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The Development of a Global Shoreland Information Database May Help Determine the Appropriate Bacteria and Nutrient Technology Needed to Remediate Oil Contaminated Shorelands

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By Tiffany A. Veeder

Abstract:

The use of oil and its derivative products is well known world-wide. The benefits of oil are many and its products, for example, range from gasoline used in our automobiles to its use in plastics, building products, and medical supplies. Oil spill hazards associated with oil extraction, production, and transportation can have devastating impacts on the environment and local economies. When oil spills happen in the water, for instance, major shorelands are placed at risk of significant harm. As oil contaminates shorelands, the impacts can range from killing sea creatures and seabirds to destroying entire ecosystems. To help mitigate the devastating effects of oil spills, access to information about impacted shoreland environments is crucial to help determine what cleanup technologies and strategies can best remediate the oil contaminated media. This paper reviews two useful and appropriate bioremediation technologies, bioaugmentation and biostimulation, that use bacteria and supplemental nutrients to assist in the degradation of petroleum contamination on beaches and aquatic shorelines. This paper also proposes the development of a global shorelands' information database relating to shorelands that are likely targets of oil spills. Before an oil spill occurs, the database would contain specific information about various shorelands' ecosystems, including their soil, bacteria, and micro flora types. In the event that an oil spill threatens a beach, the database could be consulted to predict what bioremediation technologies would be appropriate and useful for remediation purposes before the oil comes into contact with the shoreland.

Introduction:

With the overwhelming demand for petroleum products in the world, petroleum spillage during oil exploration, extraction, and transportation has led to devastating impacts on waterways and shorelines throughout the globe. Often, the spills impart a great cost to local economies that rely on businesses such as the fisheries and the tourism industries. In addition, governments, like the United States, have taken severe actions in restricting oil exploration and development permits for oil companies when their operations have led to a massive release of crude oil into the environment and devastating damage of shorelands. A prime example of a massive release occurred when the oil drill rig, Deepwater Horizon, experienced a catastrophic explosion that led to the release of millions of gallons of crude oil into the Gulf of Mexico causing substantial shoreland damage.[28] The oil exploration and development restrictions can cause significant economic losses for local communities that depend on the oil operations. For decades, major efforts have been taken by oil companies and governments to reduce the negative impacts of oil spills on the environment. Various remediation technologies, such as soil removal, soil washing, dispersants and solidifiers application, for example, have been employed to reduce, mitigate, and dispose of contaminated media (soil & water) impacted by petroleum hydrocarbons. Among these technologies, bioremediation has proven to be successful at a lower cleanup cost. Two types of bioremediation are addressed in this paper: biostimulation and bioaugmentation.

Biostimulation utilizes the addition of nutrients, such as nitrogen and phosphorous, to stimulate growth in the population of an environment's indigenous bacteria. If present, specific bacteria in these populations consume and degrade petroleum hydrocarbons into harmless byproducts. A major challenge to biostimulation is overcoming an oxygen deficient environment created when indigenous micro flora populations rapidly increase after being treated with nutrients. These enhanced micro flora populations compete for oxygen reducing the viability and effectiveness of oil metabolizing bacteria.

Another bioremediation technology used to degrade oil contamination is bioaugmentation. Bioaugmentation is the application of laboratory enhanced bacteria to contaminated media which can mediate petroleum hydrocarbons degradation. The use of petroleum eating bacteria for mitigating the impacts of crude oil contamination is desirable because it can reduce cleanup costs. The importance of petroleum degrading bacteria was recognized by the scientific community as playing a key role in degrading the crude oil that entered the environment as a result of the Deepwater Horizon oil spill.[50] Bioaugmentation uses a variety of naturally existing and laboratory enhanced bacteria to produce a highly efficient petroleum mitigating microorganism that consumes oil in saturated waters and shorelands without the need to remove soils. Although bioaugmentation is often used to cleanup oil spills, it is challenging to identify the correct bacteria or consortium of bacteria that will thrive in local indigenous bacteria populations. It is also important to provide sufficient nutrients to feed bacteria to sustain their growth and survival without causing overgrowth of indigenous micro flora which can consume the available oxygen source leading to bacteria death. In addition, local governments of oil impacted shorelands must review and authorize the use of these bioremediation strategies which often results in costly delays. Providing useful shoreland and bioremediation data would help government agencies who review and approve oil spill cleanup plans. To meet these needs, I propose the development of a global shoreland's ecological information database that would contain pre-oil spill shoreland data. This would help with the development of a preemptive strategy that could provide governments, oil companies, and oil contamination cleanup responders with valuable information on the types of bioremediation technologies that would work best for oil impacted environments before an oil spill occurs.

Crude Oil Comprised of Total Petroleum Hydrocarbons Harm Shoreland Ecosystems

Crude oil releases into the environment are toxic and can cause significant, harmful effects on established ecosystems.[45] The harmful impacts of petroleum hydrocarbons in a crude oil release that affects shorelands and destroys their ecosystems are visually disturbing and obvious as shown in Figure 1 and Figure 2.

IMPACT OF OIL ON SEABIRDS

Figure 1: Seabirds covered in oil, like this pelican, are the casualties of crude oil spills. Source: Marine Defenders. [cited 2022 Apr 27]. Available from:https://www.marinedefenders.org/impact-on-mammals-birds-and-fish.html.

REFUGIO BEACH OIL SPILL

Figure 2: Crude oil impacts human use of natural resources as seen in a 2015 photo of the Refugio State Beach oil spill in Santa Barbara County, California. Source: NOAA. 2015 May [updated 2021 Jun 29; cited 2022 Apr 27]. Available from: https://darrp.noaa.gov/oil-spills/refugio-beach-oil-spill.

