

BIOREMEDIATION OF OIL SPILLS: CURRENT STATUS, CHALLENGES, AND FUTURE PROSPECTS

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Review



ABSTRACT

The petrochemicals produced from refining oil have become a large part of human life, making oil a valuable and expensive commodity. As a non-renewable resource, extraction and transport efforts have intensified to keep up with the demand, increasing the occurrences of oil spills. Such accidents have devastating impacts on the environment, the health of organisms, and a country's economy like the Philippines, and thus, need to be resolved immediately. One way to deal with oil spills is through bioremediation but the process is still facing several challenges. For one, the toolbox for bioremediation is limited. About 79 genera of bacteria were observed to degrade oil but there are only a small number of bacterial species and/or strains that have been recognized as useful for bioremediation. Second is that most oil-degrading bacteria found have low oil degradation efficiencies. Another challenge is keeping the bacteria alive to carry out the process. Fortunately, progress has been made in solving these challenges. Researchers are now testing different consortia, including bacteria-bacteria, bacteria-fungi, bacteria-microalgae, that can complement each other such as biosurfactant-producing bacteria with different oil-degrading microorganisms or microalgae or fungi that enhance the growth of oil-degrading bacteria. A consortium like this improves the survivability of each microorganism and enhances the oil-degrading efficiency. Moreover, the search for additional oil-degrading and biosurfactant-producing bacteria and other microorganisms to add to the bioremediation toolbox has been improved with the emergence of high-throughput sequencing. Aside from microorganisms, seaweeds have shown potential for bioremediation. The seaweed *Caulerpa prolifera* has been demonstrated to degrade diesel up to a certain concentration with the help of the bacteria growing on its surface. Bioremediation has a long way to go, but recent developments have shown promise and it remains to be the cheapest, most environment-friendly, and most effective way of dealing with oil spills.

Keywords: bioremediation, crude oil, marine environment, oil degradation, petroleum, bacteria

INTRODUCTION

A naturally-occurring combustible mixture of hydrocarbons and organic compounds – petroleum or crude oil – is derived from mostly decayed plants and tiny marine animals exposed to the intense heat and immense pressure underneath the Earth's surface for millions of years (Atlas and Hazen, 2011). When refined, it yields different products that often run our modern society from gasoline, diesel, kerosene, etc. which are used for energy generation and transportation to other petrochemicals used in making plastics and other industrial products (Favennec, 2022). This makes petroleum a valuable and expensive commodity as it is a non-renewable resource that occurs only in select areas around the world, is often found beneath the ocean floor, and requires specialized equipment to extract (Kaiser and Snyder, 2013; Cordes et al., 2016; Yang et al., 2022). As a trillion-dollar industry, petroleum often impacts the economies of nations and has become part of an individual's everyday life through the years. Currently, petroleum and its derivatives remain the main source of energy even after announcements of the shift to renewable energy (Gharib et al., 2021; Yang et al., 2022). To keep up with the demand, extraction activities have been intensified along with the increase in the transport of petroleum products from the sources to several sinks which increases the potential of leakages or spills occurring that put the immediate environment in danger (Deng et al., 2014; Tian et al., 2018).

The global average of oil spill occurrences in the 2010s was more than 6 (Galerikova and Materna, 2020). Now in the 2020s, the current average has decreased to 5.7 (ITOPF, 2023). Although the trend of oil spill occurrences is decreasing, the degree of damage an oil spill can deal to an area remains the same. A country that often experiences oil spills in the marine environment is the Philippines due to it being an archipelago (Licuanan et al., 2019; Alea et al., 2022). There are more than 7,600 islands in the Philippines and since only a few of the islands are connected by bridges, products including oil are usually distributed to the islands via maritime transport (Salison and Vergel, 2021; Licuanan et al., 2019). According to the data gathered by Alea et al. (2022), there have been 14 massive oil spills out of the total 467 oil spills that occurred from 2000 to 2021. The most recent massive oil spill was near the island of Mindoro. The tanker, MT Princess Empress which carried 800,000 liters of oil, sank near Naujan, Oriental Mindoro last February 2023. The oil spill quickly spread to

different areas from Batangas in the North to Antique in the South (Agaton et al., 2023). Marine oil spills directly hinder the exchange of O₂ and CO₂ resulting in the depletion of oxygen and the alteration of the chemistry of the seawater. The anoxic environment becomes inhospitable for many organisms leading to their deaths. Aside from anoxia, some of the chemicals in the oil also poison the organisms. Oil also drenches organisms at the surface of the water which causes them to drown or experience hypothermia. Marine plants and algae also suffer due to the reduced penetration of sunlight from the surface which limits their photosynthetic activity (Xue et al., 2015). Moreover, marine oil spills pose a threat to the health of the people and the economy of the nation (Eklund et al., 2019; Zhang et al., 2018; Alvernia et al., 2021) which is why they need to be cleared as soon as possible. One of the ways to deal with aquatic oil spills is through bioremediation. The general process employs the services of oil-degrading microorganisms which are naturally found in the seawater to breakdown the oil and convert them to other products that are less toxic and can be consumed by other microorganisms (Das & Chandran, 2011; Chuah et al., 2022; Pandolfo et al., 2023). In terms of microorganisms with oil-degrading capabilities, fungi are the most abundant with 103 genera followed by bacteria with 79 genera. Although less abundant, there are currently more studies regarding oil-degradation of bacteria than fungi but studies on fungi-mediated oil degradation are catching up (Xue et al., 2015; Dell' Anno et al., 2021). Bioremediation can be carried out in two ways: biostimulation, where needed nutrients are added to the water to encourage the growth of indigenous microbes that will breakdown the oil, and bioaugmentation, where exogenous microbes with oil-degrading capabilities are added to the water (Rojas-Vargas et al., 2022). With this, bioremediation is seen as the most environmentally-friendly method in dealing with oil spills as there are no harmful chemicals involved and the process is also cheaper to carry out (Hii et al., 2009). However, the bioremediation process is not yet fully developed. In this brief review, we explored the different bacteria involved in the bioremediation process and the current state of bioremediation as well as the challenges encountered and future prospects.

CURRENT STATUS OF BIOREMEDIATION USING BACTERIA AND OTHER ASSOCIATED ORGANISMS

Bacteria Used in Bioremediation

The ocean is home to a plethora of microorganisms such as bacteria, fungi, microalgae, and cyanobacteria. They are important components of the marine ecosystem as they perform several services like food production and carrying out the decomposition process of organic matter. They are also involved in nutrient cycling and breaking down of harmful chemicals that may have contaminated the ocean (Pandolfo *et al.*, 2023). According to Xue *et al.* (2015), there are 79 genera of bacteria with oil-degrading capabilities. These include *Acinetobacter*, *Bacillus*, *Pseudomonas*, etc. The complete list compiled by Xue *et al.* (2015) from Bartha & Atlas (1977), Giebel *et al.* (2011), and Singh (2006) is shown in Table 1. The review of Xu *et al.* (2018) and Dell' Anno *et al.* (2021) have added more genera and thus were included in the list.

Table 1 List of some bacterial genera with oil degrading capabilities.

