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Abstract

Oil is still one of the major global energy sources as well as used for the production of products such as plastics and solvents. Due to the large global oil consumption, oil spills are difficult to avoid. The environmental impacts of these spills can be huge, especially in the marine environment. Oil floating on the ocean surface is harmful to marine life and it can be washed up to pollute coastal and shoreline areas damaging sensitive ecosystems. Therefore, it is very important to examine oil spill response strategies to reduce possible adverse consequences to protect the environment. There exist multiple oil spill response strategies all with advantages and drawbacks. Bioremediation plays an important role in oil spill cleanup and thus there has been a lot of research focusing on how bioremediation in marine environments can be improved. Biostimulation is stimulation of oil degrading through fertilizer addition. Oil dispersants such as Corexit can be applied to disperse oil into the water column. The aim is to stimulate removal by biodegradation by making the petroleum hydrocarbons more bioavailable to oil-degrading microorganisms, and to prevent oiling of shorelines and animals. A combination of conventional oil recovery methods along with dispersant is often preferrable. The methods used for cleanup are dependent on the location and nature of the spill. The use of dispersants often involves difficult choices, as they move the oil contamination into the water column which may harm the fish and benthic species etc. In addition, the chemical dispersants themselves may be toxic. This review examines dispersants and their efficiency on the enhancement of oil spill cleanup, toxicity and environmental impacts from dispersant use and dispersed oil, and possible alternatives to toxic dispersants. There are conflicting results on how dispersants enhance biodegradation, with the need for more research under different environmental conditions. Precautions should be taken during oil spill response as dispersants can be toxic to aquatic life like corals. Environmentally friendly dispersants can be applied such as food-grade-, biological- and biosurfactant-based dispersants, but aquatic species can still be harmed by the dispersed oil. Different environmental conditions with realistic and possibly declining concentrations of oil and dispersant should be used to test both the enhanced biodegradation by dispersants and on broad environmental impacts from the use of dispersants. More research is desirable on dispersants and the greener dispersants and their effects, to understand if and when they should be implemented.

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List of Abbreviations

BBDs	Biosurfactant-Based Dispersants
BTEX	Benzene, toluene, ethylbenzene, and xylene
DOR	Dispersant-to-Oil Ratio
DOSS	Dioctyl sodium sulfosuccinate
DWH	Deepwater Horizon
EPA	Environmental Protection Agency
HLB	Hydrophilic-Lipophilic Balance
LC_{50}	Lethal Concentration to 50% of a test population
MOS	Marine Oil Snow
MOSSFA	Marine Oil Snow Sedimentation and Flocculent Accumulation
OWR	Oil-to-Water Ratio
PAH	Polycyclic Aromatic Hydrocarbon

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1 Introduction

The world today is dependent on oil, with a global consumption of approximately 98.3 million barrels (15.6 million m^3) per day in 2019 (Sönnichsen, 2021). In 2020 the number was approximately 88.5 million barrels (14.1 million m^3) as consumption decreased due to the COVID 19 pandemic. During oil extraction and refining, it is probable there will be occasional accidents. The larger the oil spill, the more adverse environmental consequences it may have. The *Deepwater Horizon* (DWH) explosion in the Gulf of Mexico in 2010 lead to a catastrophic oil spill, releasing an estimated 5 million barrels (around 795 million liters) of crude oil into the water (Atlas & Hazen, 2011; McNutt et al., 2012).

Crude oil is composed of different hydrocarbons that are major pollutants in the marine environment (Chandra et al., 2013; Rojo, 2009). These organic pollutants can have carcinogenic and mutagenic effects and cause considerable damage to the environment and human life (CDC, 2009; Hart et al., 2012). Oil spilled at sea is harmful to many species and is easily spread to shorelines where it can do damage and persist for years (Atlas & Hazen, 2011; Prince, 2015). Efficient methods of cleaning up oil spills are therefore important to explore, with the purpose of reducing the environmental impact of these spills. The preferred methods for cleanup can differ from site to site, and are dependent on the conditions at the individual spill site which can vary greatly (Al-Majed et al., 2012; Michel et al., 2013). These factors include weather conditions at the site, composition and amount of the crude spilled, the water surface and soil, presence of oil-degrading microbes etc. Cleanup methods include containment by physical barriers to avoid shoreline stranding or to control it for further treatment such as recovery or burning directly at sea, it can be absorbed by sorbents, dispersed to enhance natural biodegradation among others (Al-Majed et al., 2012).

In addition to anthropogenic releases during the extraction and utilization of the oil, sources of oil in the marine environment include natural leakages from oil seeps (Kvenvolden & Cooper, 2003). There is therefore numerous microorganisms that can biodegrade the hydrocarbons arising from oil pollution, converting them into less harmful compounds (Al-Majed et al., 2012). Microbial biodegradation of the oil played an important role after large oil spills such as the DWH and *Exxon Valdez* incidents where different types of cleanup efforts including dispersion and bioremediation were applied (Lehr et al., 2010; McNutt et al., 2012; Wolfe et al., 1994). The biodegradation of petroleum hydrocarbons can be stimulated through fertilizer addition (biostimulation) if limiting nutrients are missing (Atlas, 1995a; Harayama et al., 2004). Another method of increasing the biodegradation rate is by making the substrate (petroleum hydrocarbons) more bioavailable by dispersing the oil into the water column (Al-Majed et al., 2012; Kleindienst et al., 2015; Xuan & Bing, 2017). Oil dispersion and both its effects on the biodegradation of oil and environmental impacts will be the focus of this review. Oil dispersion may increase the biodegradation rate and other natural weathering processes and therefore reduce the amount of oil ending up in sensitive shorelines and wetlands.

The use of oil dispersants also has its drawbacks. Dispersants such as Corexit may possibly play an important role on the enhancement of oil biodegradation however some chemical dispersants have been shown toxic to aquatic species especially corals (Shafir et al., 2007). Oil dispersion also moves the toxic compounds into the water, exposing sensitive aquatic species. Corexit has therefore been banned in many countries including Sweden and the United Kingdom. Due to these advantages and disadvantages more research needs to be conducted on the actual effectivity of dispersant use in the enhancement of oil spill cleanup and their toxic effects. This review attempts to examine effectivity of dispersants on the enhancement of oil spill cleanup, environmental impacts from dispersant use and possible alternatives to toxic dispersants.

2 Oil Spills in the Marine Environment

About half of the nearly nine million barrels crude oil released into the oceans each year is due to anthropogenic activity (Cohen, 2013; M. Fingas, 2012; Kvenvolden & Cooper, 2003). The rest is released through natural sources, such as seeps where oil and gas leak through cracks in the seafloor. It is though believed that natural oil seeps actually account for more oil spilled into the sea than the anthropogenically sources (Committee on Oil in the Sea et al., 2003). Still, the anthropogenic ones are the ones that are important to observe and act on. Huge amounts of money and time are associated with oil spill cleanup. Even after cleanup, the remaining environmental consequences from an oil spill may be adverse.

Anthropogenically spilled crude or refined oil can come from multiple sources during extracting, refining, consuming and transportation of the oil. The most common thought of types of anthropogenically spilled oil is often the large tanker and oil rig accidents, however marine oil pollution is not only associated with these. Diverse smaller releases make up large volumes of oil pollution in total, and the tanker and rig accidents normally only account for a relatively small fraction of the spilled oil in total (Prince, 2015; Schröder, 2010). Some other sources of petroleum pollution include spills during refining and shipping of oil, runoffs from activities on land, discharge of produced water, illegal discharges and dumping at sea. Though large volumes in short time such as tanker and oil rig accidents usually have more adverse environmental consequences. Table 2.1. Overview of large marine oil spills, the estimated amount of oil spilled, and the major response efforts that were performed.Table 2.1 shows an overview of some important large oil spills that have occurred around the world.

Table 2.1. Overview of large marine oil spills, the estimated amount of oil spilled, and the major response efforts that were performed.

Oil spill	**Year**	**Location**	**Estimated amount of oil spilled (m^3)**[1]	**Estimated amount in barrels**	**Major response efforts/cleanup methods**	**References**
Torrey Canyon tanker	1967	Seven Stones reef, English Channel	135 000	748 500	- Dispersants - Burning and bombing - Use of powdered chalk to sink oil	(Law, 2011; Wells, 2017)
The *IXTOC-I* well blowout	1979	Bay of Campeche, Gulf of Mexico	556 000	2.5 million	- Mechanical recovery and confinement - Burning at well site - Dispersant	(Atlas & Hazen, 2011; Jerneloev & Linden, 1981)
Exxon Valdez tanker	1989	Prince William Sound, Alaska	42 000	250 000	- Bioremediation (fertilizer to treat shoreline oiling)	(Atlas & Hazen, 2011; Wolfe et al., 1994)
The Persian Gulf War Oil Spill	1991	Persian Gulf	954 000	6 million	- Recovery - Moved to pits in the desert	(Randolph et al., 1998)
Sea Empress tanker	1996	Milford Haven, West Wales	81 000 (+480 tons fuel oil)	-	- Dispersants at sea and on beaches - At-sea recovery	(Law & Kelly, 2004)
BP *Deepwater Horizon* /DWH explosion	2010	Gulf of Mexico, United States	795 000	5 million	- Dispersant at sea and subsea - Recovering at the wellhead	(Atlas & Hazen, 2011; Lehr et al., 2010; McNutt et al., 2012)

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[1] Some volumes are converted from weights using petroleum calculators to easier compare the size of the spills.

The *Torrey Canyon* supertanker accident in 1967 in the English Channel was the first large oil-tanker accident with an estimated amount of 135 000 m^3 (Table 2.1) (Law, 2011; Wells, 2017). The fate of petroleum pollutants in different environments was for real starting to be stressed after this incident, and has since been studied a lot (Atlas, 1981). It was the first known oil spill where large-scale use of chemical dispersants was used in an attempt to aid oil spill cleanup. Other response efforts include bombing to enhance evaporation of oil, and the use of powdered chalk to enhance sedimentation of oil. Hopanes were used as a reference marker due to the resistance to biodegradation. They were biodegraded at very slow rates compared to most other compounds in the oil (Atlas, 1995b). More than a decade after the spill, hopanes were still found in the sediments.

The *IXTOC-I* well blowout in 1979 in the Bay of Campeche was considered the largest blow out in the Gulf of Mexico (Table 2.1), until the DWH spill occurred (Atlas & Hazen, 2011; Jerneloev & Linden, 1981). An estimated 556 000 cubic meters of light crude were released subsea into the waters of the Gulf of Mexico before the well was capped 290 days after the spill started. Response efforts were made although, with disappointing results. The oil spill response attempted to confine the oil using high sea booms and recover oil by skimmers and absorbent devices. Bad weather conditions and trouble with the equipment made the recovery and confinement rather unsuccessful. Other response efforts that were made included; spraying of dispersant from airplanes and boats, as well as *in-situ* burning of oil at the well site. The main removal of oil was assumed to be by evaporation (about 50%). After substantial weathering it is assumed that most of the remaining higher density oil sank (25%). 12% was assumed to be removed through biodegradation and photooxidation. The biodegradation was mainly happening in the water column. Biodegradation was expected to be higher at the beaches because of favorable conditions and especially the presence of oil-degrading organisms. Great biodegradation potential had been seen earlier in the area (Atlas, 1995b). However, the formation of resistant emulsions/mousse made the biodegradation at the beaches challenging. Even though the microbes were there, their substrates were not bioavailable.

The environmental effects after the *IXTOC-I* spill in 1979 (Table 2.1) could be seen by looking at the littoral crab populations along several hundred kilometers which were almost completely eliminated (Jerneloev & Linden, 1981). Nine months after the spill, only a small percentage of the normal crab population on nearby coral islands was observed. It is assumed that shrimp and fish populations also were adversely affected, however appropriate studies were not completed.

Given the size of the spill it is reasonable to believe that the spill resulted in more adverse environmental effects to the nearby ecosystems than what was reported. The total environmental impact of the spilled oil is still unknown.

Another well-known oil spill is the *Exxon Valdez* spill in 1989, where a tanker was stranded on a reef and released about 42 million liters of crude oil in Prince William Sound (Table 2.1) (Atlas & Hazen, 2011; Wolfe et al., 1994). Large amounts of the oil spilled from the tanker ended up ashore because of wind and waves and failed attempts to contain the oil at sea. The spill gained large focus in the news due to its impact on Alaskan wildlife. Heavy shoreline oiling was observed in the area near Prince William Sound and the Gulf of Alaska. The cleanup focused on the shorelines with the use of oleophilic fertilizer that enhanced the rate of biodegradation 3-5 times (Atlas, 1995b; Atlas & Hazen, 2011; Bragg et al., 1994). The DWH spill was, when it occurred in 2010, compared to this spill, even though the volumes of spilled was much lower for the *Exxon Valdez* spill it was the largest spill in the U.S. until the DWH spill. Other details about the spills also differ, such as that the tanker released oil at the ocean surface, while in the DWH spill the spill source was at 1500 meter subsea. Still, biodegradation played an important role in reducing the oil pollution after both accidents.

The Persian Gulf War Oil Spill in 1991 is estimated to be the largest spill in history as shown in Table 2.1 (Randolph et al., 1998). The volume of spilled oil released was roughly estimated to be around 954 000 cubic meters or 6 million barrels. This spill was a result of war activities and was due to a deliberate release. As a response measure, more than one million barrels were moved and buried into pits in the desert. Coastlines were also highly polluted in the area with an estimated 905 million barrels ending up there. Temperature played a significant role in the weathering of the oil as warm climates such as in the Persian Gulf are associated with favorable conditions for oil removal. The warm climate facilitated relatively fast weathering and especially evaporation of the oil, and roughly half of the oil was estimated to have been removed via evaporation (Randolph et al., 1998). The biodegradation rate was also assumed to be relatively high because of the climate.

Also shown in Table 2.1 is the tanker accident of *Sea Empress* from 1996. The accident happened in Milford Haven in West Wales and released about 81 000 m^3 of crude oil and 480 tons fuel oil (Law & Kelly, 2004). The major response efforts include at-sea recovery, and dispersants use both at sea and on affected beaches.

The *Deepwater Horizon* (Table 2.1) drilling rig explosion in 2010 ended as a catastrophic oil spill with 11 fatalities releasing an estimated 5 million barrels (795 million liters) of crude oil into the Gulf of Mexico making it the worst oil spill in US history (Atlas & Hazen, 2011; McNutt et al., 2012; Michel et al., 2013). Oil leaked from multiple locations (the wellhead and along the riser pipe that was collapsed at the sea bottom) for 87 days before the wellhead was finally closed. Both physical (due to the high pressure at the sea bottom) and intentional chemical dispersion of the oil contributed to preventing large oil slicks from forming at the water surface above the wellhead. Although oil was still washed up ashore and polluted many beaches and shorelines in the area. In addition to the massive use of dispersants, other cleanup methods that were applied included recovery directly from the wellhead, offshore skimming, containment booms and burning (Michel et al., 2013). Dispersion and recovery of oil were the major cleanup methods (Table 2.1). Despite the massive cleanup effort huge environmental consequences are associated with the DWH spill. The oil spill and the use of dispersants are reviewed in more detail in Chapter 4.5.

The environmental consequences of an oil spill and choices of different response efforts depend on factors such as properties and composition of the oil, the magnitude and location of the spill, weather conditions at the site including waves and currents, and weathering processes that determine how the oil behaves in the marine environment (Al-Majed et al., 2012; M. Fingas, 2012).

2.1 Fate of oil at sea

To understand how humans can help to clean up after an oil spill, it is important to understand what happens to the oil that is spilled in the marine environment. How this spilled oil behaves is dependent on several physical and biological factors, describing its fate, as shown in Figure 2.1. When oil is spilled in the marine environment it undergoes several weathering processes, changing the composition of the oil over time (Harayama et al., 2004; Schröder, 2010). Weathering processes that occur in the early stages after an oil spill are spreading, evaporation, dispersion and emulsification (ITOPF, 2011). The remaining ones (biodegradation, sedimentation, and photo-oxidation) are long-term weathering processes. These weathering processes act together at different rates, and depend on different factors, to determine the short-term and long-term fate of the spilled oil. They can also influence each other. Some result in the removal of oil from the sea surface while others lead to persistence of oil in or near the marine environment.

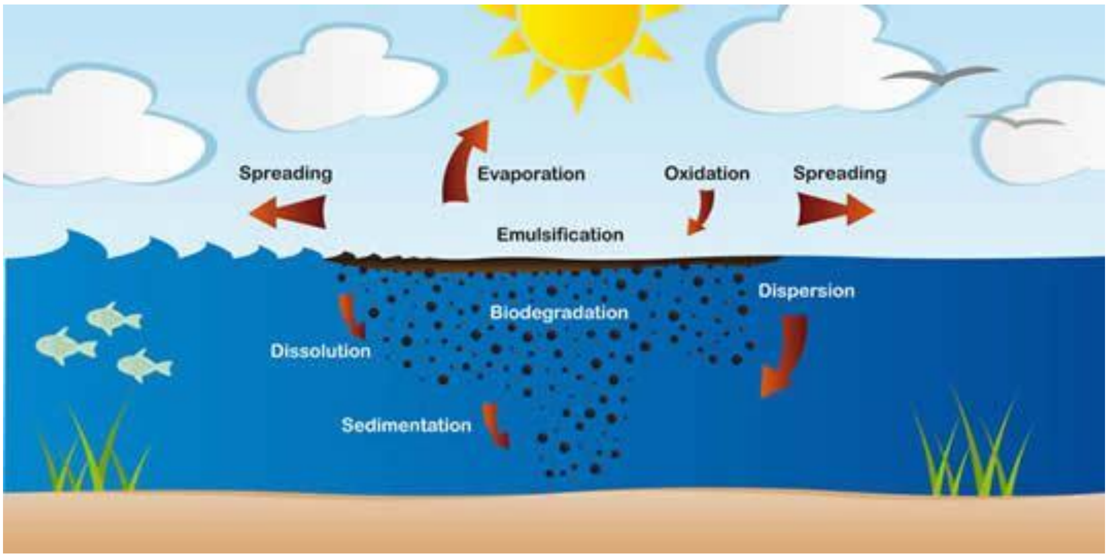

Figure 2.1. Weathering processes of oil at sea. Obtained from (ITOPF, 2011).

2.1.1 Spreading

Spreading refers to how the oil spreads out at the ocean surface (Figure 2.1) (ITOPF, 2011). It is dependent on the viscosity of the oil and the size of the spill, where high viscosity oil is associated with low spreading. The rate of oil spreading is also influenced by natural forces such as wind, waves, tidal streams, turbulence and currents (Schröder, 2010). Consequently, the oil is spread out and can cover large areas of the water surface. This can also lead to the oil being washed up ashore to pollute shorelines and wetlands.

