolized through oxidative decarboxylation, the citric acid cycle (Krebs cycle), and the electron transport chain to yield water, carbon dioxide, and energy in the form of adenosine triphosphate (ATP). The preceding metabolic pathways require oxygen. If there is not enough oxygen available, glucose metabolism in microorganisms will occur anaerobically through lactic acid fermentation and other forms of fermentation [10]. However, the energy produced is much small.

Energy is required for cell growth. Microbial cell growth leads to increase in microbial cell population. Without increase in cell population, the amount of enzymes produced to breakdown substrates not normally utilized by the cells will be limited. Microbial cell growth depends on the type of substrates available and consumed by the microorganism. Petroleum hydrocarbons are not necessary substrates for hydrocarbon-degrading microorganisms; they utilize the hydrocarbons as alternative carbon and energy sources in the absence of their preferable substrates [11]. Therefore petroleum hydrocarbons are energetically demanding substrates for microbial utilization, and a long duration of time will be required for synthesis of enzymes required for hydrocarbon degradation due to requirements of growth and anabolism. It may therefore be necessary to provide an easily utilizable co-substrate that will enable hydrocarbon degrading microorganisms in crude-oil polluted environments to achieve high population within adequate duration so as to synthesis adequate amount of the enzymes required for hydrocarbon degradation within a fairly short duration. Therefore, the aim of this study was to determine the effect of glucose, an easily utilizable substrate, on biodegradation of hydrocarbons in crude-oil polluted soil undergoing bioremediation. The outcome of the study will reveal concentration of glucose below which bioremediation of hydrocarbon polluted soil will be enhanced, and above which bioremediation will be retarded.

2. MATERIALS AND METHODS

2.1. Acquisition of Hydrocarbon-Utilizing Microorganisms

Klebsiella aerogenes, *Micrococcus halobius*, *Pseudomonas fluorescence*, *Mucor* sp., *Aspergillus flavus*, and *Fusarium* sp. were obtained from stock cultures in the Department of Microbiology, Rivers State University, Nigeria. These organisms are hydrocarbon utilizers as revealed in previous studies [12-16]. The acquired microorganisms were subjected to colonial characterization, microscopy, and some physicochemical/biochemical tests so as to confirm their identity.

2.2. Identification of Hydrocarbon Utilizing Fungi used in the Study

The hydrocarbon utilizing fungi were sub-cultured onto potato dextrose agar plates. Sub-cultured plates were incubated for 5 days at ambient temperatures (27–32 °C). After incubation, fungal growths were identified through macroscopic and microscopic examination of colonial and cell morphology respectively. In the microscopic examination, stain slides of the fungi were prepared using lactophenol cotton blue stain. The stained slides were then observed under the Light Microscope using 40× objective lens. Observed macroscopic and microscopic characteristics were compared with fungal macroscopic and microscopic characteristics in Zafar, et al. [17] so as to determine the identity of the fungi.

2.3. Identification of Hydrocarbon Utilizing Bacteria used in the Study

The hydrocarbon utilizing bacteria were sub-cultured onto nutrient agar plates, and incubated at 35 °C for 24 hours. After incubation, ensuing bacterial colonies were subjected to Gram staining and microscopic examination. Subsequently, the bacteria were subjected to the following biochemical/physicochemical tests: catalase, oxidase,

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motility, citrate utilization, indole production, MRVP (Methyl Red-Vogues Proskauer), 7 % salt (NaCl) tolerance, casein hydrolysis, and fermentation tests using glucose, lactose, mannitol, sucrose, and glycerol. Results obtained from the tests and microscopic examinations were inputted into the search dialogue of the online bio-database software "Advanced Bacterial Identification Software" (available at https://www.tgw1916.net/bacteria_logare.html), so as to confirm the identity of the bacteria.