Bacterial Genus	Reference/s
<i>Achromobacter</i>	Bartha & Atlas, 1977; Singh, 2006; Giebel <i>et al.</i> , 2011; Ma <i>et al.</i> , 2014; Xue <i>et al.</i> , 2015
<i>Acinetobacter</i>	Bartha & Atlas, 1977; Singh, 2006; Giebel <i>et al.</i> , 2011; Xue <i>et al.</i> , 2015
<i>Aeribacillus</i>	Mnif <i>et al.</i> , 2014; Xu <i>et al.</i> , 2018
<i>Alcaligenes</i>	Dell' Anno <i>et al.</i> , 2021; Duran <i>et al.</i> , 2019
<i>Alcanivorax</i>	Dell' Anno <i>et al.</i> , 2021; Yakimov <i>et al.</i> , 2007; Hara <i>et al.</i> , 2003
<i>Actinomycetes</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Archrobacter</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Bacillus</i>	Dell' Anno <i>et al.</i> , 2021; Wang <i>et al.</i> , 2019; Xu <i>et al.</i> , 2018; Xu <i>et al.</i> , 2018; Jahromi <i>et al.</i> , 2014; Tavassoli <i>et al.</i> , 2012
<i>Citrobacter</i>	Xu <i>et al.</i> , 2018; Jahromi <i>et al.</i> , 2014
<i>Coryneforms</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Chromobacterium</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Cycloclasticus</i>	Dell' Anno <i>et al.</i> , 2021; Kasai <i>et al.</i> , 2002
<i>Dietzia</i>	Xu <i>et al.</i> , 2018; Wang <i>et al.</i> , 2011
<i>Enterobacter</i>	Dell' Anno <i>et al.</i> , 2021; Ramasamy <i>et al.</i> , 2017; Xu <i>et al.</i> , 2018; Jahromi <i>et al.</i> , 2014
<i>Flavobacterium</i>	Dell' Anno <i>et al.</i> , 2021; Chaudhary <i>et al.</i> , 2019
<i>Geobacillus</i>	Xu <i>et al.</i> , 2018; Abbasian <i>et al.</i> , 2015
<i>Gordonia</i>	Xu <i>et al.</i> , 2018; Brown <i>et al.</i> , 2016
<i>Klebsiella</i>	Rodriguez <i>et al.</i> , 2023
<i>Lysinibacillus</i>	Xu <i>et al.</i> , 2018; Jahromi <i>et al.</i> , 2014
<i>Marinobacter</i>	Dell' Anno <i>et al.</i> , 2021; Chernikova <i>et al.</i> , 2020
<i>Micrococcus</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Microbacterium</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Mycobacterium</i>	Xu <i>et al.</i> , 2018; Zhang <i>et al.</i> , 2013
<i>Novosphingobium</i>	Xu <i>et al.</i> , 2018; Ghosal <i>et al.</i> , 2016
<i>Neptumonas</i>	Xu <i>et al.</i> , 2018; Hedlund <i>et al.</i> , 1999
<i>Oleispira</i>	Dell' Anno <i>et al.</i> , 2021; Yakimov <i>et al.</i> , 2003
<i>Pseudomonas</i>	Dell' Anno <i>et al.</i> , 2021; Pacwa-Plociniczak <i>et al.</i> , 2014; Xu <i>et al.</i> , 2018; Jahromi <i>et al.</i> , 2014; Sugiura <i>et al.</i> , 1997; Mukherjee <i>et al.</i> , 2010; Venkateswaran <i>et al.</i> , 1995; Tavassoli <i>et al.</i> , 2012
<i>Rhodococcus</i>	Xu <i>et al.</i> , 2018; Zhukov <i>et al.</i> , 2007; Lee & Cho, 2008
<i>Sarcina</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Serratia</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Sphingobium</i>	Xu <i>et al.</i> , 2018; Ghosal <i>et al.</i> , 2016
<i>Sphingomonas</i>	Xu <i>et al.</i> , 2018; Ghosal <i>et al.</i> , 2016
<i>Staphylococcus</i>	Xu <i>et al.</i> , 2018; Jahromi <i>et al.</i> , 2014
<i>Streptomyces</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006
<i>Thalassolituus</i>	Dell' Anno <i>et al.</i> , 2021; Mahjoubi <i>et al.</i> , 2018
<i>Vibrio</i>	Xue <i>et al.</i> , 2015; Bartha & Atlas, 1977; Giebel <i>et al.</i> , 2011; Singh, 2006

Note: Bacterial genus in colored boxes are those that are usually utilized in bioremediation.

The study of Rodriguez *et al.* (2023) also showed the potential of *Klebsiella* sp. in oil degradation. According to Dell' Anno *et al.* (2021), the 10 genera of bacteria that are usually involved in bioremediation efforts are *Alcaligenes*, *Bacillus*,

Enterobacter, *Flavobacterium*, *Pseudomonas*, *Alcanivorax*, *Thalassolituus*, *Cycloclasticus*, *Oleispira*, and *Marinobacter* (Kasai *et al.*, 2002; Yakimov *et al.*, 2003, 2007; Pacwa-Plociniczak *et al.*, 2014; Ramasamy *et al.*, 2017; Mahjoubi *et al.*, 2018; Chaudhary *et al.*, 2019; Duran *et al.*, 2019; Wang *et al.*, 2019; Chernikova *et al.*, 2020; Dell' Anno *et al.*, 2021; Rodriguez *et al.*, 2023).

Petroleum and its derivatives are a complex mixture of hydrocarbons, and no bacterial species can degrade all of the hydrocarbon components. Bacteria exhibit hydrocarbon specificity and it depends on the enzymes they can produce to metabolize specific hydrocarbon components (Gao *et al.*, 2021; Rajasekar *et al.*, 2007; Dell' Anno *et al.*, 2021; Pandolfo *et al.*, 2023). For instance, an alkane is a simple hydrocarbon composed of carbon atoms singly bonded to hydrogen atoms with no functional groups attached. The bacterium *Alcanivorax* sp. strain 24 can break apart the alkane by producing enzymes such as oxygenase and dehydrogenase which convert the hydrocarbon into fatty acids which would then be metabolized by the bacterium forming carbon dioxide and water at the end (Xue *et al.*, 2015; Zadjelovic *et al.*, 2020; Dell' Anno, 2021). Another component of petroleum and its derivatives is the polycyclic aromatic hydrocarbon (PAH), which is a diverse group of organic compounds known to cause cancers and birth defects. The strain 78-ME of *Cycloclasticus* sp. can metabolize PAHs using oxygenase and hydrolase enzymes (Xue *et al.*, 2015; Messina *et al.*, 2016; Dell' Anno *et al.*, 2021). Dell' Anno *et al.* (2021) made a list of some bacterial species/strains and the type of hydrocarbon they are capable of metabolizing (Table 2).