2.1.2 Evaporation

The second weathering process shown is evaporation (Figure 2.1), which is a very important weathering process (M. Fingas, 2012). Via evaporation the most volatile compounds in the oil are evaporated into the atmosphere and are consequently removed from the water surface (Schröder, 2010). The temperature, sea roughness, initial spreading rate and wind speed affect the rate of the evaporation. Under temperate conditions, most of the light compounds with boiling points below 200 °C are evaporated 24 hours after a spill (ITOPF, 2011). The fraction evaporated from light crude oils is higher than for the heavier types. The various types of crude oil thus evaporate at different extents, depending on their composition of light and heavy compounds. As a result of the evaporation process, the oil residue acquires a higher density and viscosity than it had initially.

2.1.3 Dispersion

Rough sea conditions such as breaking waves can result in splitting parts of an oil slick into droplets in suspension in the upper parts of the water column (M. Fingas, 2012; Lewis et al., 2010). This formation of oil droplets is called dispersion (Figure 2.1). The oil viscosity affects dispersion; oils with lower viscosities are more easily dispersed. The smallest droplets stay in suspension, while the bigger ones rise back up and can gather again or start spreading out. The movement of the oil droplets are influenced by currents or turbulence in the water, and the force of gravity wanting them to rise back up. When the droplets are small enough (less than approximately 70 micrometer in diameter) these forces are balanced so the droplets remain in suspension (Beyer et al., 2016; ITOPF, 2011). Dispersion results in an increase in the interface area between the water and oil, enhancing other processes such as biodegradation (Schröder, 2010), dissolution and sedimentation. The process of dispersion and chemicals such as Corexit that are used for this purpose are discussed through Chapter 4.

2.1.4 Emulsification

If the spilled oil is able to take up water, emulsification can occur which results in the formation of water-in-oil emulsions; a droplet of water surrounded by an oily surface (Figure 2.1) (Schröder, 2010). High viscosity oils are associated with slower uptake of water than less viscous ones (ITOPF, 2011). A consequence of emulsification is that a larger area is polluted, as the volume increases when the water-in-oil emulsions are formed. In addition, this emulsification affects other weathering processes negatively. Stable emulsion are often semi-solid and are extremely persistent in the environment, resisting weathering and especially biodegradation as the bioavailability is reduced (Atlas, 1995b). This can lead to persistence of oil on the sea surface and shorelines.

2.1.5 Dissolution

Dissolution can also occur when oil spilled is in the marine environment (Figure 2.1), and its rate is dependent on oil composition, spreading, the water temperature, turbulence and dispersion (M. Fingas, 2021). Even though oil is generally not soluble in water, some lighter compounds in the oil is slightly soluble. However, these compounds are also volatile and are more likely to evaporate than dissolve. Dissolution therefore contributes very little to the removal of the oil, especially if the oil is released at the water surface (M. Fingas, 2021; Gros et al., 2014).

2.1.6 Photo-oxidation

The oxidation of oil can form soluble products or persistent tars (ITOPF, 2011). It is stimulated by sunlight (photo-oxidation as shown in Figure 2.1) but results in little spreading of the oil compared to other weathering processes even under intense sunlight. Tarballs that are formed as a result of the oxidation can be observed stranding on shorelines. The outer layer of the tarballs is usually solid as a result of formation of heavy compounds due to the oxidation of viscous oils or water-in-oil emulsions, with a less solid interior mass (ITOPF, 2011; Schröder, 2010).

2.1.7 Sedimentation

Most oils have lower density than seawater and therefore float on the sea surface (Madigan et al., 2019). However, there are multiple occurrences that can lead to sedimentation and sinking of oil particles (Figure 2.1). Dispersed oil particles in the water can react with organic matter, organisms or suspended sediment particles/minerals to increase their density and form marine oil snow (MOS) that is also ultimately sinking and transporting oil to the seafloor (Gregson et al., 2021; ITOPF, 2011). Brackish water can increase the chance of oil sinking because of the lower salinity and therefore lower specific gravity of the water in those areas. Some heavy crude oils and water-in-oil emulsions can be more pre-disposed of sinking as their specific gravities are almost as high as for seawater, and therefore minimal interactions with other particles or sediments can cause them to sink (ITOPF, 2011). Fire can also trigger sinking and sedimentation of oil. The lightest compounds are removed because of the heat, leaving residues of heavier compounds as well as some pyrogenic products formed during the fire which can then sink. Sedimentation is a long-term process and oil can consequently accumulate at the sea bottom in exposed areas. Oil stranding on sand shorelines poses a major risk of sedimentation of the oil there.

2.1.8 Biodegradation

Oil-degrading microorganisms are naturally present in the marine environment, especially where natural oil seeps occur, at other places where petroleum hydrocarbons may occur naturally or near *e.g.* runoffs from land (Atlas, 1981; Madigan et al., 2019; Schröder, 2010). Biodegradation of oil (Figure 2.1) happens at the interface between the oil and water, and is affected by factors such as temperature, nutrient availability, oxygen content and oil composition. This process plays an important role in cleaning up many oil spills and is described in more detail in Chapter 3.

2.2 Cleanup methods

After the anthropogenic release of oil in the marine environment, large amounts of oil typically gather as an oil slick floating at the water surface. This due to the properties of oil most are less dense than water as well as being immiscible in water (Atlas & Hazen, 2011; Madigan et al., 2019). Cleanup of the spilled oil is instrumental in reducing the negative consequences on the environment from the spilled oil, where the time and efficiency of the response actions are the key (M. Fingas, 2012). The efficiency and choice of cleanup methods may be influenced by several factors such as sea conditions like wave height and wave period, winds and currents, air and water temperature, and composition and behavior of the spilled oil (Al-Majed et al., 2012). When determining cleanup methods, these factors and more are considered and choices for the specific situation are made. It is desirable with a net environmental benefit (M. Fingas, 2012).

Several different cleanup methods are used for a single oil spill (Al-Majed et al., 2012). Many of the cleanup methods focus on preventing the oil from drifting ashore to avoid adverse consequences on the shorelines. One such consequence is shown in Figure 2.2a, where a pelican is covered in oil. Mammals and birds are sensitive to oil pollution, especially birds (Beyer et al., 2016; NOAA, 2020). The insulating and water repellency properties of their plumage are damaged if covered in oil, where large amounts of oil lead to an almost certain death.

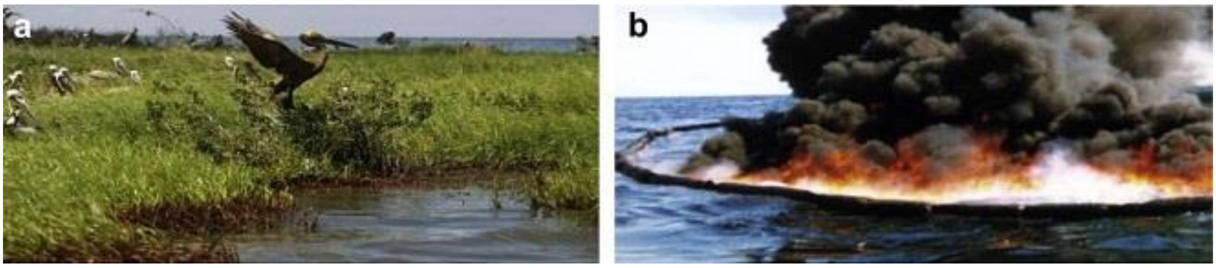

Figure 2.2. (a) A consequence of shoreline oiling, a pelican covered in oil (AP Photo/Gerald Herbert). (b) Containment of oil by a fireproof boom for treatment by controlled in-situ burning (Office of Response and Restoration, National Ocean and Atmospheric Administration). Copied from (Al-Majed et al., 2012).

2.2.1 Oil containment

To prevent the oil from drifting away from the site, oil spill response teams attempt to contain the oil and to keep it controlled at sea. The aim is usually to stop the oil from reaching a sensitive area such as shorelines areas with sensitive ecosystems (Figure 2.2a) , and to keep the oil still or thicken it for further treatment such as burning (Figure 2.2b) or recovery (M. Fingas, 2012; Shigenaka et al., 2010). Physical barriers such as containment booms like the one shown in Figure 2.2b is most frequently used for this purpose. They can be for example placed around a leaking ship. A boom consists typically of an upper part that floats (Figure 2.2b), with a barrier under the water surface. Weather conditions especially sea conditions such as wave height influence the use of this equipment. Booms are the most sensitive when it comes to weather conditions compared to other cleanup equipment (Al-Majed et al., 2012; M. Fingas, 2011b). The effects of weather could be observed after the *IXTOC-I* well blowout (Table 2.1) where the response team attempted to confine and recover the spilled oil however poor weather conditions made the response efforts difficult (Jerneloev & Linden, 1981).

2.2.2 *In-situ* burning

Burning of oil on water is a common cleanup method used for oil spilled in the marine environment to enhance evaporation of the oil components (Al-Majed et al., 2012). This is called *in-situ* burning and is shown in Figure 2.2b. Typically, the oil is first gathered or confined by equipment such as fireproof booms to concentrate it and control the burning as much as possible (M. Fingas, 2012). Time is an important factor affecting the efficiency of *in-situ* burning. It is important with rapid actions before the oil weathers much naturally, especially by evaporation and dispersion.

2.2.3 Oil recovery

Recovery of oil is another common cleanup method and refers to physical removal of oil directly from the water surface, often after the containment and thickening by *e.g.* booms (M. Fingas, 2012). Equipment used for this includes skimmers (Figure 2.3), and sorbents among others (Al-Majed et al., 2012). Skimmers are devices that collect oil from the water surface typically by a rotating device such as a brush, belt or drum, and gathers it in a collection well, as shown in Figure 2.3 (M. Fingas, 2012). Figure 2.3 shows one type of skimmer, but there also exist many different types such as vacuum devices, weir skimmers etc. Skimmers typically work best if the oil first has thickened on the water surface with for instance a boom. If the oil slick is too thin, skimmers work less efficiently with more water being taken up by the skimmer. Also, waves and currents limit the use of skimmers (Guidi et al., 2016).

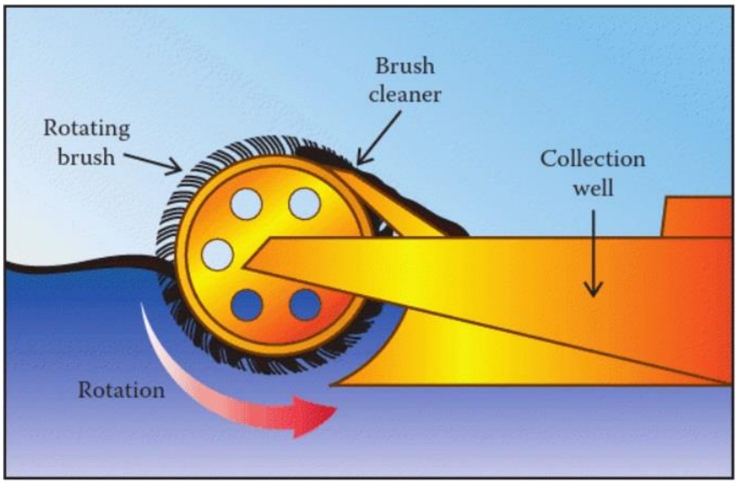

Figure 2.3. A scheme of a type of skimmer; a recovery device with a rotating brush drum to collect spilled oil from the water surface. Obtained from (M. Fingas, 2012).

2.2.4 Absorption or adsorption by sorbents

Sorbents are recovery devices that recover oil by absorption or adsorption, or both (Guidi et al., 2016). There exist many types and they are made from insoluble materials with water-repellency and oleophilic properties making the sorbents able to soak up the oil. They are most often used later in a large oil spill to clean up the remaining oil after the use of skimmers, but also as the main or only cleanup method in smaller spills. The use of sorbents is a relatively inexpensive and environmentally friendly cleanup method. Though they have limitations - they may function bad in open seas, or to soak up heavy fuel oil (Guidi et al., 2016). Recovery using skimmers or sorbents is a very common oil recovery method.

2.2.5 Dispersing agents

Chemical dispersants are used in oil spill cleanup to move the oil contamination from the water surface and into the water column. Dispersants are based on surfactants that contain one hydrophobic and one hydrophilic side, and work to reduce the surface tension between the oil and water to help disperse the oil into the water column (Al-Majed et al., 2012; Lewis et al., 2010). The aim of dispersants use is to avoid shoreline stranding, enhance biodegradation by increasing the bioavailability of the petroleum hydrocarbons as substrate for the microorganisms, and to avoid water-in-oil mousse formation. The composition, large-scale use, pros, and cons of dispersants are reviewed and discussed in Chapter 4.

2.2.6 Unconventional methods

Other less conventional cleanup methods include some of the actions that were taken after the *Torrey Canyon* tanker accident in 1967 (Table 2.1) (Law, 2011). Here large amounts of powdered chalk were applied to sink the oil at sea. The use of chalk was successful for its purpose, but the possible environmental effects of this process were not thoroughly thought through or further investigated. The accident site was also intentionally bombed by the Royal Air Force to enhance evaporation by burning of oil. Even with addition of aviation fuel to keep the fire alive this response action was somewhat unsuccessful as the fires stopped fast. Even though every oil spill situation differ, the most effective cleanup method in the marine environment is normally oil recovery and application of dispersants, followed by bioremediation at the shorelines (Dave & Ghaly, 2011).

2.3 Components and toxicity of crude oil

Crude oil is composed of many organic compounds, mainly hydrocarbons, that over millions of years have been made from decaying organic material. The composition and viscosity of different crude oils vary. This is determined by the ratio of its heavy and light constituents. The constituents are divided into four groups; saturates, aromatics, resins and asphaltenes (Harayama et al., 2004). Within each of these groups there exists numerous compounds. The saturates include alkanes and cycloalkanes which are the two major types of constituents and account for most of the crude oil, though the composition varies with the type of crude oil. The remaining part is made up of aromatics, resins and asphaltenes. Aromatic hydrocarbons are benzene ring based molecules with or without alkyl substitutions, such as polycyclic aromatic hydrocarbons (phenanthrenes, chrysenes, pyrenes, etc.), that are known for their toxicity (Walker et al., 2012). Resins and asphaltenes are large molecules able to resist biodegradation (Atlas & Hazen, 2011; Haritash & Kaushik, 2009).

2.3.1 Polycyclic aromatic hydrocarbons

When discussing the toxicity of crude oil, the focus is often on the polycyclic aromatic hydrocarbons (PAHs). These are an important part of crude oil, even though they account for a relatively small fraction of the oil, they account for the most toxic compounds in oil to animals and plants (Alloy et al., 2015; Atlas & Hazen, 2011; ATSDR, 2011; Vignier et al., 2016), and some are known to have carcinogenic and mutagenic effects (CDC, 2009; Hart et al., 2012). PAHs are organic molecules composed of fused benzene rings that can be substituted with different functional groups, they exist as pale/colorless solids at standard conditions (Walker et al., 2012). Most of the PAHs in crude oil are hydrocarbons without substitution. PAHs are also present in coal and gasoline, and they are produced when burning substances such as coal, tobacco, wood, and barbequed meat. Chemical structures of some PAHs are shown in Figure 2.4.

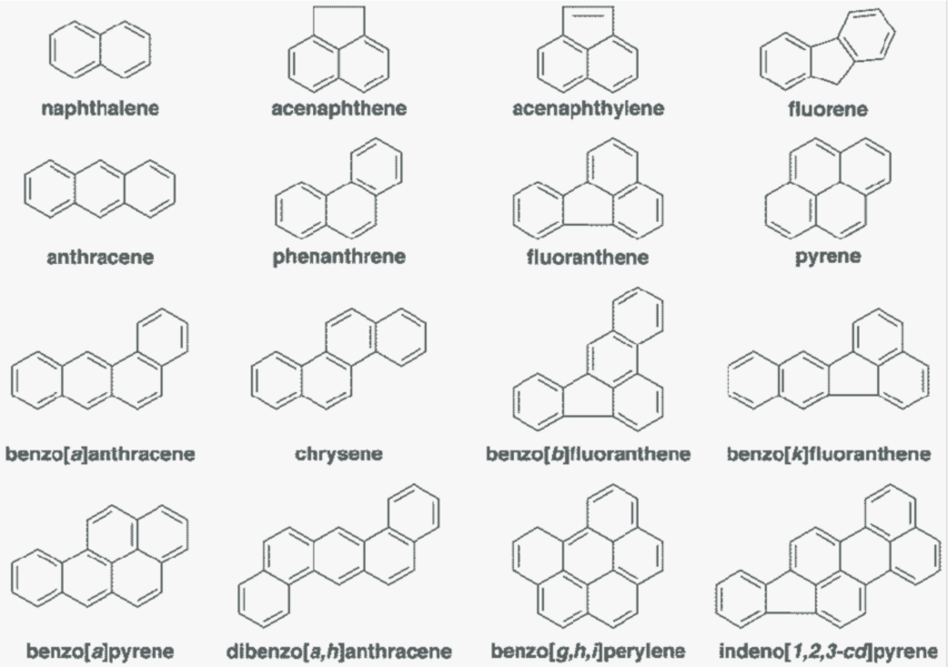

Figure 2.4. Chemical structure of the 16 PAHs on EPA's priority pollutants list. Obtained from *(Honda & Suzuki, 2020)*.

Figure 2.4 shows the chemical structure of the 16 PAHs on EPAs (Environmental Protection Agency) priority pollutants list (EPA, 2014; Honda & Suzuki, 2020). Naphthalene, ($C_{10}H_8$), is commonly known as the main ingredient of mothballs. High concentrations of this chemical in the air can irritate eyes and airways, and exposure to the liquid or vapor form can cause blood and liver abnormalities (CDC, 2009). Benzo[a]pyrene is found in petrochemical waste and is categorized as an important pollutant with very toxic and carcinogenic properties (Haritash & Kaushik, 2009).

PAHs are priority pollutants due to their carcinogenic, mutagenic and toxic properties (EPA, 2014; Haritash & Kaushik, 2009). They also have the potential to bioaccumulate and can be resistant towards biodegradation. Microbial degradation is the main process to remove these pollutants from the environment, however larger molecules are more difficult to degrade and may therefore stay longer in the environment, and those with three or more rings is barely, if at all, biodegradable (Atlas, 1995b; Harayama et al., 1999; Haritash & Kaushik, 2009; Ollivier & Magot, 2005).

2.3.2 Alkanes

Alkanes and cycloalkanes are saturates (i.e. no double bonds) that normally account for the major part of the crude oil (Harayama et al., 2004; Walker et al., 2012). Alkanes have the general formula C_nH_{2n+2} and can exist as gases, liquids and solids depending on the size of the compound, hence the number n (Walker et al., 2012). Alkanes are generally not a big threat in an oil spill as they tend to be easily biodegraded, and they are less toxic than the PAHs (Harayama et al., 1999). Also, the smaller alkanes that can be toxic to some bacteria evaporate easily (Atlas, 1981).