2.4. Preparation of the Microorganisms for Bioremediation Experiment

Inoculums from stock cultures of the bacteria and fungi were grown in nutrient broth and potato dextrose broth respectively, from where they were inoculated in a confluent manner on sterile nutrient agar (NA) and potato dextrose agar (PDA) plates accordingly, in triplicates. Inoculated NA and PDA plates were incubated at ambient temperatures (27–32 ℃) for 2 and 5 days respectively. After incubation, the ensuing growths were scooped and transferred into sterile 400 ml mineral-salts solution with the aid of sterile spatula. The composition (g/L) of the mineral-salts solution is as follows: $MgSO_4.7H_2O$ - 0.42, KH_2PO_4 - 0.83, NaCl - 10.0, KCl - 0.29, Na_2HPO_4 - 1.25, $NaNO_3$ - 0.42, Agar – 15.0 [18]. The resulting microbial-mineral-salts suspension was stored at 4 ℃ in a refrigerator until required for use.

2.5. Bioremediation Experiment

Soil was collected from the Rivers State University school farm. About 3 kg of the soil was placed in a glass trough, and artificially polluted with 450 ml of crude-oil. The artificially polluted soil was allowed undisturbed for a week at ambient temperatures (27–32 °C), after which the total hydrocarbon concentration (THC) in the soil was determined. The soil was then divided into five silver pans; 500 g per pan. The pans were tagged CT (control), S1, S2, S4, and S8. About 50 ml of the microbial-mineral-salts suspension was added to the polluted soil in each pan. Then 5, 10, 20, and 40 g glucose were added to the pans tagged 1, 2, 4, and 8 respectively: equivalent to 1, 2, 4, and 8 % glucose concentration. Tilling was carried out thrice in a week. Soil moisture content was maintained at 10-20 % by adding mineral-salts solution once in a week. The experimental setup was maintained for 21 days. Populations of total heterotrophic bacteria (THB), hydrocarbon utilizing bacteria (HUB), total fungi (TF), and hydrocarbon utilizing fungi (HUF) were determined on day 1 and 21. Also, on day 21 THC was determined.

Determination of THC: The spectrophotometric method described in Peekate, et al. [19] was used in determination of THC in the soil samples. Determination of populations of THB, HUB, TF, and HUF: About 1 g of the soil samples were placed in 10 ml sterile normal saline, separately. The resulting mixtures were subjected to 10-fold serial dilution, using sterile normal saline, to obtain 10^{-5} dilutions. About 0.1 ml of 10^{-3}, 10^{-4}, and 10^{-5} dilutions were spread inoculated on NA plates, in duplicates; 0.1 ml of 10^{-2}, 10^{-3}, and 10^{-4} dilutions on mineral salts agar (MSA) plates containing 100 mg/ml ketoconazole, PDA plates, and MSA plates containing antibiotics (50 µg/ml streptomycin and 50 µg/ml tetracycline). Inoculated NA plates were incubated at 35 °C for 24 hours. Inoculated MSA plates were supplied with petroleum hydrocarbons using the vapour phase transfer technique [20], and incubated at ambient temperatures (27 – 32 °C) for 5 days. Inoculated PDA plates were also incubated at ambient temperatures (27 – 32 °C) for 5 days. After incubation, ensuing colonies on NA plates, MSA plates containing ketoconazole, PDA plates, and MSA plates containing antibiotics were counted and used to calculate the populations of THB, HUB, TF, and HUF, respectively.

3. RESULTS

3.1. Macroscopic and Microscopic Characteristics of the Fungi

The macroscopic and microscopic characteristics of the acquired fungi (*Mucor* sp., *Aspergillus* sp., and *Fusarium* sp.) are presented in Table 1. Comparison of the characteristics with fungal description in Zafar, et al. [17] confirmed the identity of the obtained fungi.

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3.2. Reaction Pattern of the Bacteria to Identification Tests

The results of the physicochemical and biochemical tests carried out on the bacteria (*Klebsiella aerogenes*, *Micrococcus halobius*, and *Pseudomonas fluorescence*) are presented in Table 2. The identity of the acquired bacteria as confirmed through the "Advanced Bacterial Identification Software" (https://www.tgw1916.net/bacteria_abis.html) are as follows: *Klebsiella aerogenes* (99 % similarity), *Micrococcus halobius* (83.4 % similarity), and *Pseudomonas fluorescence* (89 % similarity).

