New Insights into Microbial Responses to Oil Spills from the Deepwater Horizon Incident

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Background

On April 20, 2010 a catastrophic eruption of methane caused the Deepwater Horizon exploratory drill rig drilling the Macondo well in Mississippi Canyon Block 252 (MC252) to explode. This accident killed 11 people and went on to become the worst off-shore drilling disaster in US history. The rig burned for 2 days and then sank severing the riser near the surface, which also sank to the bottom at ~1500 m or 5000 ft. All of the emergency shutoff systems failed. For the next 84 days, as much as 60,000 barrels of oil were introduced per day at ~1500 m in the deep-sea in the Gulf of Mexico, for a total of 4.9 million barrels (±10%) (The Federal Interagency Solutions Group: Oil Budget Calculator Science and Engineering Team (FRTG), 2010) (Figures 1 and 2). This volume of oil ranks this as the second largest oil spill in the world (the first being the deliberate release of oil in the marine environment by Iraq during the Gulf War). In an effort to mitigate the effects of the enormous input of oil 0.78 million gallons of COREXIT 9500A was injected directly at the wellhead (Operational Science Advisory Team Report 1 (OSAT 1, 2010)) (Figure 1). This mitigation strategy resulted in the dispersal of approximately 770,000 barrels of oil in the deep-sea (OSAT 1, 2010). As a consequence of both chemical (i.e. dispersant) and physical processes a deep-sea plume developed at 900-1300 meters below sea level (mbsl) (OSAT 1, 2010) (Figure 1). The fate of the hydrocarbon plume, which persisted for several months, has been the subject of several recent articles. The primary focus of these initial scientific articles has been the biodegradation of plume constituents by deep-sea microorganisms.

The Deepwater Horizon oil spill was unprecedented for several reasons: the volume of oil released, the spill duration, the well depth, the distance from the shoreline (50 miles), the type of oil (light crude), and the injection of dispersant directly at the wellhead (Figure 1). Historically, however, the Gulf of Mexico experiences multiple, natural, episodic "oil spills" each year. Satellite synthetic aperture radar (SAR) images have been used to inventory the volume of oil that emanates from ~400 different natural oil seep sources across the Gulf of Mexico (MacDonald et al. 2005). Estimates from these images indicate that ~850 sq. km (northern) and ~150 sq. km (southern) of water is oiled from naturally occurring oil seeps in the Gulf of Mexico (MacDonald et al. 2005), with an average of 70,000 tonnes of hydrocarbons input into the Gulf of Mexico annually from offshore seeps (National Academy of Sciences, 2003). In fact, offshore seeps contribute 95 percent of the total oil input to offshore region in the Gulf of Mexico (National Academy of Sciences, 2003). Given that the Gulf of Mexico experiences frequent, episodic, natural "oil spills" the microbial community may have, in effect, been primed or have a "memory" for biodegrading constituents introduced into the deep-sea as a result of the Deepwater Horizon oil spill.

Natural attenuation

The Environmental Protection Agency (EPA) refers to the "reliance on natural processes to achieve site-specific remedial objectives" as natural attenuation (Pope and Jones 1999). Naturally attenuating processes can be physical, chemical, or biological and "act without human intervention to reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil or ground water" (Pope and Jones 1999) (Figure 3).

The fate of Macondo oil (e.g. naturally attenuating processes such as biodegradation) has been the subject of several recent studies; however, owing to the chemical composition of Macondo oil (MC252) (up to 40% gaseous hydrocarbons (Joye et al. 2011a)) much attention has been paid to the *in situ* biodegradation of C_1 to C_3 alkanes (Valentine et al. 2010; Kessler et al. 2011) in the deep-sea plume. In fact, Kessler et al (2010) reported that the CH_4 emitted over the course of the oil spill was quantitatively consumed by microorganisms by August 2010. Hazen, et al. (2010) reported oil half-lives (alkanes) of 1.2 to 6.1 days, with much of the disappearance of this oil attributed to biodegradation rather than attenuation by dilution and mixing. These studies verify that the microbial disposition of oil constituents in the deep-sea plume was an extremely important process in determining the ultimate fate and consequences of this oil spill.

