Lessons from the 2010 *Deepwater Horizon* Accident in the Gulf of Mexico

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Contents

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Abstract

The 2010 *Deepwater Horizon* (DWH) accident in the Gulf of Mexico had many unique aspects to it not seen in previous marine spills. Indeed, research related to the DWH response phase, Natural Resource Damage Assessment, Gulf of Mexico Research Initiative (GoMRI), National Academy of Sciences, US agencies: NOAA, EPA, Fish & Wildlife, DOE, and Coast Guard have made this the most studied marine oil spill in the world. There are many oil biodegradation lessons learned from this experience and these will undoubtedly continue for many years.

1 Introduction

On April 20, 2010, the *Deepwater Horizon* (*DWH*) an ultra-deepwater, dynamically positioned, semi-submersible, mobile offshore drilling rig owned by Transocean caught fire while drilling at the *Macondo* prospect in the Mississippi Canyon Block 252 lease and exploded 77 km off the coast of Louisiana in the Gulf of Mexico with the loss of 11 lives. Several attempts to activate the blowout prevention device and the blind sheer ram failed. Two days later on April 22, 2010, the *DWH* sank to the seafloor at 1500 m, with the 53 cm riser pipe detaching from the rig it collapsed into a convoluted heap on the seafloor and began leaking oil in at least 3 sections. This caused the largest marine oil spill in United States history and the second largest marine oil spill in the world (Fig. 1). On June 3, 2010, the riser was cut off at the top of the blowout prevention device. After several attempts to stem the flow of oil failed, the well was successfully capped on July 15, 2010, and declared dead by the National Incident Commander on September 19, 2010. The government estimate of the amount of oil that came from the *Macondo* well directly into the environment was 4.1 million barrels with an additional 820,000 barrels captured via siphon tubes (Fig. 2) (FISG 2010). The cleanup effort was the largest ever in the world with more than 31,800 people involved (Fig. 2) (*Deepwater Horizon* Unified Command, 2010).

The *DWH* accident had many unique aspects to it not seen in previous marine spills. Indeed, research related to the *DWH* response phase, Natural Resource Damage Assessment, Gulf of Mexico Research Initiative (GoMRI), National Academy of Sciences, US agencies: NOAA, EPA, Fish & Wildlife, DOE, and Coast Guard have made this the most studied marine oil spill in the world. There are many oil biodegradation lessons learned from this experience and these will undoubtedly continue for many years.

2 Lesson 1. Marine Oil Biodegradation Like All Politics Is Local and *DWH* Had Many Unique Aspects

Marine oil biodegradation is affected by a large number of parameters, e.g., oil type, currents, weather, temperature, pressure, limiting nutrients, water depth, input of oil (leak, spill, failure of blowout prevention device), season, risk receptors, and ability

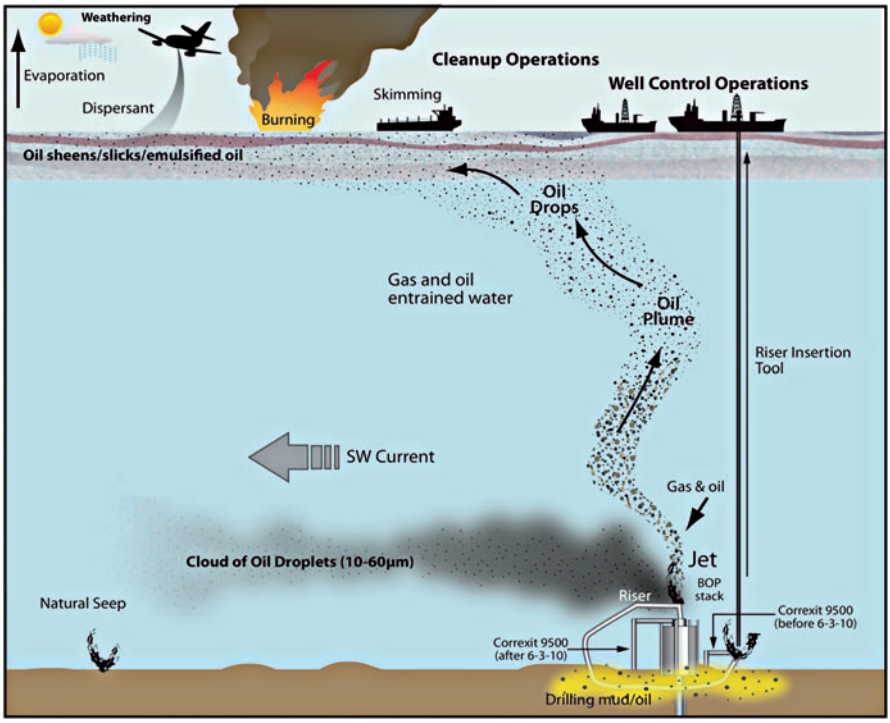

Fig. 1 Graphic depiction of Deepwater Horizon spill and cleanup, showing oil droplets rising to the surface and small droplet forming a deepwater cloud, oil sheens, and slicks the surface, with burning skimming, weathering, and blowout prevention (BOP) device that failed and injection of dispersant in the deep and at the surface. (After Atlas and Hazen (2011))

