

Degradation of Dispersants and Dispersed Oil

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Introduction

Chemical oil dispersants are proprietary mixtures of surfactants and solvents which are directly applied to a spill in order to reduce the natural attractive forces of the oil. When oil treated with dispersants is exposed to mixing energy, typically from wind and wave action, it is broken up into small droplets which may then become entrained in the water column (Li et al., 2009a; Li et al., 2009b; Li, 2008; Lunel, 1995). Many of these droplets are small enough to be neutrally buoyant, and therefore, advection and diffusion forces dilute the plume and transport the droplets far from the site of the original spill. As compared to a surface oil slick or larger and more buoyant physically dispersed oil droplets, these chemically dispersed droplets are much easier for oil-degrading bacteria to colonize and break down (Venosa and Holder, 2007; Venosa and Zhu, 2003). In addition, small droplets enhance dissolution of soluble and semi-volatile compounds into surrounding waters, wherein biodegradation is carried out by aqueous phase microbes. Under these conditions, oil concentration are effectively reduced below toxicity threshold limits, and biodegradation becomes the most important process in reducing the total mass of petroleum hydrocarbons in the environment. By enabling rapid dispersion and biodegradation of surface oil slicks at sea, the use of chemical oil dispersants can be effective in preventing heavy oiling of sensitive coastal environments such as beaches and wetlands, and consequently mitigates risk associated with marine and terrestrial wildlife coming into direct contact with a slick.

Biodegradation of Oil

Any discussion on the biodegradation of chemically-dispersed oil must consider the degradation of the oil itself. A variety of microorganisms in both terrestrial and marine environments have the capacity to utilize petroleum hydrocarbons as the sole source of carbon and energy (Head et al., 2006; Leahy and Colwell, 1990; Atlas, 1981, 1984; ZoBell, 1973). Recently a 181 genera of bacteria, 163 genera of filamentous fungi and yeast, and 22 genera of algae have been identified to have the ability to degrade hydrocarbons by metabolizing them in order to grow (Prince, 2010a,b). These findings are not surprising considering the fact that marine microorganisms have long been exposed to significant quantities of petroleum hydrocarbons from natural seepages.

From 1990 to 1999, approximately 600,000 tons of petroleum were released into the world's oceans per year from natural seepages (NRC, 2003; Stout and Wang, 2008). Biodegradation by indigenous microbial communities is the major process responsible for the weathering and eventual removal of oil from natural seeps that enters the marine

environment (Atlas, 1995; Atlas and Bartha, 1992; Leahy and Colwell, 1990). Within the marine environment, bacteria are the predominant hydrocarbon degraders (Head et al., 2006; Venosa and Zhu, 2003). Studies from tropical to cold Antarctic and Arctic environments have verified their ubiquitous distribution and their ability to multiply rapidly upon the introduction of oil (Atlas, 1995).

Biodegradation rates have been shown to be the highest for saturates, followed by light aromatics, with high-molecular-weight aromatics and polar compounds exhibiting extremely low biodegradation rates (Prince, 2010c). Co-metabolism plays an important role in oil biodegradation and may require microbial consortia or syntrophic interspecies cooperation (McInerney et al., 2008). Many complex branched, cyclic, and aromatic hydrocarbons, which otherwise would not be biodegraded individually, can be oxidized through co-metabolism in an oil mixture due to the abundance of other substrates that can be metabolized easily within the oil (Atlas, 1981).

It is important to note that microorganisms produce extracellular biosurfactants to promote the formation of oil-in-water emulsions that aid in the uptake and subsequent degradation of hydrocarbons (Desai and Banat, 1997). The hydrophilic and hydrophobic components within the biosurfactants emulsify hydrophobic hydrocarbons, and allow for transport into the hydrophilic intracellular space for biodegradation (Southam et al., 2001). In addition, the fatty acid moieties of biosurfactants promote the growth of microorganisms on the surface of oil droplets (Rosenberg et al., 1979). Nikolopoulou and Kalogerakis (2008) reported that the use of rhamnolipid biosurfactants increased removal of weathered petroleum hydrocarbons (96% removal of C19–C34 n-alkanes within a period of 18 days) and reduced the lag phase prior to the onset of biodegradation. Saeki et al. (2009) showed that addition of biosurfactant JE1058BS to seawater stimulated the degradation of weathered Alaska North Slope 521 crude oil by stimulating the activity of the indigenous marine bacteria and facilitating the removal of oil from the surface of contaminated marine sediments.

In terms of the influence of environmental factors controlling natural oil biodegradation rates, field studies have shown that active microorganisms living in low-temperature environments are dominated by two groups: psychrophilic and psychrotolerant, which are sometimes called psychrotrophic (Atlas, 1984). As defined by Morita (1975), psychrophiles experience optimum growth at less than 15°C, with a maximum growth temperature below 20°C and a minimum growth temperature at or below 0°C. Despite living at these low temperatures, psychrophiles often have metabolic rates comparable to those displayed by the mesophiles adapted to more moderate temperatures. For example, Delille et al. (2009) reported that a temperature of 4°C in the Antarctic had little effect on biodegradation efficiency and that the nutrients, nitrogen and phosphorus, were the limiting factors. Results obtained by Siron et al. (1995) indicated that the temperature threshold for observing significant oil biodegradation was around 0°C. Decreases in solubility associated with low temperatures were considered to be a causal factor for the cases of observed recalcitrance of hydrophobic compounds in cold-water. However, recent reports have indicated that some bacteria may have adapted to the low solubility of hydrophobic environmental chemicals (Deppe et al., 2005; Wick et al., 2002). Indeed there is now evidence that hydrocarbon-degrading microbes may have novel uptake

mechanisms that enable them to degrade hydrocarbons at rates that exceed their rates of dissolution in the aqueous phase (Leahy and Colwell, 1990; Thomas et al., 1986).

Throughout the world, the salinity of seawater averages about 35‰ (parts per thousand). Salinity variations, albeit small, are mainly caused by such factors as melting of ice, inflow of river water, evaporation, rain, snowfall, wind, wave action, and ocean currents that cause horizontal and vertical mixing of the saltwater (Lagerloef et al., 1995). Most marine species have an optimum salinity range of 25–35‰ (ZoBell, 1973) and species living in the transition environments are well adapted to fluctuations in salinity. Microorganisms requiring salt for growth are referred to as halophiles. Whereas halophilic hydrocarbon-metabolizing bacteria perform well in this salinity range, there have been reports of the isolation of bacteria capable of degrading hydrocarbons above a salinity of 35‰. Bertrand et al. (1990) reported the isolation from a salt marsh of an extremely halophilic archaea bacterium capable of degrading hydrocarbons in 204‰ NaCl, but not below 105‰. Diaz (2008) reported the isolation of a bacterial consortium, which mainly included members of the genera *Marinobacter*, *Erwinia* and *Bacillus*, from a crude oil sample from the Cormorant field in the North Sea. This consortium was able to metabolize petroleum hydrocarbons in a salinity range from 0 to 220‰ NaCl. Total oil degradation ranged from 48% to 75%, with the greater degradation occurring at the lower salinities.