Table 1. Macroscopic and microscopic characteristics of the fungi.

Fungi	Characteristics/Morphology	
	Macroscopic	Microscopic
Mucor sp.	White fluffy growth with reverse white colour	Non-septate hyphae; numerous sporangiphores in random arrangement within round head sporangium
Aspergillus flavus	Yellow-green granular-cottony growth with extending white hazy periphery; pale yellow reverse colour	Septate hyphae; long conidiophores with round phialide and numerous conidia in radial arrangement
Fusarium sp.	Off-white wrinkled cottony growth with pale yellow reverse colour	Septate hyphae; banana shaped macroconidia

Table 2. Physicochemical/biochemical reaction patterns of the bacteria.

Tests	Klebsiella aerogenes	Micrococcus halobius	Pseudomonas fluorescence
Gram stain	-	+	-
Cell morphology	Rods	Cocci	Rods
Catalase test	+	+	+
Oxidase test	-	+	+
Motility test	-	-	+
Citrate utilization	+	+	+
Indole production	-	+	-
Methyl red test	-	-	-
Vogues-Proskauer test	+	+	-
7 % salt tolerance	+	+	+
Casein hydrolysis	-	-	+
Glucose fermentation	Acid & Gas	Acid & Gas	Acid
Lactose fermentation	Acid & Gas	Acid & Gas	-
Mannitol fermentation	Acid	Acid	Acid
Sucrose fermentation	Acid	Acid	-
Glycerol fermentation	Acid	Acid	-
Identity similarity (%)	99	83.4	89

3.3. Populations of THB and HUB in the Experimental Setup

The populations of total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) in the setups are presented in Figure 1. In the Figure, it can be seen that from day 1 to 21 there was moderate reduction in average THB population in setup CT (from 7.4 to 6.6 Log_{10}CFU/g), and minor reduction in setup S1 (from 6.5 to 6.4 Log_{10}CFU/g). The THB populations in the both setups were significantly different on day 1 ($p < 0.05$), but not on day 21 ($p > 0.05$); logically implying that the extents of THB population reduction in the both setups were significantly different. On the other hand, there were slight increases in average THB populations in setup S2, S4, and S8 (from between 6.5-6.7 to between 6.7-6.8 Log_{10}CFU/g). The THB populations in the setups were not significantly different on day 1 and day 21 ($p > 0.05$); logically implying that the extents of THB population increase in the 3 setups were not significantly different. Also in Figure 1, it can be seen that from day 1 to 21 there were reduction in average HUB populations in setup C, S2, S4, and S8 (from between 4.5-5.5 to between 3.5-3.9 Log_{10}CFU/g). On the other hand, there was increase in average HUB population in setup S1 (from 4.8 to 5.2 Log_{10}CFU/g). The HUB populations in the setups were significantly different on day 1 and day 21 ($p < 0.05$); implying that the comparison is significant.