to apply remediation (dispersants, siphon tubes, booms, skimmers, burns). Many of these can work synergistically to impact oil biodegradation: (1) chemical dispersants + mineral fines can enhance formation and transfer of oil from the surface into the water column (Li et al. 2007), (2) autoinoculation from gyres + "memory response" of oil degraders leads to an increase in microbial abundance and accelerated oil biodegradation (Valentine et al. 2012), (3) oil droplet size + dispersion + biodegradation rates + dissolution enhances biodegradation, dissolution and dispersion rated oil hydrocarbons (Brakstad et al. 2015a), (4) cometabolic biodegradation + dispersion + secondary electron donors enhances biodegradation, dissolution, and dispersion rates of oil hydrocarbons even when the oil itself cannot be a suitable electron donor (Hazen et al. 2016), and (5) biosurfactants from multiple microorganisms can enhance bioavailability of poorly soluble hydrocarbons in the oil (Singh et al. 2007; McGenity et al. 2012).

DWH had many unique aspects, it was the deepest oil well blowout that has ever occurred, and it was the first time that dispersants were applied at the well head. It was not controlled for 84 days. It had deep water temperatures of 4 °C and simultaneous surface water temperatures of over 30 °C (Hazen et al. 2010).

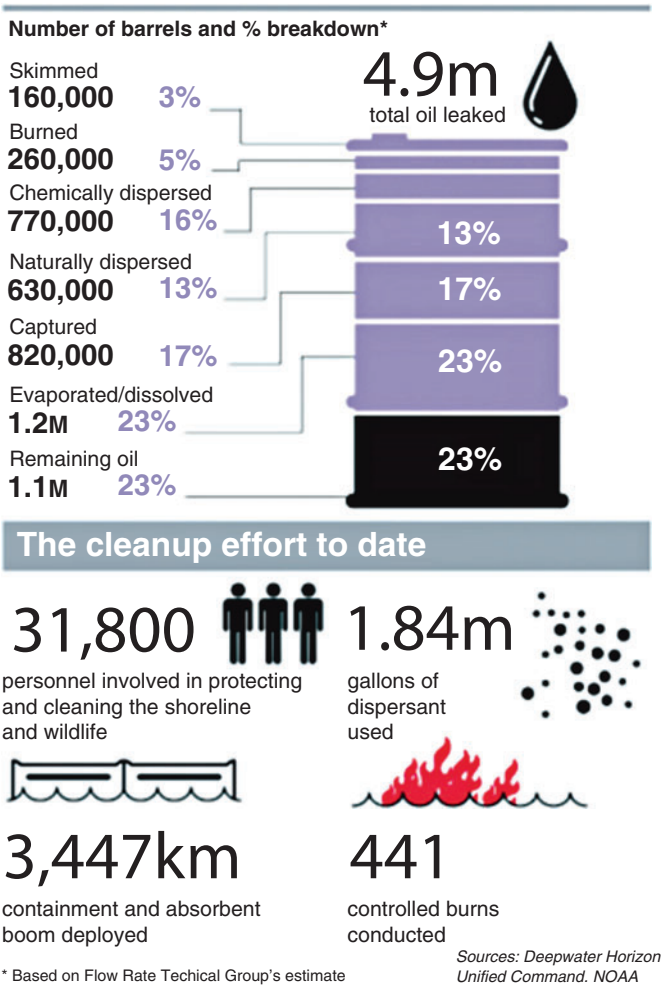

Fig. 2 Where the oil went? The Federal Interagency Solutions Group, Oil Budget Calculator Science and Engineering Team (November, 2010)

It occurred during the hurricane season, but only two major storms occurred during the period. There was a deepwater gyre at 1100 m that went from the *Macondo* well head out 15 km to the SW before turning back (Valentine et al. 2012). Deep water plumes occurred at four depths: 25, 265, 865, 1175 m, oil at the surface was moving to the North East while oil in the 1100 m plume was moving to the South West, the other three water column plumes moved to the SE, and NW (Spier et al. 2013). The Gulf of Mexico has more natural seeps than any other deepwater basin being considered for deepwater oil production (NAS 2003). *Macondo* oil is a very light crude, the *Macondo* well was jetting oil at high temperature (200 °C) and high pressure (676 bars) at the well head (pressure of the ocean at 1500 m was 152 bars).

The *Macondo* well was also one of the deepest wells; thus, the hydrostatic pressure may have had an effect on oil degraders like we have not seen before (Marietou et al. 2018). The Macondo oil had a high proportion of methane (Kessler et al. 2011). Nutrients from the Mississippi River made the overall nutrients higher near the spill (Hazen et al. 2010), and many hydrocarbons found in the *Macondo* oil and in the CORREXIT dispersant used were also found in Mississippi River and drainage into the Gulf of Mexico from non DWH sources (Kujawinski et al. 2011; King et al. 2014a).

