

**A Comparison of Response Technologies for Offshore Oil Spills**  
**Exxon Valdez and BP Gulf of Mexico Oil Spills**

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## ABSTRACT

The *Exxon Valdez* oil spill (EVOS) in Prince William Sound, Alaska in 1989 and the BP *Deepwater Horizon* (DWH) oil well blow-out in the Gulf of Mexico in 2010 are considered the two worst oil spills in North American history. The released oil caused devastating impacts to the marine ecosystem. The nature of these two oil spills and the spill locations were vastly different; therefore, their environmental impacts and remediation technologies are also different. The purpose of this research is to conduct a literature review to compare and contrast these two catastrophic oil spills and determine which long-term remediation treatments (beside initial response approaches, e.g., booms, control ignitions) were/are most effective and least-invasive given the different environments in which the oil spills occurred. After analyzing and comparing the environmental conditions and remediation treatments of these two oil spills, bioremediation, the acceleration of degradation processes through stimulation of microorganisms, is considered most effective over the long term. Bioremediation is a non-invasive and cost-effective process compared to the traditional physical oil removal methods. However, a combination of approaches is often most effective for response approaches and longer-term remediation strategies. Ultimately, the most effective approach is to avoid oil spills.

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## INTRODUCTION

Every year, it is estimated that over 40 million gallons of crude oil are released into the marine environment (Kvenvolden & Cooper, 2003). Nearly half of this is caused by natural oil seepage, and the remainder is the result of human activities such as petroleum extraction and transportation (Kvenvolden & Cooper, 2003). When oil is released into water, it immediately undergoes a series of changes in both physical and chemical properties. The changes include evaporation or volatilization, emulsification, dissolution, and oxidation (abiotic: chemical, photo-degradation; and biotic: microbial). This combination of degradation reactions is called the oil weathering process (Fingas, 2012; Board, 2003).

When oil seepage occurs slowly, microbial communities are exposed to slow diffusive hydrocarbon fluxes (Joye *et al.*, 2010). In this manner, the microorganisms indigenous to natural oil seeps are physiologically adapted to this environment and use these hydrocarbons as their energy (carbon) source (Hazen *et al.*, 2010). As a result, ecosystems are generally capable of adapting to the natural oil seeps' presence and thus, of maintaining ecological balance. However, oil spills caused by human activities typically rapidly release oil in quantities that overwhelm the threshold and adaptation capabilities of the ecosystem (Eykelbosh, 2014). As such, damage may occur at the various ecosystem levels including at the individual, population, community and overall ecosystem levels. The problem becomes more complex when the spatial, temporal scales and the inherent variability of the system are also considered (Board *et al.*, 2003).

### Composition and Toxicity of Petroleum Hydrocarbons

Petroleum oil is a complex mixture of organic compounds including hydrocarbons, nitrogen- and sulfur-containing molecules, and some heavy metals such as vanadium (Saadoun, 2015). Petroleum hydrocarbons are comprised of a wide range of molecular weights and structures that are divided into four classes (Fig1): 1) the saturates (alkanes), 2) aromatics: monocyclic aromatics (BTEX) (benzene, toluene, ethylbenzene and xylene) and Polycyclic aromatic hydrocarbons (PAHs) (e.g., naphthalene, anthracene and phenanthrene), 3) nonhydrocarbon, high-molecular-

weight polycyclic organic substances that contain N, S and O atoms including asphaltenes and waxes (phenols, fatty acids, ketones, esters, and porphyrins), and 4) resins (pyridines, quinolines, carbazoles, sulfoxides, and amides) (Colwell *et al.*, 1977; Tissot & Welte, 1984).

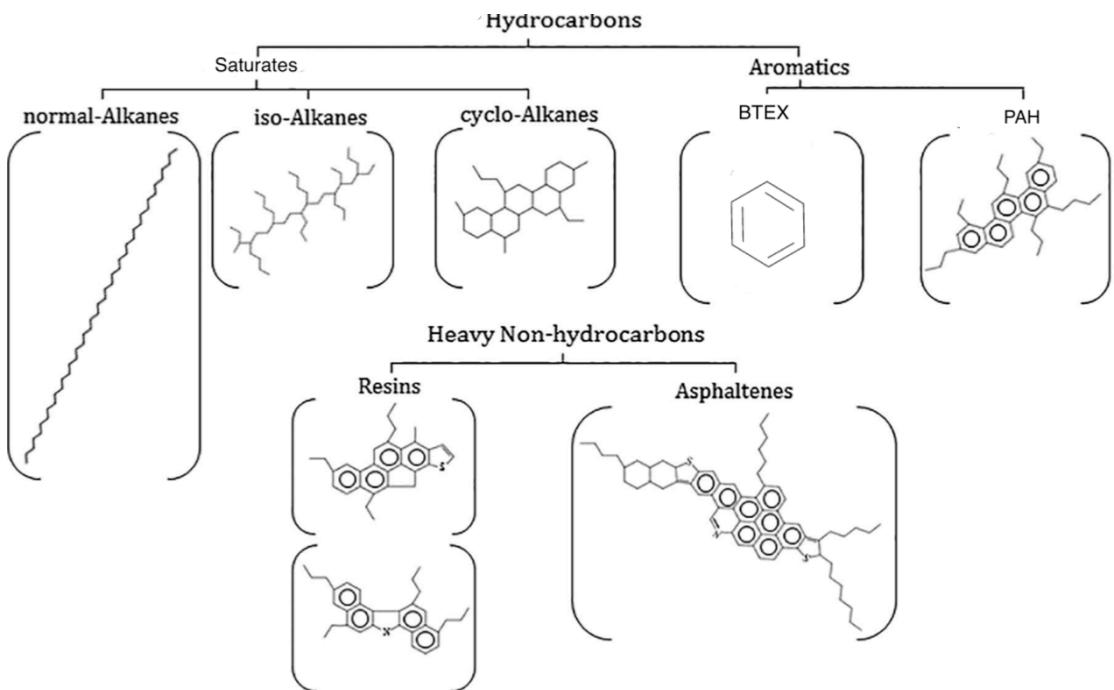


Figure 1. The four classes of petroleum hydrocarbons (Brown *et al.*, 2017)

Generally, all oil components can exhibit both acute and chronic toxicity effects on the marine ecosystem and marine organisms. The relative concentrations drive the potential for adverse effects. When the oil components are floating on the ocean surface as well as entrained in the water column (i.e., either present in the dissolved aqueous phase and/or entrained with particulate material or dispersants), they are acutely toxic because they are readily bioavailable to aquatic organisms. If the weathering process is inhibited, oil can persist when buried in anoxic, nutrient-limited sediments (Boufadel *et al.*, 2010), this can lead to chronic biological exposures. As a result, adverse effects on the reproductive systems of various organisms can result and indirectly suppress the population recovery of exposed animals for decades. For example, fish embryos exposed to weathered oil over the long-term could experience deformities, reproductive mutations or even mortality (Teal & Howarth, 1984; Bodkin *et al.*, 2002; Culbertson *et al.*, 2007; Esler & Iverson, 2010; Charles *et al.*, 2011). A

decadal database of herring biomass showed that adult herring are especially vulnerable to damage from oil spills, in one instance this was observed as a population decline over a 5-year period (Harwell & Gentile, 2006; Throne & Thomas, 2007).

The 1) Saturates (Alkanes) with the lowest molecular weights are volatile at low environmental temperatures. They may display acute toxicity, but they evaporate so quickly on the surface of the ocean that their impacts are generally small. However, when released in deep water the saturates lead to greater toxicity because of their water solubility, even though they biodegrade rapidly. The 2) mono-aromatics and polycyclic aromatic hydrocarbons (PAHs) represent the most toxic fraction, and as such possess the greatest potential for ecological and toxicological adverse effects. The 3) nonhydrocarbon (asphaltenes) and 4) resins are resistant to biodegradation and are very persistent, so they are more chronically toxic (IPIECA, 2015). Among these four classes, the monocyclic and polycyclic aromatics are typically the most toxicologically relevant.

Aromatics are comprised of mono- and poly-aromatic hydrocarbons. The mono-aromatic hydrocarbons are collectively referred to as BTEX (benzene, toluene, ethylbenzene, and xylenes). BTEX comprise a significant percentage of petroleum precursor chemicals (Doherty & Otitoloju, 2016). They are used extensively in a number of manufacturing processes, including the production of synthetic materials, fuel additives, and consumer products (Wilbur & Bosch, 2004). About 90% of BTEX are released into the atmosphere through the combustion of fossil fuels (Bolden *et al.*, 2015). BTEX have relatively high in water solubility, and they tend to evaporate into the air from soil. Because they are relatively hydrophilic, they do not tend to sorb to soil particles and can be transported long distances (Wilbur & Bosch, 2004). BTEX compounds are listed as priority pollutants by the U.S. Environmental Protection Agency (Fay *et al.*, 2007). Prolonged exposure to BTEX compounds may have adverse effects on both human health (e.g., damage the central nervous system (ATSDR, 1993) as well as ecosystem functions (Khodaei *et al.*, 2017). Some studies indicate that benzene can cause acute leukemia, and ethylbenzene is carcinogenic in organism tissues (Wilbur & Bosch, 2004).

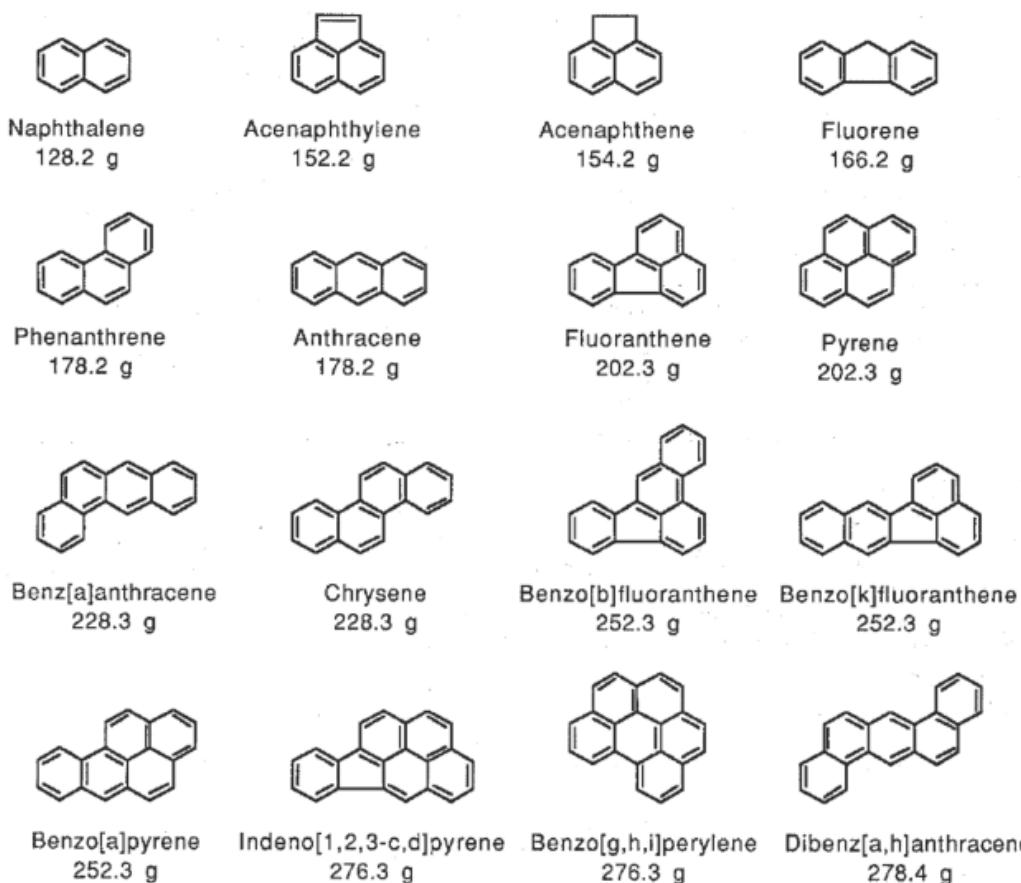


Figure 2. The molecular structure of the 16 polycyclic aromatic hydrocarbons (PAHs) selected as priority pollutants by the U.S. Environmental Protection Agency (EPA). Numbers are molecular weights (Henner *et al.*, 1997).