2.3.3 BTEX

BTEX is the abbreviation for the compounds benzene, toluene, ethylbenzene, and xylenes, or sometimes BTX without the ethylbenzene (Leusch & Bartkow, 2010). This mixture of aromatic hydrocarbons is important in marine oil spills because the chemicals can dissolve in and pollute water if not evaporated into the air. The compounds are toxic to many organisms (M. Fingas, 2012). BTEX compounds are also central in the manufacturing of paints, adhesives, thinners, solvents etc. The chemicals are produced in large quantities each year. Releases into the ocean though are mainly from oil spills and make up a small portion compared to what is released into the atmosphere. Crude oil can contain high levels of benzene, which is listed as number 6 in ATSDR’s Substance Priority List, before PAHs which are listed as number 9 (ATSDR, 2011). Benzene can thus be found in seawater in areas near oil seeps or oil pollution. It is much more toxic to humans than toluene is, and can cause liver damage and cancer (Hart et al., 2012). Humans are mostly in contact with these chemicals after breathing them in from car exhaust or cigarette smoke, and not from contaminated water (Leusch & Bartkow, 2010).

2.3.4 Resins and asphaltenes

Crude oil also contains heavy components such as resins and asphaltenes; large, heavy compounds that contain trace amounts of nitrogen, sulfur, and/or oxygen (Harayama et al., 2004). They make the crude oil thicker, and with relatively high amounts of asphaltenes the oil appears as a thick liquid almost like asphalt, versus light oil which appears thinner and hence less viscous. These large molecules are important because they resist biodegradation and therefore tend to persist in the environment for long periods of time (Atlas & Hazen, 2011).

Thick crude oil that has large fractions of these heavy compounds is also more poorly dispersed, both naturally and by chemical dispersants, possibly reducing other weathering processes (M. Fingas, 2012). Relatively large fractions of asphaltenes are also associated with emulsion formation, but it is suggested that resins also can contribute to this (M. Fingas et al., 1996). Again, this leads to the persistence of oil pollution in nature and can be visible or hidden as tar or tarballs at beaches and in the sediments.

2.4 Environmental Effects of spilled oil

Oil spills are a threat to the environment and the effects can last for decades. This includes the marine-, shoreline and coastal life. It is especially visible at beaches and shorelines after oil slicks at sea travel by waves, wind and currents to reach the beach and shoreline areas (Schröder, 2010). Although dispersed and sedimented oil may also adversely affect the marine life. The affected ecosystems can consequently be in acute and long-term danger. Exposure may lead to acute toxicity or long-term or chronic toxicity and may be lethal or sublethal to the organism. Species in early life-stages such as juvenile animals, fish eggs and larvae are more sensitive to the effects of pollution like petroleum hydrocarbons (DeLorenzo et al., 2018; M. Fingas, 2012; Jerneloev & Linden, 1981; McIntosh et al., 2010). Immobile species are at higher risk of oil exposure.

How adverse the local environment will be affected by an oil spill is dependent on several factors. Removal by weathering of the oil is important, much influenced by temperature and sea energy. The amount of oil entering or coating an organism can determine its fate, and heavy oiling has the potential to kill most species (M. Fingas, 2012). The sensitivity of the affected ecosystems to oil pollution is also key. Some organisms or ecosystems may recover after a certain time. Arctic regions are in general more sensitive to pollution (Walker et al., 2012). Not only are persistent pollutants traveling towards those regions, but the colder temperatures reduce the removal of oil through evaporation and lower biodegradation rates. Salt marches, mangroves and coral reefs are also particularly sensitive to oil pollution (Beyer et al., 2016; Mendelssohn et al., 2012).

2.4.1 Mammals and birds

A consequence of oil slicks and shoreline oiling is animals trapped in oil or poisoned by ingesting the oil, as often seen in the media after the occurrence of an oil spill. An example of the effects of an oil spill on pelicans can be seen in Figure 2.2a in Chapter 2.2. Diving birds and mammals are very exposed to floating oil (Prince, 2015). There have been many reports about bird mortality after oil spills (Harris, 1995). The deleterious impact of an oil spill on animals was exemplified from impact of the *Torrey Canyon* tanker accident in 1967 which resulted in the death of more than 25 000 birds (Table 2.1, Chapter 2) (Wells, 2017). Thousands of birds also died after the Sea Empress tanker release in 1996 (Sea Empress Environmental Evaluation Committee & Little, 1998). The behavior and reproductivity of birds can also be affected as some birds can stop laying eggs after being exposed to oil pollution (M. Fingas, 2012). In addition, weakened animals can easier become prays for predators.

Oil can stick to the fur of mammals and to the plumage of birds resulting in weakened isolating properties in the fur and plumage of the animals (NOAA, 2020; Schröder, 2010). The animals are highly dependent on these insulating properties to stay warm. Birds are also dependent on their water repellency of the plumage which can be damaged by oil. The effects are very deadly to the birds. Mammals also struggle, but they are not as sensitive as birds. Oil spills can also have dire effects on marine mammals such as whales and dolphins. Oil can get into their system through respiration, harming their lungs, immune function, and reproduction ability. Seals that have been exposed to oil pollution can struggle to eat (M. Fingas, 2012). Mammal deaths are not very common after oil spills, but otters are vulnerable and may die.

2.4.2 Fish

Oil spills often lead to concerns regarding the fish populations in the area. Fish is an important food source and a potential pollutant source for humans. Commercial fishing is typically stopped in the area of an oil spill as the fish and shellfish might be polluted and develop an undesired taste (M. Fingas, 2012). Floating oil is harmful to fish and fish eggs (Irie et al., 2011). When oil is mixed into the water column by dispersion or dissolution, the oil becomes even more available for fish. PAHs in the oil are toxic to fish embryos such as Pacific herring and pink salmon embryos (Al-Majed et al., 2012), and large fish such as tunas and amberjacks (Incardona et al., 2014). Oil exposure to fish can lead to both acute and chronic toxicity. Growth and reproduction abilities can be affected. Also liver enlargement, changes in heart and respiration rates, disturbances in heart development (Al-Majed et al., 2012), and fin erosion have been reported (NOAA, 2020). Oil spilled at open sea is normally not a big risk to fish, because the oil that is dispersed or dissolved in the water column is quickly diluted. The risk increases for fish that stay near land, lay floating eggs, or having other behavior that make them more likely to be exposed to oil.

2.4.3 Other aquatic species

Many aquatic species can be adversely affected by oil pollution, especially the juvenile and/or immobile species. The oil may be taken up by breathing or absorption and dispersed or sedimented oil can be mistaken for food by some species. Filter feeder species might eat the oil along with their food, some benthic species may also do if the oil sinks and becomes sedimented oil on the ocean bottom, possibly leading to deaths (Schröder, 2010). Mussels are filter feeders that are particularly sensitive to oil pollution and can be used as biosensors (Pérez-Cadahía et al., 2004). Benthic species are negatively affected by oil sedimentation, which further can affect other species that are dependent on benthic species for food (Randolph et al., 1998). An example is the case of the *IXTOC-I* oil spill in 1979. Benthos species were adversely affected by the oil (Table 2.1). Massive littoral crab mortality was observed along several hundred kilometers (Jerneloev & Linden, 1981). It took long time for the crabs to regenerate as they were nearly eliminated. Many other benthic and intertidal invertebrates are also vulnerable to oil pollution (Bejarano & Michel, 2016). Some are also food recourses for humans.

Amphipods and mysid shrimps are sensitive to oil pollution (Atlas, 1995b; Jewett et al., 1999). A toxicity study was conducted on marine amphipods by Randolph et al. (1998) 30 months after the Persian Gulf War Oil Spill in 1991. A clear connection between increased mortality amphipods and the concentration of hydrocarbon pollutants in the sediments was seen (Randolph et al., 1998). Infrared spectrometry tests were used to test for hydrocarbon pollution in the sediments, and by comparing these samples to samples of amphipods from the same locations it was found that concentrations of hydrocarbons above 1 mg/g dry sediment were toxic to the amphipods.

Corals, especially early stages of soft and hard coral species, are very sensitive to dispersed oil droplets (Epstein et al., 2000; Shafir et al., 2007). Coral reefs can take long time to regenerate after oil exposure. Corals in the area of the oil release from DWH were damaged and showed signs of stress after being covered in or exposed to dispersed oil and marine oil snow (Beyer et al., 2016). The sensitivity of corals to oil pollution is dependent on time of the year (due to reproduction and life stages of the corals), time and concentration of exposure, etc. (Shigenaka et al., 2010). Branching corals and corals in the early stages of life are the most sensitive to oil pollution.

Reptiles can also be adversely affected by oil pollution. Sand beaches are sensitive to oil washing up ashore, and beaches where turtles lay their eggs are very vulnerable (Bejarano & Michel, 2016; Schröder, 2010). Sea turtles can also be exposed to floating and dispersed oil and deaths have been reported (Antonio et al., 2011). Sea turtles, fish and mammals may be indirectly polluted by oil via the food chain. Phytoplankton and zooplankton among others exposed to oil may transport the pollution up in the food chain (M. Fingas, 2012).

2.4.4 Long-term consequences

The composition and type of oil as well as the environmental conditions determine how and if the oil is completely removed. Some oils will be almost or completely removed within a relatively short time, while others may persist for years or (almost) indefinitely (Atlas, 1995b). What may be left after severe weathering of the oil is a complex residue of a black tar made of the non-biodegradable compounds especially the asphaltenes. Typically, the residue is left in the sediments or at other environmentally unavailable places. It is assumed that the tar stays there without posing large toxic ecological effects. Petroleum hydrocarbons travel via the food chain, but most compounds rarely bioaccumulate in organisms (M. Fingas, 2012). Many petroleum hydrocarbons can be biodegraded as explained in Chapter 3. Despite the fact that many petroleum compounds are biodegraded the environmental effects of oil spills in the marine environment are not fully known.

3 Biodegradation in the marine environment

Biodegradation is the process where organic pollutants/materials are transformed or decomposed by biological organisms such as microorganisms, fungi, and plants. This process can involve the use of numerous different compounds as food (substrate) for the organisms, turning these compounds into less harmful ones (Al-Majed et al., 2012). Examples of substrate include petroleum hydrocarbons, making biodegradation an important natural cleanup process after an oil spill.

3.1 Biodegradation of petroleum hydrocarbons

Due to the natural seeping of crude oil in marine environments around the globe, and the presence of hydrocarbons from natural sources such as oil seeps, some microorganisms have evolved with the ability to degrade petroleum hydrocarbons (Madigan et al., 2019; Prince, 2010). These microorganisms are present in marine environments where there is no oil present in small numbers and in higher numbers at places with oil pollution or seeps (Atlas, 1995b; Harayama et al., 2004). There are numerous microorganisms capable of degrading hydrocarbons – hundreds of species of bacteria, archaea, and fungi. Degraders of alkanes, toluene, naphthalene and phenanthrene have often been isolated, especially from contaminated soil or sediments where they can exist in large numbers (Haritash & Kaushik, 2009). If petroleum hydrocarbons are not currently present it is assumed that the organisms utilize other carbon sources such as hydrocarbons produced by plants, algae or other living organisms, or carbohydrates, proteins, etc. depending on the type of organism and its preferences (Atlas, 1995b). A large increase growth of hydrocarbon-degrading bacteria is observed after a large sudden input of petroleum hydrocarbons in the marine environment from for instance an oil spill.

Most of the compounds found in crude oil are biodegradable under aerobic conditions (Madigan et al., 2019). Though anaerobic degradation of hydrocarbons can occur it is usually not the focus concerning petroleum hydrocarbon degradation as it does not contribute to considerably removal of oil as it is very slow (Madigan et al., 2019). The compounds are degraded differently and at different rates, depending on their size and structure as described below. Therefore, the type of crude oil is important with regards to the biodegradation potential. Many aromatic compounds are toxic but can be detoxified by bacteria and broken down completely into carbon dioxide, water, and cell biomass (Atlas, 1995b). The presence of oxygen is very important in the degradation process as it is used as a terminal electron acceptor. In addition, oxygenase enzymes are involved in the biodegradation process. These enzymes introduce oxygen to the hydrocarbons during aerobic biodegradation.

3.1.1 Biodegradation of alkanes

Most alkanes in crude oil are biodegradable under aerobic conditions. The lighter hydrocarbons are usually the easiest to degrade (Harayama et al., 1999; Kleindienst et al., 2015). However, some of the lightest and most volatile ones can be toxic to microorganisms (Atlas, 1981), but are often removed by rising and evaporation rapidly before becoming available to the microorganisms (Harayama et al., 1999). Alkanes with 10-26 carbon atoms are considered the easiest to degrade (Atlas, 1995b).

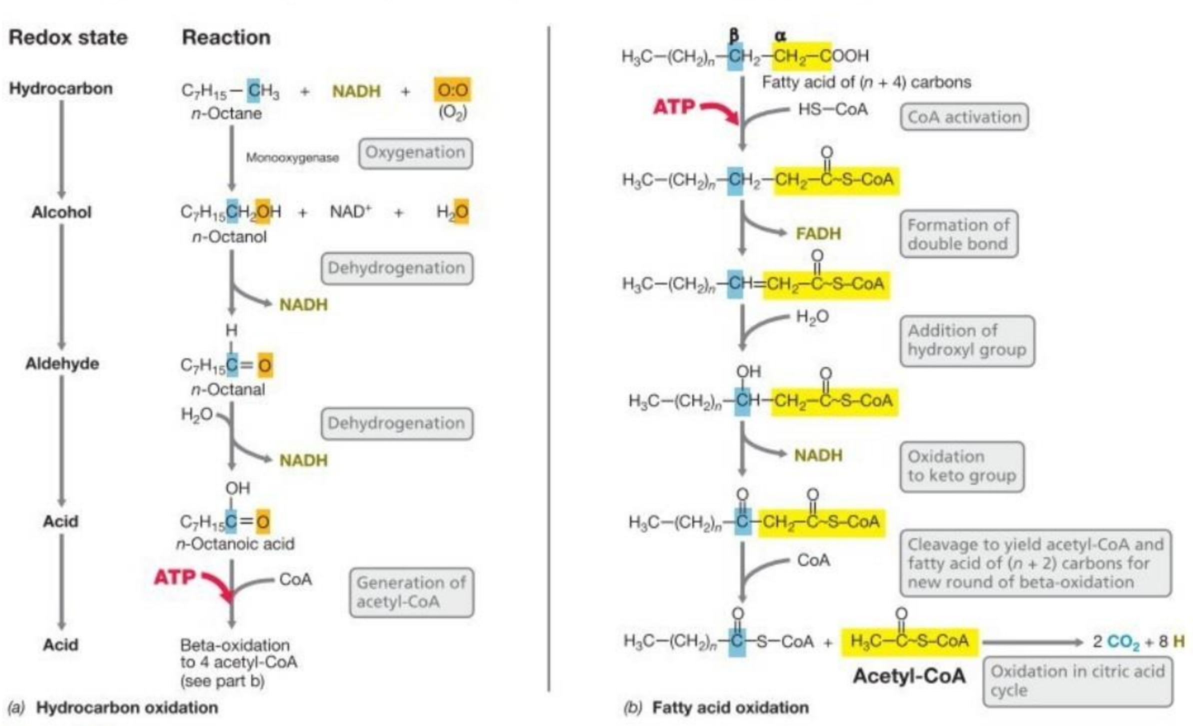

Figure 3.1. Pathway for the aerobic metabolism of an aliphatic hydrocarbon (n-octane). Obtained from (Madigan et al., 2019).

Figure 3.1 shows a suggested pathway (of the four possible) for hydrocarbon metabolism (Ji et al., 2013; Madigan et al., 2019). Oxygenase enzymes are valuable catalysists that incorporate O_2 into the hydrocarbon and are divided into two classes: *monooxygenase* enzymes like alkB and *dioxygenase* enzymes like Nah or nahA. A primary alcohol is typically formed as shown in Figure 3.1a is the first step in the hydrocarbon oxidation and involves incorporation of one oxygen atom from O_2 catalyzed by the *monooxygenase* enzyme to oxygenate the alkane into an alcohol (*n*-octanol) (Atlas, 1981; Madigan et al., 2019). Molecular oxygen is thus important (Atlas, 1995b). The alcohol is further dehydrogenated into an aldehyde and then to a fatty acid. Figure 3.1b shows the further oxidation of this fatty acid by beta-oxidation. Some fatty acids are toxic and can accumulate during this degradation process (Atlas, 1981).

3.1.2 Biodegradation of aromatic hydrocarbons

Many aromatic compounds are biodegradable and can be detoxified by bacteria (Atlas, 1995b). For instance, the BTEX compounds (benzene, toluene, ethylbenzene, and xylenes) are relatively easily degraded which is desirable due to their toxicity. Figure 3.2 shows steps for the catabolism of benzene and toluene.

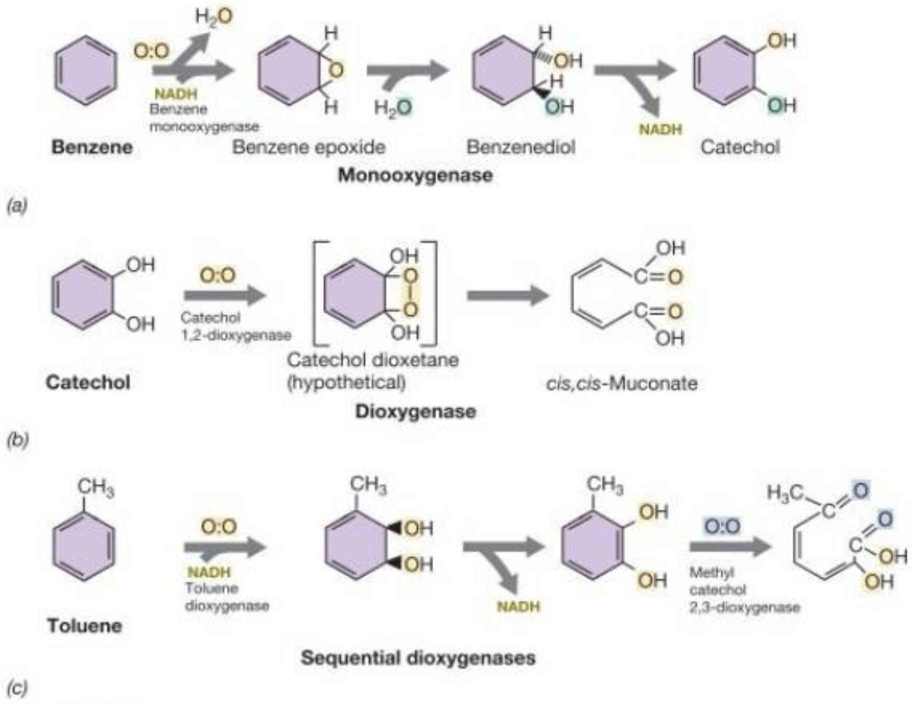

Figure 3.2. Steps in the catabolism of benzene and toluene catalyzed by monooxygenase and dioxygenase, respectively. Obtained from (Madigan et al., 2019).

As shown in Figure 3.2 monooxygenase and dioxygenase can catalyze the metabolism of benzene (a) and toluene (c), respectively (Madigan et al., 2019). Multiple steps are in the need of oxygenase enzymes. For benzene to be metabolized (a), it is here transformed into catechol after three steps, after forming a diol which is typical during bacterial degradation of aromatics (Atlas, 1981). A catechol derivative is also formed during the catabolism of toluene (c) as shown in the figure. The formation of catechol is common during the metabolism of many aromatic compounds. Catechol can be further cleaved (b) to form a diacid such as *cis*,*cis*-

muconate as shown in the figure (Atlas, 1981; Madigan et al., 2019). This compound is then degraded into *e.g.*, acetyl-CoA, such as for alkane catabolism shown in Figure 3.1, or succinate or pyruvate. These can be further oxidized in the citric acid cycle. The compounds are ultimately oxidized completely to CO_2.