3 Lesson 2. Oil in the Water Column and in Coastal Sediments Biodegraded Faster Than Expected

One of the first studies on oil biodegradation reported that the Macondo oil average half-life of alkanes in the deep water (1100) plume was 1.2–6.1 days (Table 1) (Hazen et al. 2010). The deepwater plume contained more than 80% alkanes, and four different techniques were used to make these calculations using microcosms with water and fresh *Macondo* oil at 5 °C, mixed consortia (Venosa and Holder 2007) incubations with fresh *Macondo* oil at 5 °C, and changes in alkane concentration from in the plume from the source to 10 km down gradient with split sample analyses done by two different labs and considering whether it took 2 days or 5 days to traverse that 10 km gradient (Hazen et al. 2010; Valentine et al. 2012). This surprised a lot of people. Rapid biodegradation also occurred initially of propane and ethane (Valentine et al. 2010). A more recent study again verified these findings (Thessen and North 2017). Considering that below 700 m the temperature in the Gulf of Mexico is always 5 °C or less and it has been that way for millions of years, it should not be surprising that there are true psychrophiles that can degrade oil faster at 5 °C then at 20 °C and given there potentially long period of adaption degrade it faster than in previous studies at the surface (Baelum et al. 2012; Chakraborty et al. 2012; Dubinsky et al. 2013; Brakstad et al. 2015a; Hazen et al. 2016).

Macondo oil was also deposited in the sediments especially around the well head and in some other parts closer to shore as marine snow etc. (Rahsepar et al. 2017). Numerous studies also found that the sediment microbial community was degrading the *Macondo* oil faster than initially expected (Kimes et al. 2013, 2014; King et al. 2014a; Mason et al. 2014). Studies showed that a very active microbial community in the sediment was enriched in anaerobes (*Deltaproteobacteria)* in the deeper sediment and aerobes (*Gammaproteobacteria*) at the sediment surface that was very actively degrading a variety of *Macondo* well hydrocarbons including aromatic hydrocarbons (Kimes et al. 2013; Mason et al. 2014). Key hydrocarbon degradation pathways were determined by ^{14}C-labeled substrates in order: propylene, glycol, dodecane, toluene, and phenanthrene (Mason et al. 2014).

Many studies along the coast where emulsified and weathered *Macondo* oil washed ashore also found that degradation rates of the Macondo oil were faster than previous studies at other sites around the world had shown (King et al. 2012, 2014a, b). Beach samples collected during the response phase and after showed a

Table 1 MC-252 alkane half-life (days) from field and laboratory with currents of 2–5 days to move 10 km from source. (After Hazen et al. (2010))

		Plume samples	Plume samples	BP data	BP data	Mixed Consortia 5°C	Microcosm water, 5°C
	Average	**2.4**	**6.1**	**1.2**	**2.9**	**3.5**	**2.2**
n-Tridecane	C13alk	1.6	4.0	1.4	3.5	3.1	2.1
n-Tetradecane	C14alk	1.5	3.8	1.4	3.4	3.5	2.3
Pentadecane	C15alk	1.5	3.8	1.0	2.4	3.6	2.1
n-hexadecane	C16alk	1.6	4.0	2.0	5.0	3.6	2.2
n-heptadecane	C17alk	1.7	4.3	1.1	2.8	3.6	2.3
Pristane	C19teralk	1.6	4.1	1.3	3.2	3.0	2.3
n-octadecane	C18alk	2.1	5.2	1.0	2.6	4.2	2.3
Phytane	C20teralk	1.8	4.6	1.4	3.4	3.6	2.3
n-Nonadecane	C19alk	2.1	5.4	1.0	2.6	3.6	2.3
eicosane	C20alk	3.2	7.9	1.0	2.5	3.7	2.3
Heneicosane	C21alk	3.7	9.3	1.9	4.7	3.5	2.6
n-Docosane	C22alk	3.8	9.5	1.0	2.5	3.7	2.2
Tricosane	C23alk	3.7	9.2	1.0	2.5	3.6	2.2
tetracosane	C24alk	3.2	8.0	0.9	2.2	3.5	2.3
n-Pentacosane	C25alk	2.8	7.0	0.8	1.9	3.6	2.0
n-hexacosane	C26alk	3.1	7.8	0.6	1.6	3.1	1.7

dominance of *Alphaproteobacteria* and *Gammaproteobacteria* (Kostka et al. 2011; Lamendella et al. 2014). Taxonomic diversity decreased in the sands for first few months but rebounded 1 year after the oil came ashore and much of the oil had been degraded (King et al. 2014a). Initially Pensacola Beach sands oil-degraders increased two orders of magnitude within the first week, while diversity decreased 50% (Huettel et al. 2018). Half-lives of the aliphatic and aromatic hydrocarbons were less than 25 days. Aerobic oil degradation was significantly promoted by tidal pumping. In the coastal salt marsh (Mobile Bay), the oil degrading community increased in richness and abundance especially among the *Proteobacteria*, *Bacteroidetes*, and *Actinobacteria* (Beazley et al. 2012). This study also suggested that marsh rhizosphere microbial communities could be contributing to the hydrocarbon degradation since there was a greater decrease in Macondo oil in marsh grass sediments than in inlet sediments that lacked marsh grass (King et al. 2014a). Studies in marshes in Barataria Bay, Louisiana, also showed increases in the bacteria *Rhodobacterales* and *Sphingomonadales* and the fungi *Dothideomycetes* (Mahmoudi et al. 2013). Another study that included 11 sites in southern Louisiana found that all studied marshes had increased abundance in *Proteobacteria*, *Firmicutes*, *Bacteroidetes*, and *Actinobacteria* during the first 4 months, but after 2 years with barely detectable hydrocarbon levels the bacteria communities were more diverse and dominated by *Alphaproteobacteria (Rhizobiales), Chloroflexi (Dehalococcoidia),* and *Planctomycetes* (Engel et al. 2017).