Table 2 Selected oil-degrading bacteria and the hydrocarbons they can metabolize

Bacteria	Hydrocarbon Metabolized	Reference/s
<i>Alcaligenes aquatilis</i> BU33N	Crude oil and phenanthrene	Mahjoubi <i>et al.</i> , 2019; Dell' Anno <i>et al.</i> , 2021
<i>Alcanivorax</i> sp. IO_7	Alkanes	Dell' Anno <i>et al.</i> , 2021; Sinha <i>et al.</i> , 2021
<i>Alcanivorax</i> sp. 24	Alkanes	Zadjelovic <i>et al.</i> , 2020; Dell' Anno <i>et al.</i> , 2021
<i>Cupriavidus metallidurans</i> CH34	Toluene	Tofalos <i>et al.</i> , 2018; Dell' Anno <i>et al.</i> , 2021
<i>Cycloclasticus</i> sp. strain BG-2	Phenanthrene	Gutierrez <i>et al.</i> , 2015; Dell' Anno <i>et al.</i> , 2021;
<i>Cycloclasticus</i> sp. 78-ME	Polycyclic aromatic hydrocarbons	Messina <i>et al.</i> , 2016; Dell' Anno <i>et al.</i> , 2021;
<i>Cycloclasticus</i> sp. strain P1	Naphthalene, phenanthrene, pyrene	Dell' Anno <i>et al.</i> , 2021; Wang <i>et al.</i> , 2018
<i>Halomonas</i> sp. strain MCTG39a	Hexadecane	Dell' Anno <i>et al.</i> , 2021; Gutierrez <i>et al.</i> , 2015
<i>Halomonas pacifica</i> strain Cnaph3	Naphthalene	Dell' Anno <i>et al.</i> , 2021; Cheffi <i>et al.</i> , 2020
<i>Marinobacter hydrocarbonoclasticus</i> SdK644	Crude oil	Dell' Anno <i>et al.</i> , 2021; Zenati <i>et al.</i> , 2018
<i>Oleispira antarctica</i> RB-8	Aliphatic alkanes	Dell' Anno <i>et al.</i> , 2021; Gregson <i>et al.</i> , 2020
<i>Pseudomonas aeruginosa</i> N6P6	Phenanthrene and pyrene	Dell' Anno <i>et al.</i> , 2021; Mangwani <i>et al.</i> , 2015
<i>Pseudomonas pseudoalcaligenes</i> NP103	Phenanthrene and pyrene	Dell' Anno <i>et al.</i> , 2021; Mangwani <i>et al.</i> , 2016
<i>Pseudomonas</i> sp. sp48	Phenol, naphthalene, pentadecane	Dell' Anno <i>et al.</i> , 2021; Farag <i>et al.</i> , 2018
<i>Pseudomonas aeruginosa</i> GOM1	Hexadecane	Dell' Anno <i>et al.</i> , 2021; Muriel-Millan <i>et al.</i> , 2019
<i>Ralstonia pickettii</i>	Crude oil	Dell' Anno <i>et al.</i> , 2021; Setyo Purnomo <i>et al.</i> , 2019

Development of Various Consortia

A consortium refers to a group of different species of microorganisms that are able to coexist with each other. For instance, members of a bacterial consortium include oil-degrading bacteria as well as other bacteria that may produce biosurfactants or other nutrients (Rojas-Vargas *et al.*, 2022). Together, these bacteria become a robust unit and can degrade oil with higher efficiency (Patowary *et al.*, 2016). In the study by Yu *et al.* (2022), they used sequencing to find potential oil-degrading bacteria and eventually, they arrived at a consortium composed of *Rhodococcus* sp. OS62-1 and *Pseudomonas* sp. P35 with a high crude oil degradation efficiency of 85.75±3.21%. The consortium was found to be efficient at a pH of 5 to 11 and salinity levels from 0 to 80 g/L. Another consortium can be composed of a fungus and a bacterium. Atakpa *et al.* (2022) found the fungus *Scedosporium* sp. strain ZYY can be grown together with the Y2 strain of *Actinobacter* sp. The bacterium

produces biosurfactants and improves the growth of the fungus. When exposed to crude oil, the consortium was able to degrade total petroleum hydrocarbons (TPH) with a degradation rate of 58.61%. Aside from fungi, microalgae can also work synergistically with bacteria in degrading crude oil (Radice et al., 2023). Different hydrocarbonoclastic bacteria belonging to different genera particularly *Alcanivorax* and *Marinobacter* spp. were found in a petroleum-enriched microalgae culture of *Pavlova lutheri* and *Nannochloropsis oculata* (Chernikova et al., 2020). Bacterial growth, especially that of the aerobic ones, was supported by the products produced by the microalgae such as oxygen and organic materials. The bacteria, in turn, supported the growth of the microalgae by producing carbon dioxide and other nutrients aside from acting on the toxic compounds (Mahdavi et al., 2015; Chernikova et al., 2020; Radice et al., 2023). However, not all combinations of microalgae and bacteria would result in hydrocarbon degradation and removal of toxic compounds. The study by Tang et al. (2010) showed that the microalga *Scenedesmus obliquus* strain GH2 had a reduced PAH removal activity when paired with hydrocarbonoclastic bacterial strains (*Shingomonas* GY2B, *Burkholderia* capacia GS3C, *Pseudomonas* GP3A and *Pandoraea pnomenus* GP3B) (Radice et al., 2023). As for macroalgae, the study of Caronni et al. (2023) used the seaweed, *Caulerpa prolifera*, and tested whether it can degrade diesel in the water since seaweeds are home to various microorganisms, particularly bacteria some of which might have hydrocarbonoclastic properties. The seaweeds produce substances that favor the growth of microorganisms and the microorganisms in return produce metabolites that can influence the growth and other processes of the seaweed (Singh and Reddy, 2014). They reported that *C. prolifera* can degrade diesel hydrocarbons within a certain concentration. The microbial diversity on the exposed seaweed has been altered and favored the growth of *Vibrio* species some of which have oil-degrading capabilities (Graziano et al., 2016; Imron et al., 2019; Zhou et al., 2021; Caronni et al., 2023). Indeed, there is bioremediation potential in the various consortia but there is more work needed to be done to make them actually effective.

Search for More Oil-Degrading Bacteria

Advancements in sequencing have made it possible for more potential microorganisms with oil-degrading capabilities to be detected, classified, and eventually studied. It has enabled scientists to study microbial diversity and perhaps the functional diversity of the community (Guerra et al., 2018). Most of the current studies regarding the search for oil-degrading bacteria have included 16s rRNA gene sequencing in the protocol which uses the differences in the sequences in the regions of the highly conserved 16s rRNA gene that encodes the smaller subunit of the bacterial ribosome to identify bacteria (Kim et al., 2018; Gao et al., 2021; Yu et al., 2022). Analyzing the sequences enables scientists to link the potential oil-degrading bacterium they have found to any well-studied bacteria and gain insights into its biology (David and Berry, 2017). Oil-degrading microorganisms for bioremediation are still scarce (Gao et al., 2021) but the high throughput and relatively quick sequencing provided by the current sequencers now could help in significantly increasing the number of oil-degrading microorganisms detected and classified.

Through the years, the number of identified oil-degrading microorganisms has been increasing thanks to the efforts of scientists. However, discovering new oil-degrading microorganisms is one thing but using them efficiently for bioremediation opens a door full of challenges.