At the sea surface, wind and wave action maintain a constant supply of oxygen, thus aerobic catabolism of hydrocarbons is usually the preferred biochemical pathway (Leahy and Colwell, 1990). Oxygen may become limiting in subsurface sediments and anoxic zones of the water column. Oxygen limitation is also a concern for most fine-grained marine shorelines, freshwater wetlands, mudflats and salt marshes (Venosa et al., 2002a; Venosa and Zhu, 2003). It is commonly believed that biodegradation rates under anaerobic conditions are almost negligible, while aerobic biodegradation of hydrocarbons occurs rapidly. However, the importance of anaerobic biodegradation should not be underestimated as it has been shown to be a major process under certain conditions. In anoxic marine sediments, reductions of sulphate, Mn(IV) and Fe(III) are the primary terminal electron-accepting processes (Canfield et al., 2005; Finke et al., 2007). Hydrocarbon degradation coupled with sulphate reduction prevails in marine anoxic sediments (Lovley et al., 1997).

With recent advances in analytical methods such as genomics, we are now able to determine the potential of whole microbial communities for oil biodegradation at low temperatures. New evidence as a result of advances in the field of environmental genomics suggests that crude oils are degraded by indigenous organisms in cold water environments at a higher rate than previously reported. This is not surprising since natural oil seeps occur in the world's oceans at great depths and low temperatures – microbes have become well adapted to their surrounding environment. Studies have conclusively shown that elevated concentrations of hydrocarbons in the environment increase the number of catabolic-gene copies among the microbial community (Heiss-Blanquet et al., 2005; Stapleton and Sayler, 2000; Whyte et al., 2002).

Biodegradation of Chemically Dispersed Oil

The effect of chemical dispersion on the biodegradation rate of petroleum hydrocarbons has been studied for several decades, and it is generally agreed that chemically dispersed oil is biodegradable. However, the observed effects of chemical dispersants on the rate of oil biodegradation have varied significantly among studies (National Research Council, 2005). Whereas some studies observed stimulation of biodegradation rates by the use of chemical dispersants (Swannell and Daniel, 1999; Traxler and Bhattacharya, 1978), chemical dispersion inhibited the biodegradation rate or had no effect in other studies (Foght and Westlake, 1982; Lindstrom and Braddock, 2002). The effect of chemical dispersion on the rate of oil biodegradation has been further complicated by substrate-dispersant interactions associated with differences in the experimental test conditions, which caused the biodegradation of individual hydrocarbons to be stimulated by some dispersants and inhibited by others (Foght et al., 1987; Van Hamme and Ward, 1999). As a result, it is difficult to predict the effect of dispersants on the biodegradation of specific hydrocarbons based on chemical class (e.g., aliphatic vs. aromatic) (Foght et al., 1987; Lindstrom and Braddock, 2002). Similarly, the effects of specific dispersants on biodegradation cannot be predicted based on the chemical characteristics of the surfactants or the hydrophile-lipophile balance (HLB) of the mixture (Van Hamme and Ward, 1999; Varadaraj et al., 1995).

Attempts have been made to predict the rate of oil biodegradation in the environment based on the results of laboratory studies using scalable, quantitative biodegradation kinetics models that treat oil as droplets suspended in water rather than as homogenous solutions of hydrocarbons in water and consider the growth of the organisms responsible for the biodegradation of oil (National Research Council, 2005). To date only two studies have made an attempt to estimate biodegradation kinetic parameters (Venosa and Holder, 2007; Zahed et al., 2011), by measurement of first-order (in oil concentration or the concentrations of specific oil components) rate coefficients to enable comparison among treatments. However as only one independent rate coefficient was estimated for each treatment, treatment effects could not be rigorously evaluated.

Conducting representative biodegradation studies on dispersed oil in microcosm-scale test systems has at least two important challenges that researchers need to consider as they develop test protocols (Lee et al., 2011). One challenge is to conduct tests at the low dispersed oil concentrations representative of field conditions. Many previous biodegradation studies were conducted at unrealistically high concentrations of dispersed oil in closed microcosms. Prior research either failed to recognize the rapid dilution that occurs at sea or employed methods that were not sufficiently sensitive to study low concentrations. Studying dispersed oil biodegradation at concentrations several orders of magnitude above expected at-sea concentrations in closed systems could limit biodegradation rates and total degradation by exhausting the available nutrients. Some researchers attempted to address this by adding nutrients to the system, but this can lead to unrepresentative modification of the microbial community.

The second challenge with studying dispersed oil biodegradation in a closed system is the difficulty of maintaining a stable dispersion in the laboratory. Dispersed oil in the water

column exists as small droplets that neither surface nor sink, and the droplets become so scattered that they cannot coalesce. It is challenging to maintain stable dispersions of oil in closed systems during the often multi-week test periods required to conduct biodegradation studies. To simulate the dispersion of oil at sea, biodegradation studies require formation of a stable dispersion containing droplets no greater than 70 - 100 microns and enough mixing energy to keep droplets from resurfacing during an experiment.

A recent Joint Industry Program (McFarlin et al., 2011) on the biodegradation of oil under Arctic conditions has assessed biodegradation of chemically and physically dispersed Alaska North Slope oil as indicated by both primary degradation and hydrocarbon mineralization. The study was conducted under low ambient temperature conditions (-10C – 20C) and relatively low oil concentrations (10-12 ppm crude oil in seawater) and under low level nutrients (0.5 – 1% of the OECD recommended volume of Bushnell Haas Broth). The results of the study demonstrated that the use of dispersant increased the primary biodegradation of fresh oil from 37% to 56% and the mineralization from 12% to 27%. Further increment was due to the addition of nutrients by 10 % for both primary degradation and mineralization.