4 Lesson 3. Long-Term Adaption to Natural Seeps Played an Important Role in DWH Oil Biodegradation

Natural seeps in the Gulf of Mexico are the most abundant of any deepwater marine basin being considered for petroleum exploration and production (Fig. 3) (NAS 2003). A 10-year average showed that 400,000–1,000,000 barrels oil go into the Gulf of Mexico every year from natural seeps. These seeps are episodic and are primarily due to the major salt domes in the Gulf of Mexico which allow leakage from deeper petroleum reservoirs intersected by the salt domes. Recent use of satellite imagery and Fourier transform-ion cyclotron resonance-mass spectrometry may enable an even more detailed quantification of natural seeps in the Gulf of Mexico (Krajewski et al. 2018). Natural seeps in North America are estimated to exceed 160,000 tons and 600,000 tons globally each year. Over 60% of the petroleum entering North American waters comes from natural seeps, but only 45% of the petroleum entering the marine environment worldwide is from natural seeps (NAS 2003) See ▶ "Oil Biodegradation in Deep Marine Basin" chapter in this book.

It is not surprising then that microbes have become very well adapted to oil biodegradation in the Gulf of Mexico since it is the major long-term carbon and energy source that has been episodically released over millions of years (Kimes et al. 2013; Hazen et al. 2016). This long-term adaption to episodic release of oil provided a "memory" response that allowed oil-biodegraders to respond rapidly whenever oil was being seeped. Indeed, a significant increase in *Oceanospirillaceae* was seen only 1 km from the well head, and calculations suggest that it would only take the prevailing several hours to reach this area (Fig. 4) (Hazen et al. 2010).

5 Lesson 4. Jetting and Dispersants at the Well Head Increased Oil Biodegradation

The pressure of the *Macondo* well at the well head was 676 bars at >200 °C while the ambient pressure in the water around the well head was 152 bars at <5 °C. This would cause jetting a well-known phenomenon for oil well blowouts (Agbaglah et al. 2011). This would form oil droplets that would increase biodegradation primarily because of the change in the ratio of surface/volume (Fig. 4). Microbes can biodegrade oil hydrocarbons dissolved in water or are present at the oil/water interface. During *DWH* it was decided that even though jetting was occurring, there was too much oil coming to the surface close to the well control operations. This presented a major safety concern since the high methane content and relative flammability of the oil increased the risk of a fire and or explosion. So, for the first time ever permission to inject Corexit 9500 at the well head was given. It was also hoped that this would increase dispersion and biodegradation of oil so that less would reach the surface. Within 4 h after subsurface injection of dispersant was started, the oil coming to the surface was much farther away from the well control operations and every time that dispersant injection was stopped within 4 h the surface slick would move closer to the well control operations. Corexit 9500 was also used at the surface by spraying on