BIOREMEDIATION CHALLENGES

One of the challenges hounding bioremediation efforts is the low degradation efficiency exhibited by the microbes. Degradation efficiency can be seen as how efficient the microbes are in breaking down the components of petroleum into simpler, less toxic compounds. The efficiency depends on many factors given that the petroleum components are complex and that the surrounding environment is not stable and the parameters can freely change (Xu et al., 2015, 2018; Galitskaya et al., 2021). But generally, degradation efficiency is highest if the components have lower molecular weights or simple structures (Xue et al., 2015). Currently, scientists are still searching for bacterial strains that have high degradation efficiencies. Gao et al. (2021) tried to find novel strains in Jiaozhou Bay, which is an area in Shandong, China that can efficiently degrade diesel. They isolated a strain of *Bacillus megaterium*, which they called MJ4, that has shown promise in the controlled laboratory set-up. The *B. megaterium* MJ4 was able to achieve a degradation efficiency of almost 71%. A similar study was done in Taean, South Korea where they searched for novel strains of oil-degrading bacteria. Kim et al. (2018) found the Co17 strain of *Gordonia* sp. to have the highest efficiency owing to it producing the enzyme, alkane hydroxylase, more than the other strains. Both studies have suggested that these strains should be used for bioremediation and it should be carried out as soon as possible in order to know their true degradation efficiency. Most of the time, the environment where they will be deployed will not have the conditions or parameters similar to those in the laboratory which can possibly dampen their degradation efficiencies (Xu et al., 2018). Additionally, most of the studies done tend to focus on the degradation of specific oil components but in reality, the bacteria would be exposed to a complex concoction of hydrocarbons

to which some cannot be metabolized by the specific bacteria or can even be toxic (Varjani, 2017; Pandolfo et al., 2023).

Another challenge with bioremediation is the bioavailability of the target compounds. Hydrocarbons are typically hydrophobic which makes them clump together when they are in water. This makes it harder for bacteria to facilitate the breakdown of hydrocarbons through the action of enzymes such as oxygenases as the enzymes would need to contact the substrate but the bacteria cannot access most of the hydrocarbons (Xu et al., 2018). Surfactants which are chemicals that reduce surface tension are usually added to improve the emulsification of the hydrocarbons with water and allow bacteria to access more substrate (Mohanty et al., 2013). However, surfactants can be toxic to bacteria (Kleindienst et al., 2015). The task now is finding bacterial species that can produce biosurfactants without endangering the oil-degrading bacteria in the water. Bacteria under the genera *Bacillus* and *Pseudomonas* can produce biosurfactants that are more effective, more stable, and less toxic when compared to synthetic surfactants (Desai and Banat, 1997; Pardhi et al., 2022).

Yet another challenge with bioremediation is keeping the bacteria alive for a period of time to ensure the degradation of the contaminant. Enzyme production of bacteria meant for oil degradation may take some time as bacteria are not often exposed to petroleum or they may not use it readily given the availability of other substrates they naturally use. Oil degradation depends on the activity of the bacteria which is linked to the availability of nutrients and suitable environmental conditions (Xu et al., 2018).

Lastly, the toolbox for bioremediation is still limited. Oil-degrading microorganisms with high degradation efficiency are still scarce (Gao et al., 2021).

FUTURE PROSPECTS

Nations that often experience oil spills should invest in bioremediation research. The future of oil spill bioremediation rests on improving the degradation efficiency of bacteria and other microorganisms. Improvement can be carried out by finding new or existing microorganisms with better degradation efficiencies as well as those that produce useful compounds for microbial growth and petroleum degradation such as nutrients and surfactants, respectively. Aside from searching for microorganisms, scientists should study further the different interactions of various bacteria with other bacteria and with other microorganisms. These interactions will become the basis for which microorganisms can be grouped to form a consortium that can be used for bioremediation. Currently, there are promising consortia of bacteria, bacteria-fungi, and bacteria-microalgae but there should be more in the future. Additionally, conducting bioremediation research in a country, including the Philippines that has experienced massive oil spills may lead to the discovery of a natural consortium of bacteria with hydrocarbon degradation potential. The study of Rodriguez et al. (2023) showed the hydrocarbon degradation potential of different bacterial consortia from the samples taken from Guimaras, Philippines, a small island that experienced an oil spill last 2006. One consortium was dominated by *Klebsiella* sp., a bacterium that was not listed in the different reviews regarding hydrocarbon degradation. More research should also be done on the oil degradation potential of different seaweeds that grow in each country. As previously mentioned, seaweeds are home to many bacteria and Caronni et al. (2023) have demonstrated that certain species of macroalgae can degrade hydrocarbons to some degree with the help of the epibionts. For the Philippines, seaweeds are economically important commodities and comprise most of the bulk of aquaculture production. The main seaweeds grown in the Philippines are *Kappaphycus alvarezii*, *Gracilaria* spp., *Eucheuma denticulatum*, and *Caulerpa lentilifera* (BFAR, 2010). Scientists could look into studying the seaweed-associated bacteria in these species and perhaps an oil-degrading bacterium with high degradation efficiency or a biosurfactant-producing bacterium may be thriving in the seaweeds which can be a lot of help in future bioremediation efforts. With this potential venture, the use of metagenomics which is the culture-independent analysis of the entire genome of all organisms in an environmental sample (Jackson et al., 2015) can help in identifying and characterizing bacteria present on seaweeds exposed to oil as well as determining the bacterial diversity. The information gathered could greatly help in developing and/or refining bioremediation strategies (Guerra et al., 2018).

CONCLUSION

Bioremediation remains a viable choice for removing petroleum products in the water as it does not involve the use of toxic substances and is relatively cheaper. However, there are still challenges that need to be overcome particularly in the improvement of the degradation efficiency of the microbes and the search for more oil-degrading bacteria in which high throughput sequencing is a useful tool. Increasing the degradation efficiency may be achieved by establishing different consortia of microorganisms. Each microorganism involved in the consortium may produce useful compounds or may be directly involved in the degradation of petroleum products. As for the search for additional hydrocarbonoclastic bacteria, seaweeds can also be a potential source of these types of bacteria. Despite the current limitations of bioremediation, its utility during oil spills is valued and its potential for further improvement is recognized.