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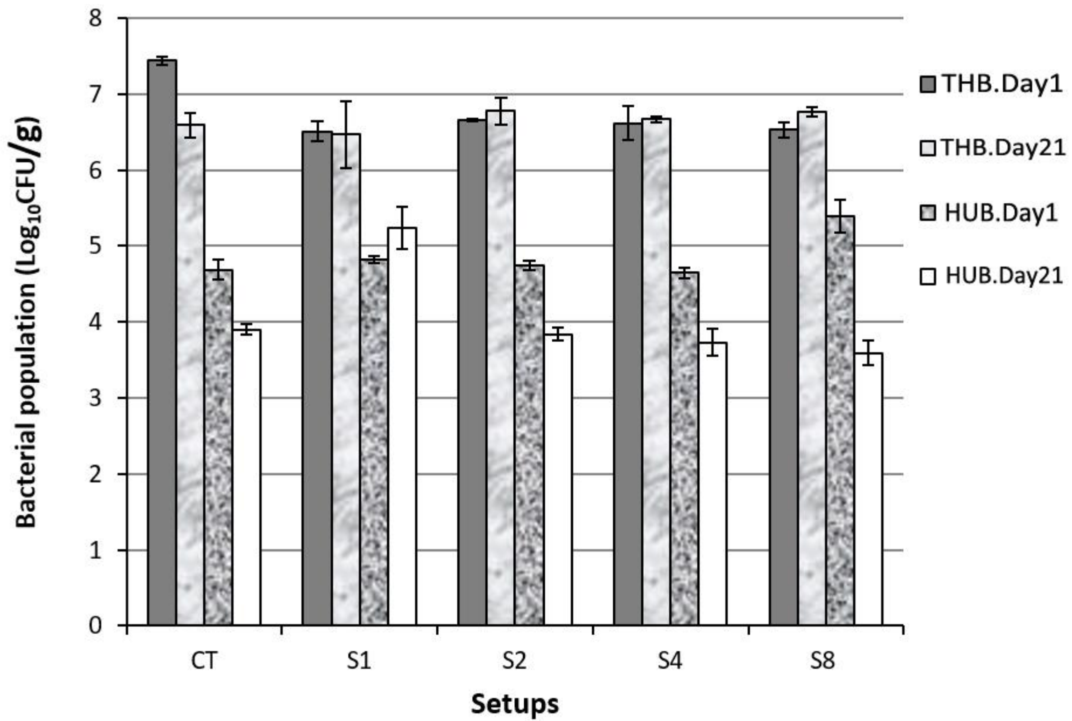

Figure 1. Populations of total heterotrophic bacteria (THB) and hydrocarbon utilizing bacteria (HUB) in the setups.

3.4. Populations of TF and HUF in the Experimental Setup

The populations of total fungi (TF) and hydrocarbon utilizing fungi (HUF) in the setups are presented in Figure 2. In the Figure it can be seen that from day 1 to 21 there were reductions in the average populations of TF and HUF in all the setups, except in setup S4 where there was a little increase from 4.65 to 4.67 $Log_{10}CFU/g$. The TF populations in the setups were significantly different on day 1 and day 21 ($p < 0.05$); logically implying that the extents of TF population decrease in the setups were significantly different. On the other hand, the HUF populations in the setups were significantly different on day 1 ($p < 0.05$), but not on day 21 ($p > 0.05$).