Polycyclic aromatic hydrocarbons (PAHs) are a class of fused-ring multi-aromatic ring compounds that are ubiquitous environmental pollutants caused by fossil fuel combustion (Brooijmans *et al.*, 2009). They are comprised of two or more fused aromatic rings with a diverse range of branching types. Although aromatic molecules are naturally occurring compounds (Dong *et al.*, 2014) (Fig 2), they are also environmental contaminants, which are somewhat resistant to environmental degradation due to their hydrophobic nature (Gan & Ng, 2009). The PAHs found in oil are also particularly resistant to microbial degradation, by the intrinsic stability of the aromatic ring. PAHs are toxic and/or carcinogenic (depending on the specific analyte) and persist in the oil-polluted environments long after the (linear) alkanes are degraded. There are several hundreds of chemically related compounds, with various structures and varied toxicity in PAHs (Abdel-Shafy & Mansour, 2016). The most

common aromatic hydrocarbons with two rings are naphthalenes. The three-ring compounds are anthracene and phenanthrenes (National Academies of Sciences, Engineering, and Medicine, 2016). PAHs can cause carcinogenic and mutagenic effects and are potent immune-suppressants (Armstrong *et al.*, 2004) (Table 1). After marine animals ingest PAHs, the enzymes in liver activate PAHs making them more toxic as reactive metabolites. These aliphatic hydrocarbons in PAH metabolites are highly oxidative and carcinogenetic because of their ability to bind to DNA and proteins (Lin & Tjeerdema, 2010). PAHs containing up to six fused aromatic rings are called “small” PAHs, and those containing more than six aromatic rings are called “large” PAHs. Small PAHs are more predominant in the environment, such as phenanthrene and anthracene (Armstrong *et al.*, 2004).

	No. Rings	Log K <sub>ow</sub>	Toxicity	Reference
Naphthalenes	2	3.29	Acute toxic, Carcinogenic	EPA, 1982
Anthracene	3	4.45	Not Carcinogenic	Hansch <i>et al.</i> , 1995
Phenanthrenes	3	4.46	Not Carcinogenic	Hansch <i>et al.</i> , 1995

Table 1. Selected chemical properties and example toxicities of 2- and 3- ring PAHs

These four classes of hydrocarbons are subject to different rates of microbial biodegradation, due to the susceptibility of hydrocarbons to microbial attacks, driven in large part by their chemical composition, structures, and physico-chemical properties. In general, the biodegradability of the oil components generally decreases in the following order: *n*-alkanes > branched-chain alkanes > branched alkenes > low-molecular-weight *n*-alkyl aromatics > monoaromatics > cyclic alkanes > polycyclic aromatic hydrocarbons (PAHs) ≫ asphaltenes (Van Hamme *et al.*, 2003). Some high molecular weight compounds, such as asphaltenes, may not be degraded at all, depending on the surrounding environment and whether they are present in surficial sediment or buried (Atlas & Bragg, 2009a). The persistence of petroleum hydrocarbons in the environment is a critical factor for determining the magnitude of adverse effects.

### Degradation Pathways

There are two natural degradation pathways for oil spills, divided broadly into abiotic

(chemical) and biotic (biological) degradation. Abiotic pathways, such as photo-degradation, transform higher molecular weight alkanes and PAHs, especially larger PAHs, rapidly by sunlight into more water-soluble oxygenated compounds or into smaller degraded organic molecules (Yu, 2002). This is an extremely important initial step in promoting bioavailability and further degradation of the resistant petroleum compounds (Kings *et al.*, 2014). Biotic degradation is facilitated by microbes, which metabolize petroleum hydrocarbons as a food (i.e., carbon) source (Brooijmans *et al.*, 2009). Biodegradation is very effective in degrading small molecule petroleum components, such as alkanes, alkenes, and small polycyclics. For high-molecular-weight hydrocarbon classes, microorganisms produce extracellular enzymes and biological surfactants (such as glycolipids, lipopeptides, lipoproteins or heteropolysaccharides) to assist natural oil degradation (Ziervogel *et al.*, 2012; Desai & Banat, 1997). Anaerobic biodegradation of petroleum components has frequently been observed in nature (Widdel & Rabus, 2001). Anaerobic microbial processes are also significant for degrading aromatic compounds such as BTEX compounds, by using either nitrate or sulfate as an electron acceptor (Boopathy *et al.*, 2012). The anaerobic transformation of benzene and toluene under methanogenic conditions is similar to fermentation, in which the substrate is partially oxidized and partially reduced, yielding carbon dioxide and methane as end products (Mihelcic & Luthy, 1988). The rate of biotic biodegradation depends mainly on the physiological capabilities of the microorganisms and the hydrocarbon classes. In general, aerobic processes are faster than anaerobic processes, and low-molecular-weight hydrocarbons are degraded more quickly (Kleindienst *et al.*, 2015). The rates of biodegradation also depend on the physical state and concentration of the oil and environmental conditions, such as temperature, oxygen level, nutrients (fertilizer), and the presence of biosurfactants, (Brooijmans *et al.*, 2009; Leahy & Colwell, 1990).

### Response and Remediation Approaches

Traditional (i.e., historical) oil cleanup operations involve the use of toxic cleaning agents or inappropriate methods that may cause more harm to the ecosystem than no treatment. For example, the consequences of applying pressurized aqueous washing to oily, rocky, intertidal communities contaminated by *Exxon Valdez* oil were tested. Studies revealed that pressurized hot-water washing induced greater macro-algal and

invertebrate mortality than the oiling itself (Mearns, 1996; Peterson, 2011). Therefore, it is important to select response approaches and remediation strategies to treat oil spills that are as environmentally safe and effective as possible with the ultimate goal of eventually restoring ecosystem balance. The most common oil spill remediation methods involve physical oil containment and skimming with floating booms, and chemical or biological approaches such as applying chemical dispersants or biological agents to enhance oil degradation (Fingas, 2012). The DWH spill, which was the largest emergency response to a marine oil spill in history included dispersant application, controlled burn, skimming, siphoning from the wellhead, containment booms, shoreline scavenging/berms, and beach sand mixing to mitigate the spill's impact.

Biodegradation of petroleum hydrocarbons by natural populations of microorganisms represents one of the primary mechanisms to eliminate oil spill contamination from the environment (Das & Chandran, 2011). The rates of biodegradation of oil spills are dependent on both environmental and biological factors. In addition, applying biodegradable dispersants and surfactants instead of toxic chemical-based solvents helps to accelerate the biodegradation process, and hence, reduce the environmental impact of the oil spill (Nyankson *et al.*, 2016).

## DISCUSSION

As the introduction has demonstrated, while generalizations can be made regarding petroleum hydrocarbon behavior in the environment following an oil spill, the location of the actual spill can better inform appropriate response approaches, which remediation technologies are most effective, and the potential for adverse effects. Factors that influence fate and transport, as well as effects of spilled oil are discussed below. Comparisons are drawn between the high latitude *Exxon Valdez* oil spill in Prince William Sound, Alaska (1989) and the low latitude BP *Deepwater Horizon* oil well blowout in the Gulf of Mexico (2010) (Table 2).

Table 2. Comparison of *Exxon Valdez* Spills, and BP *Deepwater Horizon* Spills

	<i>Exxon Valdez (EVOS)</i>	<i>BP Deepwater Horizon (DWH)</i>
1. Location	Prince William Sound, Alaska (1989)	Gulf of Mexico (2010)
2. Latitude	60° 49' 59.99" N (GeoHack - <i>Exxon Valdez</i> oil spill, n.d.)	28° 44' 11.99" N (GeoHack - <i>Deepwater Horizon</i> oil spill, n.d.)
3. Avg. Water Temperature	0-12 °C	20-27°C
4. Nutrients	Sufficient in open water (Atlas & Bragg, 2009a), but deficient on rocky beaches (Schmidt, 2012).	Deficient (Atlas & Hazen, 2011). A large bloom of carbon-metabolizing microbes due to oil spills could deplete the surrounding nutrients (American Academy of Microbiolog, 2011).
5. Marine Environment	Vulnerable and pristine (Riedel, 2014). More enclosed and shallower than open ocean.  Sub-Arctic region. Latitude just south of the arctic circle.	Robust system. Open ocean. Responds rapidly to the influx of oil, because indigenous microbiota are adapted to natural oil seeps in marine environment (National Research Council, 1985).  Subtropical region. Latitude just north of the equator.
6.Oil Type and Degradation Speed	North Slope Heavy Crude Oil (API gravity 29.6) (Atlas & Hazen, 2011). Contained heavy, polar compounds such as resins, hopanes, and asphaltenes. Slow biodegradation rates (Atlas & Hazen, 2011).	Light Louisiana Crude Oil, and methane gas ( $\text{CH}_4$ ) (API gravity 35.2) (Atlas & Hazen, 2011). Lower molecular weight hydrocarbons are more biodegradable initially than heavy crude oil, which also biodegrades more rapidly than heavy crude oil (Atlas & Hazen, 2011).
7. Spill Volume	11 million gallons. Enclosed and shallower (Wolfe <i>et al.</i> , 1994).	206 million gallons estimated by the National Incident Command's Flow Rate Technical Group. Deeper and open ocean (Atlas & Hazen, 2011; Nyankson <i>et al.</i> , 2006).
8. Spill Position in Water Column	Discharged as a surface spill (Atlas & Hazen, 2011).	Discharged at the wellhead in 1500 m water. As oil became weathered during ascent, color appeared reddish –brown and was less cohesive than crude oil when reaching the surface (Atlas & Hazen, 2011).
9. Spill Location	On Bligh Reef near island shorelines (Skinner & Reilly, 1989).	77 km offshore US southeast coastal states (Atlas & Hazen, 2011)
10. Spill Types	The traditional shore-	Novel, deep-ocean persistence of

	bound surface spill characterized by buoyant oil, fouling and killing organisms at the sea surface and eventually grounding on and damaging sensitive shoreline habitats (Peterson <i>et al.</i> , 2012).	intrusions of finely dispersed oil, gas, and dispersants at 1500 m water depth with high pressure (Peterson <i>et al.</i> , 2012). Impacted deep-sea, marshes and sandy beach shorelines with fine droplets of low concentration oil. (Atlas & Hazen, 2011).
11. Degradation Speed	It was estimated that there had been a 22% per year decline from 1991 to 2001 in the amount of oil remaining on the shore (Short <i>et al.</i> , 2004).	Unbranched chain hydrocarbons can be degraded quickly in a matter of days or weeks. (American Academy of Microbiology, 2011).
12. Degradation Results	By 2003, more than 80% of alkanes are depleted, and more than 70% of PAHs are lost (Atlas& Bragg 2009a).	By August 2010, 78% of the oil is disposed off by either human intervention (direct recovery, <i>in situ</i> , burning, skimming, and dispersal) or natural processes (naturally dispersed, evaporated, and dissolved) (Ramseur, 2010), whereas the fate of the remaining 22% of the oil spill was uncertain.
13. Initial Response	Not applicable due to storms (Council, 2013).	Direct recovery from the wellhead, Control burning, skimming (Lubchenco <i>et al.</i> , 2010)
14. Cleanup Strategies	Bioremediation and physical removal methods such as water washing were used extensively. Applied fertilizers containing Nitrogen. Nutrients increased the degradation process (Atlas & Hazen, 2011).	Photo-degradation significant (Nicodem <i>et al.</i> , 1997)  Microbes played a major mitigating role. Aerial and subsurface dispersants used extensively. Physical methods such as booming, skimming, and controlled burns were used to mitigate spill's impact. (Atlas & Hazen, 2011).
15. Role of Photo-degradation	Less important (Amon and Meon, 2004).	Very important (Nicodem <i>et al.</i> , 1997)
16. Use of Dispersant	Only 4000 gallons of dispersants were used Lack of waves and regulation concerns of	Aerial and subsurface dispersant used immediately and extensively (Atlas & Hazen, 2011).