Aromatic compounds made of two rings can also be biodegraded. Despite the toxicity associated with polycyclic aromatic hydrocarbons (PAHs), the ones made from up to three rings are theoretically able to be completely degraded by bacteria into biomass, CO_2 and H_2O (Atlas & Hazen, 2011). However polycyclic aromatic hydrocarbons containing more than three rings are very resistant to biodegradation and are therefore more likely to remain in the environment over time (Harayama et al., 1999).

The simplest PAH, naphthalene, is easily soluble and biodegradable and is often used as a basis to investigate degradation pathways of PAHs by bacteria (Figure 3.3) (Goyal & Zylstra, 1997; Seo et al., 2009). Many naphthalene-degrading bacteria have been isolated though *Pseudomonas* strains are typically used for laboratory testing of the bacterial degradation of naphthalene (Goyal & Zylstra, 1997). A suggested pathway for bacterial degradation of naphthalene is reviewed by Seo *et al*. and shown in Figure 3.3 (Seo et al., 2009). First, *cis*-1,2-dihydroxy-1,2-dihydronaphthalene is formed and dehydrogenated to 1,2-dihydroxynaphthalene. After several more steps this compound is metabolized to salicylate as shown in Figure 3.3. Salicylate is then decarboxylated to catechol for further metabolism via the catechol pathway (Figure 3.2).

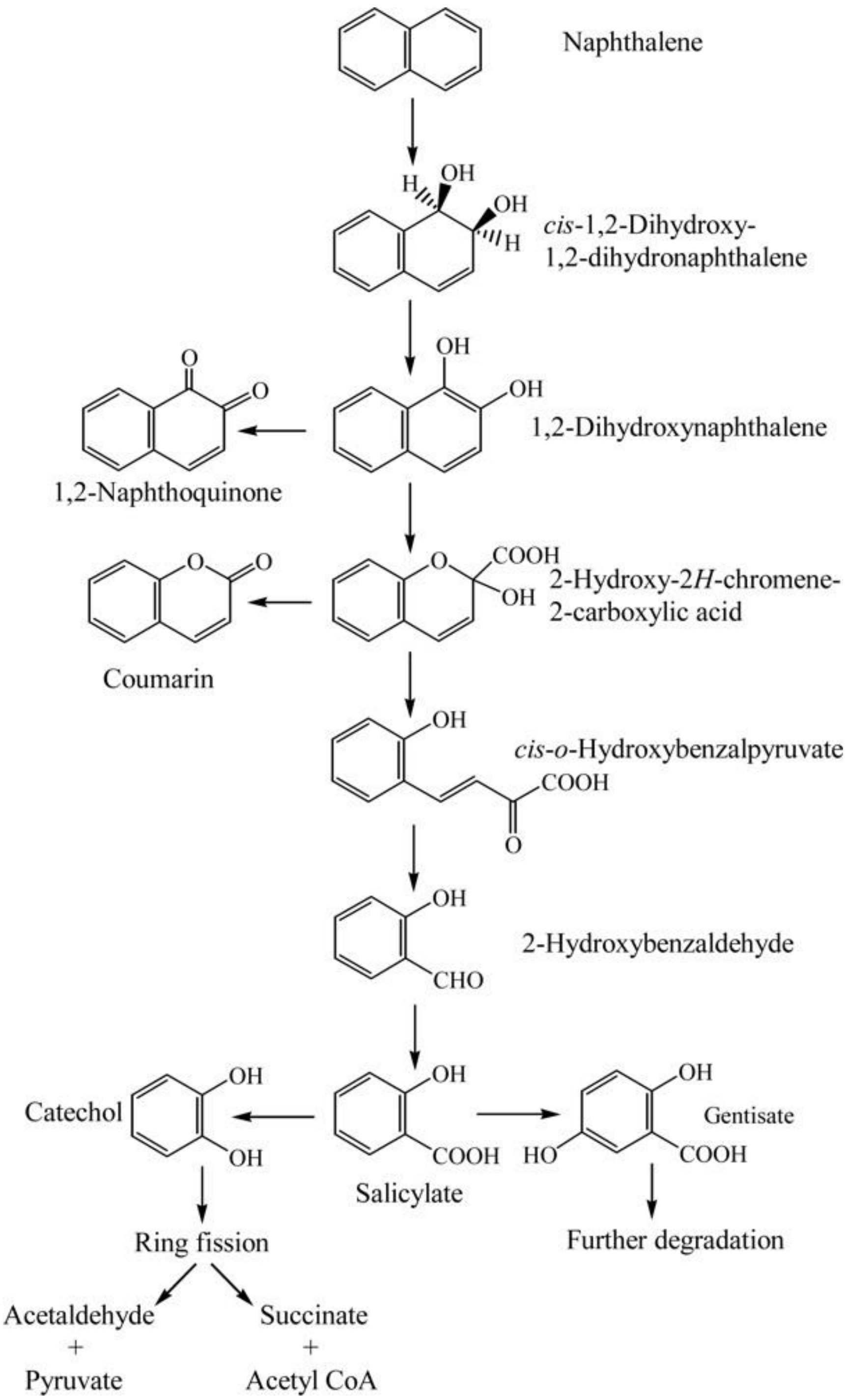

Figure 3.3. Suggested pathway for the bacterial degradation of the simplest PAH made from two fused benzene rings: naphthalene. Copied from (Seo et al., 2009).

3.1.3 Heavy compounds

The heavier hydrocarbons such as resins, polar molecules and asphaltenes are often not biodegradable, or if they degrade it is at a much slower rate (Atlas & Hazen, 2011; Haritash & Kaushik, 2009). The more complex and heavy the compound is, the more difficult it is for microorganisms to degrade it (Atlas, 1995b). Nzila and Musa reviewed the bacterial biodegradation of asphaltenes and resins from oil and found no indications for the complete mineralization of these compounds (Nzila & Musa, 2021). Parts of the compounds were assumed to be degraded, but large complex and recalcitrant compounds remain in the environment. In addition, the heavy compounds respond poorly to natural and enhanced dispersion (M. Fingas, 2012).

3.1.4 Anaerobic biodegradation

In the mud or sediment and other places where oxygen might not be present, anaerobic degradation of the petroleum hydrocarbons can occur. Anaerobic degradation occurs at a much slower rate than the aerobic degradation (Atlas, 1995b; Kleindienst et al., 2015; Madigan et al., 2019). Sulfate-reducing bacteria can degrade petroleum hydrocarbons if enough sulfate (SO_4^{2-}) is present, or denitrifying bacteria using nitrate (NO_3^-) as electron acceptor can degrade the petroleum hydrocarbons. Sulfate-reducing bacteria are mainly responsible of anoxic biodegradation of hydrocarbons in marine sediments (Kleindienst et al., 2015). Anaerobic biodegradation overall contributes negligibly to the removal of the oil due to its slow rate (Atlas, 1995b). Despite this Harayama *et al*. proposed that there should be more research on the anaerobic biodegradation of petroleum hydrocarbons as less human involvement is needed for microbial cleanup (Harayama et al., 2004).

3.2 Oil-degrading organisms

Many microorganisms can degrade petroleum hydrocarbons and they are broadly distributed in the marine environments around the globe (Q. Liu et al., 2015). Bacteria, archaea and fungi (and even some algae) can degrade petroleum hydrocarbons aerobically, some examples include the hydrocarbon-degrading yeast *Yarrowia lipolytica* isolated from the Persian Gulf (Hassanshahian et al., 2012) and phenanthrene-degrading yeast *Rhodotorula glutinis* (Romero et al., 1998). Bacteria are though believed to be the best oil-degraders in marine environments (Atlas, 1995b). More than 75 species of bacteria can grow on hydrocarbons and many more can degrade hydrocarbons, though not as sole energy and carbon source but in need of other substances to grow (Q. Liu et al., 2015). Several more species of oil-degraders are assumed to exist and as of yet remain undiscovered (Harayama et al., 2004).

3.2.1 Oil-degrading bacteria

Bacteria are the organisms mainly responsible for the biodegradation of petroleum hydrocarbons (Atlas, 1995b). There exist bacteria that can degrade hydrocarbons as well as other substrates such as carbohydrates, and bacteria that are specialized hydrocarbon degraders. Specialized hydrocarbon degraders are called hydrocarbonoclastic bacteria (Harayama et al., 2004). There also exists *obligate* hydrocarbonoclastic bacteria that are vitally dependent on hydrocarbons and do not use other carbon sources, however they are rare (Atlas, 1995b). Some bacteria are specialized in degrading alkanes, like the bacterium *Alcanivorax borkumensis* (Table 3.1) that is unable to degrade other carbon sources than alkanes, fatty acids and pyruvate (Madigan et al., 2019; Schneiker et al., 2006; Yakimov et al., 1998). There are others that are specialized as degraders of aromatics like toluene, naphthalene and phenanthrene (*e.g. Cycloclasticus*) (Dyksterhouse et al., 1995). Other examples of isolated bacteria that can degrade phenanthrene or chrysene include *Vibrio*, *Pseudoalteromonas*, *Marinomonas* and *Halomonas* (Melcher et al., 2002).

Table 3.1 shows a selection of petroleum hydrocarbon-degrading bacteria, the location of the collection, and examples or preferences of substrate they can degrade and possibly grow on (Bruns & Berthe-Corti, 1999; Dyksterhouse et al., 1995; Fernández-Martínez et al., 2003; Gauthier et al., 1992; C. Liu & Shao, 2005; Ortega-González et al., 2015; Peralta et al., 2022; Romero et al., 1998; Schneiker et al., 2006; Yakimov et al., 1998). *Alcanivorax* species dominate the table as they are the most common oil-degrading bacterial genus. Therefore the most attention is often given to *Alcanivorax borkumensis* (Yakimov et al., 1998). These bacteria are valuable oil-degraders that produce their own surfactant chemicals that facilitate dispersion of the oil, making it more bioavailable. Bacteria can have various properties or specialties. Marine salts are required for growth for the bacterium *Alcanivorax venustensis* (Fernández-Martínez et al., 2003). *Marinobacter hydrocarbonoclasticus* also need salt, they have an extreme halo tolerance (obligate halophiles) and require high salt concentrations (Gauthier et al., 1992).

Table 3.1. Some selected species of hydrocarbon degrading bacteria

Bacterial species	**Location collected**	**Substrate**	**Reference(s)**
Alcanivorax borkumensis	North Sea (Ise of Borkum)	Aliphatic hydrocarbons (and fatty acids, pyruvate)	(Schneiker et al., 2006; Yakimov et al., 1998)
Alcanivorax dieselolei	China	Alkanes	(C. Liu & Shao, 2005)
Alcanivorax jadensis	German North Sea coast /Mediterranean Sea (Alicante, Spain)	Hexadecane	(Bruns & Berthe-Corti, 1999; Fernández-Martínez et al., 2003)
Alcanivorax venustensis	Mediterranean Sea (Alicante, Spain)	Organic acids and alkanes	(Fernández-Martínez et al., 2003)
Amycolatopsis sp. (Poz14)	Veracruz, Mexico	PAHs	(Ortega-González et al., 2015; Peralta et al., 2022)
Cycloclasticus	Washington, United States	Aromatics, PAHs	(Dyksterhouse et al., 1995)
Gordonia sp. (Poz20)	Veracruz, Mexico	PAHs	(Ortega-González et al., 2015)
Marinobacter hydrocarbonoclasticus	Gulf of Fos, French Mediterranean coast	Aromatic and aliphatic hydrocarbons	(Gauthier et al., 1992)
Pseudomonas aeruginosa	Argentina	Phenanthrene	(Romero et al., 1998)
Rhodocuccus sp. (Poz54)	Veracruz, Mexico	PAHs	(Ortega-González et al., 2015)

3.2.2 Genes for hydrocarbon degradation

For a microorganism to be able to degrade petroleum hydrocarbons, they must have genes required for the metabolism of hydrocarbons. Hydrocarbon degrading genes are often located on a plasmid within the bacteria (Atlas, 1995b). Two hydrocarbon-degrading genes for the metabolisms of petroleum hydrocarbons are alkane monooxygenase (*alkB*) and naphthalene dioxygenase (*Nah* or *nahA*) (Q. Liu et al., 2015). *AlkB* encodes a gene for the metabolism of alkanes (van Beilen & Funhoff, 2007). While *Nah* is a gene used for the metabolism of aromatics such as naphthalene. The genes are involved in the first step of the hydrocarbon metabolism, encoding for the enzymes involved in the degradation (Figure 3.1, Figure 3.2 and Figure 3.3). When microbes have these hydrocarbon-degrading genes and are present at a polluted site multiple factors can further affect the rate of the petroleum biodegradation.

3.3 Factors affecting biodegradation

The potential and rate of petroleum hydrocarbon biodegradation is reliant on several other factors other than just presence of the oil-degrading organisms near the site of oil contamination. Biodegradation may continue for years after an oil spill, or can, under favorable conditions be accomplished within a matter of days (Atlas, 1981; Schröder, 2010). The rate is thus dependent on several factors and can therefore help to determine the environmental consequences of an oil spill. It can be important to understand these factors that can facilitate or impede the biodegradation rate.

Crucial environmental factors that affect the biodegradation of oil are highlighted below and include temperature, mixing, bioavailability and nutrient availability (Atlas, 1981). Other factors that affect the biodegradation of petroleum hydrocarbons include pre-spill microbial population types and sizes, structure and concentration of the substrate, pH, salinity, pressure, oxygen, water availability, and cellular transport properties (Atlas, 1981; Haritash & Kaushik, 2009).

3.3.1 Temperature

Temperature is an important factor affecting biodegradation. High temperatures stimulate bacterial activity in general, increasing the biodegradation rates by increasing the enzymatic reactions (Atlas, 1981; Madigan et al., 2019). This is also true for petroleum hydrocarbon biodegradation. If the temperature is too high or too low, it prevents the growth of microorganisms or it can kill them. These temperature ranges depend on the species in question.

Each species has their own cardinal temperatures; minimum, optimum and maximum temperatures for growth.

Temperature changes also affect the state of the oil (Schröder, 2010). A temperature increase makes the petroleum hydrocarbons more soluble in the water. It also makes the hydrocarbons evaporate easier. Both outcomes can change the types of hydrocarbons that are present in the water available to the hydrocarbon degrading microorganisms. As different compounds are degraded at different rates, this can affect the biodegradation rate. The biodegradation rate of the spilled oil from the Persian Gulf oil spill was assumed to be relatively high due to the favorable climate with high temperatures, though much of the oil was removed by evaporation (Randolph et al., 1998).

The viscosity of oil is also affected by temperature. Low temperature is associated with higher viscosity. Higher viscosity oil spreads to a lesser extent, making the oil less available to the microorganisms in the soil and water (Whyte et al., 1998). Furthermore, some alkanes form crystals at low temperatures (below 0 – 5 °C), decreasing their bioavailability which again leads to a reduction in the biodegradation rate.

3.3.2 Mixing

Mixing of oil into the water column makes the substrates physically more available for the microbes, hence can contribute to enhancing biodegradation. Mixing and breaking waves can also disperse the oil droplets, making the surface area larger. This interface between the water and oil is where the biodegradation happens (Atlas & Hazen, 2011), and by increasing this, the bioavailability of the substrate is increased. Nutrients including oxygen are also more spread out by the mixing, becoming more available for the microbes that need them for their growth. Especially aeration can be important (Atlas, 1981). Consequently, an increase in the biodegradation rate can be seen.

3.3.3 Bioavailability

Bioavailability refers to the availability of substrate for biodegradation by microorganisms. This is very important for the success of pollutant biodegradation such as petroleum hydrocarbons. Figure 3.4 shows how bacteria are located at the interface between the oil droplet and aqueous environment and not inside of the oil droplet (Madigan et al., 2019). When oil forms a slick at the water surface, biodegradation happens around this, as the microorganisms are located in the water phase. Dispersing the oil into droplets makes the interface areas larger and hence the substrate more bioavailable (Tremblay et al., 2017). Physical dispersing by harsh weather conditions can contribute to this dispersing of oil, so can certain microorganisms as well as chemical dispersants applied by humans. One aim of dispersants as an oil spill response is thus to enhance bioavailability and thereby enhance natural biodegradation, and this is reviewed in more detail in Chapter 4.

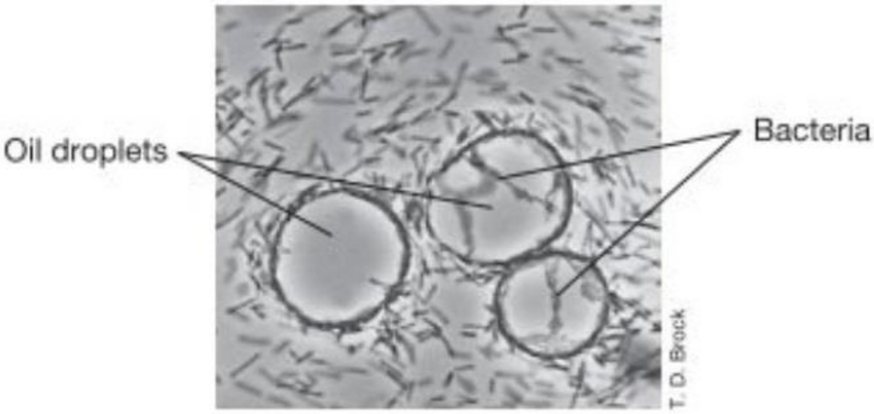

Figure 3.4. Oil droplets surrounded by oil-degrading bacteria. Obtained from (Madigan et al. 2019).

3.3.4 Nutrient availability

Nutrient availability refers to the access of nutrients needed by the microorganisms for incorporation into cellular biomass during aerobic biodegradation of petroleum hydrocarbons (Atlas, 1995b). An oil spill supplies the site with an abrupt large supply of petroleum hydrocarbons, leading to a growth in the population of hydrocarbon-degrading microorganisms, especially bacteria (Harayama et al., 2004). As the oil-degrading microorganisms often need nutrients other than carbon for growth, these can be depleted due to the sudden growth. This depletion is associated with a decrease in the biodegradation rate.

The two most commonly limiting nutrients are nitrogen and phosphorus (Atlas, 1995b; Harayama et al., 2004). Molecular oxygen and iron as well as some other mineral nutrients may also be limiting (Atlas, 1981; Ollivier & Magot, 2005). However, marine environments are often well enough aerated so that oxygen is not limiting (Atlas, 1995b). The main limiting factor of oil biodegradation in marine environments is therefore the absence phosphorus and nitrogen (Atlas, 1995b). Sediments and mud are typically places that can be oxygen depleted, making anaerobic biodegradation the only alternative (Atlas, 1995b). To increase the rate of degradation in areas with the nitrogen and phosphorus depletion, various fertilizers are available for remediating polluted areas as explained in Chapter 3.5.