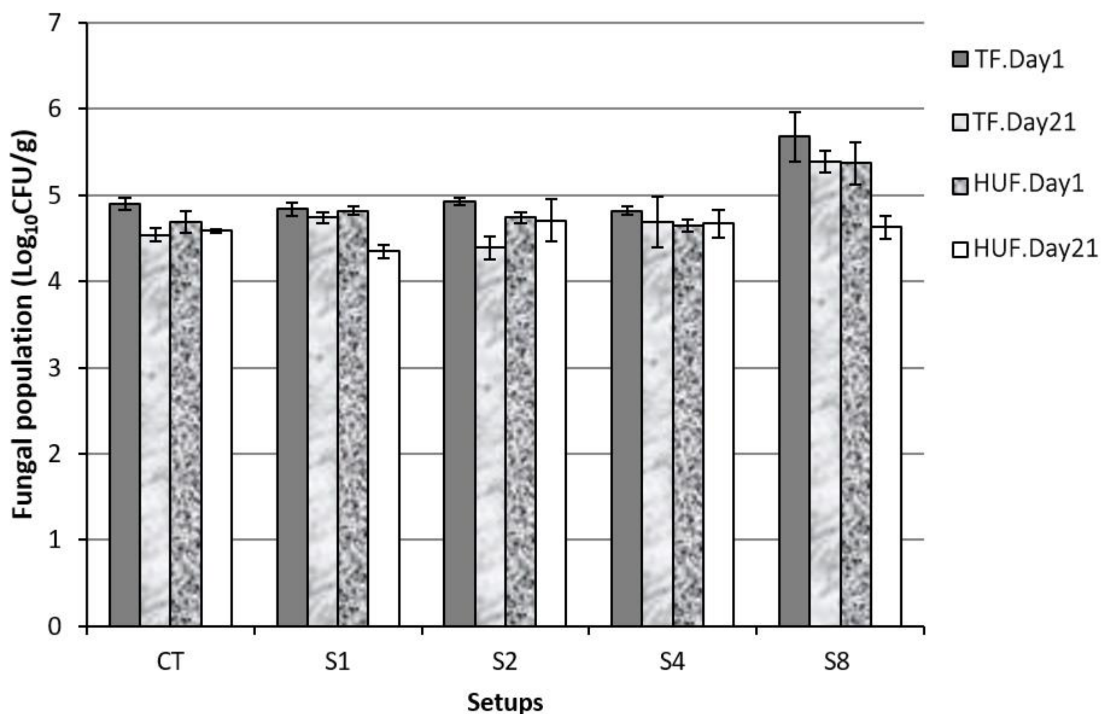

Figure 2. Populations of total fungal (TF) and hydrocarbon utilizing fungi (HUF) in the setups.

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3.5. Total Hydrocarbon Concentrations in the Experimental Setup

The total hydrocarbon concentrations (THC) in the setups are presented in Figure 3. In the Figure it can be seen that from day 1 to 21 there was decrease in average THC in all the setups. The decrease was more in setup S1 (18.9 % reduction) and least in setup S8 (0.8 % reduction). THC in the setups were significantly different on day 21 ($p < 0.05$); implying that the extents of THC degradation in the setups were significantly different.

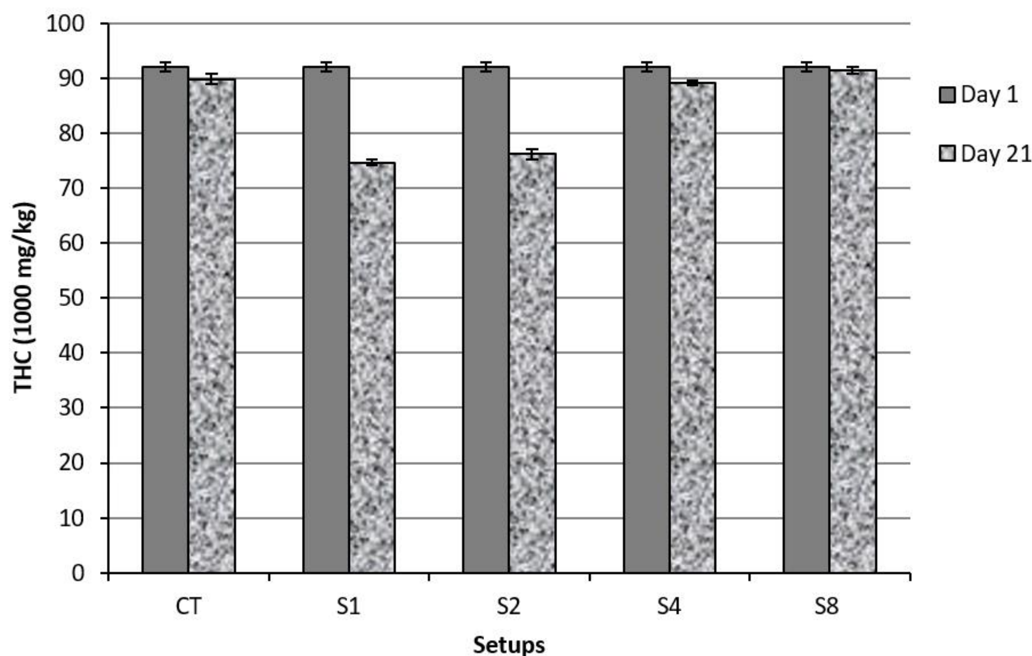

Figure 3. Total hydrocarbon concentration in the setups.