Biodegradation of Dispersants

Most studies on surfactant biodegradation focus on surfactants that are used in high-volume consumer products, such as laundry detergents (e.g., linear alkyl sulfonates), or other cleaning agents which have known environmental health and safety concerns (e.g., alkylphenol ethoxylates). In general, the results of these have shown that most surfactant formulations are fairly readily biodegraded under aerobic (oxygen present) conditions by marine bacteria (Lee et al., 1985; Liu, 1983; Una and Garcia, 1983). The rate of biodegradation under anaerobic (absence of oxygen) conditions tends to be much lower (Berna et al., 2007; Ying, 2006). Research into the anaerobic biodegradability of sulphonate-based (anionic) surfactants has shown that chemical composition and molecular orientation can play an important role in biodegradability of a particular compound. Through the use of anaerobic digesters and analyses of bacterial biogas production, Garcia et al. (2009) conducted a series of batch degradation experiments which revealed that the anaerobic biodegradability of branched alkyl sulphosuccinates such as those used in Corexit were much lower ($\leq 50\%$ mineralization after 50 days) than that of linear alkyl sulphosuccinates ($\geq 80\%$ mineralization after 50 days). It is clear that different types of surfactants, and even individual surfactants of the same class, can biodegrade at very different rates depending on the structural complexity of chemical branching.

A report by the Fraunhofer Institute (2003) discusses in detail the anaerobic biodegradation of detergent surfactants such as those used in household cleaners. Unfortunately, little attention is paid to the surfactants commonly found in chemical oil dispersants. Generally speaking, the report states that non-ionic surfactants are readily biodegradable under both aerobic and anaerobic conditions. While anionic surfactants based on sulphosuccinate are readily biodegraded under aerobic conditions, data is limited concerning their anaerobic biodegradability.

The biodegradation of surfactants used oil spill treatment agents has been studied for years (Baumann et al., 1999; García et al., 2009; Lindstrom and Braddock, 2002; Liu, 1983; Odokuma and Okpokwasili, 1992; Una and Garcia, 1983). Surfactant biodegradation studies usually distinguish between primary biodegradation, which was calculated from the overall mass balance of surfactant in the reactors (García et al., 2009; Una and Garcia, 1983), and ultimate biodegradation, which also considers the removal of the intermediate products, usually based on oxygen consumption (Odokuma and Okpokwasili, 1992) or carbon dioxide production (García et al., 2009; Lindstrom and Braddock, 2002) relative to the amount expected based on the compound structure. Extensive, but incomplete, primary biodegradation of the ethoxylated non-ionic surfactants used in Corexit 9527 and 9500, Tween 80 and Tween 85, was observed in pure cultures of marine bacteria isolated from an estuary in Spain (Una and Garcia, 1983). Primary biodegradation of Span 80, the unethoxylated non-ionic surfactant used in both Corexit products, was less than 20% in the same study, but the authors suggested that the poor biodegradation may have been caused by substrate inhibition due to the extremely high surfactant concentration (5 g/liter), which likely would have impacted the integrity of bacterial membranes. Ultimate biodegradation of Tween 80 was about 50% in another study (Baumann et al., 1999), and DOSS (dioctyl sodium sulfosuccinate) was extensively biodegraded by activated sludge bacteria (García et al., 2009), but the observed oxygen consumption or carbon dioxide production were much lower than expected for Corexit 9527 (Odokuma and Okpokwasili, 1992) and Corexit 9500 (Foght et al., 1987), respectively, suggesting that biodegradation was incomplete within the testing period. Some studies have suggested that partial biodegradation of Tween 80 involves metabolism of the oleic acid portion of the molecule, leaving the polyethoxy groups untouched or only partially metabolized (Baumann et al., 1999; Kim and Weber Jr., 2003). Note, however, that enzymatic oxidation and subsequent metabolism of polyethoxylate groups has been described (Nguyen and Sigoillot, 1997; Owen et al., 1997).

These results suggest that the fate of dispersant surfactants is highly dependent on the concentration and chemical characteristics of the surface-active compounds, the microbes available, the methods used to monitor biodegradability (as the separation of surfactants and the crude oil hydrocarbons remains a challenge in analytical chemistry), and hence again the critical importance of testing biodegradability at environmentally relevant substrate concentrations. Unfortunately, until the recent Deepwater Horizon oil spill in the Gulf of Mexico, little information is available on the fate of surfactants in the presence of natural microbial seawater communities at concentrations expected during actual spill response operations. .

Toxicity and Bioaccumulation of Dispersant Surfactants

The toxicity of dispersants may influence trophic level dynamics including microbial processes responsible for oil degradation (Lee et al., 1985). The premise of dispersant use is based on the reduction of oil to concentrations below toxicity threshold limits. Based on the results of toxicity tests for EPA-approved dispersants such as Corexit 9527 and 9500 listed on the National Contingency Plan (NCP) Product Schedule and the

recommended dispersant-to-oil (DOR) application rates, major environment impacts were not expected at the concentrations to be encountered during their operational use for the treatment of oil spills. A report from the Centers for Disease Control and Prevention (2010) also concluded that “because of the strict guidelines that must be followed to utilize dispersants, it is unlikely that the general public will be exposed (directly) to (the) product.” The report further states that “ingredients are not considered to cause chemical sensitization; the dispersants contain proven, biodegradable and low toxicity surfactants.”

Despite the development of a regulatory approval mechanism for the support of their operational use, a considerable amount of ongoing research has been funded to evaluate the toxicity of chemical oil dispersants in the marine environment. Public concerns remain high in regards to this topic as the data collected to date has been highly variable due to factors such as differential sensitivity between species, the particular dispersant formulation used, and experimental conditions used (George-Ares and Clark, 2000; Lyons et al., 2011).

Ramachandran et al. (2004) reported that chemically-dispersing different crude oils increased the exposure of fish to the constituents of oil by 10 to 1000-fold in comparison to undispersed oil. The enhanced exposure was demonstrated by an increased activity of liver enzymes that oxygenate compounds accumulated from water containing chemically-dispersed oil droplets. The implication of this work is that the toxicity of oil to fish increased following chemical dispersion in proportion to the extent to which the oil was dispersed. It was noted that the amount of a solution of chemically-dispersed oil that caused toxicity was 100 times less than the amount of undispersed oil required to cause the same effect. However, the measured concentrations of hydrocarbons that were toxic was virtually the same between solutions of dispersed and undispersed oil. Thus, the effect of chemical dispersion was to transfer more compounds from oil to water, and not to make these compounds more toxic.

A study conducted by Fuller et al. (2004) using two fish species, *Cyprinodon variegatus* and *Menidia beryllina*, one shrimp species, *Americamysis bahia* (formerly *Mysidopsis bahia*), and the luminescent bacteria *Vibrio fischeri* indicated that the toxicity of chemically dispersed oil preparations was equal or less toxic than that of the oil alone. A separate study by Hemmer et al. (2010) looking at toxicity of Louisiana Sweet Crude (LSC), chemical oil dispersants, and chemically dispersed LSC on *M. beryllina*, and *A. bahia* reported that the toxicity of the dispersant alone was lower than that of LSC or dispersed LSC which both showed moderate to high toxicity. Similarly, Milinkovitch et al. (2011) found that while containment and recovery of spilled oil is optimal, there is no significant difference between the toxicity of naturally and chemically dispersed oil when looking at a series of biomarker responses in the gills of golden grey mullet (*Liza aurata*). Further work by Judson et al. (2010) has investigated the potential for chemical oil dispersants to interfere with hormone and other bio-chemical processes in marine organisms. The results of this study concluded that while some dispersants did show low potential for endocrine disruption, most (including Corexit) did not show any significant effect.