It is well known that humans can be negatively impacted from petroleum hydrocarbons toxicity that emanates from contaminated media where humans may interact with an oil impacted environment.[10,28] Although oil impacted shorelines show clear signs of destruction, what goes unnoticed is the potential destruction of beneficial microorganism colonies that exist on the shorelands that are interfacing with toxic petroleum hydrocarbons that are present in crude oil.[34,38]

Crude oil is a complex liquid that is flammable, sticky, and varies in chemical composition.[44] Environmental damage from crude oil releases are caused by many factors. Although the public usually becomes aware of only massive crude oil spills, there are many other releases of oil that have detrimental impacts to sensitive environmental media. Accordingly, there are eight major causes of crude oil spills that contribute to the contamination of waterways and shorelands as shown in Figure 3.

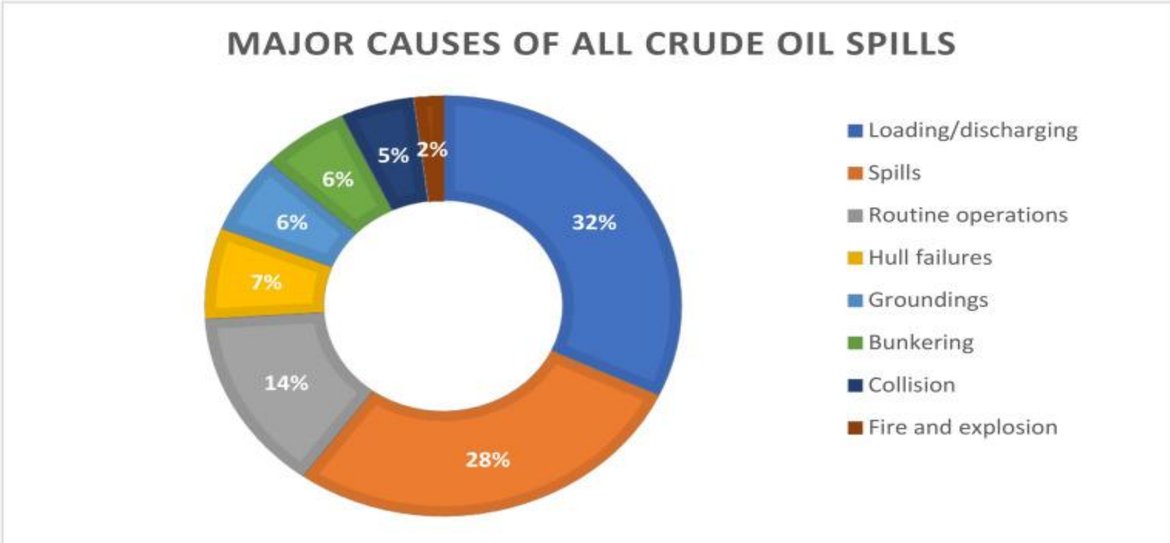

Figure 3: Oil tanker groundings, oil tanker hull failures, and routine oil operations account for the largest percentage of all oil spills that negatively impact shorelands.[44]

In order to cleanup shorelands contaminated with oil, three contamination cleanup techniques are generally considered for implementation during remediation. Depending on the significance of the release and the type of the impacted media, physical, chemical, and/or bioremediation technology may be employed during the remediation process as shown in Figure 4.

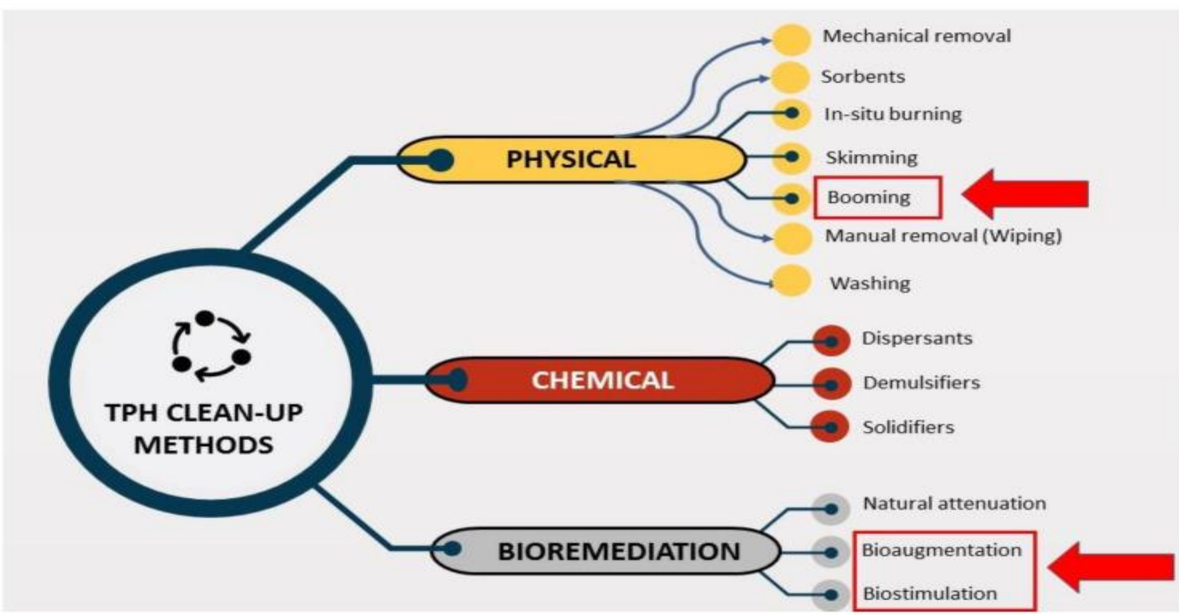

Figure 4: Total Petroleum Hydrocarbons cleanup methods include bioremediation processes involving bioaugmentation (the use of enhanced bacteria) and biostimulation (the application of nutrients to a shoreland's indigenous bacteria) to help remediate oil contaminated soils. While booming activities, a physical approach, help stop or redirect floating oil from reaching the shoreline.[44]

A Shoreland's Indigenous Bacteria Can Reduce the Toxicity of Petroleum Hydrocarbons