4. DISCUSSION

Addition of easily assimilated carbon sources such as glucose to hydrocarbon polluted environment during bioremediation of such environment can enhance the biodegradation of the hydrocarbons through the process of co-metabolism. However beyond a certain concentration of such easily assimilated carbon source, biodegradation of the hydrocarbon pollutant may be stalled. In this study, the effect of increasing glucose concentrations on biodegradation of hydrocarbons in crude-oil polluted soil undergoing bioremediation was investigated. The concentrations of glucose used in the study, relative to the quantity of soil used, ranged from 10 – 80 g/Kg (equivalent to 1 – 8 %). The results revealed that glucose at 10 g/Kg (1 %) enhanced biodegradation of hydrocarbons from 2.5 to 18.9 % after 21 days; beyond 20 g/Kg (2 %) there was retardation in biodegradation of the hydrocarbons Figure 3. The trend in Figure 3 indicates that enhanced biodegradation of hydrocarbons in polluted soil can be achieved at glucose concentrations lesser than 1 %. In similar studies, relatively high biodegradation of polycyclic-aromatic hydrocarbons (PAH) has been achieved within short periods with glucose concentrations ranging from 0.1 – 0.5 % [1, 21]. At 0.1 % glucose concentration, 98.7 % biodegradation of a PAH was achieved by Logeshwaran, et al. [21] after 15 days. At 0.5 % glucose concentration, 82.47 % biodegradation of another PAH was achieved by Fadilah, et al. [1] after 96 hours (4 days). However, moderate hydrocarbon biodegradation have also been achieved in other studies: Daesung, et al. [22] achieved 14.2 and 16.7 % PAH biodegradation with glucose concentrations of 500 and 2000 mg/L (equivalent to 0.05 and 0.2 %) respectively after 2 days, and Boszczyk-Maleszak, et al. [23] achieved 56 % total-hydrocarbon biodegradation with 1 % glucose concentration after 21 days. The starting hydrocarbon concentrations used in the preceding cited works are as follows: 50 mg/L (equivalent to 0.005 %) in Daesung, et al. [22], 3 % in Boszczyk-Maleszak, et al. [23], 100 mg/L (equivalent to 0.01 %) in Logeshwaran, et al. [21], and 0.02 % w/v in Fadilah, et al. [1]. These concentrations were much less compared to the starting hydrocarbon concentration (92,180 mg/Kg, equivalent to 9.2 %) used in this study. The

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large difference in starting hydrocarbon concentrations is a possible reason for the differences observed in the extent of hydrocarbon degradation; also the studies, except that of Boszczyk-Maleszak, et al. [23], were carried out in liquid media as opposed to solid heterogeneous medium (soil) used in this study.

Increase in population of hydrocarbon utilizing bacteria (HUB) occurred only in setup S1 Figure 1; setup S1 had the lowest glucose concentration (1 %). It can be reasoned that glucose was depleted soon enough in setup S1 and the enzymes produced by HUB in setup S1 for utilization of glucose were partaking in the degradation of the hydrocarbon pollutants. In a similar study [3], enhanced biodegradation of a pollutant (methyl tert-butyl ether) was reasoned to be due to catalytic activities of enzymes produced for biodegradation of the easier utilizable substrate, n-pentane. The HUB populations in the setups were significantly different on day 1 and day 21 ($p < 0.05$), indicating that the increased HUB population in setup S1 for probably increased production of such enzymes was significant. On the other hand, increase in population of hydrocarbon utilizing fungi (HUF) occurred only in setup S4 Figure 2. The increase was very little, and could not have translated to increased production of such enzymes by the HUF. Overall these indicate that HUB partakes in hydrocarbon degradation in the presence of glucose, while HUF most likely do not partakes in hydrocarbon degradation in the presence of glucose. Preferred glucose utilization to hydrocarbon degradation by HUF in the presence of glucose and hydrocarbons could be a probable reason why there is scarcity of literature on enhanced hydrocarbon degradation using fungi with glucose or any other sugars. On the other hand, there are available literatures where bacteria such as *Pseudomonas* spp., *Burkholderia cepacia*, and *Microbacterium esteraromaticum* [1, 21, 22] have been used and high extent of biodegradation recorded.