3.4 Bacterial adaption to aid biodegradation

Dorobantu *et al*. (2004) suggested that hydrophobic bacteria can stabilize oil-water emulsions (Figure 3.5) (Dorobantu et al., 2004). They attempted to coalescence two oil-in-water emulsions. There were bacteria at the oil-water interface and the droplets did not coalescence, indicating that the bacteria stabilized the emulsions. This stabilization is connected to the contact angle of the bacteria to the oil-water interface such as for fine particles (Levine et al., 1989). A intermediate contact angle was associated with the highest stability of the oil-water emulsions (Dorobantu et al., 2004). This resistance of coalescence is also a consequence of the fact that bacteria have an affinity for each other and therefore stabilize the oil-water interface by adhering to each other. Hydrophilic bacteria did not stabilize the oil-water emulsions in the tests that were taken. Hydrophobic bacteria stabilized the emulsions for a while, but the most stable ones were formed by the intermediate hydrophobic bacteria throughout this experiment.

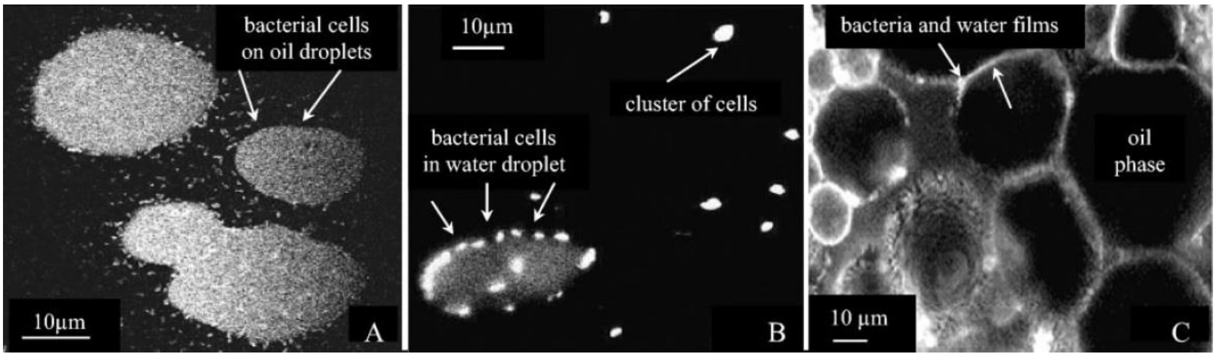

Figure 3.5. Confocal micrographs of emulsions surrounded by bacteria, stained with fluorescein. (A) Hexadecane droplets surrounded by fluorescent bacteria. (B) A water-in-oil emulsion to the left. The cluster of cells to the upper right refers to a thin water film with clusters of bacteria inside. (C) Oil-in-water emulsions gathered as an "emulsion gel", where the black parts are hexadecane drops and the surrounding films are water and bacteria. Copied from (Dorobantu et al., 2004).

Figure 3.5 shows three confocal micrographs taken by Dorobantu *et al*. showing emulsions stabilized by bacteria. Emulsions were prepared in a graduated cylinder for gravitational separation. Stable oil-in-water emulsions taken from the middle of the cylinder is shown in Figure 3.5A. The top layer in the cylinder had water-in-oil emulsions and clusters of bacterial cells surrounded by thin water films (Figure 3.5B). While 80% of the total volume in the cylinder was taken up by what is shown in Figure 3.5C, an "emulsion gel". It is suggested that the emulsion gel is a consequence of strong cell-cell interactions but also durable adherence between cells and oil droplets (Dorobantu et al., 2004). The bacterial strain *Acinetobacter* *venetianus* RAG-1, a degrader of *n*-alkanes (Vaneechoutte et al., 1999) is the bacteria helping with this stabilization seen in Figure 3.5C, after mixing the bacteria with *n*-hexadecane.

3.5 Bioremediation

Bioremediation is a process with the intention to help to improve natural biodegradation rates to clean up a polluted area, such as an oil spill site (Atlas, 1981; Ollivier & Magot, 2005). It can be performed by adding exogenous microbial populations to a site (bioaugmentation or seeding), or by helping to stimulate existing microbes by fertilizer addition (biostimulation), both with the aim to enhance natural biodegradation of oil by microorganisms (Al-Majed et al., 2012). The latter one, biostimulation, is the most applied and successful method of bioremediation, treating shoreline oiling after an oil spill.

3.5.1 Biostimulation

Rates of oil biodegradation may be limited if necessary nutrients are unavailable, especially nitrogen and phosphorus, as discussed above (Atlas, 1995b). Therefore, biostimulation by nutrient addition (with application of fertilizer) as an oil spill response strategy may be essential to stimulate the growth to increase the biodegradation rate of the oil. This was of great importance after the *Exxon Valdez* accidental oil spillage in 1989 where large amounts of nitrogen-containing oleophilic fertilizer were used on the stranded oil at the shorelines, increasing the biodegradation rate by an estimated 3-5 times (Atlas, 1995b; Bragg et al., 1994; Prince et al., 1990).

The effectiveness of *in-situ* treatment of an oil spill by biostimulation can, however, be difficult to know. Some places indications may be visible by comparing treated and untreated places near each other, to directly observe the removal of oil by color change from black to white depending on the ground type. Also, oil samples can be taken at to compare treated and untreated areas at different times. The compounds in the oil can be measured against each other to check the ratio of biodegradable vs non-biodegradable (or slowly biodegradable) compounds, such as pristane, hopanes or phytane (as internal recovery standards that resist biodegradation) against *n*-alkanes (Atlas, 1995a).

Both nitrogen- and phosphorus-containing fertilizers are easy to apply and have been proven to speed up biodegradation rates of petroleum hydrocarbons (Atlas, 1995a; Harayama et al., 2004; Madigan et al., 2019). Oil-spill cleanup is the most applied and successful type of bioremediation, as missing nutrients are a central factor that decreases biodegradation rates. Fertilizer addition is also a relatively inexpensive cleanup method and considerations must be taken as too much fertilizer may lead to eutrophication and algal bloom.

3.5.2 Bioaugmentation

Bioaugmentation using bacteria with the desired hydrocarbon-degrading abilities is also a method to stimulate natural petroleum hydrocarbon removal. Hydrocarbon-degrading microorganisms often have an affinity to degrade certain types of compounds. If the area of oil pollution lacks microorganisms that can degrade a wide range of petroleum hydrocarbons, bioaugmentation can be considered to increase biodegradation rates or extents (Atlas, 1995a). Ra *et al*, 2019 performed a study revealing that some microbes taken from non-contaminated soil have greater oil-degrading potential than those taken from the oil-contaminated area (Ra et al., 2019). Biostimulation simultaneously can be important as nutrient might also be limiting (Whyte et al., 1998). However, there are conflicting results with use of bioaugmentation (Atlas, 1995a). Microorganisms which are not naturally present in an area are often not perfectly acclimatized to the temperatures or other conditions and not adapted like the indigenous bacteria or microorganisms (M. Fingas, 2012). They are therefore not likely to be able to compete with the indigenous microorganisms and will often be outcompeted and not grow. In addition, the seed cultures must be added at a very early stage to succeed. From these reasons, bioaugmentation may not always be successful.

4 Chemical dispersants

The use of dispersants, or dispersing agents can be used to aid cleanup of oil. It has been used on various oil spills incidents (Harris, 1995; Jerneloev & Linden, 1981; Law & Kelly, 2004; O'Sullivan & Richardson, 1967), though particular attention has been given to the DWH well blowout incident due to the massive use of Corexit (Beyer et al., 2016; Lehr et al., 2010). After an oil spill in the marine environment, the oil typically gathers as an oil slick on the water surface. The components of oil are insoluble in water, because of the low density compared to seawater it mostly floats on the sea surface. To minimize environmental consequences associated with a large oil spill, various cleanup methods can be carried out, some of which are reviewed in Chapter 2.2. Recapturing of oil is often the preferred method but not always achievable, therefore dispersion of the oil is considered (Prince, 2015).

Chemical dispersants are surfactant-based mixtures of chemicals that serve to help disperse oil slicks at sea, to move the oil from the water surface into the water column (Atlas & Hazen, 2011; Stroski et al., 2019). The main goals are to protect seabirds, mammals, and sensitive shoreline area ecosystems and to enhance removal of oil by other weathering processes especially biodegradation. Moving the oil into the water column increases the bioavailability of the hydrocarbons as they become more accessible for the oil-degrading microorganisms. This can enhance biodegradation, but it also prevents the formation of persistent water-in-oil mousse. Other weathering processes such as dissolution and sedimentation of oil are also increased by dispersion of oil into the water column (Schröder, 2010). Natural dispersion happens to some extent, depending on the weather conditions. However, due to the large amounts of oil which occur during an oil spill, the addition of a dispersant can be desirable to help increase dispersion, especially when a sensitive shoreline ecosystem is threatened (M. Fingas, 2012). Consequently, moving the oil into the water column can directly and indirectly through enhanced biodegradation and other weathering processes result in less shoreline pollution.

Oil biodegradation occurs at the oil-water interface as shown in Figure 3.4 (Atlas & Hazen, 2011; Madigan et al., 2019). The bacteria (or other oil-degrading microorganisms) are not located inside of the oil droplet as they are dependent on water. The oil is therefore degraded at the area between the oil and water phases making the oil water interface extremely important for oil biodegradation. To enhance the biodegradation, it is desirable to increase the area of the interface. This area is increased significantly by dispersing the oil into small droplets (Barnes

& Gentle, 2011; Dorobantu et al., 2004). Consequently, the bioavailability of the hydrocarbons is increased. This can lead to an increase in the biodegradation rate if conditions are otherwise favorable for biodegradation.

As most other oil spill response methods, there are advantages and drawbacks to the use of dispersants. When sprayed from the air, dispersants are able to treat larger areas than many other oil spill response methods (Al-Majed et al., 2012). Dispersants can also be applied by boats and even subsea as the case after the DWH oil spill in 2010 (Lehr et al., 2010). Oil dispersed into the water column is removed much faster by different weathering processes. Laboratory and wave-tank tests have shown positive results of the use of oil dispersants (Xuan & Bing, 2017). Correct usage can prevent a large proportion of the oil from reaching sensitive shoreline areas. These shoreline areas are associated with persistence of oil for many years (Prince, 2015).

On the other hand, many dispersants have been found to be toxic especially to aquatic life, and some even make the situation worse by also increasing the toxicity or availability of the toxic compounds in the oil (Harayama et al., 1999; O’Sullivan & Richardson, 1967; Place et al., 2010; Shafir et al., 2007; Swedmark et al., 1973). Also, the “window-of-opportunity” for successful dispersant application is quite short and loss of efficiency is seen if the oil weathers too much beforehand (NRC, 2005). They do not work well for viscous oils. Real life environmental conditions are difficult to re-create in laboratories for tests. The effects of dispersants at sea can be difficult to measure as it is unknown how the situation would have been without the dispersant use, especially the biodegradation rates. In addition, little is known about the long-term effects of oil sedimentation at the sea bottom (M. Fingas, 2011a).

4.1 Composition and types of oil dispersants

Chemical dispersants are generally composed of different surfactants that are partially soluble in both oil and water, with one hydrophobic (or lipophilic) and one hydrophilic side, making up an amphipathic molecule as shown in Figure 4.1 (Al-Majed et al., 2012; Lewis et al., 2010; NRC, 2005). The most important part of the dispersant is the surfactant or also called surface-active agents, but they typically also contain solvents and other additives. The characteristics of these surfactants make it possible to partial dissolve hydrophobic compounds such as oil into water by decreasing the surface tension between the oil and water (or the interfacial tension). Combining the surfactant properties with the natural mechanical mixing of wave-motion, parts of the oil slick are changed into smaller droplets and entrained into the water column as described below.

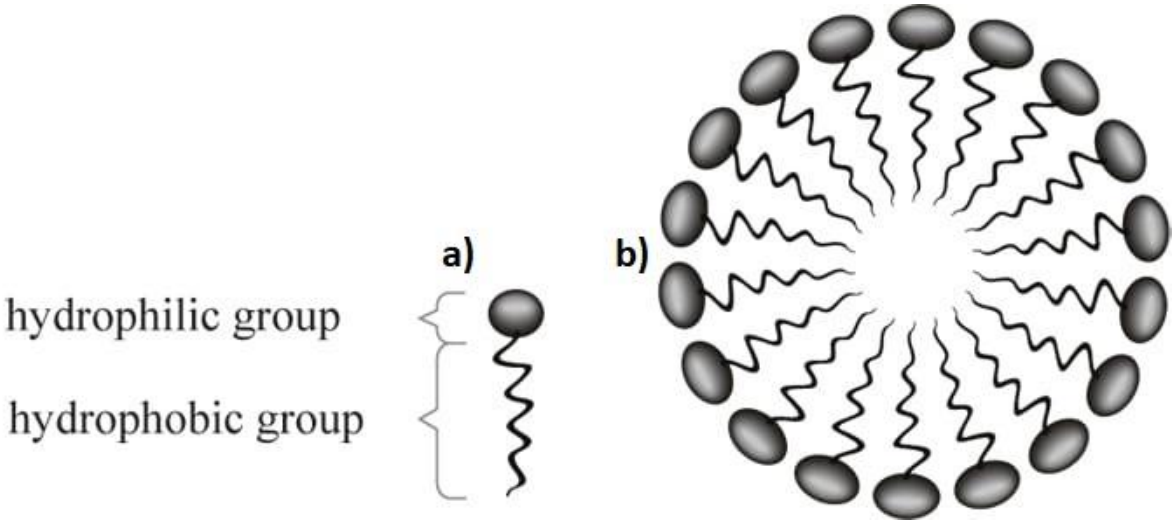

Figure 4.1. (a) Schematic explanation of a surfactant molecule with its two sides; hydrophilic group and lipophilic/hydrophobic group. And (b) a micelle formation. This is connected to how a surfactant molecule works on an oil droplet. Obtained from (Gusev et al., 2009).

Figure 4.1 shows a scheme of how a surfactant molecule (a) is containing two sides, or groups, with different properties, and how these groups work to form a micelle (b), which typically ranges in size from an average of 1-100 nm (Gusev et al., 2009; NRC, 2005). The hydrophilic, “water-loving” group illustrated by a circle is associated with dissolution in the water phase. While the lipophilic or hydrophobic, “oil-loving” or water repellent side stays in the oil phase. Figure 4.1b illustrates how these molecules move and work to form a micelle. It is surrounded by water, and oil inside, and a surfactant-coated oil droplet is formed. The lipophilic group points into the oil phase while the hydrophilic group stays in the water phase. The result is an oil droplet in water, or oil-in-water emulsion. For a water-in-oil emulsion however, the opposite can be seen. Thus, the hydrophobic tails are pointing outwards and the hydrophilic heads into the water droplet. The strength of the hydrophilic group or hydrophobic group may differ depending on the surfactant molecule, determining if it is most soluble in the water or oil phase.

Figure 4.2 illustrates how it may look when these surfactant chemicals stabilize an oil droplet. Modern dispersants typically contain more than one type of surfactant, and the properties of the surfactants can determine whether it prefers to form an oil-in-water emulsion or the opposite, a water-in-oil emulsion or mousse (NRC, 2005). A parameter which determines these different solubility properties is the hydrophilic-lipophilic balance (HLB) (Becher, 1977). The surfactants can thus have a stronger hydrophobic side and therefore tend to be more oriented in the oil phase (low HLB, compound A in Figure 4.2), or conversely have a stronger hydrophilic side (High HLB, compound B in Figure 4.2) (Porter, 1991). HLB values usually range from 0-20, from no hydrophobic group (HLB of 0) to no hydrophilic group (HLB of 20) (NRC, 2005). Surfactants with HLB value between 1-8 (low HLB) are associated with dissolution into the oil phase and the formation of water-in-oil emulsions. Whereas high HLB values of between 8-12 are associated with dissolution into the aqueous phase. Higher HLB values have also been reported, facilitating the formation of more stable oil-in-water emulsions (Becher, 1977; Porter, 1991). The overall HLB value of a dispersant is typically between 9-11. This value is important for the performance of the dispersant (M. Fingas et al., 2005). Dispersants intended for use at sea are often constructed so they work best in the average salinity of seawater (33-35 ppt) (Blondina et al., 1999).

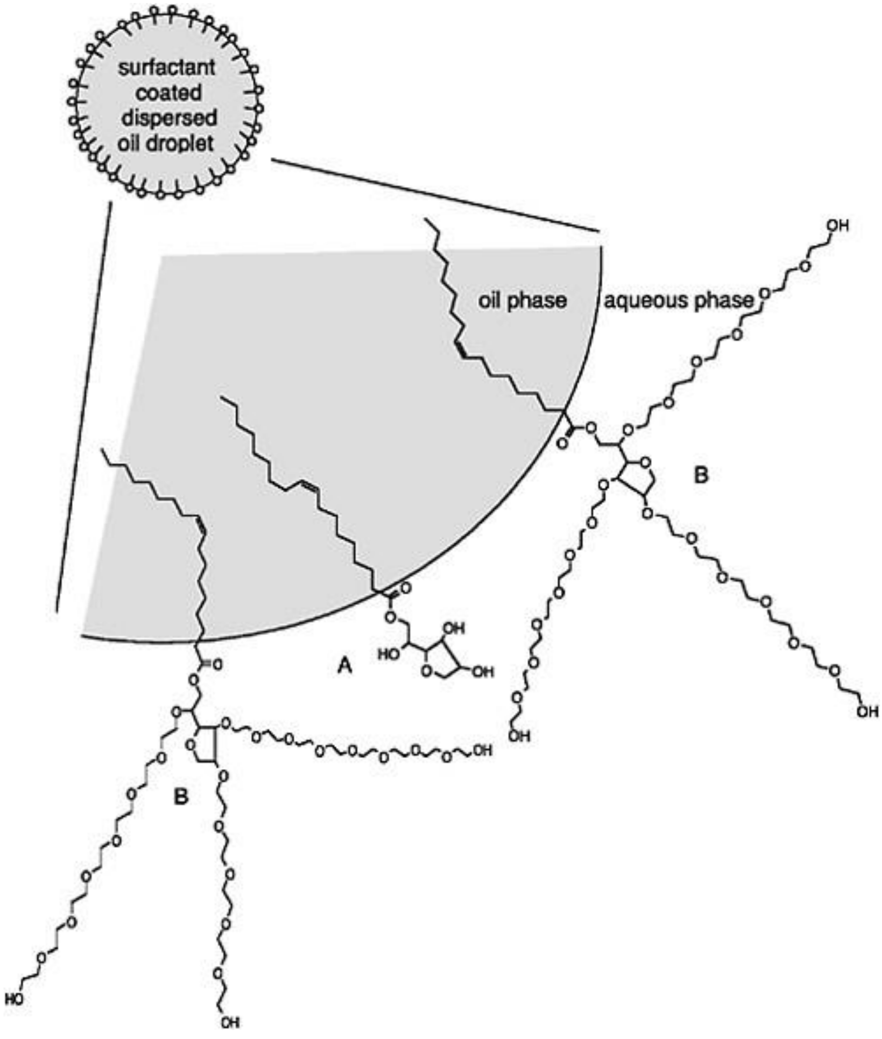

Figure 4.2. A close-up of a surfactant-coated dispersed oil droplet proposing the orientation of the surfactants at the oil-water interface. It shows two types of surfactants (A: Span 80, B: Tween 80) which combined favor the formation of oil droplets dispersed into the water. Copied from (NRC, 2005).