A shoreland's natural defense against an invading crude oil release is found in its bacteria populations. It is well documented that large populations of indigenous bacteria are known to exist in oil-rich environments.[16,58] Bacteria naturally help reduce toxic oil contamination to harmless byproducts that no longer pose a threat to delicate ecosystems within a crude oil impacted environment.[16,22] The quantity and quality of bacteria that consumes petroleum hydrocarbons during the remediation process is a function of the individual constituents that make up unique crude oil types and indigenous environmental factors.[15,53] The bacteria that degrades petroleum hydrocarbons have been identified as existing in at least 79 bacteria subdivisions.[50] The degrading bacteria have been identified as *Acinetobacter, Achromobacter, Alteromonas*, *Alkanindiges*, *Arthrobacter*, *Dietzia*, *Burkholderia*, *Marinobacter*, *Enterobacter, Kocuria*, *Mycobacterium*, *Pseudomonas, Pandoraea*, *Rhodococcus*, *Streptococcus, Staphylococcus*, and *Streptobacillus* and are essential in the petroleum hydrocarbon degradation process.[8,20,26,31,43,52,53,57] Some bacteria, such as *Alkanindiges* sp., for example, are known to exhibit infrequent to substantial bacteria shifts that are greatly influenced by environmental constraints that are observed with diesel oil contamination.[15] What the studies demonstrate is that many bacteria microorganisms possess potential that is essential for cleaning up crude oil contaminated shorelands and their respective ecosystems.[8,53] While other bacteria are simply ineffectual to assist in the remediation process.[8,53] In regard to bioremediation strategies, studies suggest that a consortium of petroleum hydrocarbons degrading bacteria would provide the most effective result for successfully remediating a petroleum hydrocarbons contaminated shoreland environment.[11] Researchers constructed a halotolerant Hydrocarbon Utilizing Bacterial Consortium (HUBC) that consisted of the bacterial isolates, *maltophilia, Stenotrophomonas, Ochrobactrum* sp., and *Pseudomonas aeruginosa* that were found to be effective at degrading crude oil where the degradation percentage reached 83.49%.[51] Other researchers used a defined co-culture of an indigenous bacteria consortium and exogenous *Bacillus subtilis* to effectually accelerate crude oil degradation.[48] Other studies similarly conclude that an indigenous bacterial consortium had higher crude oil degradation potentials and efficacy than individual bacteria in a marine ecosystem environment.[56] One field study revealed that bioaugmentation using an artificial bacteria consortium consisting of *Alcaligenes xylosoxidans, Gordonia* sp., *Pseudomonas fluorescens, Pseudomonas putida*, *Aeromonas hydrophila*, *S. maltophilia*, *Xanthomonas* sp. and *Rhodococcus equi* provided a high biodegradation efficiency rate of (89%) in a one-year treatment period of diesel oil-contaminated soil.[47] Based on the aforementioned studies, it is reasonable to conclude that using a bacteria consortium to accelerate the remediation process is an effective bioaugmentation strategy for cleaning up a crude oil contaminated shoreland.

Biostimulation is an Effective Bioremediation Process that Uses Nutrients to Increase Indigenous Bacteria Populations that Help Cleanup Oil Contaminated Shorelands

Unlike bioaugmentation, biostimulation uses nutrients to stimulate the growth of a shorelands' naturally existing indigenous bacteria population that can successfully remediate Total Petroleum Hydrocarbons (TPH) impacted media. Many factors, however, can limit or affect biodegradation in a given location, including indigenous media nutrients, moisture concentrations, pH levels, media temperature, oxygen concentrations, contaminant concentrations, and soil properties.[5,6,7] Biostimulation is the process by which environmental media is modified, through the introduction of nutrients, to promote the growth of indigenous bacteria that can help degrade petroleum hydrocarbons. The modification is the result produced when adding electron acceptors and nutrients, like nitrogen, oxygen, or phosphorus, to oil impacted media which, in a number of cases, are in quantities too insufficient to promote healthy microbial activity and growth that is needed to assist in a successful remediation process.[14,36,37] When adding electron donors, like nutrients or oxygen, to petroleum contaminated sites, indigenous, oil consuming, bacteria populations and their biochemical activity increase dramatically resulting in the growth of microorganisms necessary to promote effective bioremediation.[35] Applying nutrients, like nitrogen, or enhanced exogenous bacteria to contaminated media can be conducted using aerial disbursement as shown in Figure 5.[49,55]

DEEPWATER HORIZON OIL SPILL

Figure 5: Aerial application of chemicals on oil released from the Deepwater Horizon oil spill. Source: NRC US ecology. [cited 2022 May 3]. Available from: https://response.restoration.noaa.gov/sites/default/files/images/13/dwh-dispersant-application-from-airplane_us-air-force_980.jpg.

Limited Contact between Petroleum Hydrocarbons and Bacteria Reduce Degradation Efficacy

Biodegradation efficacy of petroleum hydrocarbons mainly relies on two characteristics: (1) bioavailability of bacteria to petroleum hydrocarbons, and (2) contact between hydrocarbon substrates and bacteria that must occur before molecular oxygen can be introduced into molecules through functional oxygenases.[18, 54] Evolving bacteria can change their surface cell components enhancing their adhesion to petroleum hydrocarbon substrates.[21,23] Effective bioremediation agents often display this potential which accelerate the removal of petroleum hydrocarbon contamination from environmental ecosystems.[21,23] Surface adhesins of bacteria are often involved in the biodegradation of hydrophobic hydrocarbon substrates.[59] Researchers discovered that adherence of hydrophobic contamination to the cells of bacteria is primarily associated to hydrophobic fibrils, fimbriae, lipids, and outer-membrane proteins to small molecules present in bacterial cell surfaces.[41] Even though bacteria cell wall adherence enhances the biodegrading effects of these organisms, it is not essential to attach bacteria cells to selected substrates.[1] Because it is not essential to attach bacteria to selected substrates, bacteria with abundant surface hydrophobicity are effortlessly aggregated and form biofilms which produce possible disease risks.[12] As a result, it was determined that using hydrophilic microorganisms to combat petroleum hydrocarbons contamination appears to be a better strategy as opposed to using hydrophobic microorganisms.[33]

Environmental Factors Can Influence Bacteria Biodegradation Rates of Petroleum Hydrocarbons