Microbial Response and Biodegradation

Thus far, a recurrent theme in the analysis of the deep-sea microbial community response to the Deepwater Horizon oil spill has been that a low diversity bloom (Figure 4) of Bacteria occurred (Hazen et al. 2010; Valentine et al. 2010; Kessler et al. 2011). The dominant members of this bloom appeared to evolve over the history of the plume. This suggests that the deep-sea in the Gulf of Mexico harbors a microbial community that possesses an impressive functional repertoire that allows for hydrocarbon degradation of a myriad of compounds.

Hazen, et al (2010) reported that in samples collected in May and early June 2010 a rapid and significant microbial response to the oil spill occurred (Hazen et al. 2010) (Figure 4). Microarray analysis of 16S rRNA genes revealed that gammaproteobacteria were enriched in the plume interval compared to non-plume samples. Cloning and sequencing of a subset of the plume samples revealed that members a single *Oceanospirillales* Operational Taxonomic Unit (OTU; 97% or more 16S rRNA gene sequence similarity) comprised more than 90% of the bacterial community, compared to 5% of the uncontaminated sample (Hazen et al. 2010). This OTU forms a clade with two distinct *Oceanospirillales* groups, one of which is comprised of known hydrocarbon degraders (Hazen et al. 2010). In the later clade are the cultivated aliphatic hydrocarbon degraders *Oeiphilus messinesis, Oleispira antarctica,* and *Thalassolituus oleivorans* (Golyshin 2002; Yakimov et al. 2003; Yakimov et al. 2004). These cultured representatives are capable of growth on n-alkanes C_{11} to C_{20}, C_{10} to C_{18}, and, C_7 to C_{20}, respectively (Golyshin 2002; Yakimov et al. 2003; Yakimov et al. 2004).

As discussed above, degradation in the plume was assessed by determining oil half-lives in field samples, which ranged from an average of 1.2 to 6.1 days (Hazen et al. 2010). A marked preference for degradation of short-chain alkanes (C_{26}/C_{15}) over a large spatial scale is consistent with the substrate range utilized by close relatives to the dominant OTU. Further, this preferential degradation, decrease in dissolved oxygen, nitrate and phosphate concentrations, with a concomitant increase in ammonium concentrations, indicates that biodegradation was responsible for the disappearance of alkanes, rather than other naturally attenuating processes (Hazen et al. 2010).

The microbial response to and attenuation of gaseous components present in the hydrocarbon pool released from the Macondo well has been the subject of several recent studies. Similar to Hazen et al (2010), Valentine, et al (2010) used cloning and sequencing and reported that a low-diversity of putative hydrocarbon degrading gammaproteobacteria bloomed in samples that exhibited propane and ethane anomalies (Valentine et al. 2010). Specifically, relatives of *Cycloclasticus* and *Colwellia* appeared to dominate these samples (Valentine et al. 2010). Although methane concentrations were as high as 180 µM in the plume (Valentine et al. 2010) and was the most abundant hydrocarbon released (Kessler et al. 2011) from the Macondo well, preferential loss of both ethane and propane relative to methane suggested that it exhibited conservative behavior (Valentine et al. 2010). Additionally, tritium tracer tests suggested a slow rate of methane oxidation (Valentine et al. 2010). In fact, a regression of propane and ethane anomalies against oxygen anomalies indicated that 70% of the oxygen anomaly could be linked to microbial respiration of these compounds initially (Valentine et al. 2010). Thus, the microbial community, and in particular *Cycloclasticus* and *Colwellia* exhibited a rapid and substantial response to, as suggested by the authors, propane and ethane in the plume interval, serving to naturally attenuate substantial quantities of these gaseous compounds.

Valentine et al (2010) reported that early in the plume history (June, 2010) methane did not appear to fuel appreciable microbial respiration. Kessler, et al. (2010), suggested that a subsequent bacterial bloom that occurred in August through October 2010, after the