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REFERENCES

- Abbasian, F., Lockington, R., Mallavarapu, M., & Naidu, R. (2015). A comprehensive review of aliphatic hydrocarbon biodegradation by bacteria. *Applied Biochemistry and Biotechnology*, 176 (3), 670–699. <https://doi.org/10.1007/s12010-015-1603-5>
- Agaton, C. B., Guno, C. S., Labog, R. A., & Collera, A. A. (2023). Immediate Socio-Economic Impacts of Mindoro Oil Spill to Fisherfolk in Naujan, Philippines. *Resources*, 12(9), 102. <https://doi.org/10.3390/resources12090102>
- Alea, C., Ruiz, C. I., Yap, J. B., Molina, E. F., Saballa, A. J., Ñuneza, J. S., & Bacharo, K. B. B. (2022). An investigation of aquatic oil spills in the Philippines from 2000 to 2021. *Marine Pollution Bulletin*, 185 Part A, 114241. <https://doi.org/10.1016/j.marpolbul.2022.114241>
- Alvernina, P., Utomo, S., Soesilo, T. E. B., & Herdiansyah, H. (2021). Studies of fishermen's economic loss due to oil spills. *IOP Conference Series: Earth and Environmental Science*, 802, 012002. <https://doi.org/10.1088/1755-1315/802/1/012002>
- Atakpa, E. O., Zhou, H., Jiang, L., Ma, Y., Liang, Y., Li, Y., Zhang, D., & Zhang, C. (2022). Improved degradation of petroleum hydrocarbons by co-culture of fungi and biosurfactant-producing bacteria. *Chemosphere*, 290, 133337. <https://doi.org/10.1016/j.chemosphere.2021.133337>
- Atlas, R. M., & Hazen, T. C. (2011). Oil biodegradation and bioremediation: A tale of the two worst spills in U.S. history. *Environmental Science and Technology*, 45(16), 6709–6715. <https://doi.org/10.1021/es2013227>
- Bartha, R., & Atlas, R. M. (1977). The microbiology of aquatic oil spills. *Advances in Applied Microbiology*, 22, 225 – 266. [https://doi.org/10.1016/S0065-2164\(08\)70164-3](https://doi.org/10.1016/S0065-2164(08)70164-3)
- Berry, D., & Gutierrez, T. (2017). Evaluating the detection of hydrocarbon-degrading bacteria in 16S rRNA gene sequencing surveys. *Frontiers in Microbiology*, 8, 896. <https://doi.org/10.3389/fmicb.2017.00896>
- Brown, L. M., Gunasekera, T. S., Striebig, R. C., & Ruiz, O. N. (2016). Draft genome sequence of *Gordonia silhwensis* strain 9, a branched alkane-degrading bacterium. *Genome Announcements*, 4(3), 10-1128. <https://doi.org/10.1128/genomeA.00622-16>
- Bureau of Fisheries and Aquatic Resources. (2010). *Fisheries Commodity Road Map: Seaweeds*. Retrieved on 28 July 2023 from https://www.bfar.da.gov.ph/files/img/photos/roadmapseaweeds_wdcorrection2008.pdf
- Caronni, S., Quaglini, L. A., Franzetti, A., Gentili, R., Montagnani, C., & Citterio, S. (2023). Does *Caulerpa prolifera* with its bacterial coating represent a promising association for seawater phytoremediation of diesel hydrocarbons?. *Plants*, 12(13), 2507. <https://doi.org/10.3390/plants12132507>
- Chaudhary, D. K., Kim, D. U., Kim, D., & Kim, J. (2019). *Flavobacterium petrolei* sp. nov., a novel psychrophilic, diesel-degrading bacterium isolated from oil-contaminated Arctic soil. *Scientific Reports*, 9(1), 4134. <https://doi.org/10.1038/s41598-019-40667-7>
- Cheffi, M., Hentati, D., Chebbi, A., Mhiri, N., Sayadi, S., Marqués, A. M., & Chamkha, M. (2020). Isolation and characterization of a newly naphthalene-degrading *Halomonas pacifica*, strain Cnaph3: biodegradation and biosurfactant production studies. *3 Biotech*, 10, 89 <https://doi.org/10.1007/s13205-020-2085-x>
- Chernikova, T. N., Bargiela, R., Toshchakov, S. v., Shivaraman, V., Lunev, E. A., Yakimov, M. M., Thomas, D. S., & Golyshin, P. N. (2020). Hydrocarbon-degrading bacteria *Alcanivorax* and *Marinobacter* associated with microalgae *Pavlova lutheri* and *Nannochloropsis oculata*. *Frontiers in Microbiology*, 11, 572931. <https://doi.org/10.3389/fmicb.2020.572931>
- Chuah, L. F., Chew, K. W., Bokhari, A., Mubashir, M., & Show, P. L. (2022). Biodegradation of crude oil in seawater by using a consortium of symbiotic bacteria. *Environmental Research*, 213, 113721. <https://doi.org/10.1016/j.envres.2022.113721>
- Cordes, E. E., Jones, D. O. B., Schlacher, T. A., Amon, D. J., Bernardino, A. F., Brooke, S., Carney, R., DeLeo, D. M., Dunlop, K. M., Escobar-Briones, E. G., Gates, A. R., Génio, L., Gobin, J., Henry, L. A., Herrera, S., Hoyt, S., Joye, M., Kark, S., Mestre, N. C., & Witte, U. (2016). Environmental impacts of the deep-water oil and gas industry: A review to guide management strategies. In: *Frontiers in Environmental Science* (Vol. 4, Issue SEP). Frontiers Media S.A. <https://doi.org/10.3389/fenvs.2016.00058>
- Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology Research International*, 2011, 941810. <https://doi.org/10.4061/2011/941810>
- Dell' Anno, F., Rastelli, E., Sansone, C., Dell' Anno, A., Brunet, C., & Ianora, A. (2021). Bacteria, fungi and microalgae for the bioremediation of marine sediments contaminated by petroleum hydrocarbons in the omics era. *Microorganisms*, 9(8), 1695. <https://doi.org/10.3390/microorganisms9081695>
- Deng, M. C., Li, J., Liang, F. R., Yi, M., Xu, X. M., Yuan, J. P., Peng, J., Wu, C. F., & Wang, J. H. (2014). Isolation and characterization of a novel hydrocarbon-degrading bacterium *Achromobacter* sp. HZ01 from the crude oil-contaminated seawater at the Daya Bay, Southern China. *Marine Pollution Bulletin*, 83(1), 79–86.
- Desai, J. D., & Banat, I. M. (1997). Microbial Production of Surfactants and Their Commercial Potential. In: *Microbiology And Molecular Biology Reviews* (Vol. 61, Issue 1).
- Durán, R. E., Méndez, V., Rodríguez-Castro, L., Barra-Sanhueza, B., Salvà-Serra, F., Moore, E. R. B., Castro-Nallar, E., & Seeger, M. (2019). Genomic and physiological traits of the marine bacterium *Alcaligenes aquatilis* QD168 isolated from Quintero bay, central Chile, reveal a robust adaptive response to environmental stressors. *Frontiers in Microbiology*, 10, 528. <https://doi.org/10.3389/fmicb.2019.00528>
- Eklund, R. L., Knapp, L. C., Sandifer, P. A., & Colwell, R. C. (2019). Oil spills and human health: contributions of the Gulf of Mexico research initiative. *GeoHealth*, 3(12), 391–406. <https://doi.org/10.1029/2019GH000217>
- Farag, S., Soliman, N. A., & Abdel-Fattah, Y. R. (2018). Statistical optimization of crude oil bio-degradation by a local marine bacterium isolate *Pseudomonas* sp. sp48. *Journal of Genetic Engineering and Biotechnology*, 16(2), 409–420. <https://doi.org/10.1016/j.jgeb.2018.01.001>
- Favennec, J. P. (2022). Economics of Oil Refining. In: *The Palgrave Handbook of International Energy Economics* (pp. 59–74). Springer International Publishing. https://doi.org/10.1007/978-3-030-86884-0_3
- Galeriková, A., & Materna, M. (2020). World seaborne trade with oil: one of main cause for oil spills?. *Transportation Research Procedia*, 44, 297–304. <https://doi.org/10.1016/j.trpro.2020.02.039>
- Galitskaya, P., Biktasheva, L., Blagodatsky, S., & Selivanovskaya, S. (2021). Response of bacterial and fungal communities to high petroleum pollution in different soils. *Scientific Reports*, 11, 164. <https://doi.org/10.1038/s41598-020-80631-4>
- Gao, J., Ming, J., Xu, M., Fu, X., Duan, L. F., Xu, C. C., Gao, Y., Xue, J. L., & Xiao, X. F. (2021). Isolation and characterization of a high-efficiency marine diesel oil-degrading bacterium. *Petroleum Science*, 18(2), 641–653. <https://doi.org/10.1007/s12182-020-00540-z>
- Gharib, C., Mefteh-Wali, S., Serret, V., & Ben Jabeur, S. (2021). Impact of COVID-19 pandemic on crude oil prices: Evidence from Econophysics approach. *Resources Policy*, 74, 102392. <https://doi.org/10.1016/j.resourpol.2021.102392>
- Ghosal, D., Ghosh, S., Dutta, T. K., & Ahn, Y. (2016). Current state of knowledge in microbial degradation of polycyclic aromatic hydrocarbons (PAHs): a review. *Frontiers in Microbiology*, 7, 1369. <https://doi.org/10.3389/fmicb.2016.01369>
- Giebel, H. A., Kalhoefer, D., Lemke, A., Thole, S., Gahl-Janssen, R., Simon, M., & Brinkhoff, T. (2011). Distribution of *Roseobacter* RCA and SAR11 lineages in the North Sea and characteristics of an abundant RCA isolate. *ISME Journal*, 5(1), 8–19. <https://doi.org/10.1038/ismej.2010.87>
- Gregson, B. H., Metodieva, G., Metodieva, M. v., Golyshin, P. N., & McKew, B. A. (2020). Protein expression in the obligate hydrocarbon-degrading psychrophile *Oleispira antarctica* RB-8 during alkane degradation and cold tolerance. *Environmental Microbiology*, 22(5), 1870–1883. <https://doi.org/10.1111/1462-2920.14956>
- Guerra, A. B., Oliveira, J. S., Silva-Portela, R. C. B., Araújo, W., Carlos, A. C., Vasconcelos, A. T. R., Freitas, A. T., Domingos, Y. S., de Farias, M. F., Fernandes, G. J. T., & Agnez-Lima, L. F. (2018). Metagenome enrichment approach used for selection of oil-degrading bacteria consortia for drill cutting residue bioremediation. *Environmental Pollution*, 235, 869–880. <https://doi.org/10.1016/j.envpol.2018.01.014>
- Gutierrez, T., Biddle, J. F., Teske, A., & Aitken, M. D. (2015). Cultivation-dependent and cultivation-independent characterization of hydrocarbon-degrading bacteria in Guaymas Basin sediments. *Frontiers in Microbiology*, 6, 695. <https://doi.org/10.3389/fmicb.2015.00695>
- Hara, A., Syutsubo, K., & Harayama, S. (2003). *Alcanivorax* which prevails in oil-contaminated seawater exhibits broad substrate specificity for alkane degradation. *Environmental Microbiology*, 5(9), 746–753. <https://doi.org/10.1046/j.1462-2920.2003.00468.x>
- Hedlund, B. P., Geiselbrecht, A. D., Bair, T. J., & Staley, J. T. (1999). Polycyclic aromatic hydrocarbon degradation by a new marine bacterium, *Neptunomonas naphthovorans* gen. nov., sp. nov. *Applied and Environmental Microbiology*, 65(1), 251–259. <https://doi.org/10.1128/AEM.65.1.251-259.1999>
- Hii, Y. S., Law, A. T., Shazili, N. A. M., Abdul-Rashid, M. K., & Lee, C. W. (2009). Biodegradation of Tapis blended crude oil in marine sediment by a consortium of symbiotic bacteria. *International Biodeterioration and Biodegradation*, 63(2), 142–150. <https://doi.org/10.1016/j.ibiod.2008.08.003>
- International Tanker Owners Pollution Federation (ITOPF). (2023). *Oil Tanker Spill Statistics 2022*. https://www.itopf.org/fileadmin/uploads/itopf/data/Photos/Statistics/Oil_Spill_Stats_brochure_2022.pdf