	toxicity (EPA, 2016)	2.1 million gallons of dispersant applied. (Nyankson <i>et al.</i> , 2006)
17. Limitation Factor	Temperature and nutrients. (Boopathy <i>et al.</i> , 2012; Boufadel <i>et al.</i> , 2010).	Nutrients and oxygen level (Nicodem <i>et al.</i> , 1997)
18. The Fate of Oil Remnants	Still under study almost 25 years after the spill (Atlas & Hazen, 2011)	Roughly 45% to 76% of the discharged hydrocarbons were constrained within five years (Joye, 2015)
19. Type of Exposure (Risk Assessment Model)	Long term, chronic persistence toxic oil components, such as PAHs, may remain buried in sediment, released by bioturbation or human activities. (McGenity <i>et al.</i> , 2012)	Selective, largely short-term, acute toxicity (McGenity <i>et al.</i> , 2012). (Perhaps with exceptions to near-shore wetland areas).
20. Microbe Community Adaption	Adapted to Cold Temperature. (American Academy of Microbiology, 2011)	At a depth of over 1500 meters, the temperature is low, and pressure is high. The microbial species are adapted to these extreme conditions. (American Academy of Microbiology, 2011)
21. Focus on Biodegradation	Shorelines, oil concentrate at the surface of the water	A unique deep-water cloud of dispersed oil

Petroleum degradation processes are very complex. There are various factors that determine the rates of biodegradation, including: 1) Physical state of the oil spill (i.e., the classes of petroleum hydrocarbons and their behavior in the marine environment; 2) the location of the spill and the ambient chemical and physical oceanographic properties of those locations (i.e. Artic Sea [high latitude] and the Gulf of Mexico [low latitude], temperature, dissolved oxygen concentrations, nutrients/nutrient limitations); 3) remediation options (i.e., use of dispersants and biosurfactants) and 4) the biological composition, especially the presence and composition of the microbial communities of the affected environments (Saadoun, 2015).

The greatest difference between DWH oil spill and EVOS was more than an order of

magnitude in a total volume of oil spills (DWH 205.8 million gallons versus EVOS 11 million gallons). DWH spill also released unprecedented amounts of methane gas into the water column ( $\text{CH}_4$ ) (Fernández - Carrera *et al.*, 2016). The rapid natural degradation of oil in the DWH spill is due to a number of factors, such as the type of crude oil, offshore, jetting of the oil in to the deep-sea, rapid dissolution, and microbial adaption (Atlas & Hazen, 2011). The EVOS occurred near shore and occurred as a surface slick, while the DWH blowout was a leak from a well 1500 m below the ocean surface, which forms both a deep-sea “cloud” and a surface water slick, more than 80 km from the nearest shore (Atlas & Hazen, 2011). The environments impacted were also very different in terms of climate, weather, and ecosystems, EVOS occurred in a sub-Arctic region and the DHW spill occurred in a subtropical region. The Gulf of Mexico has numerous natural oil seeps, greater than any other marine area in North America, contributing more than 400,000 barrels of oil a year to the Gulf of Mexico (Board *et al.*, 2003). The microbiota are better adapted to oil because of natural seeps and offshore drilling activities and as such, the biodegradation process is very rapid. This is in contrast to the relatively pristine conditions of Prince William Sound which is much more enclosed and shallower than the open ocean environment in the Gulf of Mexico (Atlas & Hazen, 2011).

Oil spilled in water tends to spread and form a slick (Berridge *et al.*, 1968). The larger and thicker the slick, the less surface area for microbes; therefore, the degradation is slower. A fine dispersion of droplets of oils in the water column in the form of oil-in-water state, called emulsions, increases the surface area of the oil. Therefore, this form is more prone to microbial attack. On the contrary, tarballs, which are large aggregates of solidified crude oil, inhibit biodegradation, due to low surface-to-volume ratios of oil to water. The lighter the oil, the faster this diffusion, making the petroleum more available to the microbes (American Academy of Microbiology, 2011). In addition, weathering factors such as precipitation and wave action highly influence the rate of degradation (Davis & Gibbs, 1975). The type of oil released from EVOS was North Slope Heavy Crude Oil (API gravity 29.6) (Atlas & Hazen, 2011). It contains high-molecular-weight, polar compounds such as resins, hopanes, and asphaltenes that have nominal biodegradation rates (Atlas & Hazen, 2011). In contrast, the type of oil released from DWH was Light Louisiana Crude Oil; This oil contains

many unbranched simpler lower molecular weight hydrocarbons and methane gas ( $\text{CH}_4$ ) (API gravity of 35.2) (Atlas & Hazen, 2011). Therefore, the light Louisiana crude oil released from DWH spill biodegraded more readily than heavy crude oil from EVOS.

Among physical factors, temperature plays the most important role in biodegradation of hydrocarbons by affecting the oil's physical properties, the chemical composition of the pollutants, solubility of hydrocarbons, as well as affecting the physiology and diversity of the microbial flora and its activities (Atlas, 1981; Das & Chandran, 2011; Foght *et al.*, 1996). Petroleum hydrocarbons can be biodegraded at temperatures below 0 ° to more than 80 °Celsius (Das & Chandran, 2011). The rate of biodegradation decreases with decreasing temperature. At low temperature, the viscosity of the oil increases and the volatilization of toxic short-chain alkanes is reduced, therefore slowing the biodegradation. Higher temperature increases the rate of enzymatic activities of the microbial community, thus increasing the biodegradation processes (Atlas & Bartha, 1972; Gibbs, 1975). The highest degradation rates occur in a range of 15–20°C in marine environments (Cooney, 1984). This indicates that relative to temperature degradation, the waters of the Gulf of Mexico would be more conducive to hydrocarbon degradation than the waters of Prince William Sound (i.e., 20-27°C vs. 0-12°C, respectively).

Generally, oil creates a slick that covers the surface of the water. This physical coating prevents seawater from absorbing oxygen from the atmosphere. In open waters, the oil spill exhibits oxygen-depleting effects from the surface waters all the way to the seafloor (Biello, 2010). However, conditions of oxygen limitation generally do not exist in the upper levels of the water column in marine environments (Floodgate, 1984). Nevertheless, the aquatic sediments are usually anoxic (Hambrick *et al.*, 1980). Moreover, the rich fertilizer carried into the Gulf of Mexico estuaries region from inland farms and runoff prompts blooms of algae, phytoplankton, and other microscopic plants. Their decomposition depletes the oxygen in seawater, creating a vast dead zone that is lethal to sea life (i.e., anthropogenic eutrophication). An oil spill could exacerbate the situation (Biello, 2010). On the contrary, the EVOS did not create such dead zones near shore. The dissolved oxygen levels were lower than optimal values for oil biodegradation (Boufadel *et al.*, 2010), but

currents promoting air- water column mixing there minimized the damage to sea life (Atlas& Bragg, 2009b).

Nutrients are very important factors for successful biodegradation of petroleum hydrocarbon pollutants. Some nutrients can act as limiting factors because microorganisms require elements in addition to carbon for growth. The concentrations of inorganic nitrates ( $\text{NO}_3^-$ ), phosphates ( $\text{PO}_4^{3-}$ ), and iron (Fe) in the marine environment can limit rates of oil biodegradation (Atlas, 2011). The marine environment was found to contain fewer nutrients than freshwater because seawater had lower levels of nitrogen and phosphorous (Floodgate, 1984). Consequently, it is necessary to add nutrients in order to facilitate biodegradation (Choi *et al.*, 2002). Studies have shown that fertilizers are most effective in the first weeks after the spill and speed up the natural biodegradation process (Pelletier *et al.*, 2004)). However, fertilizers may impose a negative impact on certain hydrocarbon biodegradation processes. For example, PAH biodegradation values were higher when nutrients were not added (Vinas *et al.*, 2005). The biodegradation activity can also be inhibited if there is excessive nutrient concentration (Chaillan *et al.*, 2006). The use of fertilizers under severe weather conditions in artic regions seems to be beneficial, as low temperatures tend to slow down bacterial growth. In EVOS, even though there were sufficient concentrations of nutrients and oxygen in the open waters to support biodegradation processes (Atlas & Bragg, 2009b), the nutrients were deficient on the rocky beaches where the majority of spilled oil washed. Consequently, the limited water flow limited biodegradation rates (Boufadel *et al.*, 2010, Li & Boufadel, 2010). The offshore waters of the Gulf of Mexico are oligotrophic in nature. Bacterial activities in the DWH oil spill show enhanced microbial respiration, but signs of phosphate stress indicated this nutrient was a factor limiting the rate of biodegradation (Edwards *et al.*, 2011). This is due to the sudden input of carbon (oil spills) to the Gulf of Mexico causing a large bloom of carbon-metabolizing microbes, and the subsequent nutrient depletion limiting microbial activity.

### Photo-degradation

Particularly in the ocean at low latitudes, where seawater receives a high solar flux but is nutrient depleted, the microbial activities are limited, making photo-degradation processes pervasive (Ehrhardt *et al.*, 1992). For example, photo-degradation was

extensively observed in the *Deepwater Horizon* oil spill. In addition, for low molecular weight volatile oil components, evaporation from seawater followed by photooxidation in the air was also a key process (Cox *et al.*, 1981; Mantoura *et al.*, 1982; Nicodem *et al.*, 1997). This was a less important mechanism for EVOS in Prince William Sound, AK, because low solar angles minimized the solar radiation availability to aquatic photo-degradation processes (Amon and Meon, 2004). Photo-degradation processes may also interfere with biodegradation, because photo-degraded PAHs may produce a toxic substance that is harmful to microorganisms (Kings *et al.*, 2014; Bertilsson & Widenfalk, 2002). Thus, photo-enhanced toxicity could contribute to the toxicity of crude oil in the field.

### Remediation Options

#### Dispersants

According to the “Oil Spill Response Field Manual” by ExxonMobil (ExxonMobil, 2014), the first response approach to an oil spill is oil containment and skimming. It is the most effective method if the spill is accessible within a few hours from onset. However, in the EVOS and DWH spills, oil containment and skimming were not applied because a storm with 80 km/h winds hit Prince William Sound within two days of the initial spill resulting in an area too large to contain with floating booms (Atlas & Hazen, 2011). Moreover, the DWH spill occurred at the wellhead in 1500m water depth, so this method was also ineffective. Thus, practical approaches to oil spill response containment strategies must be considered; booming and skimming may not always be applicable. Applying both dispersants and other biological agents to enhance oil degradation were the two major strategies implemented in these two spills. Artificial dispersants can break up oil slicks and emulsify oil in water, leading to the formation of small droplets and make the oil potentially available for biodegradation (Kleindienst *et al.*, 2015). Chemical dispersants also dissolve oil in water, and reduce oil accumulation at the water surface. Furthermore, chemical dispersants potentially stimulate microbial biodegradation of oil by increasing the surface area of oil. As a result, they make the oil more available to marine communities. However, some dispersant formulations can themselves pose impacts on the marine organisms and environment or inhibit microbial processes. Nevertheless, according to the US National Research Council, the acute lethal toxicity

of chemically dispersed oil are primarily the result of alkenes and dissolved aromatic and aliphatic fractions of the oil (Board & National Research Council, 1989). For example, most dispersed oil remains in the water column, and this fraction subsequently threatens pelagic and benthic organisms (NRC, 2005).