Environmental factors including electron acceptors, nutrients, substrates, and temperature are essential in bioremediation; and, they influence biodegradation reactions.[52] Many petroleum hydrocarbon degrading bacteria can obtain superior results during petroleum hydrocarbons degradation under laboratory conditions, but yield less than satisfactory results in field tests.[15] Some studies have found that bacteria strains possess different temperature sensitivities during the biodegradation process which suggests that temperature greatly affects biodegradation efficacy.[24,57] Another study similarly concluded that lower temperatures could account for poor field results relating to biodegradation of the petroleum hydrocarbon compounds, phenanthrene and dibenzothiophenes, which were well degraded experiments *in vitro*.[39,40] In addition to the soil matrix and manner of contamination occurrence, temperature may be a major consideration to the efficacy of sustainable bacteria growth and metabolism that are essential to biodegradation efficiency.[2]

Soil Conditions and Micro Flora Present Challenges to Biostimulation Effectiveness

To promote the growth and the activity of oil degrading microorganisms needed to stimulate the bioremediation process, the placement of additives into the shoreland soils where bacteria live may be difficult in certain soil structures - impermeable tight, clay, for example.[3] These tight soils inhibit the penetration of additives into the oil impacted media.[3] Another challenge to using biostimulation is that nutrient addition to oil impacted media intended to stimulate the bioremediation process may inadvertently cause the rapid growth of naturally existing heterotrophic microorganisms causing competition for oxygen between the petroleum degrading bacteria and the micro flora.[3] This competition can lead to an oxygen deficient condition that can lead to the death of key oil degrading bacteria jeopardizing the bioremediation process.

Time is a Limiting Factor in the Effective Use of Bacteria in the Biodegradation Process

Petroleum hydrocarbon degrading bacteria depend on their degrading enzymes to be effective in the bioremediation process.[29,46] Because population growth and metabolism is essential for effective biodegrading bacteria, adequate time is required to synthesize petroleum hydrocarbon degrading enzymes. Selected bacteria have demonstrated that petroleum hydrocarbons have degraded in as little as several days and, under laboratory culture conditions, even less than one day.[10,52] Limitations on functional petroleum hydrocarbons degrading bacteria result from their complex blend of different biological and abiotic factors.[56,60] In addition, after an oil spill occurs, screening for indigenous bacteria or flora in contaminated release zones is unrealistic because there simply is no time to assess, investigate, and select the appropriate exogenous bacteria to apply to the oil impacted media after the oil contacts a beach.[19] Moreover, securing government approval to use bioremediation technology is often time consuming.[19] Despite the limitations, microbial organism remediation technology serves an essential function in providing environmental security when interfacing with petroleum hydrocarbons contaminated environments associated with its low cost, negligible environmental influence, useful effect, and lack of secondary contamination.[13] Furthermore, petroleum hydrocarbons in crude oil can be entirely mineralized into carbon dioxide and water under various microbial actions, notwithstanding time consuming limitations symptomatic of bioremediation technologies. Therefore, to decrease the microbial remediation period and enhance the remediation rate, using a consortium of microbial remediation technology is an effective strategy to accelerate the removal of petroleum hydrocarbon pollutants from soil. Although other technologies, such as electrokinetic remediation technology,[25] photocatalytic remediation technology,[57] nanotechnology,[4] and bioreactor technology [42] may assist microorganisms degrade petroleum hydrocarbons, it is the consortium of bacteria that have proven to be the leading technology that should be considered first when implementing a bioaugmentation strategy to remediate petroleum hydrocarbons impacted media.

The Creation of a Global Shoreland Information Database Could Prove to be a Preemptive and Useful Tool in Cleaning Up Crude Oil Contaminated Shorelands with Bioremediation Technologies

Currently, there is no global shorelands database that can provide specific ecological and environmental information about a shoreland's characteristics and its delicate ecosystem that lies in and around areas possessing a high probability of being impacted by an accidental release of crude oil. As addressed in this article, biostimulation and bioaugmentation processes work by engaging specific types of bacteria populations with oil impacted shorelands. Using enhanced bacteria of a specific type to degrade oil may prove to be ineffective when applied to shorelands possessing a different type of indigenous bacteria. Similarly, using various mixtures of nutrients intended to increase oil degrading native bacteria population growth may prove to be ineffectual if native micro flora populations, for example, dramatically increase causing competition for oxygen among the native oil consuming bacteria resulting in an oxygen deficient environment causing bacteria death. With bacteria death, the bacteria bioremediation process will become ineffectual. As described in previous sections of this article, there are many types of bacteria that degrade oil as part of the bioremediation process. If a database existed that could provide detailed ecological information about shorelands in areas likely to be impacted by crude oil releases, then oil producing and transporting companies, environmental consultants, and governments would have the ability to access information on what bioaugmentation and/or biostimulation technologies would prove useful to get a head start on cleaning up crude oil impacted shorelands in the event that an oil spill were to occur. Soil and water sampling laboratory results would generate key information needed for the global database. The types of information generated from the laboratory results would include soil structure and geologic conditions, native bacteria type identification, micro flora species identification, pH levels, seasonal water and ambient location temperatures, and saline concentrations. Collecting the information for the database could be accomplished using a consortium of groups comprised of the oil industry, environmental consultants, and local governments who have a vested interest in protecting shorelands that are likely targets of petroleum contamination in the event of a crude oil spill.

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References:

[1]Abbasnezhad H, Gray M, Foght JM. Influence of adhesion on aerobic biodegradation and bioremediation of liquid hydrocarbons. Appl Microbiol Biotechnol. 2011 Oct [cited 2022 Apr 20];92, 653–675. Available from: https://link.springer.com/article/10.1007/s00253-011-3589-4.doi:10.1007/s00253-011-3589-4.