Brief pulses of chemically-dispersed oil can be just as toxic to fish embryos as prolonged exposures. McIntosh et al. (2010) recently reported that exposures to chemically-dispersed crude oil as brief as one hour prevented the fertilization of Atlantic herring embryos. Similarly, one-hour exposures immediately following egg fertilization were sufficient to cause deformities, interfere with development, and kill herring embryos at oil concentrations typical of those measured near actual marine oil spills. Thus, contrary to expectations, the primary concern about oil dispersion may not be the toxicity of chemical dispersants, nor the enhanced toxicity of oil. Rather, it is the greatly increased exposure of highly sensitive embryos to the toxic components of oil. As the result, there is the possibility that even brief exposures of fish embryos to dispersed oil can cause embryo toxicity at oil concentrations typical of actual spills. The results of these laboratory studies have highlighted the need for future research to determine effects under the environmental conditions encountered under operational response operations.

In light of the toxicity research study results obtained for chemical oil dispersants, it is suggested that factors such as toxicity of dispersed oil (rather than dispersant itself), dilution and degradation in the environment, species/resources requiring priority protection, potential adverse effects of all response options, and the potential for recovery of sensitive habitats and populations should weigh more heavily into the decision making process than dispersant toxicity alone George-Ares and Clark (2000). Supporting this view, a recent review of the use of chemical dispersants in Europe found that ongoing improvement in dispersant formulation has now reached a point where the toxicity of the dispersant itself is much less important than the toxicity of the oil it is dispersing (Chapman et al., 2007).

While the focus of studies on chemically dispersed oil has been on the induction of acute and/or chronic toxic effects for risk assessments associated with dispersant use, some consideration has also been given to the bioaccumulation of surfactants. In a study on the uptake of two linear alkylbenzene sulfonates by a freshwater oligochaete (*Lumbriculus variegatus*) and a larval insect (*Chironomus riparius*) using radioactive tracers Mäenpää and Kukkonen (2006) reported that the surfactant residue in the body of the test organisms was more highly dependent on the organic content of the test sediment than on the initial exposure concentration. It was concluded that the high organic content of the test sediment reduced body residue concentrations due to the adsorption of the surfactant to the organic material contained in the sediment. This result is consistent with earlier research that concluded surfactants of all classes are readily taken up across the gills but that environmental variables could reduce the concentration of surfactants associated with the test species (EOSCA, 2000).

Following a comprehensive review on the bioaccumulation potential of surfactants, the European Oilfield Specialty Chemicals Association (EOSCA) concluded that although surfactants and their metabolites can be found in aquatic organisms following exposure, there is no evidence to support biomagnification of surfactants through the food chain (EOSCA, 2000). There is also evidence that non-ionic and anionic surfactants (such as those found in most oil dispersants) are biotransformed and eliminated via the gall bladder (Tolls et al., 1994) As a result of surfactant metabolism, Comber et al. (2003)

suggested that linear alkylbenzene sulfonates, alcohol ethoxylates, and other structurally similar surfactants are unlikely to bioaccumulate to any significant degree.

Dispersant Use During the DWH Spill Response

The decision by the EPA, NOAA and BP to use chemical dispersants at the Deepwater Horizon spill site did not come lightly. The United States Clean Water Act specifically addresses the use of dispersants in response to oil spills in Section 311(d)(2)(G) which requires that the federal National Contingency Plan for oil spill response contain a schedule identifying:

- (i) dispersants, other chemicals, and other spill mitigating devices and substances, if any, that may be used in carrying out the Plan,
- (ii) the waters in which such dispersants, other chemicals, and other spill mitigating devices and substances may be used, and
- (iii) the quantities of such dispersant, other chemicals, or other spill mitigating device or substance which can be used safely in such waters, which schedule shall provide in the case of any dispersant, chemical, spill mitigating device or substance, or waters not specifically identified in such schedule that the President, or his delegate, may, on a case-by-case basis, identify the dispersants, other chemicals, and other spill mitigating devices and substances which may be used, the waters in which they may be used, and the quantities which can be used safely in such waters.

Although Corexit 9527 and 9500 were both pre-approved by the EPA for use in the event of an oil spill, until the incident in the Gulf, little consideration was given to the suitability of these products for subsurface application. Subsurface injection of dispersant was considered as a means to reduce VOC levels and the volume of dispersant to be used (as application at the well head would improve contact between dispersant and the oil). Thus, regulatory approval subsurface application was withheld until its efficacy and potential effect on the environment could be assessed (EPA Press Conference Call, 2010).

Following a Net Benefit Environmental Analysis (NEBA) process, a decision was made to apply dispersants as an operational countermeasure during the Deepwater Horizon response operations. In Total, 43,884 barrels of Corexit brand chemical oil dispersant was applied (1); 25,505 barrels of Corexit 9527 and Corexit 9500A at the surface (by spraying from vessels at sea and aircraft) and 18,379 barrels of Corexit 9500 via subsurface injection (Federal Interagency Solutions Group, 2010).