The USEPA has conducted acute, short-term toxicity tests on dispersants, and confirmed low to moderate toxicity (Hemmer *et al.*, 2011). Analyses of tests conducted on a variety of aquatic species showed that compared with fish, crustaceans are more sensitive to oil dispersant exposure (George-Ares & Clark, 2000). Ramachandran *et al.*, (2004) suggest that the use of oil dispersants increases the exposure and uptake of PAHs by fish. The impact of PAH toxicity on sensitive life stages, such as eggs and larvae, is enhanced by chemical dispersion (McConville *et al.*, 2018; Negri *et al.*, 2018). On the contrary, Fingas *et al.*, (1991) suggested that dispersants possess low toxicity and do not increase the toxicity of the dispersed oil, because they are present in the water column at very low concentrations (Fingas *et al.*, 1995). Toxicity is dependent on both concentration and exposure time. Wells (1984) reported that dispersants would need to be present in the water column at hundreds of parts per million for over several days to exert either lethal or sub-lethal toxicities. Wells (1984) conducted extensive laboratory studies. In addition, the effectiveness of dispersants has been shown to be extremely variable depending on the specific oil type. In general, one-part dispersant will disperse about 20–30 parts of oil. When dispersing the light oil with optimum sea energy, it could reach over 100 parts of oil per part of dispersant (Mackay, 1995). Heavy (i.e., low API gravity) and weathered oils are more resistant to dispersion and may require a higher ratio of dispersant to oil. Highly viscous, non-spreading oils and waxy oils may resist chemical dispersion altogether (Lessard & DeMarco, 2000).

During the early phases of an oil spill, applying dispersants may prevent or reduce impacts on coastal habitats, wildlife, and property. However, dispersant use is not universally recommended for all spills and habitats due to limitations in the rate or capacity for immediate mixing and dilution. So, the necessity of oil dispersant use is sometimes questioned (American Society for Testing and Materials, 1997).

In the case of the *DWH*, the application of 1.07 million gallons of dispersants onto the sea surface and injection of 0.77 million gallons of dispersant directed toward the

wellhead was controversial. Because this blowout occurred in deep offshore water (1500 m), the highly turbulent discharge of hot, pressurized oil and gas could have produced a variety of dispersed phases, including small oil droplets, gas bubbles, oil-gas emulsions, and gas hydrates (Federal interagency solutions group, 2010). This turbulent mixing induced by pressurized oil and gas into cold seawater was sufficient to induce massive dispersion of oil into fine droplets while creating emulsions of oil, gas, water, and gas hydrates. (Johansen *et al.* 2003; Federal Interagency Solutions Group, 2010). Moreover, dispersants applied to weathered oil at the sea surface during windy days had sufficient surface mixing to be effective; whereas, on calm days, the dispersants were largely ineffective (Fingas, 2001). In EVOS, dispersants were only performed in a trial application stage. Only about 4,000 gallons of dispersant were applied, and the effects were diminished due to lack of wave action to mix the dispersant with the oil in water (EPA, 2016). Weather affects dispersant application and effectiveness in three ways: 1) the amount of dispersant that contacts oil is highly wind-dependent, 2) the amount of oil dispersed on the water surface is dependent on ocean turbulence and other energy, and 3) the amount of oil remaining in the water column is also dependent on the same energy. The higher the wind, the less dispersant will be applied to the oil from an aerial platform, but the greater the mixing with the water column. The greater the sea energy, the more oil will be dispersed downwards and the more it will stay dispersed (Fingas & Ka’aihue, 2004).

During the DWH spill, over 2.1 million gallons of dispersant were applied to minimize the impact of this disaster (Nyankson *et al.*, 2006). The use of dispersants resulted in a significant reduction of oil reaching the shore, which reduced the immediate threat to beaches and salt marshes. The effects of dispersants on the biodegradation in the deep plumes are unknown (American Academy of Microbiology, 2011). However, environmental and toxicity issues arose over the usage of dispersant. After the DWH spill, various studies have been conducted to research various remediation strategies. It has been recently discovered that some environmental and biodegradable dispersants can replace the chemical dispersants in order to reduce toxicity. Nyankson *et al.*, (2006) examined the ability of dispersants formulated with hydroxylated soybean lecithin solubilized in water to disperse crude oil. The de-oiled soybean lecithin was hydroxylated and solubilized in water to procedure the dispersant. The U.S. EPA’s test showed that the hydroxylated soybean

lecithin processed near complete dispersion effectiveness: (1) reducing the rigidity of the fatty acid chain, (2) increasing interaction between the surfactant and aqueous film at the interface, and (3) increasing the hydrophilicity of soybean lecithin. The findings from this study suggest that a dispersant formulated from hydroxylated soybean lecithin has the potential of replacing the traditional chemical dispersants in oil spill remediation (Nyankson *et al.*, 2015). However, this approach has yet to be tested outside of the laboratory.

## Biodegradation and Microbial Community

### Bioremediation

Because the traditional oil cleanup operations include inappropriate methods that can cause more harm to the ecosystem, bioremediation could play an important role in restoration with minimum impact on the ecosystem. The process of bioremediation involves using microorganisms to detoxify or remove pollutants through diverse metabolic reactions. This is an evolving method to remove and degrade petroleum pollution in the marine environment (Medina - Bellver *et al.*, 2005). This bioremediation technology is considered to be noninvasive and relatively cost-effective (April *et al.*, 1999). It can effectively remove environmental contaminants, contrary to physical and chemical methods, which usually cause dispersion of the oils, are not effective in contaminant removal, and are very expensive (Khan *et al.*, 2004). Bioremediation of contaminated sites depends on the metabolic capacity of indigenous microorganisms as well as environmental conditions (Yu *et al.*, 2005). There are the two main approaches to oil spill bioremediation: (1) biostimulation, in which the growth of indigenous oil degraders is stimulated by the addition of nutrients or other growth-limiting cosubstrates. (2) bioaugmentation, in which known oil-degrading bacteria are added to supplement the existing microbial population (Das & Chandran, 2011). One of the cleanup strategies employed in EVOS involved the application of nutrients in the form of fertilizer to increase oil biodegradation rate (biostimulation). Because bioremediation was used extensively in the EVOS, it has become one of the primary mechanisms to clean up oil spills due to its effectiveness and lower cost than other remediation technologies (Leahy & Colwell, 1990). Bioremediation functions basically via biodegradation pathways, which can result in

the complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds, and cell proteins or transformation of complex organic contaminants to other simpler organic compounds by biological agents including microorganisms.

Microbes have used oil as their source of energy for hundreds of millions of years (American Academy of Microbiology, 2011). Because microbes can biodegrade up to 90% of light crude oil (American Academy of Microbiology, 2011), microbial degradation is the major and ultimate natural mechanism that can clean up oil spills (Atlas, 1984). No single microbe can break down all petroleum compounds, but the combining action of the community can degrade almost all of the petroleum components. Hydrocarbons in the environment are biodegraded primarily by bacteria, yeast, and fungi (Das & Chandran, 2011). Several bacteria are even known for feeding exclusively on hydrocarbons (Yakimov *et al.*, 2007). Floodgate (2004) listed 25 genera of hydrocarbon degrading bacteria and 25 genera of hydrocarbon degrading fungi, which were isolated from the marine environment. The genome of the obligate hydrocarbon degrading bacterium *Alcanivorax borkumensis* has recently drawn the interest of some researchers. It blooms in oil spill contaminated areas and can constitute up to 80% of the bacterial population (Schneiker *et al.*, 2006). The features of its genome reveal how this bacterium grows efficiently on alkanes. As hydrocarbons are poorly soluble in water, this bacterium encodes extensive exopolysaccharide production, and it can attach to the oil - water interface (Brooijmans *et al.*, 2009). In addition, cytochrome P450 (CYP450) alkane hydroxylases constitute a super family of ubiquitous heme-thiolate monooxygenases, which play an important role in the microbial degradation of oil (Van Beilen & Funhoff, 1997). Depending on the chain length, enzyme systems are required to introduce oxygen in the substrate to initiate biodegradation. CYP450 enzyme systems were found to be involved in biodegradation of petroleum hydrocarbons. Such CYP450's are commonly found in higher eukaryotes in general but only in a few species of microorganisms (Zimmer *et al.*, 1996). CYP450 forms are also found in several yeast species that use n-alkanes and other aliphatic hydrocarbons as a sole source of carbon (Scheller *et al.*, 1998).

Microbes can biodegrade up to 90% of light crude oil by volume but are not

significantly effective in degrading high-molecular-weight compounds such as Asphaltenes and PAHs (American Academy of Microbiology, 2011). Some bacteria can effectively break down PAHs, which involves whole communities of both bacteria and eukaryotes (Brooijmans *et al.*, 2009). *Cycloclasticus* (*Piscirickettsiaceae*) is one of the important PAH-degrading bacteria (Dyksterhouse *et al.*, 1995). It was observed in one experiment that its growth pattern increased with PAH biodegradation processes (Campeão *et al.*, 2017). Even though bacteria can convert PAHs completely to biomass, CO<sub>2</sub>, and H<sub>2</sub>O, they usually need the initial insertion of O<sub>2</sub> via dioxygenase enzymes (Atlas, 2011). Moisture content and aeration were determined to be the key factors associated with PAH bioremediation (Vinas *et al.*, 2005). For instance, the PAHs from EVOS has persisted on several beaches in Prince William Sound (PWS) for more than 20 years after the contamination. The current degradation rate of the total PAH (TPAH) is estimated at 1% per year. Low oxygen concentrations were the major factor causing this oil persistence. One bioremediation method is by injecting of hydrogen peroxide and nutrients deep into four beaches in Prince William Sound in the summers of 2011 and 2012. As a result, the TPAH biodegradation rate was between 13% and 70% during this time. The results also showed high efficiency in the delivery of oxygen and nutrients to the contaminated areas of the beach. This method is especially effective in higher latitudes regions (Boufadel *et al.*, 2016). On the contrary, the PAHs at the Gulf of Mexico deplete rapidly naturally in the open ocean, substantially due to photodegradation (King *et al.*, 2014). PAHs in the oil residues would also have undergone considerable weathering such as volatilization and dissolution when transiting from the wellhead to the shoreline (Yin *et al.*, 2015). Nevertheless, preliminary findings show that PAHs in the Light Louisiana Crude oil were rapidly depleted in the Gulf of Mexico environment (Brown *et al.*, 2011). One study showed that by 2012, 97.0% of the TPAH fraction had already been lost (Atlas *et al.*, 2015).

### Biostimulation

Biostimulation involves the identification and adjustment of factors that may affect the indigenous microorganism's biodegradation rate of the contaminants of the affected site (Swannell *et al.* 1996). These factors include type and concentration of nutrients, physical and environmental parameters, chemical composition, physical

state and concentration of the crude oil or hydrocarbons; along with the temperature, oxygen availability, salinity, pressure, water activity, and pH on the site (Leahy and Colwell 1990). Petroleum hydrocarbons are a substantial carbon source for the indigenous microorganism, whereas, the nitrogen and phosphorous nutrients, as previously mentioned are commonly limiting. Thus, biostimulation accelerates the biodegradation rate when adding these nutrients into the contaminants (Nikolopoulou and Kalogerakis 2009; Prince 1997). Biostimulation technologies for beach cleanup is the most effective (OTA , 1991). During the EVOS, biostimulation using fertilizers containing N nutrients was the major strategy (Atlas & Hazen, 2011). Soon after the incident, both the EPA and Exxon quickly conducted laboratory and field tests and confirmed that the addition of fertilizer would enhance the rate of oil biodegradation by the indigenous hydrocarbon-degrading microorganisms (Bragg *et al.*, 1992; Pritchard & Costa, 1991). As a result, rates of biodegradation in bioremediation doubled (OTA, 1991). The rate of oil degradation was a function of the ratio of Nitrogen/biodegradable oil and time. In addition, O<sub>2</sub> dissolved in water was not a rate-limiting factor (Atlas & Hazen, 2011). In all, 48,600 kg of Nitrogen in fertilizers were applied from 1989 to 1991. This represents the largest use of bioremediation in history (Zhu *et al.*, 2001). A survey in 1992 found that most of the oil had been removed from shorelines and on June 12, 1992, the U.S. Coast Guard and the State of Alaska officially declared the cleanup concluded (though residual oil remained) (Atlas & Hazen, 2011). However, this strategy wasn't widely applied in the DWH spills, located in a vast of open ocean, because it is very expensive and difficult to keep the nutrient concentrations at levels that accelerate the speed of biodegradation (Atlas & Hazen, 2011).