A dispersant is usually made of two or more surfactants with varying HLB values, comprising predominantly hydrophilic surfactants to avoid water-in-oil/mousse formation (NRC, 2005). Surfactant B (Tween 80) shown in Figure 4.2 is such a predominantly hydrophilic compound (HLB value of 15) while surfactant A (sorbitan monooleate, HLB value of 4.3) is predominately lipophilic. The dispersant mixture shown in the figure has more of compound B than A, leading to the formation of stable oil-in-water droplets as it favors this over water-in-oil emulsions.

In addition to the surfactants a dispersant also contains solvents and additives (NRC, 2005). The purpose of the solvents is to dissolve the surfactants into the dispersant mixture, making it homogenous and stabilizing the consistency of the mixture. Solvents also reduce the viscosity of the dispersant mixture which can be desirable for application. Some dispersants are pre-mixed with water for easier application. In this case the solvents in the dispersant must help to dissolve the water in the mixture as well. Dispersants with dissolved water do not work well in cold climates as they may freeze during application (NRC, 2005). Additives may or may not be present in a dispersant mixture and have purposes such as long-term stability or easier dissolution of dispersant into an oil slick. Different dispersants have been used for oil spill cleanup over the years with varying results and consequences. Examples of dispersants include Corexit® 9500 and 9527, Dispersit, Dasic Slickgone, Finasol® OSR 52, Inipol IP-90, Petrotech PTI-25, Bioreico R-93, Biosolve and Emulgal C-100 (Committee on the Evaluation of the Use of Chemical Dispersants in Oil Spill Response et al., 2020; Epstein et al., 2000). Corexit products are the most well-known commercially available oil dispersants.

4.1.1 Corexit®

The Nalco Company produces a line of oil dispersants with the brand name Corexit®. The most used one in the marine environment is Corexit EC9500A and is also the one mainly used during the DWH oil spill cleanup in 2010, followed by Corexit EC9527A (EPA, 1995a; Lehr et al., 2010). More information on the composition of Corexit 9500 and 9527 was made available after the DWH oil spill in 2010, due to the large amounts used during the cleanup and the following massive media coverage and pressure from EPA because of toxicity concerns. Prior to this, some of the ingredients and most importantly some of the surface acting agents were confidential (EPA, 1995a, 1995b).

The main surfactant ingredient in the two dispersant types (Corexit 9527 and 9500) is dioctyl sodium sulfosuccinate (DOSS) (Beyer et al., 2016; Place et al., 2010). It has been measured and estimated that Corexit 9527 has 17% DOSS while Corexit 9500 has 10% DOSS (by weight) (Kujawinski et al., 2011). The solvents used in Corexit 9527 are water, propylene glycol and 2-butoxyethanol (EPA, 1995b; Nalco Company/Product Safety Department, 2010). Propylene glycol and 2-butoxyethanol are characterized as hazardous substances in the safety data sheet. Corexit 9527 was developed first. Corexit 9500 was developed as its improved and less toxic follow-up. As Corexit 9500 does not contain 2-butoxyethanol and has an improved oleophilic solvent system compared to its predecessor, consisting of aliphatic hydrocarbons ranging from nonane to hexadecane (Gray et al., 2014). The solvent 2-butoxyethanol was removed as it indicated adverse health effects to humans in kidneys and livers from prolonged exposure (NRC, 2005). Also, it is given that no additives are included in the Corexit 9500 formula (EPA, 1995a). Other surfactant components in Corexit 9500 include two sorbitan compounds known as Span 80 and Tween 80 (HLB of 15) (Figure 4.2) (Varadaraj et al., 1995). Span 80 is sorbitan mono-oleate and has a HLB value of 4.3. Tween 80 is ethoxylated (E_{20}) sorbitan mono-oleate and has a HLB value of 15. Both Span 80 and Tween 80 are relatively easily biodegraded while DOSS takes longer to degrade and has a lag-period before being biodegraded (Brakstad, Størseth, et al., 2018; Gofstein et al., 2020).

In addition to its lower toxicity, Corexit 9500 has shown to be more efficient at dispersing higher viscosity oils than its predecessor. Because of this, it has a longer "window of opportunity" for successful dispersant application than Corexit 9527. Both dispersants work best in salt water and show limited to zero effects when the salinity decreases towards 0. However, Corexit 9500 performs better over a wider range of salinities than Corexit 9527 does (Blondina et al., 1999). Corexit 9527 showed an increase in performance with increasing salinities with the greatest efficiency at a salinity of 35 ppt. Other types of dispersants from this company include Corexit EC9500B and Corexit EC9580A. The latter one is also called a cleaner and it specializes on the cleanup of freshwater and seawater shorelines such as mangroves, marshes, rivers among others (COREXIT®, 2022).

4.2 Dispersion of oil at sea

When an oil slick is dispersed naturally or with the help of chemical dispersants at sea, oil-in-water droplets of different sizes are formed, as shown in the scheme in Figure 4.3 (Lewis et al., 2010). Physical movement such as breaking waves are needed to split the oil slick at the sea surface and are needed for the chemical dispersants to work ideally. Depending on the size of the droplets that are formed, turbulence and currents in the water, some oil droplets can float back up to the water surface, while others stay in suspension as dispersed oil-in-water droplets or emulsions (Prince, 2015).

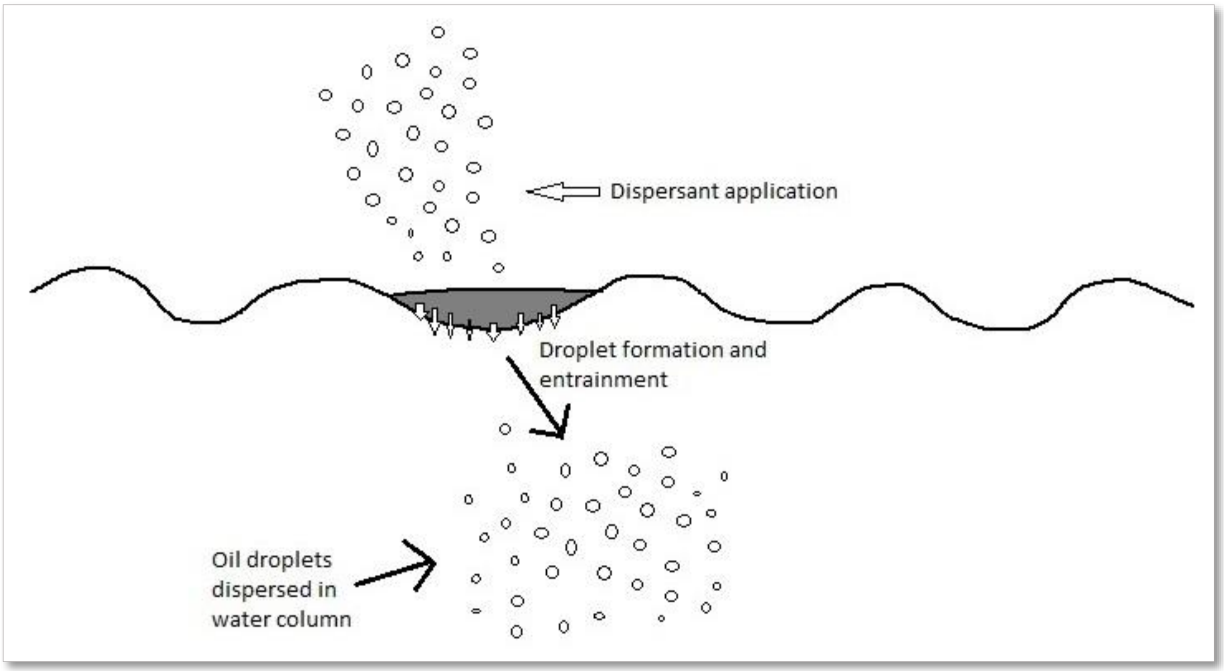

Figure 4.3. Scheme of oil dispersed at sea with the application of dispersant at the water surface. The dispersant contributes to a reduction in the surface tension between the water and oil, facilitating dispersion of oil into the water column. Recreated from (NRC, 2005).

Figure 4.3 shows a simple scheme of oil dispersant application. Dispersants are typically applied from the air onto floating oil. The dispersant is mixed with the oil, decreasing the surface tension between the two phases (Brakstad, Ribicic, et al., 2018; Chandrasekar et al., 2006; NRC, 2005). The lower surface tension favors oil droplet formation. Breaking waves push the oil mixed with dispersant into the water column, resulting in the formation of numerous small oil droplets in the water. Due to the lower density of oil compared to seawater, there is a tendency for the droplet to float back up to the water surface. Larger droplets rise faster to the water surface where they can coalescence and harm birds, shoreline ecosystems etc. (Lehr et al., 2010). Droplets below sizes of approximately 70 micrometer in diameter stay in suspension (Beyer et al., 2016; ITOPF, 2011). The stability of oil droplets in water can also be balanced by components such as surfactants or fine solid particles like silica (Levine et al., 1989), clay (Dong et al., 2014) or even bacterial cells that can produce surfactant chemicals and facilitate oil dispersion (Dorobantu et al., 2004).

After the oil is dispersed into the water column, it is more easily subject to other weathering processes such as bacterial biodegradation (Chandrasekar et al., 2006; NRC, 2005). Dissolution and volatilization (the latter one if droplets rise to the water surface but before they coalescence) can also be enhanced by dispersion. Also, sedimentation can occur more easily by interaction of dispersed oil droplets with suspended particulate material in the water to fall downwards as MOS (Gregson et al., 2021; Ross et al., 2021). While the MOS sinks, the most biodegradable compounds are typically eaten by bacteria on the way down, transporting more resistant residue to the sea bottom and in the sediments. Some of these compounds can be slowly degraded by sediment microorganisms under anoxic conditions (Kleindienst et al., 2015). It is however unclear if the use of dispersants increases the sedimentation rate of chemically dispersed oil compared to naturally dispersed oil (Passow et al., 2017). The use of dispersants can consequently have advantages and disadvantages. Oil is removed from the water surface and less harm is done to coastal and shoreline areas. The impact on less visible areas such as the seafloor and sediment ecosystems, however, is less understood. In addition, dispersants are expensive, can be toxic, and can make the situation worse in certain situations (Al-Majed et al., 2012; Frometa et al., 2017; McIntosh et al., 2010). Dispersants are often effective but also have their limitations.

4.3 Factors affecting dispersant efficiency

As for most other oil spill cleanup methods, many factors can affect the efficiency of the oil dispersants. The efficiency of an oil spill dispersant is often measured by the amount of oil that is moved into the water column compared to the amount that stays in the oil slick at the water surface (M. Fingas, 2012; Lewis et al., 2010). A brown/blackish plume visible in the water column indicates efficient dispersing of oil, while a white plume (given that the dispersant is white which it often is) indicates lower efficiency or no oil dispersion.

Various factors affect the efficiency of chemicals to disperse oil slicks at sea. The most important factors are oil composition, viscosity, and the degree of weathering. The time of response action is also of utmost importance, where a rapid response can be crucial for successful oil dispersion (NRC, 2005). These factors are highly related as the oil composition changes with time as the oil is going through weathering processes, increasing its viscosity. Other factors related to oil dispersants efficiency include mixing due to weather and wave conditions, oil-dispersant ratio, composition of the dispersant, air and water temperature, and water salinity (M. Fingas, 2012).

4.3.1 Oil composition and viscosity

The composition of the oil is the most important factor affecting dispersant efficiency, where thick oils with relatively large amounts of asphaltenes and resins are associated with high viscosity and poor dispersion efficiency, particularly if they are emulsified (M. Fingas, 2012; M. Fingas et al., 2005). As discussed in Chapter 2 and Chapter 3, high viscosity oils are associated with low spreading and are difficult to disperse, making the oil less available for the microorganisms which can lead to a decrease in removal by weathering and especially by biodegradation (Lewis et al., 2010; Whyte et al., 1998). Light oils with compounds of low molecular weight are more easily dispersed both naturally and with the help of chemicals, especially at high sea energy conditions (M. Fingas, 2012).

4.3.2 Time: Window of opportunity for efficient dispersant application

The time of action for oil spill response measures is connected to oil composition and viscosity, the time often being referred to as the window of opportunity for effective dispersant application. The highest efficiency is normally related to rapidly addition of dispersant after an oil spill, but also short contact time (D. M. White et al., 2002). This window of opportunity is roughly estimated to be around 24-72 hours in temperate conditions and 12-24 hours in colder conditions (Champ et al., 1997; NRC, 2005). The window of opportunity is also dependent on other factors as well such as oil type. If the response is too slow, more weathering of the oil will likely have occurred. This especially applies to evaporation which is increased with increasing temperatures. Highly weathered oil responds poorer to the chemical dispersants mainly because it thickens and increases its viscosity, also requiring more mixing energy (Chandrasekar et al., 2005; M. Fingas, 2011a; D. M. White et al., 2002). Stable water-in-oil emulsions (associated with lower temperatures but also other factors) also affect the dispersant efficiency by increasing the viscosity and making oil dispersion difficult. Overall, the components that are more easily dispersed are often removed by other weathering processes if too much time has passed before the addition of the dispersant. This can affect the efficiency of oil dispersants.

4.3.3 Sea energy

For most oil spill cleanup methods, rough weather conditions and especially large waves are associated with lower efficiency (M. Fingas, 2012). Almost the opposite can be seen with the use of dispersants, although the addition of chemicals is generally easier in still water and calm weather conditions. For successful use of dispersants during oil spill treatment, mixing of oil and chemical is necessary, which is highly wind-dependent (M. Fingas, 2011b). Lab experiments have shown an increase in dispersant efficiency with higher mixing energy (Chandrasekar et al., 2005). Breaking waves in the water contribute to both natural dispersion of the oil and helps chemical dispersants work. It is important that the waves are breaking as non-breaking waves are associated with much lower dispersion efficiency (Li et al., 2008). Ocean currents stimulate the dispersed oil to remain in suspension and not float back up. Therefore, weather conditions are important when considering the use of dispersants (Lewis et al., 2010). Also, physical barriers such as rain, snow, ice or fog may also interfere in the performance of the dispersant (Al-Majed et al., 2012). Frazil ice (or porous new ice) has shown to hinder the dispersant to reach and mix properly with the oil by decreasing the sea energy (Song et al., 2022).

Low sea energy conditions at the spill site require a higher dose of dispersant than for high sea energy conditions (M. Fingas, 2011a). Still water lacks the mixing potential required for the successful use of chemical dispersants (Lewis et al., 2010). Application of dispersants in calm water is therefore not always recommended. On the other hand, too rough weather conditions can push the oil subsea and make chemical dispersants use challenging, as it can be difficult for the dispersant to reach the oil for mixing. Weather conditions somewhere in-between is therefore desirable for the maximum effect of the dispersant, but unfortunately weather conditions may be difficult to predict.

Tests using Corexit 9500 have indicated that dispersants can be applied to an oil slick during calm weather. The criteria were that rougher weather and breaking waves are expected within two to six days after application. Then, depending on the thickness of the oil slick, oil and dispersant mix, allowing for successful dispersion (Lewis et al., 2010). If too much time goes by, or the oil slick is too thin, loss of dispersant from the oil can be seen before the mixing begins, reducing the effectiveness. The spreading of the oil and thickness of the slick is also important. If the slick is too thin this can result in efficiency loss as the dispersant reacts with the water instead of the oil, or does not reach the oil, not only in situations of calm water (M. Fingas, 2012). Thin slicks are therefore not easily dispersed.

4.3.4 Dispersant type and amount

For successful dispersion of oil, it is also important to consider the type of dispersant used and the amount of dispersant used, often referred to as dispersant-to-oil ratio (DOR) (M. Fingas, 2011a; NRC, 2005). Different types of dispersants have different properties and can vary in their efficiencies depending on the environmental conditions. The DOR has a direct relationship to dispersant efficiency (D. M. White et al., 2002). The ratio commonly used in laboratory experiments is 1:20-1:25 although variations exist, and the exact amount of oil in an oil spill is rarely known. The optimal DOR for the situation can also enhance biodegradation. Xia *et al.* (2018) demonstrated this by increasing the DOR and measuring the half-time of petroleum hydrocarbons in the presence of various oil-degraders, they found an optimal DOR of 30% in the study (Xia et al., 2018). The oil-to-water ratio (OWR) is also important when testing dispersant efficiencies in laboratory tests, where high OWR can make the droplets coalescence (M. F. Fingas et al., 2005).

4.3.5 Ambient temperatures

Both air and water temperature can affect the performance of chemical dispersants (Chandrasekar et al., 2005). High ambient temperatures lead to more evaporation of oil components, changing the oil composition and reducing the window of opportunity for efficient dispersant application (Champ et al., 1997). The components left on the water surface are often more difficult to disperse and therefore the dispersant efficiency is decreased (Al-Majed et al., 2012). The solubility of the dispersant increases with increasing water temperature (and it increases the oil temperature), which can lead to improved dispersion under higher temperatures if the dispersant is applied within reasonable time (Chandrasekar et al., 2006; Lehtinen & Vesala, 1984).

Lower ambient temperatures can enhance emulsification, also reducing dispersant efficiency (NRC, 2005). In laboratory tests, Corexit 9500 and 9527 have both shown a lower efficiency during subarctic temperature and salinity conditions (Moles et al., 2002). Both oil and dispersant viscosities are increased with decreasing temperatures, affecting dispersant efficiency negatively (Chandrasekar et al., 2006). Ambient temperatures lower than the spilled oil's pour point can also halt chemical dispersant efficiency. Low temperatures especially below 0 °C can affect the application of the dispersant. The mixture may freeze during application, especially if it is water-based or has been pre-mixed with water, which will greatly reduce its performance (NRC, 2005).

4.3.6 Water salinity

Water salinity affects the efficiency of dispersants, and multiple studies have shown a decline in dispersant efficiency at low salinities (Belk et al., 2005; Blondina et al., 1999; Deshpande et al., 2005; Lehtinen & Vesala, 1984). Optimal efficiency in laboratory experiments has been reported for salinities around 30-40‰ (Blondina et al., 1999; M. F. Fingas et al., 1994). The type of dispersant and other factors mentioned above also affect how the dispersants work at the different salinities. The salinity of seawater generally work to reduce the dissolution of the dispersant into the water and make the surfactant components to rather mix with the oil to achieve their purpose (Chandrasekar et al., 2006). Normal seawater salinity is associated with the highest efficiency of chemical dispersants, which is intentional as they often are designed for this application. For salinities around 0 most dispersants show very low to zero efficiency, and low dispersant effects have been reported in freshwater even for some freshwater dispersants (Belk et al., 2005). Common types of oil dispersants are therefore not recommended in freshwater environments, and more studies are necessary on the design and use of dispersant in freshwater environments.