[2] Abed RMM, Al-Kharusi S, Al-Hinai M. Effect of biostimulation, temperature and salinity on respiration activities and bacterial community composition in an oil polluted desert soil. Int Biodeterior Biodegrad. 2015 Mar [cited 2022 Apr 20];98, 43–52. Available from: https://squ.pure.elsevier.com/en/publications/effect-of-biostimulation-temperature-and-salinity-on-respiration-.doi:10.1016/j.ibiod.2014.11.018.

[3] Adams GO, Tawari-Fufeyin P, Igelenyah E. Bioremediation of spent oil contaminated soils using poultry litter. Res J Eng Appl Sci. 2014 Mar [cited 2022 Apr 20];3(2),124-130. Available from: http://citeseerx.ist.psu.edu/viewdoc/download?doi=10.1.1.675.1282&rep=rep1&type=pdf.

[4] Alabresm A, Chen YP, Decho AW, Lead J. A novel method for the bacteria. Sci Total Environ. 2018 Jul [cited 2022 Apr 20];630, 1292–1297. Available from: https://pubmed.ncbi.nlm.nih.gov/29554750/. doi:m10.1016/j.scitotenv.2018.02.277.

[5] Al-Sulaimani H, Al-Wahaibi Y, Al-Bahry S, Elshafie A, Al-Bemani A, Joshi S, Zargari S. "Experimental Investigation of Biosurfactants Produced by *Bacillus* Species and their Potential for MEOR in Omani Oil Field." Paper presented at the SPE EOR Conference at Oil & Gas West Asia, Muscat, Oman. 2010 Apr [cited 2022 Apr 20]; SPE-129228-MS. doi: https://doi.org/10.2118/129228-MS.

[6] Atagana, HI. Compost bioremediation of hydrocarbon-contaminated soil inoculated with organic manure. Afr J Biotechnol. 2008 May [cited 2022 Apr 21];*7*(10),1516-1525. Available from: https://academicjournals.org/journal/AJB/article-abstract/DD8F68C7389.doi:org/10.5897/AJB08.193.

[7] Bundy JG, Paton GI, Campbell CD. Microbial communities in different soil types do not converge after diesel contamination. J Appl Microbiol. 2002 Jan [cited 2022 Apr 21];92(2),276-288. Available from: https://sfamjournals.onlinelibrary.wiley.com/doi/pdf/10.1046/j.1365-2672.2002.01528.x.doi:10.1046/j.1365-2672.2002.01528.x. PMID: 11849356.

[8] Chaerun SK, Tazaki K, Asada R, Kogure K. Bioremediation of coastal areas 5 years after the Nakhodka oil spill in the Sea of Japan: isolation and characterization of hydrocarbon-degrading bacteria. Environ. Int. 2004 Oct [cited 2022 Apr 21];30, 911–922. Available from: https://pubmed.ncbi.nlm.nih.gov/15196839/.doi: 10.1016/j.envint.2004.02.007.

[9] Chen W, Li J, Sun X, Min J, Hu X. High efficiency degradation of alkanes and crude oil by a salt-tolerant bacterium Dietzia species CN-3. Int. Biodeterior. Biodegrad. 2017 Mar [2022 Apr 21];118, 110–118. Available from:https://www.sciencedirect.com/science/article/abs/pii/S0964830517300689?via%3Dihub.doi:10.1016/j.ibiod.2017.01.029.

[10] Díez S, Jover E, Bayona JM, Albaigés J. Prestige oil spill. III. Fate of a heavy oil in the marine environment. Environ. Sci. Technol. 2007 Mar [cited 2022 Apr 21];41, 3075–3082. Available from: https://pubs.acs.org/doi/abs/10.1021/es0629559.doi:10.1021/es0629559.