Moreover, biosurfactants can speed up the biodegradation process. Biosurfactants are a heterogeneous group of surface-active chemical compounds produced by a wide variety of microorganisms, such as yeast, bacteria, and fungi (Muthusamy *et al.*, 2008; Mahmoud *et al.*, 2008). Biosurfactants are amphiphilic compounds that attract both lipids and water and reduce the surface tension between the two substances. Biosurfactants emulsify alkanes and increase the oil - water surface area (Brooijmans *et al.*, 2009). Therefore, it enhances solubilization and removal of contaminants (Brusseau *et al.*, 1995; Bai *et al.*, 1997). Biodegradation is also enhanced by

biosurfactants due to increased bioavailability of pollutants because biosurfactants facilitate the breakdown and dispersal of lipid molecules into smaller droplets (Barkay *et al.*, 1999). The microdroplets encapsulated in the hydrophobic microbial cell surface are taken inside and degraded (Das & Chandran, 2011). Gross (2010) proposed introducing biosurfactants in sufficient quantity to assist these organisms in quickly converting the oil from the Deep Horizon spill. Currently, the major market for biosurfactants is for oil spill cleanup, the removal of oil residue from storage tanks, microbial-enhanced oil recovery, and the bioremediation of soil and water (Sobrinho *et al.*, 2013).

### Bioaugmentation

More recently, scientists have been looking for new methods that are efficient and environmentally safe to advance bioremediation. Bioaugmentation is another process that enhances bioremediation. Bioaugmentation is the supplementation of highly concentrated and specialized microbe populations (single strains or consortia) to the contaminants (Leahy and Colwell 1990; Gentry *et al.* 2004). There are two categories; 1) introduction of special assemblages of naturally occurring oil-degrading microorganisms (seeding) (Atlas, 1995), and 2) introduction of genetically engineered microorganisms (GEMs) with special oil-degrading properties (Urgun-Demirtas *et al.*, 2006). In the first seeding method, these microorganisms can be cultivated in the laboratory and then inoculated into the environment. As they are the indigenous species in the environment, these microorganisms are adapted to the native environment of the contaminated site compared with the non-indigenous groups (Orcutt *et al.*, 2010, Thavasi *et al.*, 2011). It was reported that bioaugmentation, tested in the laboratory, was a good strategy to accelerate the degradation of components like n-alkanes, branched alkanes, and PAHs during the first five days after contamination. After the early stage of the oil spill, the rate of biodegradation slows down (McKew *et al.*, 2007). This technique is most applied in the following situations when the environment (1) does not have sufficient microbial cells present or (2) the native population does not possess the metabolic routes necessary to metabolize the contaminants of concern (Tyagi *et al.*, 2011). By adding cultured microorganism strains into the subsurface, it may increase the speed of degradation of a contaminant (Herrero & Stuckey, 2015). However, in field tests, adding seed

cultures has proven less promising for treating oil spills than adding fertilizers and ensuring adequate aeration because the widely distributed hydrocarbon degrading bacteria and fungi naturally exist in the marine environment (Atlas, 1995). In addition, applications for genetically engineered microorganisms (GEMs) have received attention for improving degradation under laboratory conditions (Urgun-Demirtas *et al.*, 2006). The genetically engineered bacteria show higher degradative capacity than non-genetically engineered microorganisms. From a practical perspective, using a mixed bacterial consortium culture rather than a single-strain culture for the bioremediation is more advantageous as it is more robust and diversified (Rahman *et al.* 2002). There have been several successful laboratory experiments on GEM bacteria that resulted in major degradation of hydrocarbons, and the degradation rate was significantly increased (Tyagi *et al.*, 2011). However, due to the ecological and environmental concerns and current regulatory constraints, the testing of GEM in the field is still prohibited (Garbisu & Alkorta, 1999). Even though the field test of the seeding technology is not as promising, there have been some successful cases. For example, Raghavan & Vivekanandan (1999) reported that the application of natural seeding bacteria to the oil spill area resulted not only in rapid multiplication of bacteria but also accelerated the disappearance of the hydrocarbons. The combination of microbiological and ecological knowledge, biochemical mechanisms, and field engineering designs are essential elements for successful in-situ bioaugmentation (American Academy of Microbiology, 2011).

The DWH oil spill provides a unique opportunity for scientists to understand the significant roles that microbes play in the recovery process at oil-contaminated environments. Different microbiological methods were applied to investigate the changes and responses of bacterial communities after the spill (Zhang *et al.*, 2018). As of 2001, over 200 species of microbes (bacteria and fungi) that degrade hydrocarbons had been discovered in oil (Zhu *et al.*, EPA, 2001). Among these species, some indigenous bacterial communities in different habitats (seawater, deep-sea sediment, marshes, and beach sands) responded rapidly to the spilled oil and some bacterial groups might play significant roles in reducing this type of environmental contamination (Zhang *et al.*, 2018). A class of bacteria called hydrocarbonoclastic bacteria (HCB) have been the most active, globally, at degrading hydrocarbons in

marine environments (McKew *et al.*, 2007). Recently, scientists have successfully applied DNA synthesis and genome construction on marine bacteria that can break down the different types of hydrocarbon chemicals in oil, including PAHs. For example, *Oceanospirillales*, degraded alkanes in the Deepwater oil plume, whereas *Rhodospiralles* was responsible for degrading PAHs (Hu *et al.*, 2017). The combination of stable isotope probing (SIP) and metagenomics techniques allowed for the reconstruction of hydrocarbon degradation pathways of bacterial taxa that were present in contaminated Gulf of Mexico water with the demonstrated uptake of specific oil constituents. These data revealed a greater microbial capacity for hydrocarbon degradation than was previously known (Dombrowski *et al.*, 2016).

Studies showed that bioremediation products might be effective in the laboratory but significantly less so in the field (Mearns, 1997; Venosa *et al.*, 1996; Lee *et al.*, 1997). This is because laboratory studies cannot always simulate complicated real-world conditions including factors such as spatial heterogeneity, biological interactions, climatic effects, and nutrient mass transport limitations. For example, an engineered *Pseudomonas* sp. is capable of degrading camphor, octane, salicylate, and naphthalene. When such bacteria are introduced into the environment, they disappear quickly. Their introduction into already colonized niches is very difficult, as most fall prey to protozoa (Cases and de Lorenzo, 2005). Therefore, field studies and applications are the ultimate tests or the most convincing demonstration of the effectiveness of bioremediation products. Compared to microbial products, very few nutrient additives have been developed and marketed specifically as commercial bioremediation agents for oil spill cleanup. This is probably because common fertilizers are inexpensive, readily available, and have been shown to be effective if used properly (Das & Chandran, 2011). However, due to the limitations of common fertilizers (e.g., being rapidly washed out due to tide and wave action), several organic nutrient products, such as oleophilic nutrient products, have recently been evaluated and marketed as bioremediation agents. These nutrient products were the most well-known bioremediation agents for oil spill cleanup due to its use on the EVOS in Prince William Sound, Alaska (Ladousse & Tramier, 1999).

## CONCLUSIONS

There is no single remediation practice that can achieve 100% removal of oil, and as such, patches of highly weathered oil can easily remain in marine environments. It is also impossible to genetically engineer a superbug that can degrade all environmental oil contamination. However, comparison of the EVOS and the DWH well blowout results in important similarities and differences that can inform future approaches to oil spill response. The oil from the DWH spill biodegraded more quickly than oils from EVOS because it was a 1) light crude oil, 2) highly dispersed in the water column, 3) subjected to more extensive photodegradation processes, 4) exposed to warmer water temperatures, 5) present in an offshore location and, 6) microbial populations were well adapted to hydrocarbon exposure in the Gulf of Mexico compared to Prince William Sound, AK. In the case of the DWH spill, two weeks after the accident, no surface oil slick was observed, and concentrations of detectable oil in the water column were greatly diminished (Operational Science Advisory Team, 2010). Whereas, the EVOS oil required a significantly longer time to biodegrade. Decisions on remediation strategies such as whether to physically collect the spilled oil and/or to apply bioremediation approaches should be driven by research and assessment of both the spill situation and ecosystem conditions, not just the presence of detectable hydrocarbons. Biostimulation through fertilizer addition was shown to be effective for the EVOS and increased rates of oil biodegradation; however, the use of dispersants to accelerate oil biodegradation for the DWH spill was chosen instead of biostimulation because the DWH spill was located in open ocean, and it is very expensive and difficult to keep the nutrient levels sufficient to accelerate the speed of biodegradation. In addition, the advanced molecular techniques for modified microbial communities were not available at the time of the EVOS. Recently, considerable progress has been made in evaluating specific strains of bacteria to degrade one or several compounds of crude oil. The next step is to actually use all this information to enhance and control the degradability of spilled oil by bioaugmentation (Brooijmans *et al.*, 2009). With the advancement in microbiology and molecular engineering technology, we can predict that extensive molecular analyses of microbial communities will be developed as a result of research from the DWH, and applied to future oil spills. While scientists are working hard to find approaches that accelerate the biodegradation rate to remediate oil spill sites via

bacteria, the best method is to take care of the ecosystem and minimize the disturbances to the environment in the first place with preventive measures (i.e., avoiding spills).

## REFERENCES:

- Agency for Toxic Substances and Disease Registry Toxicological Profile Information. (1995). *Toxicology and Industrial Health*, 11(6), pp.196-203.
- Alexander, M. (1999). *Biodegradation and bioremediation*. Gulf Professional Publishing.
- American Academy of Microbiology. (2011). Microbes & oil spills FAQ. Retrieved 04 29, 2017, from American Society of Microbiology:  
<https://www.asm.org/index.php/colloquium-reports/item/4480-faq-microbes-and-oil-spills>
- American Society for Testing and Materials. (1997). Annual Book of Standards. Vol. 11.04. Philadelphia, PA (Section F)
- Amon, R. M., & Meon, B. (2004). The biogeochemistry of dissolved organic matter and nutrients in two large Arctic estuaries and potential implications for our understanding of the Arctic Ocean system. *Marine Chemistry*, 92(1-4), 311-330.
- April, T. M., Foght, J. M., & Currah, R. S. (1999). Hydrocarbon-degrading filamentous fungi isolated from flare pit soils in northern and western Canada. *Canadian Journal of Microbiology*, 46(1), 38-49.
- Atlas, R. M. (1981). Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiological reviews*, 45(1), 180.
- Atlas, R. M. (1984). Petroleum microbiology.
- Atlas, R. M. (1995). Petroleum biodegradation and oil spill bioremediation. *Marine Pollution Bulletin*, 31(4-12), 178-182.
- Atlas, R. M., & Bartha, R. (1972). Biodegradation of petroleum in seawater at low temperatures. *Canadian Journal of Microbiology*, 18(12), 1851-1855.
- Atlas, R. M., & Hazen, T. C. (2011). Oil Biodegradation and Bioremediation: A Tale of the Two Worst Spills in U.S. History. *Environmental Science & Technology*, 45(16), 6709–6715.
- Atlas, R. M., Stoeckel, D. M., Faith, S. A., Minard-Smith, A., Thorn, J. R., & Benotti, M. J. (2015). Oil biodegradation and oil-degrading microbial populations in marsh sediments impacted by oil from the deepwater horizon well blowout. *Environmental science & technology*, 49(14), 8356-8366.
- Atlas, R., & Bragg, J. (2009a). Bioremediation of marine oil spills: when and when not—the Exxon Valdez experience. *Microbial biotechnology*, 2(2), 213-221.
- Atlas, R., & Bragg, J. R. (2009b). Evaluation of PAH depletion of subsurface Exxon Valdez oil residues remaining in Prince William Sound in 2007-2008 and their likely bioremediation potential.
- ATSDR. (1993). Toxicological Profile for Toluene (Update).