- Jackson, S. A., Borchert, E., O'Gara, F., & Dobson, A. D. W. (2015). Metagenomics for the discovery of novel biosurfactants of environmental interest from marine ecosystems. *Current Opinion in Biotechnology*, 33, 176–182. <https://doi.org/10.1016/j.copbio.2015.03.004>.
- Jahromi, H., Fazelipour, M. H., Ayatollahi, S., & Niazi, A. (2014). Asphaltenes biodegradation under shaking and static conditions. *Fuel*, 117(PART A), 230–235. <https://doi.org/10.1016/j.fuel.2013.09.085>
- Kaiser, M. J., & Snyder, B. (2013). A primer on the offshore contract drilling industry. *Ocean Development and International Law*, 44(3), 287–314. <https://doi.org/10.1080/00908320.2013.780856>.
- Kasai, Y., Kishira, H., & Harayama, S. (2002). Bacteria belonging to the genus *Cycloclasticus* play a primary role in the degradation of aromatic hydrocarbons released in a marine environment. *Applied and Environmental Microbiology*, 68(11), 5625–5633. <https://doi.org/10.1128/AEM.68.11.5625-5633.2002>.
- Kim, H. S., Dong, K., Kim, J., & Lee, S. S. (2019). Characteristics of crude oil-degrading bacteria *Gordonia iterans* isolated from marine coastal in Taean sediment. *Microbiology Open*, 8(6), e00754. <https://doi.org/10.1002/mbo3.754>.
- Kleindienst, S., Seidel, M., Ziervogel, K., Grim, S., Loftis, K., Harrison, S., Malkin, S. Y., Perkins, M. J., Field, J., Sogin, M. L., Dittmar, T., Passow, U., Medeiros, P. M., & Joye, S. B. (2015). Chemical dispersants can suppress the activity of natural oil-degrading microorganisms. *Proceedings of the National Academy of Sciences of the United States of America*, 112(48), 14900–14905. <https://doi.org/10.1073/pnas.1507380112>.
- Lee, E. H., & Cho, K. S. (2008). Characterization of cyclohexane and hexane degradation by *Rhodococcus* sp. EC1. *Chemosphere*, 71(9), 1738–1744. <https://doi.org/10.1016/j.chemosphere.2007.12.009>
- Licaunan, W. Y., Cabreira, R. W., & Aliño, P. M. (2018). The Philippines. In: *World Seas: An Environmental Evaluation Volume II: The Indian Ocean to the Pacific* (pp. 515–537). Elsevier. <https://doi.org/10.1016/B978-0-08-100853-9.00051-8>.
- Ma, Y. L., Lu, W., Wan, L. L., & Luo, N. (2015). Elucidation of fluoranthene degradation characteristics in a newly isolated *Achromobacter xylosoxidans* DN002. *Applied Biochemistry and Biotechnology*, 175(3), 1294–1305. <https://doi.org/10.1007/s12010-014-1347-7>
- Mahdavi, H., Prasad, V., Liu, Y., & Ulrich, A. C. (2015). In situ biodegradation of naphthenic acids in oil sands tailings pond water using indigenous algae-bacteria consortium. *Bioresource Technology*, 187, 97–105. <https://doi.org/10.1016/j.biortech.2015.03.091>
- Mahjoubi, M., Aliyu, H., Cappello, S., Naifer, M., Souissi, Y., Cowan, D. A., & Cherif, A. (2019). The genome of *Alcaligenes aquatilis* strain BU33N: insights into hydrocarbon degradation capacity. *PLoS ONE*, 14(9), e0221574. <https://doi.org/10.1371/journal.pone.0221574>.
- Mahjoubi, M., Cappello, S., Souissi, Y., Jaouani, A., & Cherif, A. (2018). Microbial Bioremediation of Petroleum Hydrocarbon–Contaminated Marine Environments. In: *Recent Insights in Petroleum Science and Engineering*. InTech. <https://doi.org/10.5772/intechopen.72207>.
- Mangwani, N., Kumari, S., & Das, S. (2015). Involvement of quorum sensing genes in biofilm development and degradation of polycyclic aromatic hydrocarbons by a marine bacterium *Pseudomonas aeruginosa* N6P6. *Applied Microbiology and Biotechnology*, 99(23), 10283–10297. <https://doi.org/10.1007/s00253-015-6868-7>.
- Mangwani, N., Kumari, S., & Das, S. (2016). Effect of synthetic N-acylhomoserine lactones on cell-cell interactions in marine *Pseudomonas* and biofilm mediated degradation of polycyclic aromatic hydrocarbons. *Chemical Engineering Journal*, 302, 172–186. <https://doi.org/10.1016/j.cej.2016.05.042>.
- Messina, E., Denaro, R., Crisafi, F., Smedile, F., Cappello, S., Genovese, M., Genovese, L., Giuliano, L., Russo, D., Ferrer, M., Golyshin, P., & Yakimov, M. M. (2016). Genome sequence of obligate marine polycyclic aromatic hydrocarbons-degrading bacterium *Cycloclasticus* sp. 78-ME, isolated from petroleum deposits of the sunken tanker Amoco Milford Haven, Mediterranean Sea. *Marine Genomics*, 25, 11–13. <https://doi.org/10.1016/j.margen.2015.10.006>.
- Mnif, S., Sayadi, S., & Chamkha, M. (2014). Biodegradative potential and characterization of a novel aromatic-degrading bacterium isolated from a geothermal oil field under saline and thermophilic conditions. *International Biodeterioration and Biodegradation*, 86, 258–264. <https://doi.org/10.1016/j.ibiod.2013.09.015>
- Mohanty, S., Jasmine, J., & Mukherji, S. (2013). Practical considerations and challenges involved in surfactant enhanced bioremediation of oil. In: *BioMed Research International* (Vol. 2013). <https://doi.org/10.1155/2013/328608>.
- Mukherjee, S., Bardolui, N. K., Karim, S., Patnaik, V. v., Nandy, R. K., & Bag, P. K. (2010). Isolation and characterization of a monoaromatic hydrocarbon-degrading bacterium, *Pseudomonas aeruginosa* from crude oil. *Journal of Environmental Science and Health - Part A Toxic/Hazardous Substances and Environmental Engineering*, 45(9), 1048–1053. <https://doi.org/10.1080/10934529.2010.486328>
- Muriel-Millán, L. F., Rodríguez-Mejía, J. L., Godoy-Lozano, E. E., Rivera-Gómez, N., Gutierrez-Rios, R. M., Morales-Guzmán, D., Trejo-Hernández, M. R., Estradas-Romero, A., & Pardo-López, L. (2019). Functional and genomic characterization of a *Pseudomonas aeruginosa* strain isolated from the southwestern Gulf of Mexico reveals an enhanced adaptation for long-chain alkane degradation. *Frontiers in Marine Science*, 6, 572. <https://doi.org/10.3389/fmars.2019.00572>.