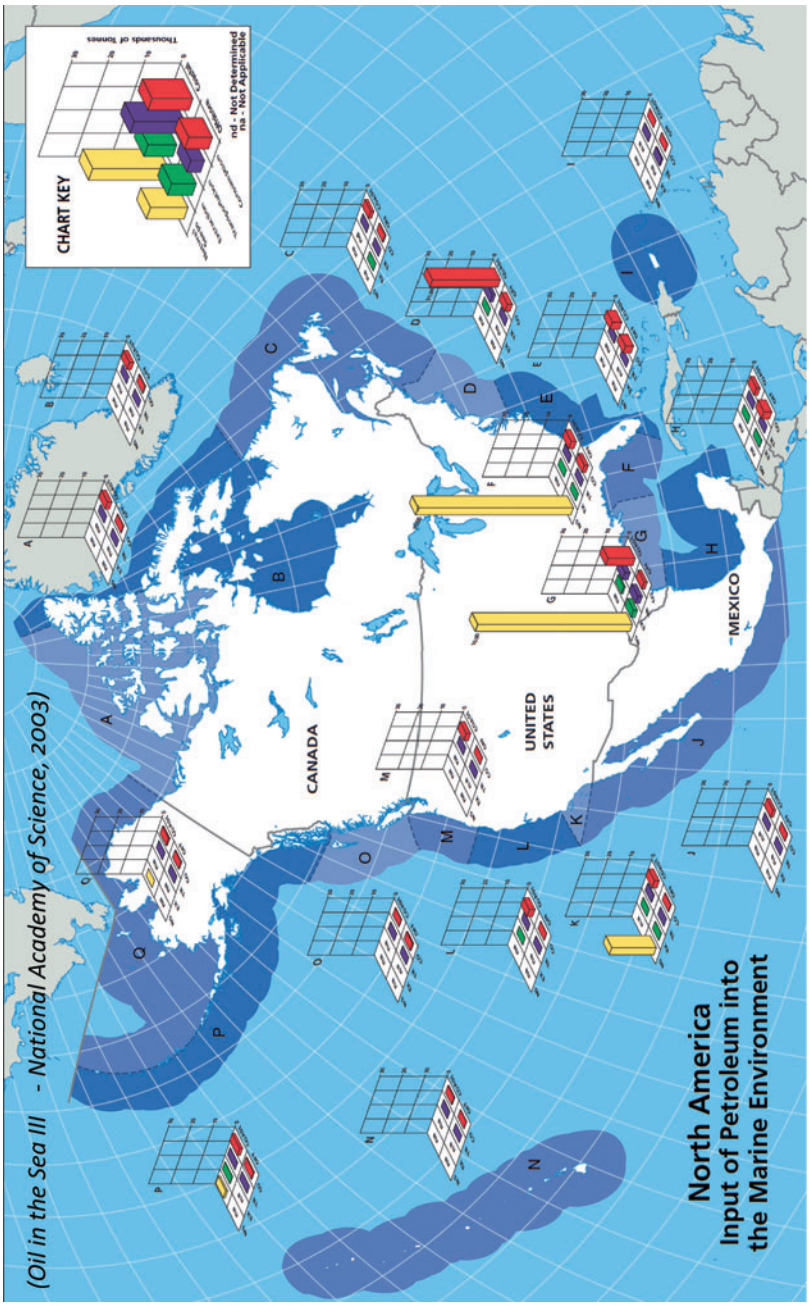

Fig. 3 Input of Oil in North America showing natural seeps, extraction, transportation, and consumption in deep water and the coast. (After NAS (2003))

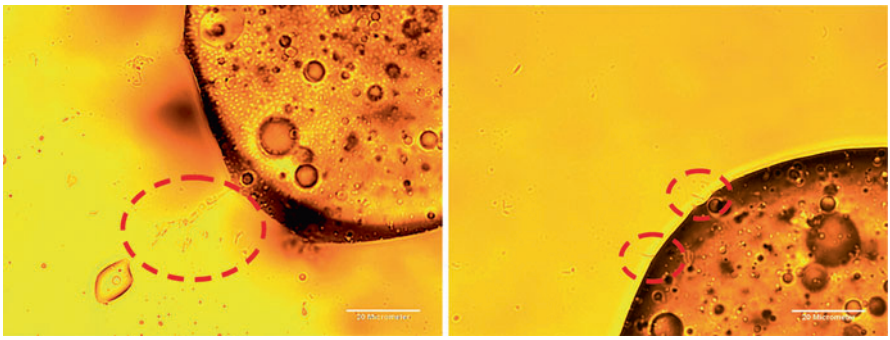

Fig. 4 Oil-degrading bacteria from 1100 m plume attached to a droplet of *Macondo* Oil. (Bright-field, 100X)

surface slicks via ships and planes. It was also used on surface slicks during the Ixtoc I blowout in the Gulf of Mexico in 1979 (Hooper and NOAA Hazardous Materials Response Project (U.S.) 1982). Corexit 9500 or analogs had been used as an oil dispersant for more than 30 years. Any droplets that were formed by jetting or dispersant that were 10–60 μm in diameter were neutrally buoyant and were entrained in the current at 1100 m. Droplets that were 300 μm or larger were positively buoyant and rose to the surface.

Several studies on the *Macondo* oil have clearly demonstrated that smaller droplets degrade faster (Baelum et al. 2012; Adams et al. 2013; Vilcaez et al. 2013; Brakstad et al. 2014, 2015a, b; King et al. 2014a). Some studies have suggested that Corexit 9500 may be directly inhibitory to some oil biodegraders (Kleindienst et al. 2015). However, the vast majority of papers has found no inhibition by Corexit 9500 (Baelum et al. 2012; Prince and Butler 2014; Brakstad et al. 2015a; Prince 2015; Hazen et al. 2016; Techtmann et al. 2017b).

6 Lesson 5. Comparisons of *DWH* with *Exxon Valdez* Oil Spill for Oil Biodegradation Were Not Appropriate

It was appalling that during the *DWH* spill the media and many scientists were comparing DWH to the *Exxon Valdez* oil spill. While the *Exxon Valdez* oil spill was the largest oil spill in US marine waters up until *DWH* it was in no ways similar (Atlas and Hazen 2011). Unlike the DWH, the *Exxon Valdez* oil spill in *Prince William Sound* was a tanker spill that was close to shore and was "dead" oil, i.e., it did not have any of the methane or volatile organic carbon that the *Macondo* oil had. The *Prudhoe Bay* oil was heavier than *Macondo* oil and inherently less biodegradable. Natural attenuation was less of an option for the *Exxon Valdez* oil spill since the oil accumulated on shore near risk receptors for birds, fish, and mammals so several biostimulation techniques were tried. Since *DWH* was nearly 50 miles off shore and was degrading rapidly in the water column, no oil could be detected only 2 weeks after the well was finally capped.