- Bai, G., Brusseau, M. L., & Miller, R. M. (1997). Biosurfactant-enhanced removal of residual hydrocarbon from soil. *Journal of Contaminant Hydrology*, 25(1-2), 157-170.
- Bao, M. T., Wang, L. N., Sun, P. Y., Cao, L. X., Zou, J., & Li, Y. M. (2012). Biodegradation of crude oil using an efficient microbial consortium in a simulated marine environment. *Marine Pollution Bulletin*, 64(6), 1177-1185.
- Barkay, T., Navon-Venezia, S., Ron, E. Z., & Rosenberg, E. (1999). Enhancement of solubilization and biodegradation of polycyclic aromatic hydrocarbons by the bioemulsifier alasan. *Applied and environmental microbiology*, 65(6), 2697-2702.
- Barron, M. G., & Ka'aihue, L. (2001). Potential for photoenhanced toxicity of spilled oil in Prince William Sound and Gulf of Alaska waters. *Marine pollution bulletin*, 43(1-6), 86-92.
- Berridge, S. A., Dean, R. A., Fallows, R. G., & Fish, A. (1968). The properties of persistent oils at sea.
- Bertilsson, S., & Widenfalk, A. (2002). Photochemical degradation of PAHs in freshwaters and their impact on bacterial growth—Influence of water chemistry. *Hydrobiologia*, 469(1), 23-32.
- Biello, D. (2010). How Will the Oil Spill Impact the Gulf's Dead Zone? *Scientific American*.
- Board, M., & National Research Council. (1989). *Using oil spill dispersants on the sea*. National Academies Press.
- Board, M., Board, O. S., & National Research Council. (2003). *Oil in the sea III: inputs, fates, and effects*. National Academies Press.
- Board, O. S., & National Research Council. (2005). *Oil spill dispersants: efficacy and effects*. National Academies Press.
- Boehm, P. D., Page, D. S., Brown, J. S., Neff, J. M., Bragg, J. R., & Atlas, R. M. (2008). Distribution and weathering of crude oil residues on shorelines 18 years after the Exxon Valdez spill. *Environmental science & technology*, 42(24), 9210-9216.
- Bolden, A. L., Kwiatkowski, C. F., & Colborn, T. (2015). New look at BTEX: are ambient levels a problem? *Environmental science & technology*, 49(9), 5261-5276.
- Boopathy, R., Shields, S., & Nunna, S. (2012). Biodegradation of crude oil from the BP oil spill in the marsh sediments of southeast Louisiana, USA. *Applied biochemistry and biotechnology*, 167(6), 1560-1568.
- Boufadel, M. C., Geng, X., & Short, J. (2016). Bioremediation of the Exxon Valdez oil in Prince William sound beaches. *Marine pollution bulletin*, 113(1-2), 156-164.
- Boufadel, M. C., Sharifi, Y., Van Aken, B., Wrenn, B. A., & Lee, K. (2010). Nutrient and oxygen concentrations within the sediments of an Alaskan beach polluted with the Exxon Valdez oil spill. *Environmental Science & Technology*, 44(19), 7418-7424.

- Bragg, J. R., Prince, R. C., Wilkinson, J. B., & Atlas, R. M. (1992). *Bioremediation for shoreline cleanup following the 1989 Alaskan oil spill*. Exxon Company.
- Brooijmans, R. J., Pastink, M. I., & Siezen, R. J. (2009). Hydrocarbon - degrading bacteria: the oil - spill clean - up crew. *Microbial Biotechnology*, 2(6), 587-594.
- Brooijmans, R. J., Pastink, M. I., & Siezen, R. J. (2009). Hydrocarbon - degrading bacteria: the oil - spill clean - up crew. *Microbial Biotechnology*, 2(6), 587-594.
- Brown, D. M., Bonte, M., Gill, R., Dawick, J., & Boogaard, P. J. (2017). Heavy hydrocarbon fate and transport in the environment. *Quarterly Journal of Engineering Geology and Hydrogeology*, 50(3), 333-346.
- Brown, J. S., Beckmann, D., Bruce, L., Cook, L., & Mudge, S. (2011, March). PAH Depletion Ratios Document the Rapid Weathering and Attenuation of PAHs in Oil Samples Collected after the Deepwater Horizon: 2011 International Oil Spill Conference, Portland, Oregon. In *International Oil Spill Conference Proceedings (IOSC)* (Vol. 2011, No. 1, p. abs336). American Petroleum Institute.
- Brusseau, M. L., Miller, R. M., Zhang, Y., Wang, X., & Bai, G. Y. (1995). Biosurfactant-and cosolvent-enhanced remediation of contaminated media. by cytochrome P450 52A3. *Journal of Biological Chemistry*, 273(49), 32528-32534.
- Campeão, M. E., Reis, L., Leomil, L., de Oliveira, L., Otsuki, K., Gardinali, P., ... & Thompson, C. C. (2017). The deep-sea microbial community from the Amazonian basin associated with oil degradation. *Frontiers in microbiology*, 8, 1019.
- Carmichael, L. M., & Pfaender, F. K. (1997). The effect of inorganic and organic supplements on the microbial degradation of phenanthrene and pyrene in soils. *Biodegradation*, 8(1), 1-13.
- Cases, I., & Lorenzo, V. D. (2005). Genetically modified organisms for the environment: stories of success and failure and what we have learned from them.
- Chaillan, F., Chaineau, C. H., Point, V., Saliot, A., & Oudot, J. (2006). Factors inhibiting bioremediation of soil contaminated with weathered oils and drill cuttings. *Environmental Pollution*, 144(1), 255-265.
- Chandrasekar, S., Sorial, G. A., & Weaver, J. W. (2006). Dispersant effectiveness on oil spills—impact of salinity. *ICES Journal of Marine Science: Journal du Conseil*, 63(8), 1418-1430.
- Choi, S. C., Kwon, K. K., Sohn, J. H., & Kim, S. J. (2002). Evaluation of fertilizer additions to stimulate oil biodegradation in sand seashore mesocosms. *Journal of microbiology and biotechnology*, 12(3), 431-436.
- Collin, G., Höke, H., & Talbiersky, J. (2000). Anthracene. *Ullmann's Encyclopedia of Industrial Chemistry*.
- Colwell, R. R., Walker, J. D., & Cooney, J. J. (1977). Ecological aspects of microbial

- degradation of petroleum in the marine environment. *CRC critical reviews in*
- Council, E. V. O. S. T. (2013). Questions and Answers About the Spill.
- Cooney, J. J. (1984). The fate of petroleum pollutants in freshwater ecosystems.
- Cox, R. A., Patrick, K. F., & Chant, S. A. (1981). Mechanism of atmospheric photooxidation of organic compounds. Reactions of alkoxy radicals in oxidation of n-butane and simple ketones. *Environmental science & technology*, 15(5), 587-592.
- Das, N., & Chandran, P. (2011). Microbial degradation of petroleum hydrocarbon contaminants: an overview. *Biotechnology research international*, 2011.
- Davis, S. J., & Gibbs, C. F. (1975). The effect of weathering on a crude oil residue exposed at sea. *Water Research*, 9(3), 275-285.
- Desai, J. D., & Banat, I. M. (1997). Microbial production of surfactants and their commercial potential. *Microbiology and Molecular biology reviews*, 61(1), 47-64.
- Doherty, V. F., & Otitoloju, A. A. (2016). Occurrence and distribution of monocyclic aromatic hydrocarbons (BTEX) and the impact on macrobenthic community structure in Lagos lagoon, Nigeria. *Environmental monitoring and assessment*, 188(10), 571.
- Dombrowski, N., Donaho, J. A., Gutierrez, T., Seitz, K. W., Teske, A. P., & Baker, B. J. (2016). Reconstructing metabolic pathways of hydrocarbon-degrading bacteria from the Deepwater Horizon oil spill. *Nature microbiology*, 1(7), 16057.
- Dong, C., Bai, X., Sheng, H., Jiao, L., Zhou, H., & Shao, Z. (2014). Distribution of PAHs and the PAH-degrading bacteria in the deep-sea sediments of the high-latitude Arctic Ocean. *Biogeosciences Discussions*, 11(9), 13985-14021.
- Dyksterhouse, S. E., Gray, J. P., Herwig, R. P., Lara, J. C., & Staley, J. T. (1995). Cycloclasticus pugetii gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium from marine sediments. *International Journal of Systematic and Evolutionary Microbiology*, 45(1), 116-123.
- Dyksterhouse, S. E., Gray, J. P., Herwig, R. P., Lara, J. C., & Staley, J. T. (1995). Cycloclasticus pugetii gen. nov., sp. nov., an aromatic hydrocarbon-degrading bacterium from marine sediments. *International Journal of Systematic and Evolutionary Microbiology*, 45(1), 116-123.
- Edwards, B. R., Reddy, C. M., Camilli, R., Carmichael, C. A., Longnecker, K., & Van Mooy, B. A. (2011). Rapid microbial respiration of oil from the Deepwater Horizon spill in offshore surface waters of the Gulf of Mexico. *Environmental Research Letters*, 6(3), 035301.
- EPA. (1982). Aquatic fate process data for organic priority pollutants. Washington, DC: U.S. Environmental Protection Agency, Office of Water Regulations and Standards. EPA440481014.
- EPA. (2016, Feb 20). Exxon Valdez. Retrieved from EPA United States Environmental Protection Agency:  
<https://archive.epa.gov/emergencies/content/learning/web/html/exxon.html>