Hydrocarbon utilizing microorganisms used as bioremediation agents in this study include *Klebsiella aerogenes*, *Micrococcus halobius*, *Pseudomonas fluorescence*, *Mucor* sp., *Aspergillus flavus*, and *Fusarium* sp. These microorganisms among others have been implicated in studies [12-16] on bioremediation of hydrocarbon polluted environments as microorganisms capable of effectively degrading petroleum hydrocarbons.

5. CONCLUSION

This study has shown that low concentration of glucose can enhance biodegradation of hydrocarbons in crude-oil polluted soil, and high glucose concentration will retard hydrocarbon biodegradation. Also, the study indicates the possibility of fungi not partaking in hydrocarbon degradation when glucose is made available. Further investigation on enhance bioremediation of crude-oil polluted soil is therefore suggested with the use of glucose at concentrations below 10g/Kg or 1 % and exclusion of fungi.

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Authors' Contributions: All authors contributed equally to the conception and design of the study. All authors have read and agreed to the published version of the manuscript.

REFERENCES

[1] M. Fadilah, S. Sitaresmi, Y. Yasman, and A. Oetari, "Biodegradation of 0.02 % naphthalene added with 0.5 % glucose by Pseudomonas aeruginosa DRK 9.1 isolated from volcanic mud in Renokenongo village, Sidoarjo," AIP Conference Proceedings 2242, 050004, 2020.

[2] C. A. Gulvik and A. Buchan, "Simultaneous catabolism of plant-derived aromatic compounds results in enhanced growth for members of the roseobacter lineage," *Applied and Environmental Microbiology*, vol. 79, no. 12, pp. 3716-3723, 2013. https://doi.org/10.1128/aem.00405-13

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[3] S. Li, S. Wang, and W. Yan, "Biodegradation of methyl tert-butyl ether by co-metabolism with a pseudomonas sp," *Strain International Journal of Environmental Research and Public Health*, vol. 13, no. 9, pp. 1-10, 2016. https://doi.org/10.3390%2Fijerph13090883

[4] X. Li *et al.*, "Enhanced biodegradation of aged petroleum hydrocarbons in soils by glucose addition in microbial fuel cells," *Journal of Chemical Technology & Biotechnology*, vol. 91, no. 1, pp. 267-275, 2016. https://doi.org/10.1002/jctb.4660

[5] A. Nzila, "Update on the cometabolism of organic pollutants by bacteria," *Environmental Pollution*, vol. 178, pp. 474-482, 2013. https://doi.org/10.1016/j.envpol.2013.03.042

[6] D. Ryoo, H. Shim, K. Canada, P. Barbieri, and T. K. Wood, "Aerobic degradation of tetrachloroethylene by toluene-o-xylene monooxygenase of Pseudomonas stutzeri OX1," *Nature Biotechnology*, vol. 18, no. 7, pp. 775-778, 2000. https://doi.org/10.1038/77344

[7] A. J. Domb, J. Kost, and D. Wiseman, "Handbook of biodegradable polymers," Retrieved: https://books.google.com/books?id=iLjhl6AvfIsC&pg=PA275. 1998.

[8] R. H. Garrett, *Biochemistry*, 5th ed. Belmont, CA: Brooks/Cole: Cengage Learning, 2013.

[9] L. M. Prescott, J. P. Harley, and D. A. Klein, *Microbiology*, 4th ed. New York: The McGraw-Hill companies, Inc, 1999.

[10] P. J. Jurtshuk, "Bacterial metabolism" in S. Baron (Ed.), medical microbiology. 4th edition. Galveston (TX): University of texas medical branch at galveston," Retrieved: https://www.ncbi.nlm.nih.gov/books/NBK7919/. 1996.