4.4 Large-scale use of dispersants in oil spill cleanup

Dispersants have been used to aid cleanup of many oil spills over the years. The effectiveness of the oil dispersion is difficult to measure in the field compared to in the lab (M. Fingas, 2011a). How much oil that is left at the sea surface measured against the oil dispersed is impossible to calculate during an oil spill. Though observations and estimations can be made. Table 4.1 shows some important oil spill incidents that have been treated with large-scale use of dispersants. Table 4.1 is partly a continuation of Table 2.1 showing important large oil spills but focuses on the use of dispersants.

Table 4.1. Situations where dispersants have been used, type of dispersant, amount applied, effectiveness and environmental effects of the dispersant.

Oil spill incident, year	**Location**	**Estimated amount of oil spilled**[2]	**Type/name of dispersant used**	**Estimated amount of dispersant applied**	**Effectiveness and environmental effects of the dispersant**	**References**
Torrey Canyon tanker, 1967	Seven Stones reef, English Channel	~135 000 m^3 (119 000 metric tons) +bunker fuel (unknown amount)	First generation mixtures of different solvent-emulsifiers and detergents	10 200 metric tons at beaches and 3 160 metric tons at sea	Limited effectiveness, ecological damage on shorelines. Oil-dispersant-mixture more toxic than just oil.	(Law, 2011; O'Sullivan & Richardson, 1967; Wells, 2017)
The *IXTOC-I* well blowout, 1979	Bay of Campeche, Gulf of Mexico	~556 000 m^3 (475 000 metric tons)	Corexit 9527 primarily	Unknown, but at least 900 metric tons	Effective. Prevented shoreline oiling.	(Jerneloev & Linden, 1981; Lindblom et al., 1981)
Braer tanker, 1993	Shetland Islands	~103 000 m^3 (84 700 metric tons)	Dispolene 34S, Dasic Slickgone LTSW, and Entersperse 1583	120 metric tons	Unknown, but the oil was prone to natural dispersion. Some dispersants missed the target. Some spilled on land.	(Forbes & Campbell, 1994; Harris, 1995)
Sea Empress tanker, 1996	Milford Haven, Southwest Wales	~87 000 m^3 (72 000 metric tons) + 480 metric tons fuel oil	Corexit Dasic	458 metric tons	Effective at reducing shoreline oil pollution. Amphipod mortality.	(M. Fingas, 2011a; Law & Kelly, 2004; Sea Empress Environmental Evaluation Committee & Little, 1998)
BP *Deepwater Horizon* explosion, 2010	Gulf of Mexico, United States	~795 000 m^3	Corexit 9500 Corexit 9527	~6 600 metric tons 7 000 m^3 (of which 40% was applied subsea)	MOS formation. Corals were affected. DOSS found in subsurface plume 64 days later. Efficiency unknown.	(Camilli et al., 2010; Lehr et al., 2010)

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[2] The amount of spilled oil is given in both metric tons and cubic meter. Papers can vary between tons, m^3, barrels etc. It has been used petroleum conversion calculators and taking into consideration the density of the type of oil (if given). It is emphasized that the amount is an estimate used for comparison not always completely accurate.

The *Torrey Canyon* tanker accident in 1967 (Table 4.1) was the first large oil-tanker accident and the first known large-scale use of chemical dispersants to treat an oil spill (Law, 2011; Wells, 2017). Dispersant was applied on the oil slick at sea and on contaminated beaches. The dispersant used was a first-generation mixture of solvents and detergents primarily used for the cleaning of harbors after small spills. The efficiency of this dispersant use after the large oil spill was not optimal. In addition, the mixture was very toxic and even contained some aromatic hydrocarbons, resulting in ecological damage at the shorelines where it was applied. Studies showed that the oil-dispersant mixture was more toxic than oil pollution without the dispersants (O'Sullivan & Richardson, 1967). Given the unfortunate consequences of the dispersant use and that it was the introduction to dispersants as an oil response, a lot of criticism regarding future use of dispersants was met at the time.

Dispersants were applied during the cleanup of the *IXTOC-I* well blowout in 1979 (Table 4.1). The spraying started on day six after the spill. Dispersants were also applied to the remaining oil several months later. At least 900 metric tons of dispersants were used, probably more (Jerneloev & Linden, 1981). Different dispersants were used but mainly Corexit 9527. The oil was successfully dispersed and assumed to prevent much oil from being washed up ashore (Lindblom et al., 1981). The acute and long-term environmental consequences of the large-scale use of dispersant is unknown.

In 1996, the *Sea Empress* tanker oil spill released about 87 000 m^3 oil plus some fuel oil (Table 4.1) (Law & Kelly, 2004). The response team applied 446 tons of dispersant sprayed from the air and 12 tons from boats, making a total of 458 tons dispersant used. The dispersant usage was successful in removing oil from the water surface, and about 52% was estimated to be dispersed into the water (Sea Empress Environmental Evaluation Committee & Little, 1998). It was estimated that it reduced large amounts of oil from being washed ashore and saving many seabirds from oiling (though thousands of birds died from un-dispersed oil). Though, the dispersed oil may have affected and possibly killed bivalves and amphipods. The amphipod population disappeared in some areas nearby. The dispersed oil was ultimately fate to biodegradation and to leave only small amounts in the sediments. Combined with an estimated removal of 40% via evaporation, and less than 1% remaining onshore one year later, the adverse long-term environmental effects were assumed to be minor.

The MV *Braer* tanker drifted aground at the Shetland Islands in 1993, releasing all of its about 103 000 m^3 crude oil in eight days (Table 4.1) (Harris, 1995). On day two, aerial dispersant spraying began. The total amount of dispersant used were estimated to 120 tons. Though the bad weather conditions at the spill site, especially the large waves, and the fact that the spilled oil had low viscosity, encouraged considerable natural dispersion (Harris, 1995). There were uncertainties about the type of dispersants that have been used in the beginning (first 48 hours). This became a controversial environmental issue due to the unknown toxicity of the dispersants and efficiency on rocky shores (Forbes & Campbell, 1994). Also, the dispersants did not reach the target oil in the beginning but ended up ashore. Though three types of dispersants used after 48 hours were namely Dispolene 34S, Dasic Slickgone LTSW, and Entersperse 1583. It is not clear if the dispersants were significantly effective. In addition, human errors in the storage of unused dispersants resulted in unintentional release into the environment onshore.

4.5 BP Deepwater Horizon oil spill

The large disaster of the DWH oil spill began by an explosion on April 22 in 2010. Macondo oil and gas leaked from the wellhead 1500 meters subsea into the Gulf of Mexico until it was capped July 15 the same year (Gray et al., 2014). About 5 million barrels or 795 000 m^3 of crude oil were released in that time (Table 2.1, Chapter 2) (Lehr et al., 2010). The response team applied large amounts of the dispersants Corexit 9500 and 9527 to protect the shorelines from oil pollution (Table 4.1) (Seidel et al., 2016). It is estimated that a total of 7 000 m^3 of dispersant were used in total (Lehr et al., 2010).

The use of dispersants during the DWH spill gained huge attention. The massive amounts of dispersants were not only applied at the water surface (4 055 m^3), but about 2 900 m^3 were used at the release source at the sea bottom wellhead. When oil is released at this depth the high pressure also contributes to increase the natural dispersion compared to a water-surface spill. The aim of this unconventional dispersant addition subsea was to increase the amount of oil staying in suspension by the formation of smaller and more stable droplets (Kujawinski et al., 2011; Lehr et al., 2010).

The intention of the oil dispersion was to avoid formation of large oil slicks at the water surface and their subsequent environmental consequences. Still, due to the size of the spill, oil slicks were formed that travelled and polluted ecosystems including salt marshes, wetlands, beaches and estuaries (Beyer et al., 2016). Large areas were contaminated, and the situation got massive media coverage. It was estimated that more than 2100 km of shoreline was affected (DWH NRDA, 2015), while Michel *et al*. (2013) observed more than 1773 km of affected shoreline (Michel et al., 2013). The efficiency of the oil dispersion is not fully understood.

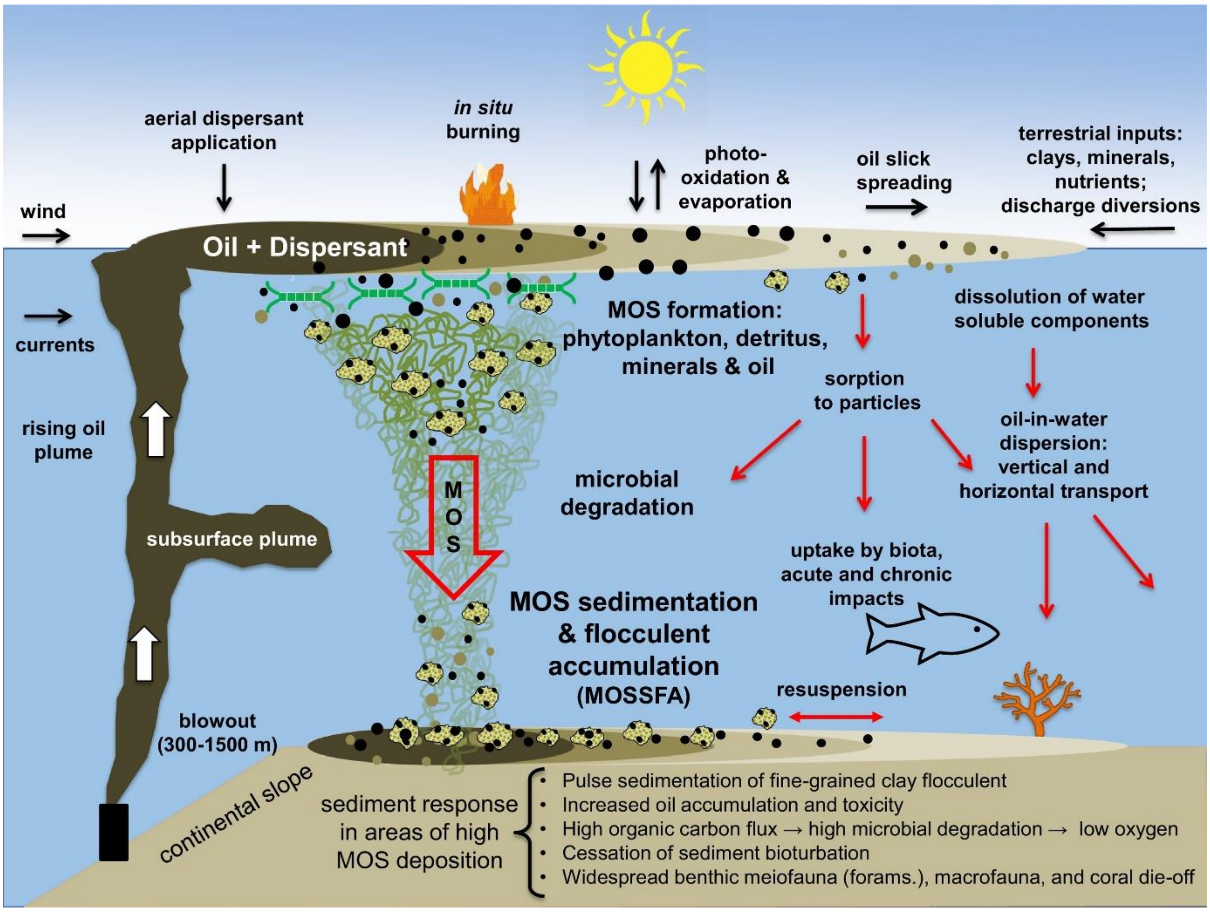
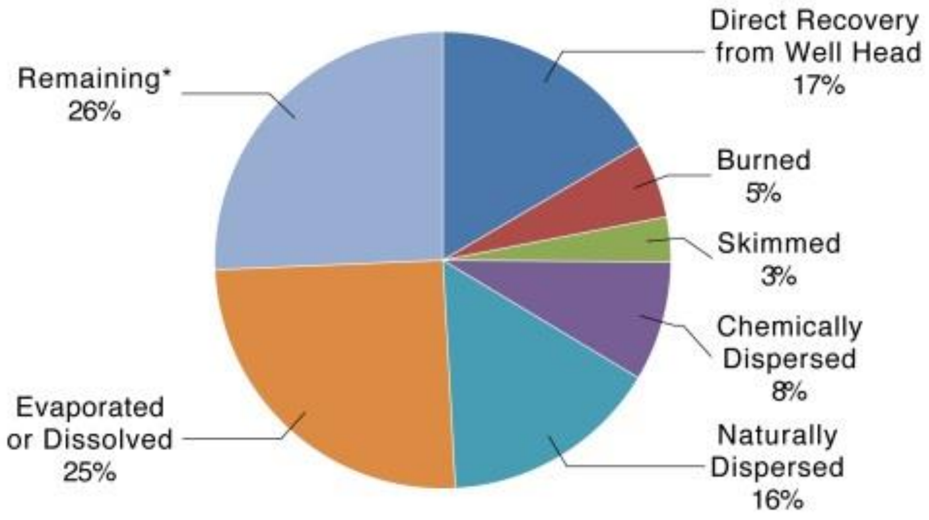


Figure 4.4. A deep-water oil blowout of crude oil showing possible consequences and fate of the oil. Oil is released from the deep, where also dispersant was injected during the DWH spill. A subsurface plume is created by natural (and possibly chemically enhanced) dispersion. Dispersed oil and the subsurface plume may interact with particles in the water to form MOS and further marine oil snow sedimentation & flocculent accumulation (MOSSFA). Copied from (Ross et al., 2021).

The subsea release of oil and possibly the application of dispersant resulted in a subsea plume (Figure 4.4) of petroleum hydrocarbons located at a depth of about 1100 meters (Camilli et al., 2010). It was estimated to have a length of 35 kilometers. How the plume was affected by the dispersant application is not fully known (Kleindienst et al., 2015). However the surfactant ingredient DOSS, the main surfactant ingredient in the Corexit dispersant that was applied subsea was found in the deep-water plume 64 days after the dispersant injection at the wellhead stopped (Kujawinski et al., 2011). The chemical was widespread and found as far as 300 km in distance from the well. A lot of bacteria were also found in this plume (Hu et al., 2017). Also, insufficient levels of dissolved oxygen for considerable oil biodegradation was found in the plume, indicating that the petroleum hydrocarbons at this depth persisted for longer than first thought and that the biodegradation rate was indeed low (Camilli et al., 2010).

Much of the oil from the DWH spill formed MOS and sank to the seafloor (Figure 4.4) (Passow et al., 2012). Chemically and naturally dispersed oil interacted with particles in the water such as phytoplankton and dead organic matter. Many benthic communities and corals were adversely affected from the dispersed and sedimented oil (Beyer et al., 2016; Prouty et al., 2016). Fragment mortality in octocorals was observed (Frometa et al., 2017). Reduction in survival and settling rate of coral larvae was also indicated (Goodbody-Gringley et al., 2013). Dispersed oil and MOS affected corals in a large area around the spill site (DeLeo et al., 2018; H. K. White et al., 2012). A decrease in seaweed and decapod crustaceans populations was also observed (Felder et al., 2014).



* Remaining oil is either at the surface as light sheen or weathered tar balls, has been biodegraded, or has already come ashore.

Figure 4.5. Oil fate estimate by cumulative percentage from three months after the DWH spill. Made from the Oil Budget Calculator. Percentages are of estimated total oil spilled. Obtained from (Lehr et al., 2010).

Three months after the DWH oil spill the fate of the oil was estimated by The Federal Interagency Solutions Group (Lehr et al., 2010). A cumulative percentage (of total oil spill estimate) is shown in Figure 4.5. It is assumed that large amounts of the chemically and naturally dispersed oil was removed by biodegradation (Prince, 2015). The Gulf of Mexico has favorable conditions for petroleum hydrocarbon biodegradation because of the natural release by many seeps and hydrocarbon-degrading organisms are well distributed there (Atlas &

Hazen, 2011). It is however unclear how the use of dispersant affected the biodegradation (Kleindienst et al., 2015; Madigan et al., 2019). The DWH had a huge environmental impact like the large fraction of oil ending up at the seafloor through MOS harming aquatic life and the shorelines that were polluted harming the ecosystems there. The total extent of the environmental impact from the DWH accident is not fully known.

4.6 Enhanced biodegradation by dispersants

It is often unclear how dispersants affect biodegradation when used to treat oil spills, such as after the DWH incident (Kleindienst et al., 2015). Tests have shown enhanced biodegradation from dispersion by Corexit 9500 (Sun et al., 2019; Varadaraj et al., 1995), and at 25 °C but not at 5 °C (Techtmann et al., 2017). Other tests have found inhibition of biodegradation from Corexit 9500 (Rahsepar et al., 2016). Some studies have found that chemically dispersed oil using Corexit 9500 not always degrades faster than fresh oil (Lindstrom & Braddock, 2002) and that dispersant application can both enhance and inhibit biodegradation (Overholt et al., 2016). Corexit has also shown to enhance biodegradation of *n*-alkanes but not PAHs (Bacosa et al., 2015). There are many variables that affect the biodegradation such as DOR, temperature and oil composition.

4.7 Toxicity of dispersants

A great concern regarding the use of dispersants is the toxicity. Many dispersants used in the early years were very toxic (Harayama et al., 1999; Swedmark et al., 1973). After the cleanup of the *Torrey Canyon* tanker accident in 1967 (Table 4.1) the oil-dispersant mixture was revealed to be more toxic than the oil pollution alone (O'Sullivan & Richardson, 1967). Modern dispersants like Corexit are much less toxic (about ten times less toxic than those in the 1960s and 1970s), but can still affect aquatic life (M. Fingas, 2011a). There are variations between the commercial chemical dispersants like the dispersant Finasol has been reported to be more toxic than Corexit 9500 (DeLorenzo et al., 2018). The toxicity of the dispersant itself is not the only concern, but also moving the contaminants. The toxic compounds in the oil become more available to sensitive aquatic species (some of which are reviewed in Chapter 2.4) when dispersed into the water. In addition, the combination of dispersant and oil can be more toxic than the oil exposure alone (Almeda, Bona, et al., 2014; Almeda, Hyatt, et al., 2014; Frometa et al., 2017; McIntosh et al., 2010).

Toxicity of a substance can be examined by laboratory toxicity tests, measuring the Lethal Concentration to 50% of the test population (LC_{50}) during a specific observation period (M. Fingas, 2011a). Species that are typically tested include invertebrates such as amphipods and crabs, or the rainbow trout, but a variety of species are used in toxicity tests. Low LC_{50} values show high toxicity of the substance tested to the species tested. Light crude oil typically has a LC_{50} value ranging from 20 to 50 mg/L to the rainbow trout while modern dispersants typically vary from 200 to 500 mg/L (M. Fingas, 2011a). Corexit 9500 has been reported with LC_{50} values like: 210 mg/L to blue crabs (24 h) (Weiner et al., 2021), 172 mg/L to early life stage fish (*C. variegatus*) (96 h) (DeLorenzo et al., 2018), 72.2 mg/L to deep-sea cold water sablefish (96 h) (McConville et al., 2018) and 70.27 mg/L to octocoral (*Swiftia exserta*) (96 h) (Frometa et al., 2017). There have also been many reports about other invertebrate species being harmed from the use of dispersants. Corexit 9500 has been reported to impact blue crab (*Callinectes sapidus*) by affecting the structure and ion transport function of the gills (Weiner et al., 2021) and on their early life stage larvae where the mobility was decreased with increasing dispersant concentration (Pie & Mitchelmore, 2015). Tests have also been conducted on the dispersants Corexit 9500 and 9527 using declining concentrations such as expected due to dilution in the marine environment. The dispersants showed low to moderate toxicity to many aquatic species such as the rainbow trout with an EC_{50} of 354 mg/L (96 h) in response to Corexit 9500, and that dispersed crude oil often is more harmful than the dispersants (George-Ares & Clark, 2000).