[11] Dombrowski N, Donaho JA, Gutierrez T, Seitz KW, Teske AP, Baker BJ. Reconstructing metabolic pathways of hydrocarbon-degrading bacteria from the Deepwater Horizon oil spill. Nat. Microbiol. 2016 May [cited 2022 Apr 21];1:16057. Available from: https://pure.hw.ac.uk/ws/portalfiles/portal/10579894/NMICROBIOL.2016.57_auproof.pdf. doi:10.1038/nmicrobiol.2016.57.
[12] Doyle R J. Contribution of the hydrophobic effect to microbial infection. Microbes Infect. 2000 Apr [cited 2022 Apr 21];2(4), 391–400. Available from: https://pubmed.ncbi.nlm.nih.gov/10817641/. doi: 10.1016/S1286-4579(00)00328-2.
[13] Dvořák P, Nikel P I, Damborský J, de Lorenzo V. Bioremediation 3.0: engineering pollutant-removing bacteria in the times of systemic biology. Biotechnol. Adv. 2017 Aug [cited 2022 Apr 21];35, 845–866. Available from: https://pubmed.ncbi.nlm.nih.gov/28789939/.doi: 10.1016/j.biotechadv.2017.08.001.
[14] Elektorowicz M. Environmental Technology Bioeemediation of petroleum-contaminated clayey soil with pretreatment. Environ Technol. 2008 Dec [cited 2022 Apr 21];15(4), 373-380. Available from: https://www.tandfonline.com/doi/citedby/10.1080/09593339409385440?scroll=top&needAccess=true.doi:10.1080/09593339409385440.
[15] Fuentes S, Barra B, Caporaso J G, Seeger M. From rare to dominant: a fine-tuned soil bacterial bloom during petroleum hydrocarbon bioremediation. Appl. Environ. Microbiol. 2016 Jan [cited 2022 Apr 21];82, 888–896. Available from: https://journals.asm.org/doi/10.1128/AEM.02625-15.doi:10.1128/AEM.02625-15.
[16] Hazen TC, Prince, RC, Mahmoudi N. Marine oil biodegradation. Environ. Sci. Technol. 2016 Mar [cited 2022 Apr 22];50, 2121–2129. Available from: https://pubmed.ncbi.nlm.nih.gov/26698270/.doi:10.1021/acs.est.5b03333.
[17] Head IM, Jones DM, Röling WF. Marine microorganisms make a meal of oil. Nat. Rev. Microbiol. 2006 Mar [cited 2022 Apr 22]; 4(3):173-82. Available from: https://pubmed.ncbi.nlm.nih.gov/16489346/.doi:10.1038/nrmicro1348.
[18] Hua F, Wang HQ. Uptake and trans-membrane transport of petroleum hydrocarbons by microorganisms. Biotechnol. Biotechnol. Equip. 2014 Mar [cited 2022 Apr 22];28(2),165-175. Available from: https://pubmed.ncbi.nlm.nih.gov/26740752/.doi:10.1080/13102818.2014.906136.
[19] Ivshina IB, Kuyukina MS, Krivoruchko AV, Elkin AA, Makarov SO, Cunningham CJ, Peshkur TA, Atlas RM, Phil JC. Oil spill problems and sustainable response strategies through new technologies. Environ. Sci. 2015 Jul [cited 2022 Apr 22];17, 1201–1219. doi: 10.1039/c5em00070j.
[20] Jin HM, Kim JM, Lee HJ, Madsen EL, Jeon CO. Alteromonas as a key agent of polycyclic aromatic hydrocarbon biodegradation in crude oil-contaminated coastal sediment. Environ. Sci. Technol. 2012 Jul [cited 2022 Apr 22];46, 7731–7740. Available from: https://pubmed.ncbi.nlm.nih.gov/22709320/.doi:10.1021/es3018545.
[21] Kaczorek E, Jesionowski T, Giec A, Olszanowski A. Cell surface properties of *Pseudomonas stutzeri* in the process of diesel oil biodegradation. Biotechnol. Lett. 2012 May [cited 2022 Apr 22];34, 857–862. Available from: https://pubmed.ncbi.nlm.nih.gov/22210557/ doi:10.1007/s10529-011-0835-x.
[22] Kleindienst S, Paul JH, Joye SB. Using dispersants after oil spills: impacts on the composition and activity of microbial communities. Nat. Rev. Microbiol. 2015 Jun [cited 2022 Apr 22];13, 388–396. Available from: https://pubmed.ncbi.nlm.nih.gov/25944491/.doi:10.1038/nrmicro3452.
[23] Krasowska A, Sigler K. How microorganisms use hydrophobicity and what does this mean for human needs? Front. Cell. Infect. Microbiol. 2014 Aug [cited 2022 Apr 22];4:112. Available from: https://pubmed.ncbi.nlm.nih.gov/25191645/doi:10.3389/fcimb.2014.00112.
[24] Li D, Xu X, Zhai Z, Yu H, Han X. Isolation and identification an n-hexadecane bacterial degrader from soil polluted by petroleum oil in Momoge wetlands and its degradation characteristics. Wetland Sci. 15, 85–91.
[25] Ma Y, Li X, Mao H, Wang B, Wang P. Remediation of hydrocarbon–heavy metal co-contaminated soil by electrokinetics combined with biostimulation. Chem. Eng. J. 2018 Jul [cited 2022 Apr 22]; 353, 410–418. Available from: https://www.cabdirect.org/cabdirect/abstract/20193122330.doi: 10.1016/j.cej.2018.07.131.
[26] Margesin R, Labbé D, Schinner F, Greer CW, Whyte LG. Characterization of hydrocarbon-degrading microbial populations in contaminated and pristine alpine soils. Appl. Environ. Microbiol. 2003 Jun [cited 2022 Apr 22];69, 3085–3092. Available from: https://pubmed.ncbi.nlm.nih.gov/12788702/ doi:10.1128/AEM.69.6.3085-3092.2003.
[27] Marine Defenders. [cited 2022 Apr 27]A vailable from: https://www.marinedefenders.org/impact-on-mammals-birds-and-fish.html.
[28] Mason OU, Hazen TC, Borglin S, Chain PSG, Dubinsky EA, Fortney JL, James H, Hoi-Ying NH, Hultman J, Lamendalla R, et al. Metagenome, metatranscriptome and single-cell sequencing reveal microbial response to Deepwater Horizon oil spill. ISME J. 2012 Sep [cited 2022 Apr 22];6, 1715–1727. Available from: https://pubmed.ncbi.nlm.nih.gov/22717885/.doi: 10.1038/ismej.2012.59.