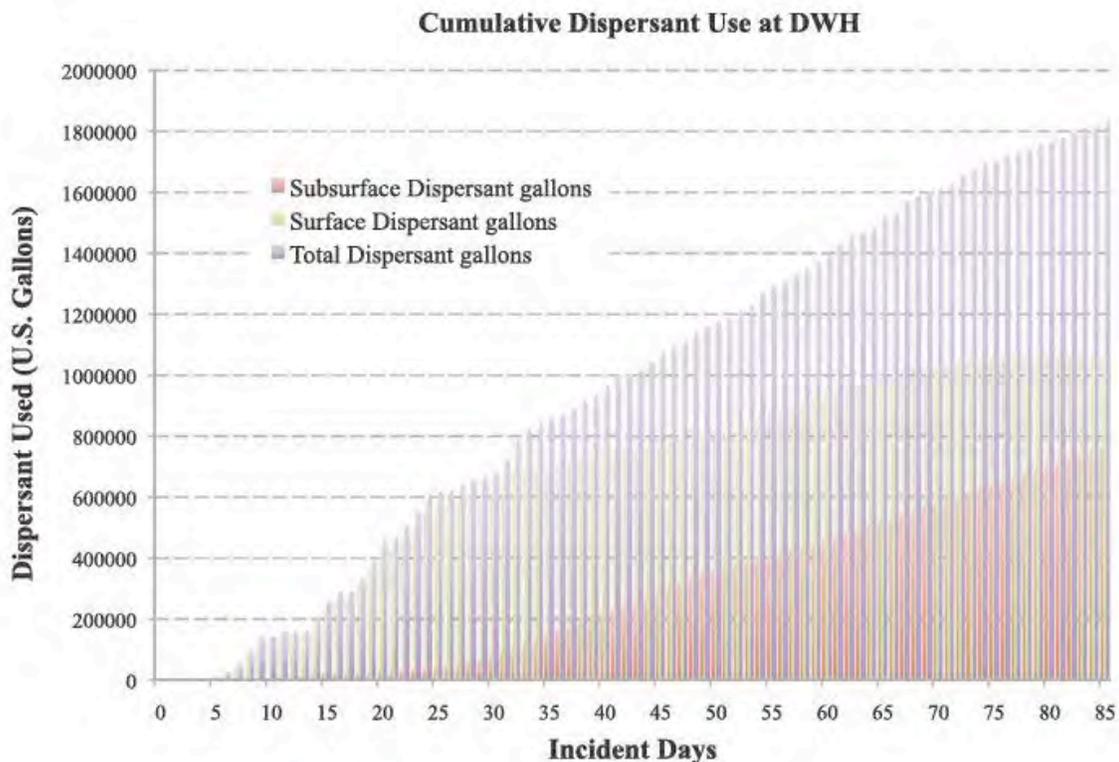


Figure 1. Cumulative surface and subsurface dispersant use by day of spill response (Federal Interagency Solutions Group, 2010).

Dispersant Transport and Fate following the DWH Spill

Corexit 9500 and 9527 were the main dispersants used in response to the BP-Deepwater Horizon oil spill. Detailed formulation and ingredient information on COREXIT dispersant products were released to the US EPA for its Gulf monitoring and environmental risk assessment program (Nalco, 2011). The surfactants in these products are similar, including several non-ionic compounds-sorbitan monooleate (Span 80), polyethoxylated sorbitan monooleate (Tween 80), and polyethoxylated sorbitan trioleate (Tween 85)—and the anionic surfactant diethylhexyl sulfosuccinate (DOSS) (Nalco, 2011), but the relative proportions of these compounds may differ somewhat between products (Kujawinski et al., 2011). The biodegradability of chemical constituents was a criterion in the selection of their formulation by the manufacturer to minimize potential for risks to the environment or public health associated with its use.

As the dispersants could be broken down rapidly in seawater; the individual chemical constituents 2-butoxyethanol, dipropylene glycol n-butyl ether (DPnB), propylene glycol, and dioctyl sodium sulfosuccinate (DOSS) were selected for analysis of water samples to determine the expanse of the Deepwater Horizon dispersed oil and Corexit (Operational Science Advisory Team, 2010). These compounds represent major constituents of Corexit, including those with known toxicology data, and also those with newly established analytical methods.

Benchmark levels for the individual compounds are established to explain the relevance of measured concentrations (i.e., concentrations above benchmark levels are “levels of concern.”) The benchmarks were based on available biological effects data were set at a conservative level to protect aquatic life. It was also recognized that the target compounds were also used in other commercial products besides Corexit. Thus, the other sources of the individual chemicals were to be considered if the benchmark values were exceeded.

Except for offshore water column samples (79% positive), the dispersant indicators were observed in a small fraction (< 10%) of the samples that were tested. DPnB was one of the most commonly observed dispersant indicator compounds (57 of the 60 positive water samples), but its concentration never exceeded 3 µg/L (Table 1).

Table 1. Samples from the Gulf of Mexico analyzed for the presence of Corexit dispersant indicators; data from the Operational Science Advisory Team (2010).

Zone	Profile	Undetected	Below Benchmark
Nearshore	water column	4790	60
	Sediment	406	6
Offshore	water column	251	199
	Sediment	242	1
Deep Water	water column	3761	353
	Sediment	119	1

* no exceedence of EPA's dispersant benchmarks were observed.

Propylene glycol was the only dispersant indicator detected in the nearshore sediments. Concentrations of DPnB decreased over time (Figure 2), with all values less than 5 µg/L by July 30. The DPnB concentration was highest at the surface and subsurface between 1000 and 1400 m (Figure 3). Deep water concentrations ranged from 0.0170 to 113.4 µg/L with a mean of 4.3 µg/L (Operational Science Advisory Team, 2010).

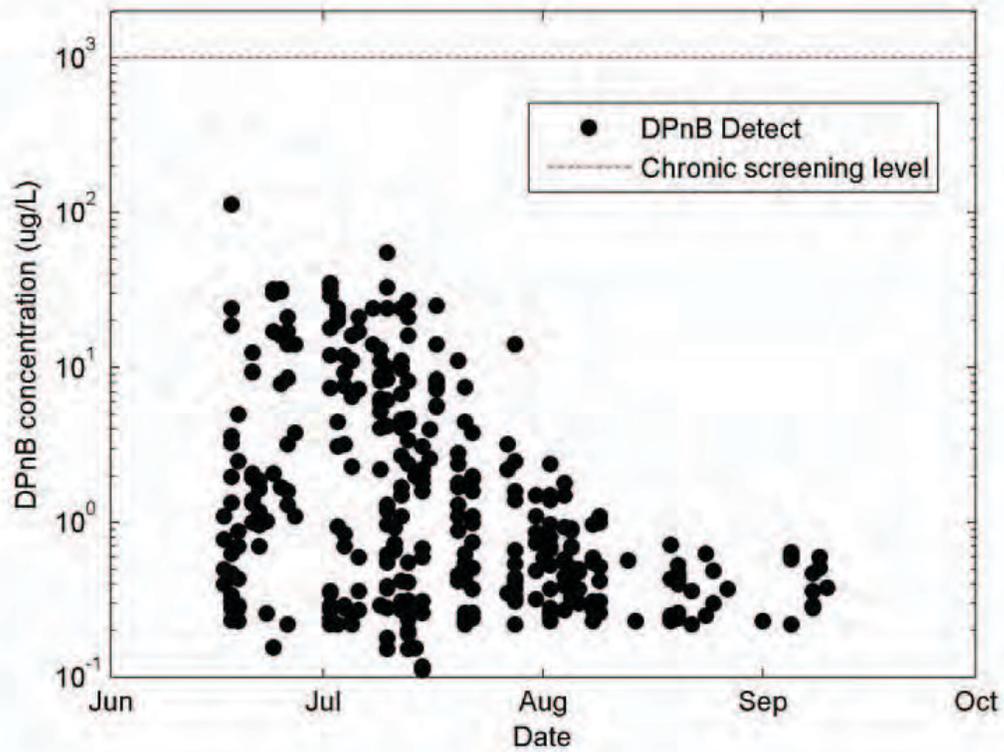


Figure 2. Offshore DPnB concentration over time; the United States Environmental Protection Agency benchmark (chronic screening level) is 1 mg/L (Operational Science Advisory Team, 2010).