- ExxonMobil. (2014). *Oil Spill Response Field Manual*. ExxonMobil Research and Engeering Company.
- Eykelbosh, A. (2014). *Short-and long-term health impacts of marine and terrestrial oil spills*. Vancouver Coastal Health.
- Fay, M., Risher, J., & Wilson, J. D. (2007). Toxicological profile for xylene.
- Federal Interagency Solutions Group, (2010). Oil Budget Calculator: Deepwater Horizon, Oil Budget Calculator Science and Engineering Team; 2010. [July 16, 2011]. Available at [http://www.restorethegulf.gov/sites/default/files/documents/pdf/OilBudgetCalc\\_Full\\_HQ-Print\\_111110.pdf](http://www.restorethegulf.gov/sites/default/files/documents/pdf/OilBudgetCalc_Full_HQ-Print_111110.pdf).
- Fernández - Carrera, A., Rogers, K. L., Weber, S. C., Chanton, J. P., & Montoya, J. P. (2016). Deep Water Horizon oil and methane carbon entered the food web in the Gulf of Mexico. *Limnology and Oceanography*, 61(S1).
- Fingas, M. (2012). *The basics of oil spill cleanup*. CRC press.
- Fingas, M. F., & Ka aihue, L. (2004). *Weather windows for oil spill countermeasures*. Prince William Sound Regional Citizens' Advisory Council.
- Fingas, M. F., Kyle, D. A., Laroche, N., Fieldhouse, B., Sergy, G., & Stoodley, G. (1995). The effectiveness testing of oil spill-treating agents. In *The Use of Chemicals in Oil Spill Response*. ASTM International.
- Fingas, M. F., Stoodley, R., Stone, N., Hollins, R., & Bier, I. (1991, March). Testing the effectiveness of spill-treating agents: laboratory test development and initial results. In *International Oil Spill Conference* (Vol. 1991, No. 1, pp. 411-414). American Petroleum Institute.
- Fingas, M., Wang, Z., Fieldhouse, B., & Smith, P. (2003). Chemical characteristics of an oil and the relationship to dispersant effectiveness. *BSEE [online]*.
- Floodgate, G. D. (1972). Microbial degradation of oil. *Marine Pollution Bulletin*, 3(3), 41-43.
- Floodgate, G. D. (1984). The fate of petroleum in marine ecosystems. p. 355-398. In R. M. Atlas (ed.), *Petroleum microbiology*. Macmillan Publishing Co., New York.
- Foght, J. M., Westlake, D. W., Johnson, W. M., & Ridgway, H. F. (1996). Environmental gasoline-utilizing isolates and clinical isolates of *Pseudomonas aeruginosa* are taxonomically indistinguishable by chemotaxonomic and molecular techniques. *Microbiology*, 142(9), 2333-2340.
- Gan, S., Lau, E. V., & Ng, H. K. (2009). Remediation of soils contaminated with polycyclic aromatic hydrocarbons (PAHs). *Journal of hazardous materials*, 172(2-3), 532-549.
- Garbisu, C., & Alkorta, I. (1999). Utilization of genetically engineered microorganisms (GEMs) for bioremediation. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 74(7), 599-606.
- Gentry, T., Rensing, C., & Pepper, I. A. N. (2004). New approaches for

- bioaugmentation as a remediation technology. *Critical reviews in environmental science and technology*, 34(5), 447-494.
- GeoHack - Deepwater Horizon oil spill. (n.d.). Retrieved from [https://tools.wmflabs.org/geohack/geohack.php?pagename=Deepwater\\_Horizon\\_oil\\_spill&params=28\\_44\\_17.30\\_N\\_88\\_21\\_57.40\\_W\\_region:US-LA\\_type:event\\_scale:700000](https://tools.wmflabs.org/geohack/geohack.php?pagename=Deepwater_Horizon_oil_spill&params=28_44_17.30_N_88_21_57.40_W_region:US-LA_type:event_scale:700000)
- George-Ares, A., & Clark, J. R. (2000). Aquatic toxicity of two Corexit® dispersants. *Chemosphere*, 40(8), 897-906.
- Gibbs, C. F. (1975). Quantitative studies on marine biodegradation of oil. I. Nutrient limitation at 14 degrees C. *Proceedings of the Royal Society of London B: Biological Sciences*, 188(1090), 61-82.
- Hansch, C., Leo, A., Hoekman, D., & Livingstone, D. J. (1995). *Exploring QSAR: hydrophobic, electronic, and steric constants* (Vol. 48). Washington, DC: American Chemical Society.
- Hambrick, G. A., DeLaune, R. D., & Patrick, W. H. (1980). Effect of estuarine sediment pH and oxidation-reduction potential on microbial hydrocarbon degradation. *Applied and Environmental Microbiology*, 40(2), 365-369.
- Harwell, M. A., & Gentile, J. H. (2006). Ecological significance of residual exposures and effects from the Exxon Valdez oil spill. *Integrated Environmental Assessment and Management: An International Journal*, 2(3), 204-246.
- Hazen, T. C., Dubinsky, E. A., DeSantis, T. Z., Andersen, G. L., Piceno, Y. M., Singh, N., ... & Stringfellow, W. T. (2010). Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science*, 330(6001), 204-208.
- Henner, P., Schiavon, M., Morel, J. L., & Lichtfouse, E. (1997). Polycyclic aromatic hydrocarbon (PAH) occurrence and remediation methods. *Analisis*, 25(9-10), M56-M59.
- Herrero, M., & Stuckey, D. C. (2015). Bioaugmentation and its application in wastewater treatment: a review. *Chemosphere*, 140, 119-128.
- Hu, P., Dubinsky, E. A., Probst, A. J., Wang, J., Sieber, C. M., Tom, L. M., ... & Andersen, G. L. (2017). Simulation of Deepwater Horizon oil plume reveals substrate specialization within a complex community of hydrocarbon degraders. *Proceedings of the National Academy of Sciences*, 114(28), 7432-7437.
- IPIECA , (2015) Impacts of oil spills on marine ecology
- Joye, S. B. (2015). Deepwater Horizon, 5 years on. *Science*, 349(6248), 592-593.
- Joye, S. B., Bowles, M. W., Samarkin, V. A., Hunter, K. S., & Niemann, H. (2010). Biogeochemical signatures and microbial activity of different cold-seep habitats along the Gulf of Mexico deep slope. *Deep Sea Research Part II: Topical Studies in Oceanography*, 57(21), 1990-2001.
- Kaplan, L. A., & Cory, R. M. (2014). Dissolved organic matter in stream ecosystems: forms, functions, and fluxes of watershed Tea. In *Stream ecosystems in a changing environment* (pp. 241-320).
- Kessler, J. D., Valentine, D. L., Redmond, M. C., Du, M., Chan, E. W., Mendes, S. D., ... & Yvon-Lewis, S. A. (2011). A persistent oxygen anomaly reveals the

- fate of spilled methane in the deep Gulf of Mexico. *Science*, 331(6015), 312-315.
- Khodaei, K., Nassery, H. R., Asadi, M. M., Mohammadzadeh, H., & Mahmoodlu, M. G. (2017). BTEX biodegradation in contaminated groundwater using a novel strain (*Pseudomonas* sp. BTEX-30). *International Biodeterioration & Biodegradation*, 116, 234-242.
- King, S. M., Leaf, P. A., Olson, A. C., Ray, P. Z., & Tarr, M. A. (2014). Photolytic and photocatalytic degradation of surface oil from the Deepwater Horizon spill. *Chemosphere*, 95, 415-422.
- Kleindienst, S., Paul, J. H., & Joye, S. B. (2015). Using dispersants after oil spills: impacts on the composition and activity of microbial communities. *Nature Reviews Microbiology*, 13(6), 388-396.
- Kostka, J. E., Prakash, O., Overholt, W. A., Green, S., Freyer, G., Canion, A., ... & Huettel, M. (2011). Hydrocarbon-degrading bacteria and the bacterial community response in Gulf of Mexico beach sands impacted by the Deepwater Horizon oil spill. *Applied and environmental microbiology*, AEM-05402.
- Kvenvolden, K. A., & Cooper, C. K. (2003). Natural seepage of crude oil into the marine environment. *Geo-Marine Letters*, 23(3-4), 140-146.
- Ladousse, A., & Tramier, B. (1991). Results of 12 years of research in spilled oil bioremediation: Inipol EAP 22. In *International Oil Spill Conference* (Vol. 1991, No. 1, pp. 577-581). American Petroleum Institute.
- Leahy, J. G., & Colwell, R. R. (1990). Microbial degradation of hydrocarbons in the environment. *Microbiological reviews*, 54(3), 305-315.
- Lee, K., Tremblay, G. H., Gauthier, J., Cobanli, S. E., & Griffin, M. (1997). Bioaugmentation and biostimulation: a paradox between laboratory and field results. In *International Oil spill Conference* (Vol. 1997, No. 1, pp. 697-705). American Petroleum Institute.
- Lessard, R. R., & DeMarco, G. (2000). The significance of oil spill dispersants. *Spill Science & Technology Bulletin*, 6(1), 59-68.
- Li, H., & Boufadel, M. C. (2010). Long-term persistence of oil from the Exxon Valdez spill in two-layer beaches. *Nature Geoscience*, 3(2), 96.
- Lin, C. Y., & Tjeerdema, R. S. (2010). Crude oil, oil, gasoline and petrol. *Ecotoxicology*, 222.
- Lubchenco, J., McNutt, M. K., Lehr, B., Sogge, M., Miller, M., Hammond, S. R., & Conner, W. G. (2010). BP Deepwater Horizon oil budget what happened to the oil.
- Mackay, D. (1995). Effectiveness of chemical dispersants under breaking wave conditions. In *The Use of Chemicals in Oil Spill Response*. ASTM International.
- Mahmoud, A., Aziza, Y., Abdeltif, A., & Rachida, M. (2008). Biosurfactant production by *Bacillus* strain injected in the petroleum reservoirs. *Journal of Industrial Microbiology & Biotechnology*, 35, 1303-1306.

- Mantoura, R. F. C., Gschwend, P. M., Zafiriou, O. C., & Clarke, K. R. (1982). Volatile organic compounds at a coastal site. 2. Short-term variations. *Environ. Sci. Technol.*, 16(1), 38-45.
- McConville, M. M., Roberts, J. P., Boulais, M., Woodall, B., Butler, J. D., Redman, A. D., ... & Bytingsvik, J. (2018). The sensitivity of a deep - sea fish species (*Anoplopoma fimbria*) to oil - associated aromatic compounds, dispersant, and Alaskan North Slope crude oil. *Environmental toxicology and chemistry*.
- McGenity, T. J., Folwell, B. D., McKew, B. A., & Sanni, G. O. (2012). Marine crude-oil biodegradation: a central role for interspecies interactions. *Aquatic Biosystems*, 8(1), 10.
- McKew, B. A., Coulon, F., Yakimov, M. M., Denaro, R., Genovese, M., Smith, C. J., ... & McGenity, T. J. (2007). Efficacy of intervention strategies for bioremediation of crude oil in marine systems and effects on indigenous hydrocarbonoclastic bacteria. *Environmental Microbiology*, 9(6), 1562-1571.
- Mearns AJ. 1996. Exxon Valdez shoreline treatment and operations: Implications for response, assessment, monitoring and research. Pages 309–328 in Rice SD, Spies RB, Wolfe DA, Wright BA, eds. Proceedings of the Exxon Valdez Oil Spill Symposium, American Fisheries Society Symposium 18. American Fisheries Society.
- Mearns, A. J. (1997). Cleaning oiled shores: putting bioremediation to the test. *Spill Science & Technology Bulletin*, 4(4), 209-217.
- Medina - Bellver, J. I., Marín, P., Delgado, A., Rodríguez - Sánchez, A., Reyes, E., Ramos, J. L., & Marques, S. (2005). Evidence for in situ crude oil biodegradation after the Prestige oil spill. *Environmental microbiology*, 7(6), 773-779.
- Mihelcic, J. R., & Luthy, R. G. (1988). Degradation of polycyclic aromatic hydrocarbon compounds under various redox conditions in soil-water systems. *Applied and Environmental Microbiology*, 54(5), 1182-1187.
- Muthusamy, K., Gopalakrishnan, S., Ravi, T. K., & Sivachidambaram, P. (2008). Biosurfactants: properties, commercial production and application. Current Science (00113891), 94(6).
- Negri, A. P., Luter, H. M., Fisher, R., Brinkman, D. L., & Irving, P. (2018). Comparative toxicity of five dispersants to coral larvae. *Scientific reports*, 8(1), 3043.
- National Academies of Sciences, Engineering, and Medicine. (2016). *Spills of Diluted Bitumen from Pipelines: A Comparative Study of Environmental Fate, Effects, and Response*. National Academies Press.
- National Research Council (US). Steering Committee for the Petroleum in the Marine Environment Update. (1985). *Oil in the sea: inputs, fates, and effects* (Vol. 1). National Academies.