[29] Mukherjee AK, Bhagowati P, Biswa BB, Chanda A, Kalita B. A comparative intracellular proteomic profiling of Pseudomonas aeruginosa strain ASP-53 grown on pyrene or glucose as sole source of carbon and identification of some key enzymes of pyrene biodegradation pathway. J. Proteomics 2017 Sep [2022 Apr 22];167, 25–35. Available from: https://pubmed.ncbi.nlm.nih.gov/28774858/doi:10.1016/j.jprot.2017.07.020.
[30] Newcott B, Kaminski K. Let us spray. Delaware Beach Life. 2019 Oct [cited 2022 Apr 30]. Available from: https://www.delawarebeachlife.com/magazine/our-content/270-let-us-spray.
[31] Nie Y, Liang JL, Fang H, Tang YQ, Wu XL. Characterization of a CYP153 alkane hydroxylase gene in a gram-positive Dietzia sp. DQ12-45-1b and its "team role" with alkw1 in alkane degradation. Appl. Microbiol. Biotechnol. 2014 Jan [cited 2022 Apr 22];98, 163–173. Available from: https://pubmed.ncbi.nlm.nih.gov/23504079/.doi:10.1007/s00253-013-4821-1.
[32] NOAA. 2015 May [cited 2022 Apr 27]. Available from: https://darrp.noaa.gov/oil-spills/refugio-beach-oil-spill.
[33] Obuekwe CO, Al-Jadi ZK, Al-Saleh ES. Hydrocarbon degradation in relation to cell-surface hydrophobicity among bacterial hydrocarbon degraders from petroleum-contaminated Kuwait desert environment. Int. Biodeterior. Biodegrad. 2009 Apr [cited 2022 Apr 22];63, 273–279. Available from: https://www.researchgate.net/publication/248436668_Hydrocarbon_degradation_in_relation_to_cell-surface_hydrophobicity_among_bacterial_hydrocarbon_degraders_from_petroleum-contaminated_Kuwait_desert_environment /.doi:10.1016/j.ibiod.2008.10.004.
[34] Overholt WA, Marks KP, Romero IC, Hollander DJ, Snell TW, Kostka JE. Hydrocarbon degrading bacteria exhibit a species specific response to dispersed oil while moderating ecotoxicity. Appl. Environ. Microbiol. 2015 Nov [cited 2022 Apr 22];82, 518–527. Available from: https://pubmed.ncbi.nlm.nih.gov/26546426/ doi: 10.1128/AEM.02379-15.
[35] Perfumo A, Banat IM, Marchant R,Vezzulli L. Thermally enhanced approaches for bioremediation of hydrocarbon-contaminated soils. Chemosphere. 2007 Jan [cited 2022 Apr 23];66(1),179-184. Available from: https://pubmed.ncbi.nlm.nih.gov/16782171/. doi: 10.1016/j.chemosphere.2006.05.006.
[36] Piehler MF, Swistak JG, Pinckney JL, Paerl HW. Stimulation of Diesel Fuel Biodegradation by Indigenous Nitrogen Fixing Bacterial Consortia. Microb Ecol. 1999 Jul [cited 2022 Apr 23];38(1),69-78. Available from: https://pubmed.ncbi.nlm.nih.gov/10384011/.doi:10.1007/s002489900157.
[37] Rhykerd, RL, Crews B, McInnes K,Weaver RW. Impact of bulking agents, forced aeration, and tillage on remediation of oil-contaminated soil. Bioresour Technol. 1999 Mar [cited 2022 Apr 23];67,279-285. Available from: https://www.sciencedirect.com/science/article/abs/pii/S096085249800114X#:~:text=Tilling%20increased%20the%20rate%20and,where%20the%20TPH%20decreased%2082%25. doi.org/10.1016/S0960-8524(98)00114-X.
[38] Rivers AR, Sharma S, Tringe SG, Martin J, Joye SB, Moran MA. Transcriptional response of bathypelagic marine bacterioplankton to the Deepwater Horizon oil spill. ISME J. 2013 Dec [cited 2022 Apr 23]7:2315-2329. Available from: https://pubmed.ncbi.nlm.nih.gov/23902988/.doi:10.1038/ismej.2013.12.
[39] Röling WF, Milner MG, Jones DM, Lee K, Daniel F, Swannell RJ, Head IM. Robust hydrocarbon degradation and dynamics of bacterial communities during nutrient-enhanced oil spill bioremediation. Appl. Environ. Microbiol. 2002 Nov [cited 2022 Apr 23];68, 5537–5548. Available from: https://pubmed.ncbi.nlm.nih.gov/12406747/.doi:10.1128/AEM.68.11.5537-5548.2002.
[40] Röling WF, Milner MG, Jones DM, Fratepietro F, Swannell RP, Daniel F, Head IM. Bacterial community dynamics and hydrocarbon degradation during a field-scale evaluation of bioremediation on a mudflat beach contaminated with buried oil. Appl. Environ. Microbiol. 2004 May [cited 2022 Apr 23];70, 2603–2613. Available from: https://pubmed.ncbi.nlm.nih.gov/15128509/.doi:10.1128/AEM.70.5.2603-2613.2004.
[41]Rosenberg M, Rosenberg E. Bacterial adherence at the hydrocarbon-water interface. Oil Petrochem. Pollut. 1985 [cited 2022 Apr 23];2(3), 155–162. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0143712785901784?via%3Dihub.doi:10.1016/S0143-7127(85)90178-4.
[42] Safdari MS, Kariminia HR, Rahmati M, Fazlollahi F, Polasko A, Mahendra S, Wilding WV, Fletcher TH. Development of bioreactors for comparative study of natural attenuation, biostimulation, and bioaugmentation of petroleum-hydrocarbon contaminated soil. J. Hazard. Mater. 2018 Jan [cited 2022 Apr 23];342, 270–278. Available from: https://pubmed.ncbi.nlm.nih.gov/28843796/.doi:10.1016/j.jhazmat.2017.08.044.
[43] Sarkar P, Roy A, Pal S, Mohapatra B, Kazy SK, Maiti MK, Sar P. Enrichment and characterization of hydrocarbon-degrading bacteria from petroleum refinery waste as potent bioaugmentation agent for in situ bioremediation. Bioresour. Technol. 2017 Oct [cited 2022 Apr 23];242, 15–27. Available from: https://pubmed.ncbi.nlm.nih.gov/28533069/.doi:10.1016/j.biortech.2017.05.010.