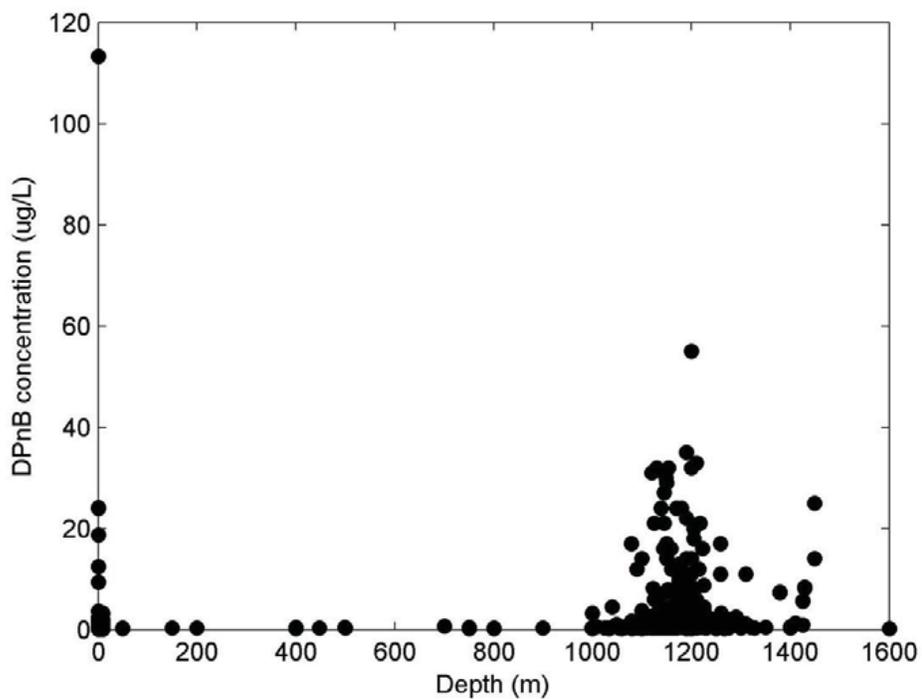


Figure 3. Concentration of DPnB with depth in water samples collected in the deep water zone of the Gulf of Mexico, defined as water depths of greater than 200 m (Operational Science Advisory Team, 2010).

Using advanced liquid chromatography Fournier transform ion cyclotron resonance mass spectrometry, DOSS was detected both during dispersant application, and up to 300 km from the wellhead 64 days after deepwater dispersant injection had ceased (Kujawinski et al., 2011). It was reported that the majority of the DOSS associated with the subsurface injection of Corexit 9500 moved to, and remained in the bottom water layer between 1000 and 1200 m depth rather than rising to the surface (Figure 4). The possibility could not be dismissed that some dissolution with subsequent vertical transport, as well as partitioning with gas or hydrate, might have occurred. The near 1:1 correlation between DOSS and methane (which was found to act as a conservative marker in this situation) and the consistency between their release rates, indicated that DOSS was not biodegraded or otherwise lost near the well head during conditions of active flow, and thus was transported to the 1000-1200 m layer (Figure 5). The measured concentrations, ranging from 0.4 to 12 $\mu\text{g/L}$, were remarkably similar to the expected estimated concentrations. Based on the data, Kujawinski et al. (2011) concluded that although biodegradation might have occurred, the most significant factor that caused a decrease in DOSS concentration at depth was dilution. They calculated that biodegradation rates would have had to be approximately ten times the dilution rate in order for it to have been observed (Kujawinski et al., 2011).

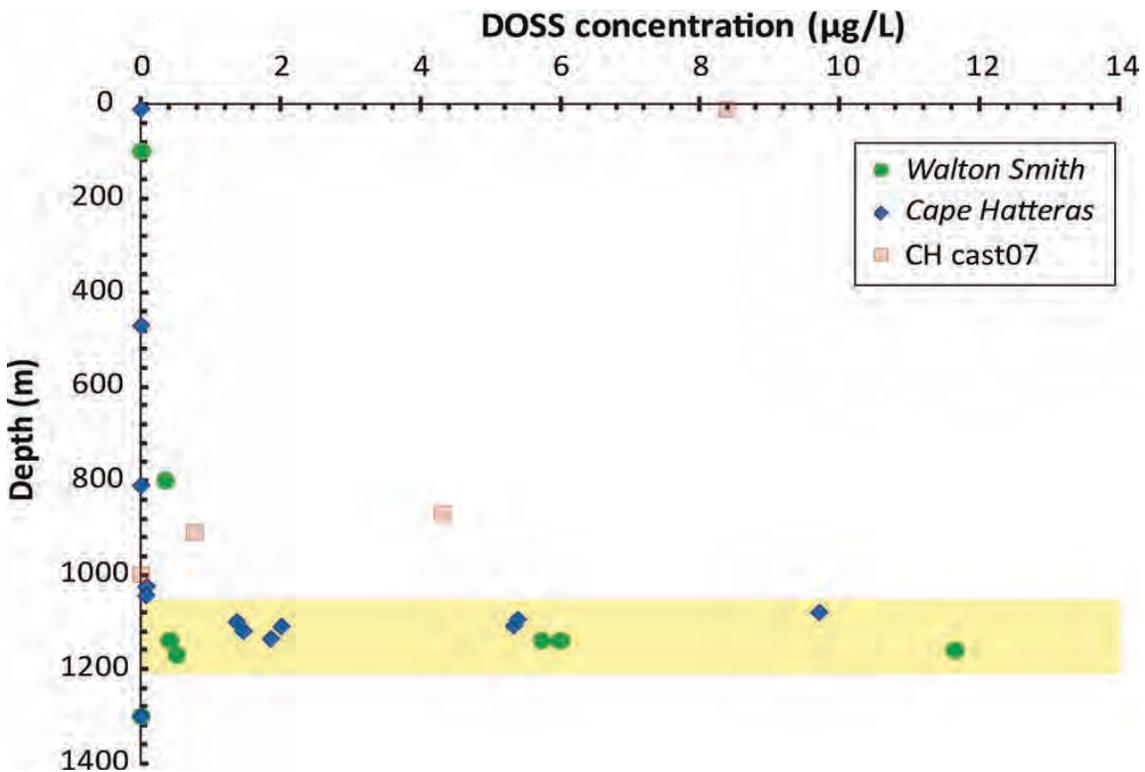


Figure 4. Depth profile of DOSS concentrations falling mainly in the high chromophoric dissolved organic matter zone which included high levels of methane (yellow area), during May and June,

2010, from samples collected by the research vessels *Cape Hatteras* (CH) and *Walton Smith*, with anomalous values from cast07 (Kujawinski et al., 2011).