- Nicodem, D. E., Guedes, C. L., Correa, R. J., & Fernandes, M. C. Z. (1997). Photochemical processes and the environmental impact of petroleum spills. *Biogeochemistry*, 39(2), 121-138.
- Nikolopoulou, M., & Kalogerakis, N. (2009). Biostimulation strategies for fresh and chronically polluted marine environments with petroleum hydrocarbons. *Journal of Chemical Technology & Biotechnology: International Research in Process, Environmental & Clean Technology*, 84(6), 802-807.
- Nyankson, E., DeCuir, M. J., & Gupta, R. B. (2015). Soybean lecithin as a dispersant for crude oil spills. *ACS Sustainable Chemistry & Engineering*, 3(5), 920-931.
- Okolo, J. C. (2005). Odu, "Effects of soil treatments containing poultry manure on crude oil degradation in a sandy loam soil. In *Applied Ecology and Environmental Research*.
- Operational Science Advisory Team. (2010) . Summary Report for Sub-Sea and Sub-Surface Oil and Dispersant Detection: Sampling and Monitoring. New Orleans: Unified Area Command. [http://www.restorethegulf.gov/sites/default/files/documents/pdf/OSAT\\_Report\\_FINAL\\_17DEC.pdf](http://www.restorethegulf.gov/sites/default/files/documents/pdf/OSAT_Report_FINAL_17DEC.pdf)(accessed Nov 1st, 2018).
- Orcutt, B. N., Joye, S. B., Kleindienst, S., Knittel, K., Ramette, A., Reitz, A., ... & Boetius, A. (2010). Impact of natural oil and higher hydrocarbons on microbial diversity, distribution, and activity in Gulf of Mexico cold-seep sediments. *Deep Sea Research Part II: Topical Studies in Oceanography*, 57(21-23), 2008-2021.
- OTA, (1991). *Bioremediation for marine oil spills*. Washington: DIANE Publishing.
- Oudot, J., Merlin, F. X., & Pinvidic, P. (1998). Weathering rates of oil components in a bioremediation experiment in estuarine sediments. *Marine Environmental Research*, 45(2), 113-125.
- Payne, J. R. (2018). Petroleum spills in the marine environment: The chemistry and formation of water-in-oil emulsions and tar balls. CRC Press.
- Pelletier, E., Delille, D., & Delille, B. (2004). Crude oil bioremediation in sub-Antarctic intertidal sediments: chemistry and toxicity of oiled residues. *Marine Environmental Research*, 57(4), 311-327.
- Peterson, C. H., Anderson, S. S., Cherr, G. N., Ambrose, R. F., Anghera, S., Bay, S., ... & Guzy, M. (2012). A tale of two spills: novel science and policy implications of an emerging new oil spill model. *BioScience*, 62(5), 461-469.
- Prince, R. C. (1997). Bioremediation of marine oil spills. *Trends in Biotechnology*, 15(5), 158-160.
- Pritchard, P. H., & Costa, C. F. (1991). EPA's Alaska oil spill bioremediation project. Part 5. *Environmental Science & Technology*, 25(3), 372-379.
- Raghavan, P. U. M., & Vivekanandan, M. (1999). Bioremediation of oil-spilled sites through seeding of naturally adapted *Pseudomonas putida*. *International biodeterioration & biodegradation*, 44(1), 29-32.

- Rahman, K. S. M., Thahira-Rahman, J., Lakshmanaperumalsamy, P., & Banat, I. M. (2002). Towards efficient crude oil degradation by a mixed bacterial consortium. *Bioresource technology*, 85(3), 257-261.
- Ramachandran, S. D., Hodson, P. V., Khan, C. W., & Lee, K. (2004). Oil dispersant increases PAH uptake by fish exposed to crude oil. *Ecotoxicology and environmental safety*, 59(3), 300-308.
- Ramseur, J. L. (2010, December). Deepwater Horizon oil spill: the fate of the oil. Washington, DC: Congressional Research Service, Library of Congress.
- Riedel, A. (2014). The Arctic Marine Environment. In *Arctic Marine Governance* (pp. 21-43). Springer Berlin Heidelberg.
- Rodrigues, E. M., Kalks, K. H., & Tótola, M. R. (2015). Prospect, isolation, and characterization of microorganisms for potential use in cases of oil bioremediation along the coast of Trindade Island, Brazil. *Journal of environmental management*, 156, 15-22.
- Saadoun, I. M. (2015). Impact of oil spills on marine life. *EMERGING POLLUTANTS IN THE ENVIRONMENT-CURRENT AND FURTHER IMPLICATIONS*, 77.
- Scheller, U., Zimmer, T., Becher, D., Schauer, F., & Schunck, W. H. (1998). Oxygenation cascade in conversion of n-alkanes to  $\alpha$ ,  $\omega$ -dioic acids catalyzed by cytochrome P450 52A3. *Journal of Biological Chemistry*, 273(49), 32528-32534.
- Schmidt, C. (2012). Exxon Valdez Vs. deepwater horizon: ES&T's top feature article 2011.
- Schneiker, S., dos Santos, V. A. M., Bartels, D., Bekel, T., Brecht, M., Buhrmester, J., ... & Goesmann, A. (2006). Genome sequence of the ubiquitous hydrocarbon-degrading marine bacterium Alcanivorax borkumensis. *Nature biotechnology*, 24(8), 997-1004.
- Short, J. W., Lindeberg, M. R., Harris, P. M., Maselko, J. M., Pella, J. J., & Rice, S. D. (2004). Estimate of oil persisting on the beaches of Prince William Sound 12 years after the Exxon Valdez oil spill. *Environmental Science & Technology*, 38(1), 19-25.
- Skinner, S. K. & Reilly, W. K. (1989). The Exxon Valdez Oil Spill: A Report to the President [PDF]. National Response Team. Retrieved from <http://www.uscg.mil/history/webshipwrecks/ExxonValdezNRT1989Report.pdf>
- Sobrinho, H. B., Luna, J. M., Rufino, R. D., Porto, A. L. F., & Sarubbo, L. A. (2013). Biosurfactants: classification, properties and environmental applications. *Recent Developments in Biotechnology*, 11, 1-29.
- Swannell, R. P., Lee, K., & McDonagh, M. (1996). Field evaluations of marine oil spill bioremediation. *Microbiological Reviews*, 60(2), 342-365.
- Tate, P. T., Shin, W. S., Pardue, J. H., & Jackson, W. A. (2012). Bioremediation of an experimental oil spill in a coastal Louisiana salt marsh. *Water, Air, & Soil Pollution*, 223(3), 1115-1123.

- Thavasi, R., Jayalakshmi, S., & Banat, I. M. (2011). Effect of biosurfactant and fertilizer on biodegradation of crude oil by marine isolates of *Bacillus megaterium*, *Corynebacterium kutscheri* and *Pseudomonas aeruginosa*. *Bioresource technology*, 102(2), 772-778.
- Thorne, R. E., & Thomas, G. L. (2007). Herring and the “Exxon Valdez” oil spill: an investigation into historical data conflicts. *ICES Journal of Marine Science*, 65(1), 44-50.
- Tissot, B. P. (1984). WELTED. H.(1984) Petroleum Formation and Occurrence. *Berlin (Springer-Verlag)*.
- Tyagi, M., da Fonseca, M. M. R., & de Carvalho, C. C. (2011). Bioaugmentation and biostimulation strategies to improve the effectiveness of bioremediation processes. *Biodegradation*, 22(2), 231-241.
- Ulrici, W. (2000). Contaminated soil areas, different countries and contaminants, monitoring of contaminants. Biotechnology Set, Second Edition, 5-41.
- Urgun-Demirtas, M., Stark, B., & Pagilla, K. (2006). Use of genetically engineered microorganisms (GEMs) for the bioremediation of contaminants. *Critical reviews in biotechnology*, 26(3), 145-164.
- Van Beilen, J. B., & Funhoff, E. G. (2007). Alkane hydroxylases involved in microbial alkane degradation. *Applied microbiology and biotechnology*, 74(1), 13-21.
- Van Hamme, J. D., Singh, A., & Ward, O. P. (2003). Recent advances in petroleum microbiology. *Microbiology and molecular biology reviews*, 67(4), 503-549.
- Venosa, A. D., Suidan, M. T., Wrenn, B. A., Strohmeier, K. L., Haines, J. R., Eberhart, B. L., ... & Holder, E. (1996). Bioremediation of an experimental oil spill on the shoreline of Delaware Bay. *Environmental science & technology*, 30(5), 1764-1775.
- Vinas, M., Sabaté, J., Espuny, M. J., & Solanas, A. M. (2005). Bacterial community dynamics and polycyclic aromatic hydrocarbon degradation during bioremediation of heavily creosote-contaminated soil. *Applied and environmental microbiology*, 71(11), 7008-7018.
- Weaver, J. W. (2004). Characteristics of spilled oils, fuels and petroleum products: 3a. *Simulation of oil spills and dispersants under conditions of uncertainty. EPA [online]*.
- Wells, P. G. (1984). The toxicity of oil spill dispersants to marine organisms: a current perspective. In *Oil Spill Chemical Dispersants: Research, Experience, and Recommendations*. ASTM International.
- Welte, D. H. (1984). Petroleum formation and occurrence-a new approach to oil and gas exploration.
- Widdel, F., and Rabus, R. (2001) Anaerobic biodegradation of saturated and aromatic hydrocarbons. *Curr Opin Biotechnol* 12: 259–276.
- Wilbur, S., & Bosch, S. (2004). Interaction profile for: benzene, toluene, ethylbenzene, and xylenes (BTEX). *Agency for Toxic Substances & Disease Registry (ATSDR)*.

- Williams, Marc, et al. *Wildlife toxicity assessments for chemicals of military concern*. Elsevier, 2015.
- Wolfe, D. A., Hameedi, M. J., Galt, J. A., Watabayashi, D., Short, J., O'Clair, C., Rice, S., Michel, J., Payne, J. R., Braddock, J., Hanna, S., & Sale, D. (1994). Fate of the oil spilled from the T/V Exxon Valdez in Prince William Sound, Alaska. *Environmental Science & Technology*, 28, 561A–568A.
- Yakimov, M. M., Timmis, K. N., & Golyshin, P. N. (2007). Obligate oil-degrading marine bacteria. *Current opinion in biotechnology*, 18(3), 257-266.
- Yin, F., John, G. F., Hayworth, J. S., & Clement, T. P. (2015). Long-term monitoring data to describe the fate of polycyclic aromatic hydrocarbons in Deepwater Horizon oil submerged off Alabama's beaches. *Science of the Total Environment*, 508, 46-56.
- Yu, H. (2002). Environmental carcinogenic polycyclic aromatic hydrocarbons: photochemistry and phototoxicity. *Journal of Environmental Science and Health, Part C*, 20(2), 149-183.
- Yu, K. S. H., Wong, A. H. Y., Yau, K. W. Y., Wong, Y. S., & Tam, N. F. Y. (2005). Natural attenuation, biostimulation and bioaugmentation on biodegradation of polycyclic aromatic hydrocarbons (PAHs) in mangrove sediments. *Marine pollution bulletin*, 51(8-12), 1071-1077.
- Zhang, S., Hu, Z., & Wang, H. (2018). A Retrospective Review of Microbiological Methods Applied in Studies Following the Deepwater Horizon Oil Spill. *Frontiers in microbiology*, 9, 520.
- Zhu, X., Venosa, A. D., Suidan, M. T., & Lee, K. (2001). Guidelines for the bioremediation of marine shorelines and freshwater wetlands. US Environmental Protection Agency.
- Ziervogel, K., McKay, L., Rhodes, B., Osburn, C. L., Dickson-Brown, J., Arnosti, C., & Teske, A. (2012). Microbial activities and dissolved organic matter dynamics in oil-contaminated surface seawater from the Deepwater Horizon oil spill site. *PLoS One*, 7(4), e34816.
- Zimmer, T., Ohkuma, M., Ohta, A., Takagi, M., & Schunck, W. H. (1996). The CYP52 Multigene Family of *Candida maltose* Encodes Functionally Diversen-Alkane-Inducible Cytochromes P450. *Biochemical and biophysical research communications*, 224(3), 784-789.