The combined toxicity of dispersants and dispersed oil is also important. It has been reported that both the oil in suspension and the dispersant are harmful to early stages of soft and hard coral species, and that the combination of these were more toxic than the crude oil alone (although Corexit was not tested but six other commercial dispersants) (Shafir et al., 2007). That the combination of dispersant (Corexit 9500) and oil is more toxic than exposure to oil alone has also been indicated for octocorals (Frometa et al., 2017). Tests of Corexit 9500 on meroplanktonic larvae (Almeda, Bona, et al., 2014) and microzooplankton (Almeda, Hyatt, et al., 2014) have shown similar effects. Coral reefs are in particular very sensitive to both dispersed oil and dispersant chemicals, and can take long time to regenerate such as after the DWH spill (M. Fingas, 2011a; Shafir et al., 2007; Shigenaka et al., 2010).

4.8 Alternatives to Corexit

Due to the environmental effects of chemical dispersants such as Corexit, the need for greener dispersants that are less toxic, and more biodegradable is present. Hydrolysis products from various types of proteins can have emulsifying capabilities, thereby work as greener surface-acting agents in dispersants. Hydrolyzed shrimp waste has been tested as an alternative and green dispersant for oil spill cleanup in seawater (K. Zhang et al., 2018). In the test, the dispersant efficiency of the hydrolyzed shrimp waste was comparable to that of Corexit 9500. Water can be used as solvent, reducing the toxicity compared to other solvents. The dispersant was efficient all over the salinity range of 0-50 psu, with the highest efficiency at 20 and 30 psu. This dispersant (and other types of protein-based waste products such as lobster wastes) is thus an option for a more environmentally friendly dispersant for oil spill cleanup in the marine environment (Saleh et al., 2021; K. Zhang et al., 2018). Bio-waste is biodegradable and relatively inexpensive. Although, more research about its application in the offshore oil industry is necessary.

An alternative to reducing the toxicity of e.g., Corexit 9500 is by displacing its most persistent and criticized surfactant component DOSS. A combination of different surfactants normally gives the highest dispersant efficiency. Athas *et al*. (2014) found an efficient replacement by combining food-grade amphiphiles to disperse oil in seawater. The combination found was lecithin (soybean) and Tween 80 in a 60:40 ratio (Athas et al., 2014). Lecithin and Tween 80 based dispersants can be efficient dispersant alternatives to Corexit, and work well in temperature ranges from 10 to 40 °C, also in deep water (Jin et al., 2019; Nyankson et al., 2015). Other blends such as Tween 80 with a low-toxicity surface-active ionic liquid (1-butyl-3-methylimidazolium lauroyl sarcosinate) (Nazar et al., 2021) and biosurfactants with ionic liquids (Hassan Shah et al., 2021) have also shown great results at dispersing crude oil in seawater and are promising alternatives to toxic dispersants. Dispersants based on environmentally friendly surfactants such as bio-waste products, food-grade and biosurfactants is highly relevant today, and their efficacy in the field deserves further research (Zhu et al., 2022).

4.8.1 Biosurfactants

Biosurfactants, or microbial surfactants, are surfactants produced by bacteria, yeasts and fungi (Mulligan, 2005). It has since early years been observed increasing hydrocarbon solubility in the presence of bacteria (Thomas et al., 1986). An increase in the solubility of octadecane with the presence of rhamnolipid biosurfactant produced by *Pseudomonas aeruginosa* has been observed many years ago, already then indicating the possible application of biosurfactants to help remediate hydrocarbon-contaminated soil and aqueous environments (Y. Zhang & Miller, 1992). Although their application as components in dispersants to treat oil spills cleanup is still emerging (Cai et al., 2021; Zhu et al., 2022).

Biosurfactant-based dispersants (BBDs) have lower toxicity and are more biodegradable than chemical dispersants such as Corexit 9500 (Cai et al., 2021; Shekhar et al., 2015). Examples of biosurfactants include: surfactins produced by e.g., *Bacillus subtilis* (Zhi et al., 2017); trehalose lipids produced by e.g., *Rhodococcus erythropolis, Mycobacterium sp*., and *Gordonia sp*. (Cai et al., 2016; Franzetti et al., 2010); rhamnolipids produced by e.g., *Pseudomonas aeruginosa* mutants (Cai et al., 2021; Romero et al., 1998; Y. Zhang & Miller, 1992); and exmulsins produced by e.g., *Exiguobacterium* sp. (Cai et al., 2017, 2021). Cai *et al*. (2021) found that surfactin and trehalose lipids showed comparable oil dispersion efficacy to Corexit 9500. They also found that a combination of rhamnolipids and exmulsins was effective but not these biosurfactants on their own (Cai et al., 2021). Toxicity tests showed lower toxicity for all biosurfactants tested (surfactins, trehalose lipids, rhamnolipids and exmulsins) than for Corexit 9500 (Cai et al., 2021). Trehalose lipids and exmulsins were the least toxic of the biosurfactants.

Biosurfactants can possibly enhance biodegradation by increasing the bioavailability of pollutants (Mulligan, 2005). They can also be effective under large ranges of temperature, pH and salinity (Shekhar et al., 2015). The application of BBDs as a greener alternative to chemical dispersants in oil spill cleanup is promising (Cai et al., 2021; Freitas et al., 2016; Mulligan, 2005). To compete with commercial chemical dispersants, the production of BBDs should be improved, and the costs lowered (Cai et al., 2021). BBDs should show competing efficiency to commercial dispersants and not be biodegraded to quickly before they fulfill their purpose. More research on development of BBDs and their efficiency of oil spill cleanup in the marine environment is desirable.

5 Discussion

With the large demand for fossil fuel, occasional oil spills are inevitable. Oil spilled in the marine environment has large spreading potential and the potential to do considerable damage. Floating oil can harm diving birds, sea turtles, marine mammals, and other exposed species (Antonio et al., 2011; Prince, 2015). Spilled oil can also be transported and washed up to harm sensitive coastal and shoreline ecosystems (Bejarano & Michel, 2016; Pérez-Cadahía et al., 2004). Benthic ecosystems and particularly coral reefs are harmed by dispersed, dissolved, and sinking oil (Shafir et al., 2007; Shigenaka et al., 2010). To protect the environment, response teams aim to remove the oil by different oil spill cleanup methods. Oil spill response is performed with focus on saving the seabirds, sea turtles, and removing or preventing shoreline oil pollution. Still, it is important to consider the total environmental impact and not just the visible impacts like oiled birds and beaches.

There have been many large catastrophic oil spills globally over the years such as the *Sea Empress* tanker (1996) and the DWH rig explosion (2010). Even with a large effort attempting to clean up the oil, the environmental impacts can be huge. The extent the environment is affected is not only determined by the amount of spilled oil, but the composition of the oil, the location, and environmental conditions etc. (Al-Majed et al., 2012). The location and weather conditions may determine how hard sensitive ecosystems are hit. The desired cleanup method is often to remove the oil from the environment by oil recovery, but is not always easy (Prince, 2015). Oil recovery, and most other cleanup methods like confinement, *in-situ* burning, and the use of sorbents are influenced by weather and sea conditions as bad weather often limit their efficiency (M. Fingas, 2011b; Guidi et al., 2016; Jerneloev & Linden, 1981). Dispersants, however, require breaking waves and mixing to successfully work (Chandrasekar et al., 2005; Li et al., 2008). It can be challenging to determine which methods to use for oil spill cleanup as every situation differ and it is nearly impossible to save all nearby ecosystems.

Crude oil is composed of different hydrocarbons like alkanes and PAHs. PAHs can have carcinogenic and mutagenic effects and are priority pollutants (Alloy et al., 2015; CDC, 2009; Vignier et al., 2016). Biodegradation is however an important removal process of many of these pollutants. Most compounds in crude oil are biodegradable under aerobic conditions (Atlas, 1981; Madigan et al., 2019). Bacteria can mineralize most of the compounds, but the larger compounds (more than three ring PAHs, asphaltenes, resins etc.) resist biodegradation

(Haritash & Kaushik, 2009). Bacteria also require oxygen, nitrogen, and phosphorus to degrade the petroleum hydrocarbons (Atlas, 1981). Nitrogen and phosphorus are often limiting factors for biodegradation in the marine environment and at shorelines where oil is washed up (Atlas, 1995b; Harayama et al., 2004). Therefore, biostimulation by fertilizer addition is a process to remediate oil spill cleanup by stimulating the bacteria to degrade the oil. Biodegradation of the oil components are also dependent other factors such as temperature, mixing, and bioavailability of the substrate (Atlas, 1981). The bioavailability of the petroleum hydrocarbons can be enhanced by oil dispersion (Varadaraj et al., 1995; Xuan & Bing, 2017).

The use of chemical dispersants like Corexit is based on moving the oil from the water surface and into the water column to enhance removal by increasing the bioavailability of oil to enhance biodegradation to protect shorelines and aquatic surface wildlife (Lehr et al., 2010; Stroski et al., 2019). Dispersants contain surfactants that are partly soluble in oil and water and that work to reduce the surface tension between the two phases (NRC, 2005). Sea energy is important as breaking waves help the oil into the water column as surfactant-coated dispersed oil droplets (Chandrasekar et al., 2005; Li et al., 2008). The petroleum hydrocarbons subsequently become more bioavailable to the microorganisms which can enhance biodegradation (Chandrasekar et al., 2006; NRC, 2005). It is not always clear if the dispersants have enhanced the biodegradation in the field (Kleindienst et al., 2015). It is difficult to know how the situation would have been without the use of dispersants, even though they sometimes show promising results in laboratory tests. Conditions in the marine environment are difficult to perfectly recreate in laboratory experiments such as in experiments conducted in a wave tank.

Dispersants have limitations. They do not work in calm water or on thin oil slicks and are not effective on viscous oils (Chandrasekar et al., 2005; Lewis et al., 2010). In calm water, other response techniques like oil recovery can be efficient. Oil weathers naturally by evaporation etc. when released into the environment. This removes the lightest compounds and increases the oil viscosity. It is therefore important to act quicky after an oil spill within the window of opportunity for successful dispersion (NRC, 2005). Too slow response can result in thinner oil slicks and more viscous oil from weathering. Also, more oil is transported towards shorelines which is wanted to avoid by using dispersants.

Corexit is most known for its massive use during the DWH spill in 2010. About 7 million liters of Corexit 9500 and 9527 were used, of which 40% was applied 1500 m subsea at the seafloor next to the wellhead where the oil was released (Lehr et al., 2010). The efficiency of the subsea dispersant injection is not understood. It may have contributed to the subsea plume that was formed, but the environmental conditions at the depth of the release would have resulted in a plume also without the dispersant application (Camilli et al., 2010). Although DOSS from the dispersant was found in the plume after 64 days and up to 300 km from the well (Kujawinski et al., 2011). A lot of bacteria was also found in the plume (Hu et al., 2017). The biodegradation rate in the plume is not fully understood and low levels of oxygen indicated slow biodegradation (Camilli et al., 2010). Although, it was difficult to obtain correct measurements and recreate conditions from the plume which was at a depth of 1100 meter.

Despite the massive use of dispersants during the DWH accident, oil slicks formed due to the large amount of oil and polluted large areas of shorelines and beaches, harming the affected ecosystems (Beyer et al., 2016). The seafloor was also highly polluted by chemically and naturally dispersed oil interacting with particles to form MOS (Passow et al., 2012). This had detrimental effect to the corals like fragment mortality (Frometa et al., 2017) and decrease in survival and settling for coral larvae (Goodbody-Gringley et al., 2013). The effects on corals from the DWH indicate that precautions must be taken before using dispersants in sensitive areas like near coral reefs. This was also indicated from toxicity tests even before the DWH spill (Epstein et al., 2000; Shafir et al., 2007). The environmental effects of dispersants used in the deep have not been properly studied as it is rather unusual. The DWH dispersant application is the largest use of dispersants, and unique in terms of the deep subsea application. The total environmental impact of the deep-water dispersant injection is uncertain (Gray et al., 2014). Sensitive ecosystems nearshore and at the shorelines may be protected by using dispersants. The effects of sinking and dispersed oil on marine sediment ecosystems are however difficult to investigate as they are hard to reach and monitor, and it can be difficult to know the pre-spill conditions. The total environmental impact from dispersants use deserves more attention. When deciding upon the use of dispersants, it is crucial to not make the situation worse.

The need for efficient environmentally friendly dispersants is present. Dispersants based on surfactants such as food-grades, waste products, ionic liquids and biosurfactants are developing (Mulligan, 2005; Saleh et al., 2021; K. Zhang et al., 2018; Zhu et al., 2022). Many have shown high efficiency in dispersing oil in seawater. The biological surfactant ingredients are much more easily biodegraded than DOSS. It is however important that they are not biodegraded before they fulfill their purpose which can lead to the oil rising to coalescence. It is also important that dispersants are efficient at low DORs so that smaller volumes make the transportation and application easier. Still, the toxicity of the oil is not changed by using a greener dispersant. Dispersed oil can be toxic, and moving the oil into the water column moves the pollution and affects the fish and benthic species which can be harmed by dispersed oil (Almeda, Hyatt, et al., 2014; DeLorenzo et al., 2018; Weiner et al., 2021). Therefore precautions must be taken especially in sensitive areas where e.g. coral reefs can be affected (Epstein et al., 2000; Shafir et al., 2007; Shigenaka et al., 2010).

When deciding which cleanup methods to apply after oil spills, there are many factors that need to be considered. It can be difficult to remove the oil, but it can be moved to protect certain sensitive areas. It is probable that it results in that some species will get affected from the pollution either way. Choices are then made to have the least environmental impact (M. Fingas, 2012). This is very difficult to predict beforehand and can be also difficult to investigate afterwards as e.g., deep waters are difficult to research, and the environmental state of the area before the spill may be unknown. It is also crucial to act quickly.

If biodegradation of oil in the marine environment is enhanced by dispersant application is not understood completely. Biodegradation is assumed to have removed much oil from the DWH oil spill, but the dispersant applications are not proven to directly enhance the biodegradation (Kleindienst et al., 2015). Laboratory tests have shown both enhancement and inhibition, and no change at all, in biodegradation rates of oil using dispersants (Overholt et al., 2016; Rahsepar et al., 2016; Sun et al., 2019). Various factors influence the biodegradation of oil like the DOR, temperature, type of microorganisms, type of oil, etc. Dispersants can also facilitate the biodegradation of some compounds but not all (like *n*-alkanes but not PAHs) (Bacosa et al., 2015). Many different results are reported and there are still need for more tests. Tests of biodegradation of dispersed oil under different environmental conditions and in e.g., wave tanks that reconstruct the marine environment in the best way possible can help to get a better understanding. Although it is not possible to reconstruct the conditions perfectly, test give an indication of dispersant efficiency that can be considered when evaluating cleanup techniques during oil spills.

Controversy exists about the use of dispersants due to toxicity concerns. Early dispersants were very toxic (Harayama et al., 1999). Modern dispersants like Corexit 9500 are less toxic than early ones but can still affect aquatic life (DeLorenzo et al., 2018; M. Fingas, 2011a; Weiner et al., 2021). Many tests show detrimental effects of dispersed oil and dispersants like Corexit on corals, crabs, etc. (Almeda, Hyatt, et al., 2014; Frometa et al., 2017; Pie & Mitchelmore, 2015; Weiner et al., 2021). Tests have shown reduction in survival and settling of coral larvae (Goodbody-Gringley et al., 2013), changes in the structure and ion transport function of the gills of blue crab (Weiner et al., 2021), and decreased mobility of blue crab larvae (Pie & Mitchelmore, 2015). In addition, the combination of Corexit and dispersed oil have been reported as more toxic than oil alone in multiple tests (Frometa et al., 2017; McIntosh et al., 2010). Coral reefs and coral species are very sensitive to some dispersant chemicals and to dispersed oil droplets. Use of dispersants near coral reefs is not recommended (Epstein et al., 2000; Shafir et al., 2007).

The effects and environmental impacts of dispersants and dispersed oil deserves more research, like the long-term effects of chemically dispersed oil, and effects on sensitive areas such as tropical regions with coral reefs. There is a need to continue the toxicity tests to different aquatic species from both dispersants, dispersed oil, and the combination. Conditions are difficult to reconstruct, like salinity and sea energy, especially those of large accidents at sea. It is unknown how it would look without the use of dispersants, especially in the case of the DWH accident. It is important to test different environmental conditions and realistic surfactant concentrations to simulate real situations in the best way possible (Brakstad, Størseth, et al., 2018; Stroski et al., 2019). In addition, dispersed oil and dispersant chemicals are relatively quickly diluted in the water (Lee et al., 2013; Prince, 2015). Decreasing concentrations like expected in the water of dispersed oil and dispersants and their effects on aquatic species should be researched more in toxicity tests. Different environmental conditions should be used to test both the enhanced biodegradation by dispersants and on broad environmental impacts from the use of dispersants.

6 Conclusion

Oil spills have disastrous ecological consequences and therefore efficient cleanup of oil spills is of utmost importance. Bad weather can make oil recovery in the marine environment challenging. Remediation techniques to stimulate the growth of indigenous oil-degrading microorganisms is of great importance. Dispersants like Corexit may protect sensitive shorelines by dispersing the oil into the water. Dispersed oil droplets increase the bioavailability of the petroleum hydrocarbons to the oil-degrading microorganisms which can enhance biodegradation. There are however conflicting laboratory test results on the efficiency of dispersants to enhance biodegradation, and monitoring enhancement of oil biodegradation in the marine environment is difficult. In addition, the chemical dispersants themselves like Corexit can damage the environment and be toxic to aquatic life, but less toxic dispersants based on environmentally friendly ingredients are developing. Though, oil dispersion exposes sensitive aquatic organisms to oil, and they can be harmed from the toxic compounds. Difficult choices are made during oil spill response, and every oil spill situation is different and may require different strategies. Some species will probably get affected from large oil spills despite cleanup efforts. Precautions should be taken during oil spill response and the strategy harming the environment the least is desirable, giving a net environmental benefit. More research needs to be conducted on the efficiency of environmentally friendly dispersants on oil spill cleanup in the marine environment. Further investigation with different environmental conditions should be performed to test the enhanced biodegradation by dispersants as well as more toxicity testing to various aquatic organisms with realistic oil and dispersant concentrations.

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