Since Prince William Sound had no natural seeps and no exposure to oil prior to completion the Trans Alaskan pipeline it was not surprising that Prudhoe Bay oil biodegraded much more slowly than the Macondo oil. For a thorough comparison of *DVH* with the *Exxon Valdez* oil spills, see Atlas and Hazen (2011).

7 Lesson 6. Models for *DWH* Were Inappropriate at First

Because of the uniqueness of the *DWH* oil spill few, if any, models were prepared to simulate what happened especially in terms of oil biodegradation. The SINTEF OSCAR model was tried initially but failed to predict the oil biodegradation rates in the deep plume, primarily because it used a Q(10) algorithm that assumed that for every 10 °C change in temperature, there would be a proportional change in biodegradation rate (Bagi et al. 2013). This did not take into consideration that the dominant bacteria in the deep were psychrophiles (Hazen et al. 2010, 2016; Baelum et al. 2012; Chakraborty et al. 2012). Droplet break-up models include Equilibrium correlations (Johansen et al. 2013; Li et al. 2016) and Dynamic models (Zhao et al. 2017). SINTEF since 2010 has developed several updates to the original Oil Spill Contingency and Response (OSCAR) model. The Structured Learning in Microbial Ecology (SLiME) model was found to predict the concentration of oil in DWH deep plume almost perfectly from the microbial community structure (Smith et al. 2015).

8 Lesson 7. Cometabolic Oil Biodegradation May Be Important in Deep Marine Basins

The aerobic cometabolic biodegraders are dependent upon oxygenases, e.g., methane monooxygenase, toluene dioxygenase, toluene monooxygenase, and ammonia monooxygense. These enzymes are extremely strong oxidizers, e.g., methane monooxygenase is known to transform over 1000 different compounds. However, like any bioremediation process, the proper biogeochemical conditions are necessary to maximize and maintain biodegradation, e.g., maintaining oxygen levels or other terminal electron acceptors that the cometabolic biodegrader is dependent (Hazen 1997, Hazen and Sayler 2016), and chapter on ▶ "Cometabolic Bioremediation" in this book. In addition, co-metabolic biostimulation may require pulsing of electron donor or electron acceptor to reduce competitive inhibition between the substrate the microbe can use and the contaminant. Pulsing of methane was found to significantly improve biodegradation of trichloroethylene rates by methanotrophs (Hazen 2010). Indeed, during the *DWH* leak (Hazen et al. 2010), there was evidence that in the Gulf of Mexico where episodic releases of methane have occurred for millions of years from natural seeps this pulsing of methane may be removing oil and other organics via cometabolic biodegradation. The methane oxidizers bloomed during the *DWH* leaked above 400 m once the well was capped (Reddy et al. 2012; Redmond and Valentine 2012; Dubinsky et al. 2013). This suggests that intrinsic cometabolic bioremediation or cometabolic natural attenuation may be a serious phenomenon

in the ocean (Stackhouse et al. 2017). Methanotrophs, methane-oxidizing bacteria, oxidize methane via a series of enzymes that are unique to this group. The primary enzyme in this oxidation chain is methane monooxygenase. Methane monooxygenase is an extremely powerful oxidizer, thus giving it the capability of oxidizing a wide variety of normally recalcitrant compounds including oil constituents (Cardy et al. 1991). See ► "Cometabolic Bioremediation" in this book.

9 Lesson 8. Blooms of Oil Degraders in the Deep Led to a Temporal Succession of Other Bacterial Communities with Unknown Effects on Trophic Levels

Once the oil was undetectable in the water column, many thought that the total biomass that would drastically decrease immediately and the microbial community diversity would increase to prespill levels (Hazen et al. 2010). However, once the oil degraders lost their competitive edge in using oil as a carbon and energy source, they began to die back, but there was a succession of bacteria that could use daughter products from direct oil degraders, i.e., "cheaters" bacteria that could not use the oil directly but could use some daughter product (Techtmann et al. 2016). As time progressed, even the "cheaters" could not compete so bacteria that could use the dead bacteria as a nutrient flourished (Fig. 5) (Dubinsky et al. 2013). So, the total microbial biomass slowly subsided over several months. The diversity of the microbial biomass also changed dramatically with the oil with the prespill having 951 subfamilies in 62 bacterial phyla (Fig. 6). The *DWH* deep oil plume had only 16 subfamilies in the Gammaproteobacteria (Hazen et al. 2010). Though bacteria do not sequester oil hydrocarbons like some organisms they basically convert oil hydrocarbons to bacterial compounds, this change in diversity could have had dramatic effects on the subsequent trophic levels since the size, shape, and compound composition of the food source had changed. This could also have a long-term effect even though the oil was gone! To date only a few studies have been published considering this (Graham et al. 2010; Abbriano et al. 2011; Chanton et al. 2012; Jung et al. 2012; Carassou et al. 2014; Walsh et al. 2015).

10 Lesson 9. Molecular Techniques Led to a More Thorough Understanding of *DWH* Oil Biodegradation

Unlike previous major oil spills molecular techniques, especially sequencing had advanced significantly allowing a near real-time assessment of oil biodegradation microbial community structure and function, in the water column, surface, sediment and coastal areas (Hazen et al. 2010, 2013, 2016; Kostka et al. 2011; Baelum et al. 2012; Beazley et al. 2012; Dubinsky et al. 2012, 2013; Lu et al. 2012; Mason et al. 2012, 2014; King et al. 2014a). It also allowed storing of samples shipboard by freezing allowing the safe transport and subsequent analysis and archiving of critical samples (Fig. 7).