wellhead was capped (July 15, 2010), consumed nearly all of the methane released in ~120 days. Although methane was not detected in samples collected in September methylotrophic bacteria were detected and, in some samples, abundant. The authors suggested that they may have observed a remnant population of methanotrophs. The consumption of nearly all of the methane that emanated from the MC252 well is significant for several reasons, the most important of which is to aide in our understanding of the role microorganisms may have played in consuming significant quantities of methane, a potent greenhouse gas, released from the marine environment over time. This methane has been implicated in ancient climate change (Dickens 2001). Though the data presented by Kessler et al (2011) suggest that microorganisms may have played a more significant role in modulating climate change in the past and today, their extrapolation in this example has been met with some controversy (Joye et al., 2011b). For over 30 years methylotrophs have been stimulated in groundwater and soil to bioremediate chlorinated solvent and variety of other hydrocarbons (Hazen, 2009; Hazen et al, 2009). Methane monooxygenases will fortuitously, or cometabolicaly, degrade over 300 compounds including most of the compounds in oil. Given that these monooxygenases do not require petroleum hydrocarbons as an electron donor they will scavenge and utilize any available hydrocarbon down to parts per trillion concentrations. This would suggest that the late surge of methanotrophs in this oil spill may have further enhanced the degradation of any of the remaining oil hydrocarbons even when they were below sustainable electron donor concentrations.

Beyond the Deep-Sea Plume

Since August 2010 less than 1% of water samples exceeded the EPA's aquatic life benchmarks for polycyclic aromatic hydrocarbons (PAHs) (OSAT 1, 2010). Approximately 1% of sediment samples, collected within 3 km from the wellhead, were determined to exceed these benchmarks since this time (OSAT 1, 2010). In December, 2010 it was determined that there was no "actionable" oil in the deep-sea water column or sediments (OSAT 1, 2010). However, in oiled nearshore sediments and shorelines removal activities were to continue (OSAT 2, 2011). As of February 2011 nearshore cleanup activities were ongoing at several locales (OSAT 2, 2011)

Summary

Taken together, these studies clearly demonstrated that there was a profound and significant response by certain members of the *in situ* microbial community in the deep-sea in the Gulf of Mexico. In particular putative hydrocarbon degrading Bacteria appeared to bloom in response to the Deepwater Horizon oil spill, even though the temperature at these depths is never >5°C. As the plume aged the shifts in the microbial community on a temporal scale suggested that different, yet metabolically important members of the community were able to respond to a myriad of plume constituents, e.g. shifting from propane/ethane to alkanes and finally to methane. Thus, the biodegradation of hydrocarbons in the plume by Bacteria was a highly significant process in the natural attenuation of many compounds released during the Deepwater Horizon oil spill.

Future work

The molecular microbial ecology and systems biology studies discussed above provided a unique insight regarding the microbial response to a large-scale oil spill at higher resolution than that of previous oil spills, largely due to recently developed molecular techniques. Yet, much of this research has focused on the gaseous components that emanated from the Macondo well. Outstanding questions remain, such as the fate of several components that can be toxic at very low concentrations, such as PAH compounds, that were released during the oil spill. Much research remains to be carried out on the benthic community in sediments impacted by the Deepwater Horizon oil spill. Finally, determining the ultimate fate of oil that was deposited in nearshore sediments and shoreline will require an ongoing effort that will likely last for several years. Going forward whether the oil in nearshore environments is actionable or whether naturally attenuating processes, and in particular biodegradation by microorganisms, is the appropriate course of action, has yet to be determined. The vigorous microbial response in the deep-sea in the Gulf of Mexico to oil emanating from the Macondo well provide strong evidence that biodegradation may be a sufficient remedial strategy for certain Macondo oil constituents.

Acknowledgments

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Figures

Figure 1. Overview of the Deepwater Horizon oil spill. This figure shows both the response to mitigate the oil spill and the behavior of the oil.

Figure 2. Photos of the oil spill from the Gulf of Mexico.

Figure 3. Diagram of naturally attenuating processes for petroleum hydrocarbons. Brightfield image of cells (dashed red circle) interacting with Maconodo oil.

Figure 4. Epifluorescent images of plume and non-plume samples. Non-plume sample BM92 was collected from 1092 meters below sea level (mbsl). Plume sample BM58 was collected from 1179 mbsl. The longest cells in the plume samples were 20 µm.