[11] X. Xu *et al.*, "Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: A perspective analysis," *Frontiers in Microbiology*, vol. 9, p. 2885, 2018. https://doi.org/10.3389/fmicb.2018.02885

[12] R. Akwukwaegbu, P. Okerentugba, G. Okpokwasili, H. Stanley, and C. Ugboma, "Hydrocarbon degradation potential of heterotrophic bacteria isolated from oil polluted sites in Sakpenwa community in Rivers State," *South Asian Journal of Research in Microbiology*, vol. 4, pp. 1-12, 2019. https://doi.org/10.9734/sajrm/2019/v4i230102

[13] N. Ali, M. Eliyas, H. Al-Sarawi, and S. Radwan, "Hydrocarbon-utilizing microorganisms naturally associated with sawdust," *Chemosphere*, vol. 83, no. 9, pp. 1268-1272, 2011. https://doi.org/10.1016/j.chemosphere.2011.03.052

[14] M. D. Asemoloye *et al.*, "Hydrocarbon degradation and enzyme activities of Aspergillus oryzae and Mucor irregularis isolated from Nigerian crude oil-polluted sites," *Microorganisms*, vol. 8, no. 12, pp. 1-19, 2020. https://doi.org/10.3390/microorganisms8121912

[15] C. Chikere, G. Okpokwasili, and O. Ichiakor, "Characterization of hydrocarbon utilizing bacteria in tropical marine sediments," *African Journal of Biotechnology*, vol. 8, no. 11, pp. 2541-2544, 2009.

[16] K. L. Njoku, E. O. Ude, T. O. Jegede, O. Z. Adeyanju, and P. O. Iheme, "Characterization of hydrocarbon degrading microorganisms from Glycine max and Zea mays phytoremediated crude oil contaminated soil," *Environ Analysis Health and Toxicology*, vol. 37, no. 2, pp. 1-16, 2022. https://doi.org/10.5620/eaht.2022008

[17] A. Zafar, K. Jabeen, and J. Farooqi, "Practical guide and atlas for the diagnosis of fungal infections," Retrieved: https://ecommons.aku.edu/books/62. [Accessed 5-11-2022], 2017.

[18] L. Odokuma and A. Dickson, "Bioremediation of a crude oil polluted tropical mangrove environment," *Journal of Applied Sciences and Environmental Management*, vol. 7, no. 2, pp. 23-29, 2003. https://doi.org/10.4314/jasem.v7i2.17207

[19] P. Peekate, G. Abu, and C. Ogugbue, "Investigating the effectiveness between using Pseudomonas fluorescens and its biosurfactant in bioremediation of petroleum hydrocarbon contaminated soil," *Asian Journal of Biotechnology and Bioresource Technology*, vol. 3, no. 2, pp. 1-10, 2018. https://doi.org/10.9734/ajb2t/2018/40528

[20] O. A. T. Ebuehi, I. B. Abibo, P. D. Shekwolo, K. I. Sigismund, A. Adoki, and I. C. Okoro, "Remediation of crude oil contaminated soil by enhanced natural attenuation technique," *Journal of Applied Sciences and Environmental Management*, vol. 9, pp. 103-106, 2005.

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[21] P. Logeshwaran *et al.*, "Polycyclic aromatic hydrocarbons biodegradation by fenamiphos degrading Microbacterium esteraromaticum MM1," *Environmental Technology & Innovation*, vol. 27, pp. 1-13, 2022. https://doi.org/10.1016/j.eti.2022.102465

[22] L. Daesung, L. Minwoo, and J.-m. Park, "Effects of salicylate and glucose on biodegradation of phenanthrene by Burkholderia cepacia PM07," *Journal of Microbiology and Biotechnology*, vol. 15, no. 4, pp. 859-865, 2005.

[23] H. Boszczyk-Maleszak, A. Przestrzelska, E. Bieszkiewicz, and M. Horoch, "Biodegradation of petroleum products by microorganisms adapted to high crude oil concentration in the presence of easy assimilated carbon source," *Polish Journal of Microbiology*, vol. 53, pp. 183-187, 2004.

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