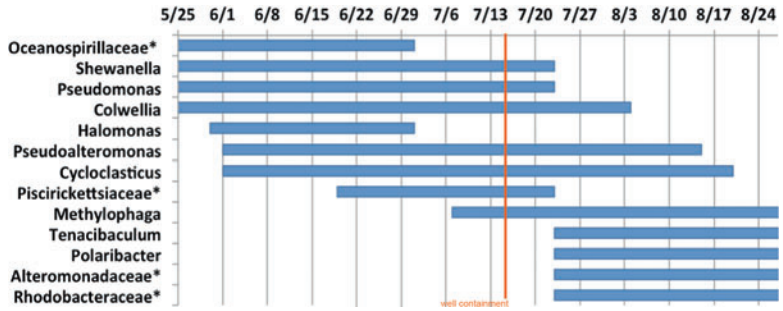

Fig. 5 Temporal Community Structure Changes showing sustained alterations in subsurface microbial communities and impacted the deep ocean for at least months after well containment. (After Dubinsky et al. (2013))

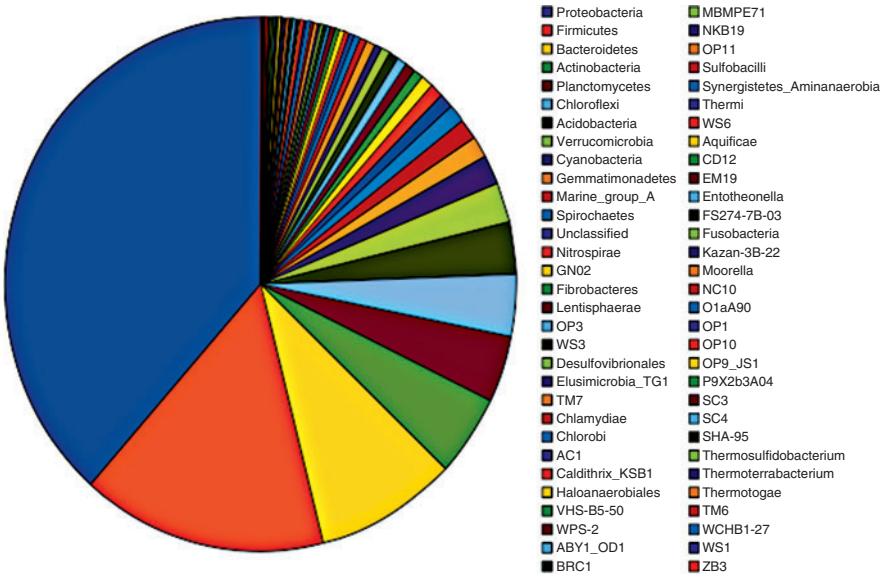

Fig. 6 951 subfamilies were detected in 62 bacterial phyla. Only 16 subfamilies in gammaproteobacteria significantly enriched in plume. (After Hazen et al. (2010))

11 Lesson 10. Hydrostatic Pressure Had Little Effect on *DWH* Oil Biodegradation

Because of the depth of the *Macondo* well (1500 m), it was thought by many that the hydrostatic pressure might reduce biodegradation and/or promote biodegradation by piezophiles. Recent studies used water collected at depth during the response phase of *DWH* and preserved hydrostatic pressure as much as possible for simulations in the laboratory. In the laboratory simulations, these samples were exposed to 0.1, 15, and 30