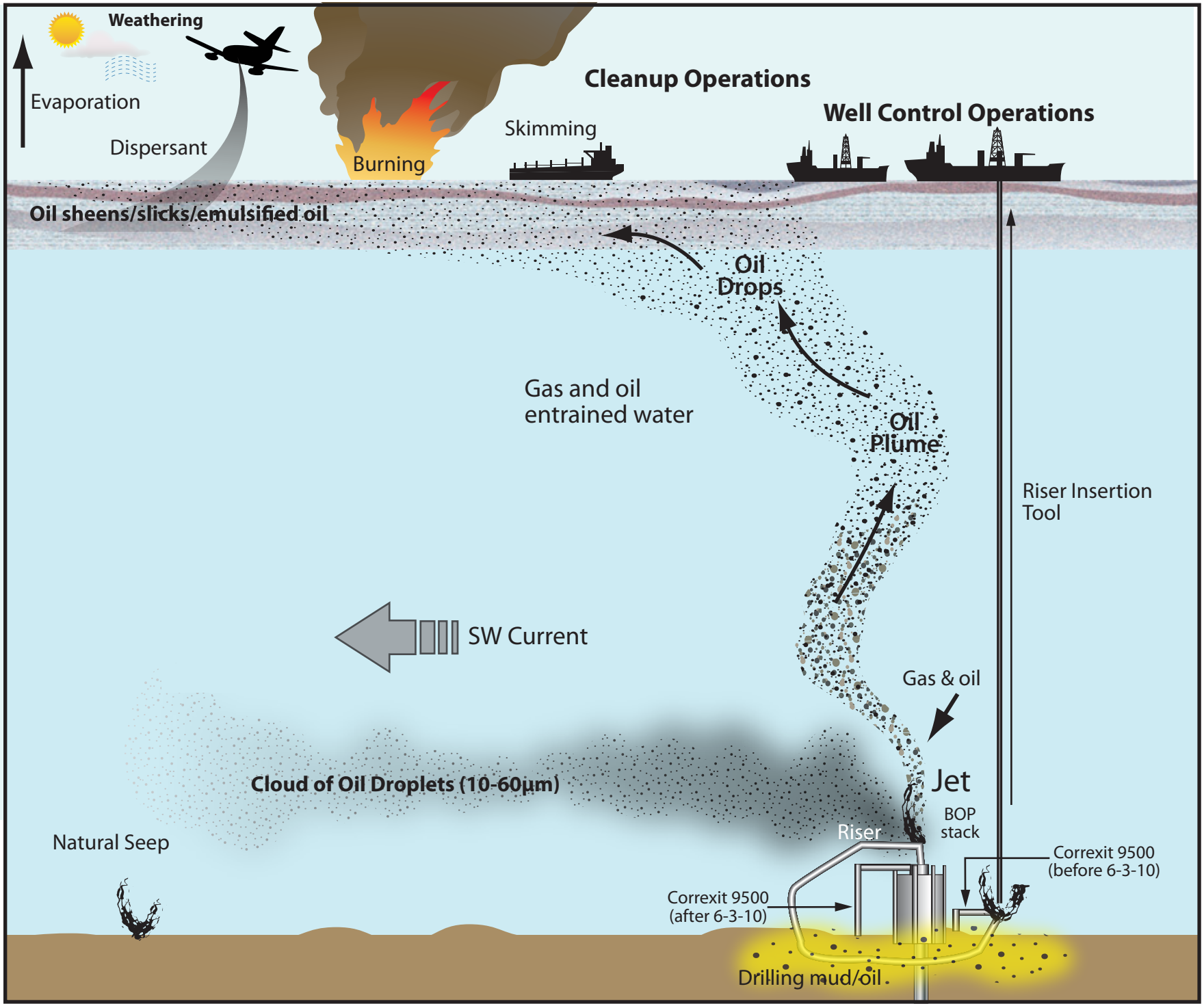

Weathering
Evaporation
Dispersant
Burning
Cleanup Operations
Skimming
Well Control Operations
Oil sheens/slicks/emulsified oil
Oil Drops
Gas and oil entrained water
Oil Plume
Riser Insertion Tool
SW Current
Gas & oil
Jet
Cloud of Oil Droplets (10-60µm)
BOP stack
Riser
Natural Seep
Correxit 9500 (before 6-3-10)
Correxit 9500 (after 6-3-10)
Drilling mud/oil

Figure 1.