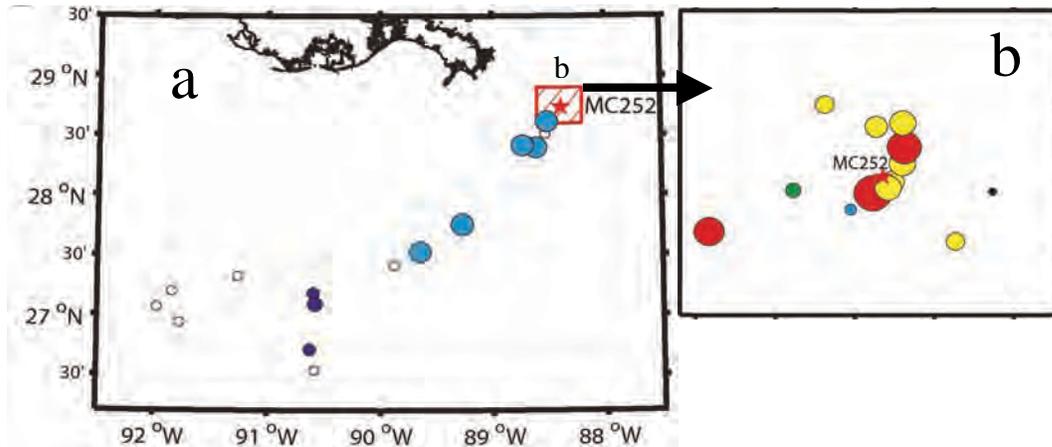


Figure 5. Bird's-eye view of DOSS concentrations at the plume depth of 1000-1200 m in September (a) and May/June (b) with circle size and colour indicative of concentration: white is below detection; blue < 0.01 µg/L; cyan 0.011-0.1 µg/L; green 0.11-1.0 µg/L; yellow 1.0-9.0 µg/L; red > 9.1 µg/L; black indicates the samples were not taken in the plume layer; the star indicates the Deepwater Horizon oil spill site (Kujawinski et al., 2011).

Oil Degradation following the DWH Spill

The recent *DWH* spill has highlighted the importance of natural oil degradation in the recovery of marine ecosystems impacted by crude oil spills. Hazen et al. (2010) reported that the disappearance of residual oil in the Gulf of Mexico from the *DWH* spill was associated microbial degradation processes based on the results of metagenomics. Temperature did not appear to be a major limiting factor as significant rates of oil degradation were observed within the subsea plume of dispersed oil at a depth of 1300 m and temperature below 4°C. Unlike oil spills occurring at the sea surface, during the *DWH* spill, petroleum hydrocarbons experienced a prolonged, buoyancy-driven ascent through the 1500 m water column (Hazen et al., 2010). Consequently a unique set of processes affected the released hydrocarbons during their trajectory in the deep sea. Some oil and gas never reached the sea surface, but instead formed hydrocarbon-rich plumes within the cold waters present at about 1100 m depth, supporting an active deep-sea microbial community (Hazen et al., 2010; Valentine et al., 2010). A combination of integrated chemical, physical and biological processes regulated the transport and fate of hydrocarbons in the deep marine environment. Considering the natural levels of variability and the availability of data at this point of time, it is difficult to disentangle the role of natural processes from the effects of countermeasures such as the use of dispersants.

A considerable amount of research has been focused on the resultant plume of dispersed oil and gas released from the well blow-out that extended southwest from the wellhead between about 1000 and 1200 m below the surface (Camilli et al., 2010; Diercks et al., 2010; Hazen et al., 2010; Kessler et al., 2011; Valentine et al., 2010). This plume was identified based on fluorescence (Camilli et al., 2010; Diercks et al., 2010; Hazen et al.,

2010), light scattering (Diercks et al., 2010), or the concentrations of specific hydrocarbons (Camilli et al., 2010; Diercks et al., 2010; Kessler et al., 2011; Valentine et al., 2010) and was detectable up to 35 km from the MC252 wellhead (Camilli et al., 2010). The average temperature in the plume was about 5 °C (Camilli et al., 2010; Hazen et al., 2010). Most of these studies also observed a local dissolved oxygen (DO) minimum in the vicinity of the hydrocarbon plume. Camilli and colleagues attributed this minimum to hydrocarbon interference with the *in-situ* DO probes that were used because Winkler titration data did not show oxygen depletion within the plume (Camilli et al., 2010). Other studies, however, showed good agreement between data from the *in-situ* DO probe and Winkler titrations (Kessler et al., 2011; Valentine et al., 2010), suggesting that the rate of aerobic microbial metabolism within the plume was higher than in the surrounding water. Evidence supporting biodegradation of gaseous alkanes (e.g., methane, ethane, propane) (Kessler et al., 2011; Valentine et al., 2010) and higher molecular weight normal alkanes (Kessler et al., 2011) was obtained based on compositional changes that reflected preferential utilization of specific compounds and (for ethane and propane) changes in $\delta^{13}C$ (Valentine et al., 2010). One study estimated that about 70% of the oxygen depletion that was observed within the plume was due to microbial metabolism of ethane and propane (Valentine et al., 2010). Microbial degradation of other hydrocarbons, including butane and longer chain alkanes, was responsible for the additional oxygen depletion. Hazen and colleagues estimated half-lives between about 1.2 and 6.1 days for higher molecular weight normal alkanes based on *in-situ* and microcosm data (Comber et al., 2003). Because their biodegradation rate model did not include biomass concentration, however, and the *in-situ* half-lives did not consider dilution as a factor contributing to the observed changes in compound concentration, the similarity among the observed half-lives should not be over interpreted.

Flocs from samples collected within the plume between May 25 to June 2 were rich in microbes, oil, and oil degradation products, and bacterial counts were elevated within the plume (Hazen et al., 2010). The abundance of genes involved in hydrocarbon degradation were significantly enhanced ($p < 0.05$ or 0.01) in plume samples, and there was a positive correlation with the concentration of low molecular weight components in the oil, suggesting that the composition of the bacterial community changed in response to the presence of oil (Hazen et al., 2010). Cloning and sequencing of 16S rRNA genes showed that the relative abundance of 16 taxa of α -Proteobacteria, including representatives of known psychrophilic and psychrotolerant hydrocarbon degraders, were higher inside the plume. The most abundant species in samples from within the plume (comprising about 90% of sequences) belonged to a single operational taxonomic unit that was closely related to *Oceanospirillales* (Hazen et al., 2010). Note that observations of samples collected in the same area by another research group about two weeks later, while oil was still being released from the wellhead, did not confirm high levels of *Oceanospirillaceae*, but the samples were dominated by other putative hydrocarbon degraders, especially relatives of *Colwellia* and *Cycloclasticus*, which were thought to be growing on propane, ethane, and butane (Valentine et al., 2010).