- Pacwa-Plociniczak, M., Plaza, G. A., Poliwoda, A., & Piotrowska-Seget, Z. (2014). Characterization of hydrocarbon-degrading and biosurfactant-producing *Pseudomonas* sp. P-1 strain as a potential tool for bioremediation of petroleum-contaminated soil. *Environmental Science and Pollution Research*, 21(15), 9385–9395. <https://doi.org/10.1007/s11356-014-2872-1>.
- Pandolfo, E., Barra Caracciolo, A., & Rolando, L. (2023). Recent advances in bacterial degradation of hydrocarbons. *Water*, 15, 375. <https://doi.org/10.3390/w15020375>.
- Pardhi, D. S., Panchal, R. R., Raval, V. H., Joshi, R. G., Pocai, P., Almalki, W. H., & Rajput, K. N. (2022). Microbial surfactants: A journey from fundamentals to recent advances. *Frontiers in Microbiology*, 13, 982603. <https://doi.org/10.3389/fmicb.2022.982603>.
- Patowary, K., Patowary, R., Kalita, M. C., & Deka, S. (2016). Development of an efficient bacterial consortium for the potential remediation of hydrocarbons from contaminated sites. *Frontiers in Microbiology*, 7, 1092. <https://doi.org/10.3389/fmicb.2016.01092>
- Radice, R. P., de Fabrizio, V., Donadoni, A., Scopa, A., & Martelli, G. (2023). Crude Oil Bioremediation: From Bacteria to Microalgae. *Processes*, 11(2), 442. <https://doi.org/10.3390/pr11020442>.
- Rajasekar, A., Babu, T. G., Pandian, S. T. K., Maruthamuthu, S., Palaniswamy, N., & Rajendran, A. (2007). Role of *Serratia marcescens* ACE2 on diesel degradation and its influence on corrosion. *Journal of Industrial Microbiology and Biotechnology*, 34 (9), 589–598. <https://doi.org/10.1007/s10295-007-0225-5>.
- Ramasamy, S., Arumugam, A., & Chandran, P. (2017). Optimization of *Enterobacter cloacae* (KU923381) for diesel oil degradation using response surface methodology (RSM). *Journal of Microbiology*, 55(2), 104–111. <https://doi.org/10.1007/s12275-017-6265-2>.
- Rodriguez, K. N. D., Santos, R. T., Nagpala, M. J. M., & Opulencia, R. B. (2023). Metataxonomic characterization of enriched consortia derived from oil spill-contaminated sites in Guimaras, Philippines, reveals major role of *Klebsiella* sp. in hydrocarbon degradation. *International Journal of Microbiology*, 2023, 3247448. <https://doi.org/10.1155/2023/3247448>
- Salison, J. A. P. & Vergel, N. K. B. (2021). Estimation of Philippine Domestic Maritime Transportation Energy Demand. *Philippine Transportation Journal*, 4 (1), 10-31. https://www.doe.gov.ph/sites/default/files/pdf/energy_statistics/ebt-primer.pdf.
- Setyo Purnomo, A.S., Rizqi, H. D., Harmelia, L., Diah Anggraeni, S., Melati, R. E., Damayanti, H., Mar'atus Shafwah, O., & Kusuma, F. C. (2019). Biodegradation of crude oil by *Ralstonia pickettii* under high salinity medium. *Malaysian Journal of Fundamental and Applied Sciences*, 15, 377–380.
- Singh, H. (2006). *Mycoremediation: Fungal Bioremediation*. New Jersey: John Wiley & Sons.
- Sinha, R. K., Krishnan, K. P., & Kurian, P. J. (2021). Complete genome sequence and comparative genome analysis of *Alcanivorax* sp. IO_7, a marine alkane-degrading bacterium isolated from hydrothermally-influenced deep seawater of southwest Indian ridge. *Genomics*, 113(1), 884–891. <https://doi.org/10.1016/j.ygeno.2020.10.020>.
- Sugiura, K., Ishihara, M., Shimauchi, T., & Harayama, S. (1997). Physicochemical properties and biodegradability of crude oil. *Environmental Science & Technology*, 31(1), 45–51. <https://doi.org/10.1021/es950961r>
- Tang, X., He, L. Y., Tao, X. Q., Dang, Z., Guo, C. L., Lu, G. N., & Yi, X. Y. (2010). Construction of an artificial microalgal-bacterial consortium that efficiently degrades crude oil. *Journal of Hazardous Materials*, 181(1–3), 1158–1162. <https://doi.org/10.1016/j.jhazmat.2010.05.033>
- Tavassoli, T., Mousavi, S. M., Shojasadat, S. A., & Salehizadeh, H. (2012). Asphaltene biodegradation using microorganisms isolated from oil samples. *Fuel*, 93, 142–148. <https://doi.org/10.1016/j.fuel.2011.10.021>
- Tian, X., Wang, X., Peng, S., Wang, Z., Zhou, R., & Tian, H. (2018). Isolation, screening, and crude oil degradation characteristics of hydrocarbons-degrading bacteria for treatment of oily wastewater. *Water Science and Technology*, 78(12), 2626–2638. <https://doi.org/10.2166/wst.2019.025>.
- Tofalos, A. E., Daghighi, M., González, M., Papacchini, M., Franzetti, A., & Seeger, M. (2018). Toluene degradation by *Cupriavidus metallidurans* CH34 in nitrate-reducing conditions and in bioelectrochemical systems. *FEMS Microbiology Letters*, 365 (12), fny119. <https://doi.org/10.1093/femsle/fny119>.
- Varjani S. J. (2017). Microbial degradation of petroleum hydrocarbons. *Bioresource Technology*, 223, 277–286. <https://doi.org/10.1016/j.biortech.2016.10.037>.
- Venkateswaran, K., Hoaki, T., Kato, M., & Maruyama, T. (1995). Microbial degradation of resins fractionated from Arabian light crude oil. *Canadian Journal of Microbiology*, 41, 418 – 424. <https://doi.org/10.1139/m95-055>
- Wang, D., Lin, J., Lin, J., Wang, W., & Li, S. (2019). Biodegradation of petroleum hydrocarbons by *Bacillus subtilis* BL-27, a strain with weak hydrophobicity. *Molecules*, 24(17), 3021. <https://doi.org/10.3390/molecules24173021>.
- Wang, W., Wang, L., & Shao, Z. (2018). Polycyclic aromatic hydrocarbon (PAH) degradation pathways of the obligate marine PAH degrader *Cycloclasticus* sp. strain P1. *Applied and Environmental Microbiology*, 84(21), e01261-18. <https://doi.org/10.1128/AEM.01261-18>.