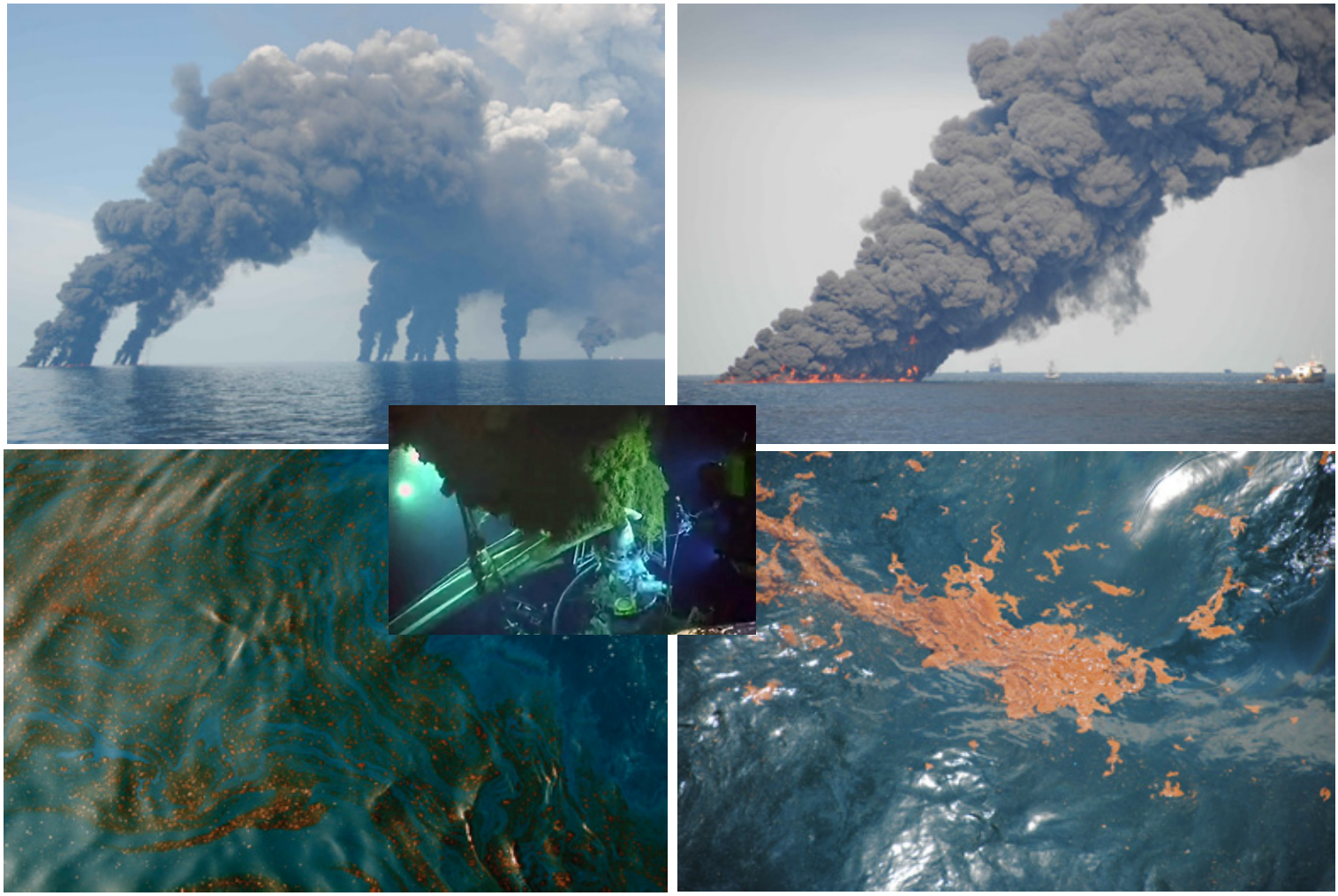