Recently, Lu et al. (2011) showed that the microbial community functional composition and structure were dramatically altered in the deep-sea from the Deepwater Horizon spill.

A variety of metabolic genes involved in aerobic and anaerobic hydrocarbon degradation were highly enriched in the plume than outside the plume. Various other microbial functional genes that are associated with carbon, nitrogen, phosphorous, sulfur, and iron cycling, metal resistance, and bacteriophage replication were also enriched in the plume. The authors suggest that the indigenous marine microbial communities could have a significant role in biodegradation of oil spills in deepwater.

In summary, the size and composition of the Gulf of Mexico microbial community was altered as microbes responded to the presence of oil. Bacterial cell densities were significantly higher in the plume, 10^5 cells mL^{-1} , as compared to numbers outside the plume, which was 10^3 cells mL^{-1} (Atlas and Hazen, 2011). As the community responded, hydrocarbon degraders dominated, resulting in reduced community diversity. DNA surveys for bacterial 16S rRNA genes from samples collected in June revealed dominance of *Cycloclasticus* and *Colwellia*, likely degrading propane and ethane preferentially (Kessler et al., 2011; Valentine et al., 2010). Sixteen taxa of the γ -proteobacteria dominated by the order *Oceanospirillales* occurred in high numbers and dominated the community in plume samples collected in the same time frame (Hazen et al., 2010). Among these were *Oliespira antarctica*, *Thalassolituus oleivorans*, and *Oliphilus messinensis*, bacteria known to degrade hydrocarbons and tolerate low temperatures that occur in the deep sea. Samples collected later (September) indicated a shift away from these hydrocarbon degraders to methanotrophs, including *Methylococcaceae*, *Methylophaga*, and *Methylophilaceae*. The enhanced abundance of methanotrophs and bacteria containing the particulate methane monooxygenase gene (*pmoA*) indicated that methane was consumed later in the spill sequence by a different bacterial assemblage (Kessler et al., 2010).

Propane and ethane were degraded relatively rapidly and likely before alkanes >5 carbons in length (Valentine et al., 2010). The occurrence of natural seeps in the area of the spill may have supported the development and persistence of microbial communities capable of degrading hydrocarbons. Dissolved propane and ethane may promote rapid hydrocarbon degradation and low diversity communities that can degrade other hydrocarbons as the nature of remaining hydrocarbons changes. Hazen et al. (2010) estimated biodegradation rates for hydrocarbons in the plume based on observed concentrations of C13-C26 alkanes from samples collected near the MC252 plume and from laboratory degradation studies at 5 °C. Based on these observations, degradation of alkanes was estimated to be 1.2–6.1 days. Rapid rates of biodegradation may be expected for alkanes, the least recalcitrant fraction among the complex mixture of compounds that makes up Sweet Louisiana Crude oil. Rapid degradation rates reported for Sweet Louisiana Crude in the region of the MC252 oil spill may be related to its relatively light character, containing a large volatile component and a large fraction of alkanes, both more amenable to degradation than heavier crude oil. Edwards et al. (2011) reported that microbes within the surface slick showed higher rates alkaline phosphate activity, indicating enhanced phosphate stress. Microbial respiration and lipase activity rates were also higher with the slick and the degradation of hydrocarbons was fairly rapid and supported the majority of respiration. The authors suggest that the microbial community possessed the potential to respire hydrocarbons at an unprecedented rate, potentially great enough to keep pace with the flux of oil reaching the surface from the Macondo well, and

the observed differences in microbial respiration and activity between stations within the slick and outside the slick is a testament to the rapid response of the microbes in surface waters of the Gulf of Mexico to oil from the Deepwater Horizon spill.

The composition of the oil fraction from MC252 estimated by Reddy et al. (2011) indicated 74% saturated hydrocarbons, 16% aromatic hydrocarbons and 10% polar hydrocarbons. Gas chromatographic analysis for several monoaromatic compounds indicated benzene, toluene, ethylbenzene and total xylenes (BTEX) concentrations exceeding $50 \mu\text{g L}^{-1}$ within the plume in June 2010 (Camilli et al., 2010). Estimates for hydrocarbon degradation in the plume range from $2\text{--}7 \mu\text{g L}^{-1}\text{d}^{-1}$, which translates to an estimated half-life of about 1 month for petroleum hydrocarbons (Reddy et al., 2011). Methane was estimated to take longer to degrade. Kessler et al. (2011) estimated the oxidative lifetime of methane resulting from the spill to be 120 days.

Lessons Learned from Deepwater Horizon Spill Response and Future Challenges

About 2.1 million gallons of dispersant were used during the *Deepwater Horizon* spill response, and about 8% of the oil that was released is thought to have been chemically dispersed (Lubchenco et al., 2010). Based on current knowledge, it is believed that most of the chemically dispersed oil, including the MC252-derived hydrocarbons in the deep plume, may have been biodegraded within the environment following its release. Indirect evidence consistent with the expected biodegradation included identification of genes known to be involved in hydrocarbon biodegradation, enrichment of 16S rRNA sequences related to known hydrocarbon degraders, and depletion of dissolved oxygen within the deep dispersed oil plume. In light of the large uncertainties associated with measurements of hydrocarbon fate following accidental spills, fate and transport modeling may offer the best means for evaluating the relative impacts and benefits of chemical dispersion in spill response.

Due to the low concentrations of dispersant following its application in the Gulf of Mexico, as the result of physical dispersion and dilution processes, as well as the intrinsic levels of variability within an open-ocean environment, it is impossible to extract concrete evidence to support the hypothesis that the dispersant surfactants biodegraded rapidly in subsurface waters. Additional research is warranted to enhance better and more quantitative understanding of the fate of dispersants and chemically dispersed oil, particularly in subsurface. In terms of potential environmental risk, it is important to note that all of the surfactants used in Corexit 9500 and Corexit 9527 are known to be at least partially biodegradable under appropriate conditions. Indeed, dispersants themselves can enhance the initial rate of petroleum hydrocarbon degradation by being the first substrate utilized by the hydrocarbon degrading bacteria to grow and colonize dispersed oil droplets (Varadaraj et al., 1995).

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