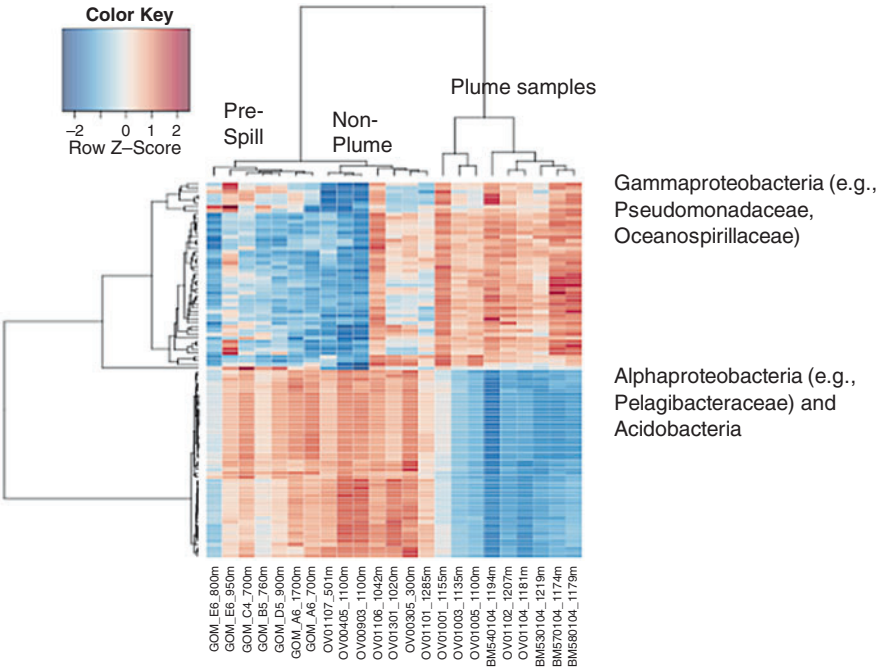

Fig. 7 The microbial community in the deep-water plume was distinct from the microbial community in uncontaminated waters at the same depth. Hierarchical clustering identified similarities between microbial communities. Uncontaminated deep-water samples showed a higher relative abundance of *Alphaproteobacteria* and *Acidobacteria*, while the deep-water oil plume had lower abundance of *Alphaproteobacteria and Pelagibacteraceae* and much higher abundance of *Gammaproteobacteria* including *Oceanospirillaceae* and *Pseudomonadaceae.* (After Hazen et al. (2010))

Megapascals (MPa) pressure (the Macondo well was at 15 Megapascals) (Marietou et al. 2018). Their results suggest that pressure acts synergistically with low temperature to slow microbial growth and change microbial community structure and thus oil degradation in deep-sea environments. This only happened with *DWH* when the water collected was exposed to 30 MPa, since *DWH* was actually at 15 MPa and there was little effect of pressure. However, if deep basin oil exploration continues, it is bound to get deeper and more attention should be paid to getting samples collected in situ at pressure from these deeper strata to determine the effect that pressure is having on the oil degrading microbiome (Hazen et al. 2016; Hazen and Techtmann 2018).

12 Research Needs

Because of all the new techniques that were demonstrated with *DWH*, Standard Operating Procedures (SOP) were in dire need during the response phase, during the subsequent investigations for National Resource and Damage Assessment (NRDA),

and during long-term investigations of effects of the *DWH* accident. We need a dynamic set of SOPs that are put together and peer reviewed by a multidisciplinary group of experts that can be used by the scientific community for oil biodegradation research.

In Situ Sampling and Characterization. During the response phase of the *DWH* accident, it was difficult to find many in situ sampling and characterization devices that were useful for taking critical samples. A lot of SOPs were developed on the fly many of the response phase ships used standard CTD sampling rosettes out fitted with 2 UV fluorometers which used fluorescence to detect hydrocarbons and captured water at depth with Niskin bottles. The UV fluorometers (Quantech/Thermo Scientific) were employed in tandem to determine fluorescence intensity ratios (FIRs). One fluorometer was equipped with a pair of wavelength filters allowing excitation at 280 nm and emission at 340 nm. The second fluorometer was equipped with the same 280 nm excitation filter and a longer (445 nm) wavelength. The Niskin bottles were cleaned internally with distilled water and detergents between samplings. The sampling crews were sensitive to the problem of contamination from surface oil and used physical methods to disperse the surface slick before initiating sampling by the CTD, e.g., prop wash at the back of the ship before deployment and recovery, and detergent if prop wash was insufficient. For side deployments, the surface of the water was sprayed with freshwater to disperse surface oil; if this was insufficient, detergent was applied to the surface of the water then sprayed with freshwater to disperse surface oil. From each sample 800–2000 ml of water was filtered through sterile filter units containing 47 mm diameter polyethylsulfone membranes with 0.22 μm pore size (MO BIO Laboratories, Inc., Carlsbad, CA) and then immediately frozen and stored at −20 °C for the remainder of the cruise. Filters were shipped on dry ice to Lawrence Berkeley National Laboratory and stored at −80 °C until DNA and PLFA extraction (Hazen et al. 2010). We also saw deployment of new in situ physical/chemical characterization devices like a subsurface hydrocarbon survey using an autonomous underwater vehicle and a ship-cabled sampler (Camilli et al. 2010). Recently it has also been demonstrated that oil seeps and spills can be linked to their origin by Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (Krajewski et al. 2018). For sampling microbiomes in situ we need more development of devices that can be triggered remotely to filter and/or sample at depth like the large volume Stand Alone Particle Sampler (SAPS, Challenger Oceanic, UK, with controller, battery, and pump upgrades by Oceanlab, University of Aberdeen, Scotland) which can filter 62 and 123 L of seawater at depth through a 292 mm diameter nylon filter with a pore size of 0.2 μm (Techtmann et al. 2015) and the commercially available McLane Pump Large Volume water sampler (McLane Labs, Falmouth MA) which can filter 10.3 and 27 L of water per sample (Techtmann et al. 2017a).

Mesocosms/Microcosms. Bottle effects are real, as are sampling with consideration of ambient temperature and pressure and travel time of the sampling device (Marietou et al. 2018). It has been found that on-board ship microcosms/mesocosms start with different community structures and give different results in terms of function and diversity than water samples taken back over some days of travel for laboratory mesocosms (Liu et al. 2017). Too many times during *DWH* the media

interviewed scientists that did not have data or did not have data tied to rigorous SOPs and peer review, which gave the public the wrong impression of what was going on during the *DWH* accident.

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