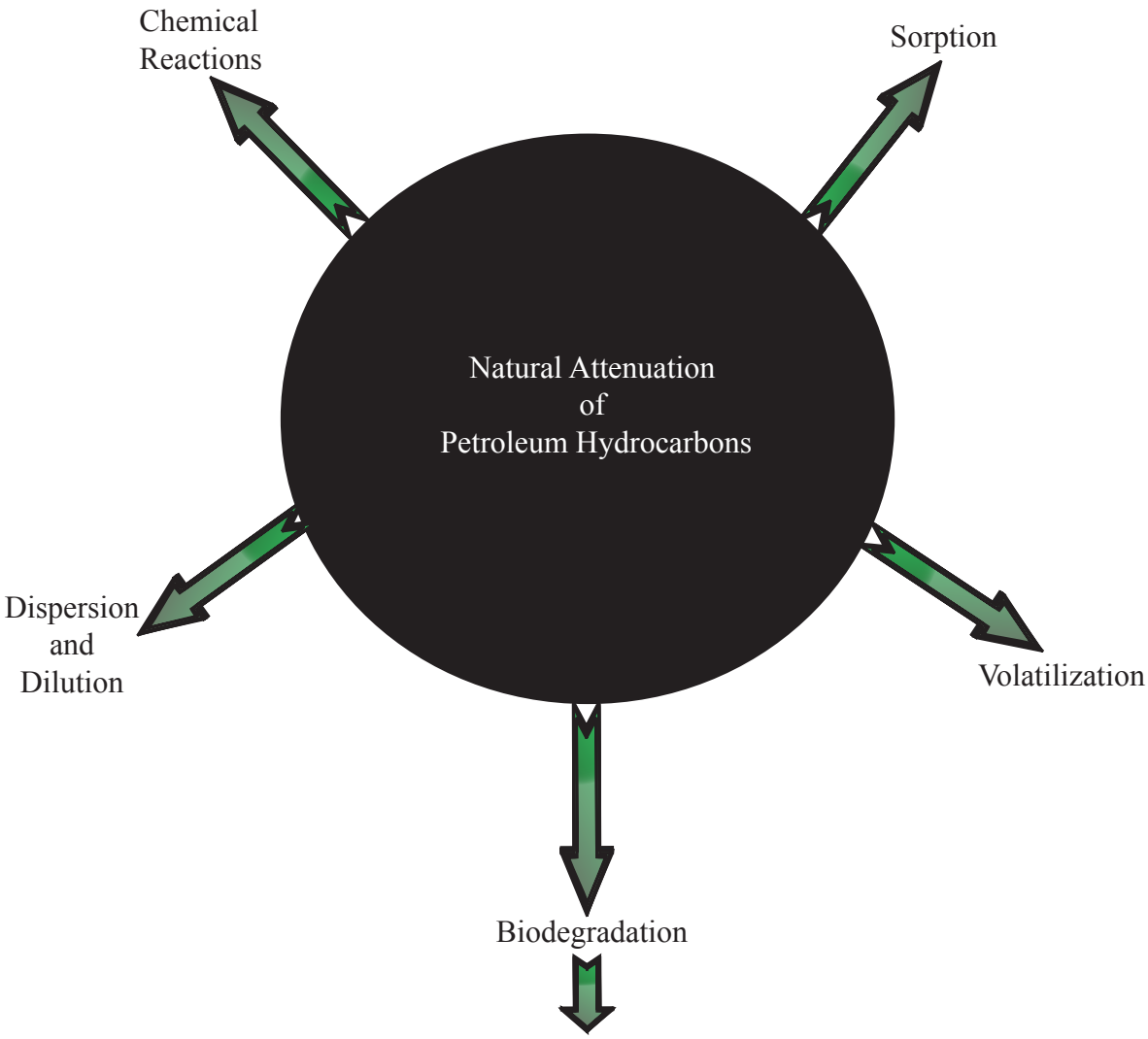