[44] Sayed K, Baloo L, Sharma NK. Bioremediation of Total Petroleum Hydrocarbons (TPH) by Bioaugmentation and Biostimulation in Water with Floating Oil Spill Containment Booms as Bioreactor Basin. Int J Environ Res Public Health. 2021 Feb [cited 2022 Apr 23];18(5), 2226. Available from: https://pubmed.ncbi.nlm.nih.gov/33668225/.doi.org/10.3390/ijerph18052226.
[45] Sikkema J, de Bont JA, Poolman B. Mechanisms of membrane toxicity of hydrocarbons. Microbiol. Rev. 1995 Jun [cited 2022 Apr 23];9(2),201–222. Available from: https://pubmed.ncbi.nlm.nih.gov/?term=Mechanisms+of+membrane+toxicity+of+hydrocarbons. doi:10.1128/mr.59.2.201-222.1995.
[46] Song M, Yang Y, Jiang L, Hong Q, Zhang D, Shen, Z, Yin H, Luo C. Characterization of the phenanthrene degradation-related genes and degrading ability of a newly isolated copper-tolerant bacterium. Environ. Pollut. 2017 Jan [cited 2022 Apr 23];220,1059–1067. Available from: https://pubmed.ncbi.nlm.nih.gov/27889087/.doi:10.1016/j.envpol.2016.11.037.
[47] Szulc A, Ambrożewicz D, Sydow M, Ławniczak Ł, Piotrowska-Cyplik A, Marecik R, Chrzanowski Ł. The influence of bioaugmentation and biosurfactant addition on bioremediation efficiency of diesel-oil contaminated soil: feasibility during field studies. J. Environ. Manage. 2014 Jan [cited 2022 Apr 23]132,121–128. Available from: https://pubmed.ncbi.nlm.nih.gov/24291585/.doi:10.1016/j.jenvman.2013.11.006.
[48] Tao K, Liu X, Chen X. Hu X, Cao L, Yuan X. Biodegradation of crude oil by a defined co-culture of indigenous bacterial consortium and exogenous *Bacillus subtilis*. Bioresour. Technol. 2017 Jan [cited 2022 Apr 23]224,327–332. Available from: https://pubmed.ncbi.nlm.nih.gov/27815043/.doi:10.1016/j.biortech.2016.10.073.
[49] Tate PT. "Significance of the effect of nitrogen application on the engineered bioremediation of crude oil in a salt marsh." LSU Historical Dissertations and Theses. 1998 [cited 2022 Apr 30]; 6871. Available from: https://digitalcommons.lsu.edu/cgi/viewcontent.cgi?article=7870&context=gradschool_disstheses.
[50] Tremblay J, Yergeau E, Fortin N, Cobanli S, Elias M, King TL, Lee K, Greer, CW. Chemical dispersants enhance the activity of oil-and gas condensate-degrading marine bacteria. ISME J. 2017 Dec [cited 2022 Apr 23];11,2793–2808. Available from: https://pubmed.ncbi.nlm.nih.gov/28800137/.doi:10.1038/ismej.2017.12.
[51] Varjani SJ, Rana DP, Jain AK, Bateja S, Upasani VN. Synergistic ex-situ biodegradation of crude oil by halotolerant bacterial consortium of indigenous strains isolated from on shore sites of Gujarat. India. Int. Biodeterior. Biodegrad. 2015 Sep [cited 2022 Apr 23];103,116–124. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0964830515001158.doi:10.1016/j.ibiod.2015.03.030.
[52] Varjani SJ, Upasani VN. Biodegradation of petroleum hydrocarbons by oleophilic strain of Pseudomonas aeruginosa NCIM 5514. Bioresour. Technol. 2016 Dec [cited 2022 Apr 23];222,195–201. Available from: https://pubmed.ncbi.nlm.nih.gov/27718402/.doi:10.1016/j.biortech.2016.10.006.
[53] Varjani SJ, Upasani VN. A new look on factors affecting microbial degradation of petroleum hydrocarbon pollutants. Int. Biodeterior. Biodegrad. 2017 May [cited 2022 Apr 23];120,71–83. Available from: https://www.sciencedirect.com/science/article/abs/pii/S0964830516308113.doi:10.1016/j.ibiod.2017.02.006.
[54] Vasileva-Tonkova E, Galabova D, Stoimenova E, Lalchev Z. Characterization of bacterial isolates from industrial wastewater according to probable modes of hexadecane uptake. Microbiol. Res. 2008 Jul [cited 2022 Apr 23];163(4), 481–486. Available from: https://www.sciencedirect.com/science/article/pii/S0944501306000954 doi: 10.1016/j.micres.2006.07.015.
[55] Walters KHD. Oil-Eating Microbes Sprayed on Spill. Los Angeles Times. 1990 Aug [cited 2022 May 5]. Available from: https://www.latimes.com/archives/la-xpm-1990-08-03-fi-1129-story.html.
[56] Wang Y, Liang J, Wang J, Gao S. Combining stable carbon isotope analysis and petroleum-fingerprinting to evaluate petroleum contamination in the Yanchang oilfield located on loess plateau in China. Environ. Sci. Pollut. Res. 2018 Jan [cited 2022 Apr 23];25,2830–2841. Available from: https://pubmed.ncbi.nlm.nih.gov/29143260/.doi: 10.1007/s11356-017-0500-6.
[57] Xu X, Zhai Z, Li H, Wang Q, Han X, Yu H. Synergetic effect of bio-photocatalytic hybrid system: g-C3N4, and Acinetobacter, sp. JLS1 for enhanced degradation of C16 alkane. Chem. Eng. J. 2017 Sep [cited 2022 Apr 23];323,520–529. Available from: https://www.sciencedirect.com/science/article/abs/pii/S1385894717307349. doi:10.1016/j.cej.2017.04.138.
[58] Yang Y, Wang J, Liao J, Xie S, Huang Y. Abundance and diversity of soil petroleum hydrocarbon-degrading microbial communities in oil exploring areas. Appl. Microbiol. Biotechnol. 2015 Feb [cited 2022 Apr 23];99,1935–1946. Available from: https://pubmed.ncbi.nlm.nih.gov/25236802/.doi:10.1007/s00253-014-6074-z.
[59] Zhang X, Zhang Q, Yan T, Jiang Z, Zhang X, Zuo YY. Quantitatively predicting bacterial adhesion using surface free energy determined with a spectrophotometric method. Environ. Sci. Technol. 2015 May [cited 2022 Apr 23];49,6164–6171. Available from: https://pubmed.ncbi.nlm.nih.gov/25898026/.doi:10.1021/es5050425.

[60] Zhao D, Kumar S, Zhou J, Wang R, Li M, Xiang H. Isolation and complete genome sequence of *Halorientalis hydrocarbonoclasticus* sp. nov., a hydrocarbon-degrading haloarchaeon. Extremophiles .2017 Nov [cited 2022 Apr 23];21,1081–1090. Available from: https://pubmed.ncbi.nlm.nih.gov/28994006/.doi:10.1007/s00792-017-0968-5.
[61] Zheng J, Feng JQ, Zhou L, Mbadinga SM, Gu JD, Mu BZ. Characterization of bacterial composition and diversity in a long-term petroleum contaminated soil and isolation of high-efficiency alkane-degrading strains using an improved medium. World J. Microbiol. Biotechnol. 2018 Feb [cited 2022 Apr 23];34(2):34. Available from: https://pubmed.ncbi.nlm.nih.gov/29426982/.doi:10.1007/s11274-018-2417-8.