- Wang, X. B., Chi, C. Q., Nie, Y., Tang, Y. Q., Tan, Y., Wu, G., & Wu, X. L. (2011). Degradation of petroleum hydrocarbons (C6-C40) and crude oil by a novel *Dietzia* strain. *Bioresource Technology*, 102(17), 7755–7761. <https://doi.org/10.1016/j.biortech.2011.06.009>
- Xu, X., Liu, W., Tian, S., Wang, W., Qi, Q., Jiang, P., Gao, X., Li, F., Li, H., & Yu, H. (2018). Petroleum hydrocarbon-degrading bacteria for the remediation of oil pollution under aerobic conditions: a perspective analysis. *Frontiers in Microbiology*, 9, 2885. <https://doi.org/10.3389/fmicb.2018.02885>.
- Xue, J., Yu, Y., Bai, Y., Wang, L., & Wu, Y. (2015). Marine Oil-Degrading Microorganisms and Biodegradation Process of Petroleum Hydrocarbon in Marine Environments: A Review. *Current Microbiology*, 71(2), 220–228. <https://doi.org/10.1007/s00284-015-0825-7>.
- Yakimov, M. M., Giuliano, L., Gentile, G., Crisafi, E., Chernikova, T. N., Abraham, W. R., Lünsdorf, H., Timmis, K. N., & Golyshin, P. N. (2003). *Oleispira antarctica* gen. nov., sp. nov., a novel hydrocarbonoclastic marine bacterium isolated from Antarctic coastal sea water. *International Journal of Systematic and Evolutionary Microbiology*, 53(3), 779–785. <https://doi.org/10.1099/ijs.0.02366-0>.
- Yakimov, M. M., Timmis, K. N., & Golyshin, P. N. (2007). Obligate oil-degrading marine bacteria. *Current Opinion in Biotechnology*, 18(3), 257–266. <https://doi.org/10.1016/j.copbio.2007.04.006>.
- Yang, T. Y., Chen, S. H., Yang, Y. T., & Chang, H. H. (2022). Relationship between crude oil price and production levels—An empirical study of OPEC and non-OPEC states. *Frontiers in Environmental Science*, 10, 933431. <https://doi.org/10.3389/fenvs.2022.933431>.
- Yu, T., Liu, X., Ai, J., Wang, J., Guo, Y., Liu, X., He, X., Deng, Z., & Jiang, Y. (2022). Microbial community succession during crude oil-degrading bacterial enrichment cultivation and construction of a degrading consortium. *Frontiers in Microbiology*, 13, 1044448. <https://doi.org/10.3389/fmicb.2022.1044448>.
- Zadjeleovic, V., Gibson, M. I., Dorador, C., & Christie-Oleza, J. A. (2020). Genome of *Alcanivorax* sp. 24: A hydrocarbon degrading bacterium isolated from marine plastic debris. *Marine Genomics*, 49, 100686. <https://doi.org/10.1016/j.margen.2019.05.001>.
- Zenati, B., Chebbi, A., Badis, A., Eddouaouda, K., Boutoumi, H., el Hattab, M., Hentati, D., Chelbi, M., Sayadi, S., Chamkha, M., & Franzetti, A. (2018). A non-toxic microbial surfactant from *Marinobacter hydrocarbonoclasticus* SdK644 for crude oil solubilization enhancement. *Ecotoxicology and Environmental Safety*, 154, 100–107. <https://doi.org/10.1016/j.ecoenv.2018.02.032>.
- Zhang, B., Matchinski, E. J., Chen, B., Ye, X., Jing, L., & Lee, K. (2018). Marine oil spills-oil pollution, sources and effects. In: *World Seas: An Environmental Evaluation Volume III: Ecological Issues and Environmental Impacts* (pp. 391–406). Elsevier. <https://doi.org/10.1016/B978-0-12-805052-1.00024-3>.
- Zhang, L., Zhang, C., Cheng, Z., Yao, Y., & Chen, J. (2013). Biodegradation of benzene, toluene, ethylbenzene, and o-xylene by the bacterium *Mycobacterium cosmeticum* byf-4. *Chemosphere*, 90(4), 1340–1347. <https://doi.org/10.1016/j.chemosphere.2012.06.043>
- Zhukov, D. v., Murygina, V. P., & Kalyuzhnyi, S. v. (2007). Kinetics of the degradation of aliphatic hydrocarbons by the bacteria *Rhodococcus ruber* and *Rhodococcus erythropolis*. *Applied Biochemistry and Microbiology*, 43(6), 587–592. <https://doi.org/10.1134/S0003683807060038>