Figure 2.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Reactions
Sorption
Natural Attenuation
of
Petroleum Hydrocarbons
Dispersion
and
Dilution
Volatilization
Biodegradation

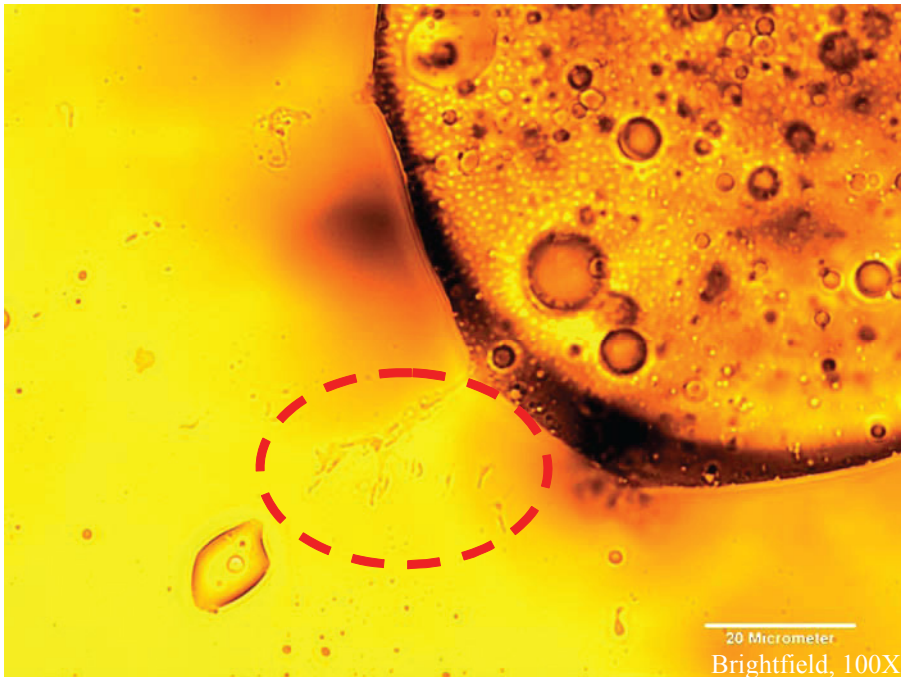
20 Micrometer
Brightfield, 100X

Figure 3.

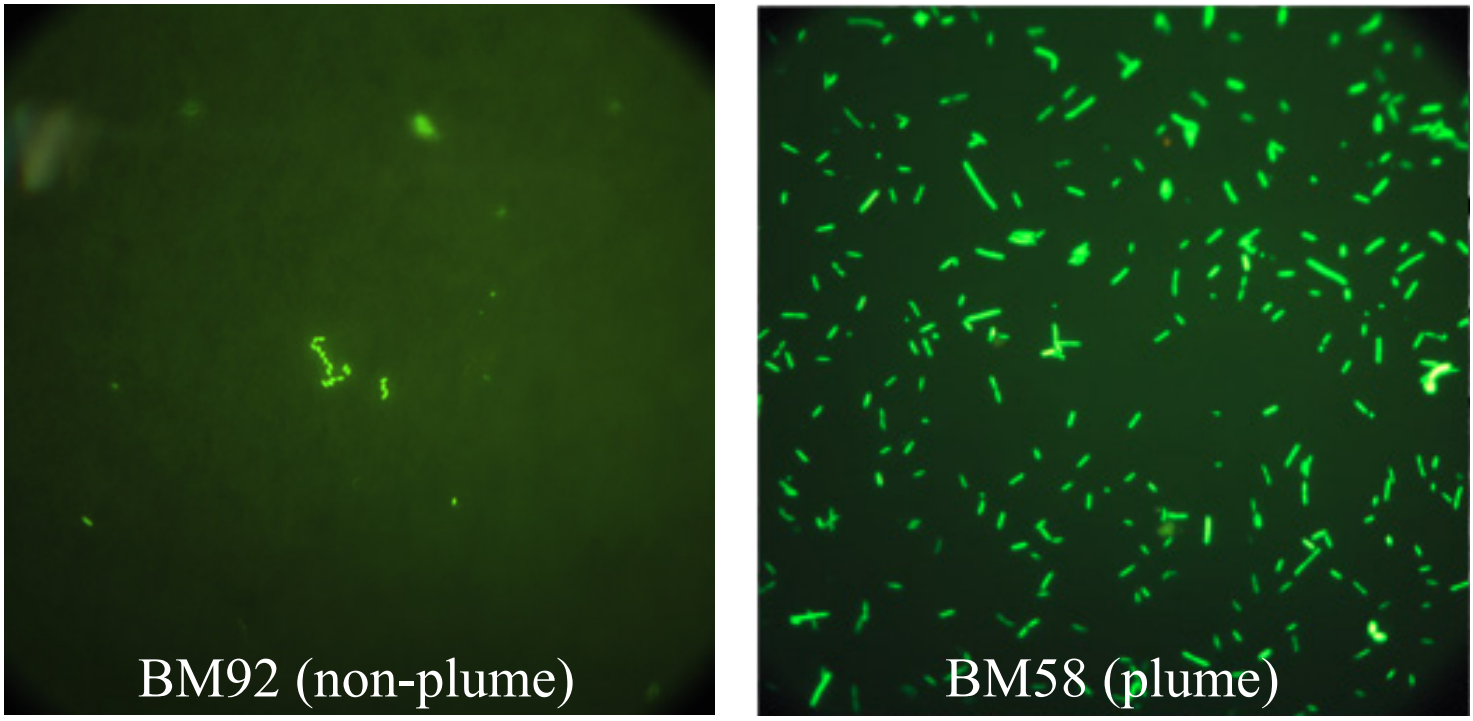

Figure 4.