

Natural Attenuation Bioremediation: Indigenous Bacterial Communities Respond to *Deepwater Horizon* Oil Spill

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WRITER'S COMMENT: *In the summer of 2010, I visited the CNN studio in Atlanta, Georgia. On the tour of the studio, I had a bird eye's view of the many cubicles where news researchers were busying themselves putting together breaking news stories. Among all the cubicles and the hustle and bustle of the researchers was a screen showing the live footage of oil, black and murky, gushing out of the broken well-head into the Gulf of Mexico. By that time, the oil had been spilling out into the Gulf for over two months, and the image had become familiar, and it seemed to me the Deepwater Horizon oil spill was losing its momentum in the news. However, that live footage of the spill in the background of the CNN news room has stayed with me and has even become more vivid in my mind today as I wonder what has happened since the news stopped reporting on the oil spill. Combining my curiosity with my biology background, in particular my knowledge from my soil microbiology class, I knew immediately what I wanted to write my literature review assignment on for my UWP 102B (writing in the biological sciences) class—I wanted to research the effects of the Deepwater Horizon oil spill on the bacterial communities living in the Gulf of Mexico.*

INSTRUCTOR'S COMMENT: *When Molly showed me a draft of this literature review, I knew immediately that it was going to be a good candidate for Prized Writing. First, it detailed research on a topic of recent interest—the BP oil spill in the Gulf of Mexico. Second, it would interest scientists, policy analysts, and lay readers. But most of all, it was written so well to accommodate those varied audiences. Molly pulled information from nine or ten sources and integrated the material into a piece that informs the scientists, points the way for policy decisions, and satisfies the public that natural processes might account for a good deal of the oil cleanup. That's quite a feat for one technical document to accomplish. There is a lot of technical information here, but it doesn't obscure the overall message of the piece.*

—Jared Haynes, University Writing Program

Introduction

On April 20, 2010 the blowout prevention device and all the emergency shutoff equipment on the *Deepwater Horizon* oil rig failed, resulting in the largest oil spill to date. For 84 days, oil escaped from the damaged well-head located on the ocean floor—1500 meters below the ocean surface. An estimated 4.9 million barrels of crude Macondo oil was released into the Gulf of Mexico (Atlas and Hazen 2011) and as early as June 2010, oil began to reach the shorelines extending from Louisiana to Florida (Beazley *et al.* 2012).

Because of the massive size of the spill and the ecologic and economic importance of the Gulf's waters and shorelines, decisions regarding the spill's clean-up were the focus of many conservation biologists and policy makers. According to Kostka *et al.* (2011), natural attenuation bioremediation, a process in which the indigenous bacteria of the Gulf of Mexico degrade—and thereby clean up—the spilled oil, will be the ultimate process that removes the majority of the spilled oil from the environment. Furthermore, natural attenuation bioremediation may be favored in the case of the *Deepwater Horizon* spill due to several unique features that characterize the spill. First, a large proportion of the Macondo oil that leaked from the well-head was composed of light weight, and thus easily degradable, hydrocarbons, including the natural gases methane, ethane, propane and butane (Valentine *et al.* 2010). Second, the oil was released 1500 meters below the ocean's surface and formed multiple oil plumes—cloud-like formations composed of oil and natural gas—that were neutrally buoyant between 900-1300 meters below the surface level (Hazen *et al.* 2010). These plumes concentrated the oil and served as microenvironments for bacteria to proliferate. Third, the bacterial communities in the Gulf of Mexico are exposed to recurrent oil leaks from natural hydrocarbon seeps in the ocean floor (Beazley *et al.* 2012) and, thus, over time may have evolved the ability to metabolize hydrocarbons. Finally, the dispersant COREXIT EC9500A was used to prevent oil slicks from forming on the ocean surface. Some research indicates that COREXIT enhances the bacteria's ability to degrade oil (Baelum *et al.* 2012); however, the biological effects of COREXIT on bacteria communities remain ambiguous (Hamdan and Fulmer 2011; Ortmann *et al.* 2012; Baelum *et al.* 2012).

Previous knowledge of bioremediation processes and hydrocarbon-degrading bacteria communities comes from laboratory incubation

experiments (Kostka *et al.* 2011) or from previous oil spills under very different environmental conditions from those of the Gulf of Mexico. Thus there is a lack of information about how *in situ* environmental conditions of the Gulf and the use of chemical dispersants like COREXIT may affect the natural bioremediation process. The *Deepwater Horizon* oil spill has given scientists the chance to study the bioremediation process *in situ*, which has led to a wealth of new knowledge regarding hydrocarbon-degrading bacterial communities present in the Gulf of Mexico.

Successional Response of Bacteria to *Deepwater Horizon* Oil Spill

The bacterial community adapted rapidly and dynamically to the influx of hydrocarbons and it is hypothesized that the bacterial community responded in a successional fashion immediately following the spill (Valentine *et al.* 2010). The community composition changed over time (and space) as the concentration and composition of hydrocarbons in oil plumes changed (Mason *et al.* 2012). There was a preferential degradation of simple and light cyclo- and n-alkanes first, followed by methane (Valentine *et al.* 2010), and finally followed by heavy n-alkanes and complex aromatics including PAHs and BTEXs (Mason *et al.* 2012).

Oil Plume Formation and Composition

Due to the depth of the spill and the slow migration of the crude oil to the surface (and possibly also due to the use of dispersant (Baelum *et al.* 2012)), some oil droplets became neutrally buoyant between 900 and 1300 meters and clustered together to form oil plumes (Atlas and Hazen 2011). Ocean currents then carried these newly formed oil plumes away from the well-head. Oil plumes were composed of natural gas hydrocarbons including methane, ethane, propane, and butane (Valentine *et al.* 2010) and non-gaseous hydrocarbons including heavier n-alkanes and toxic recalcitrant compounds such as polycyclic hydrocarbons (PAHs) and benzene toluene, ethylbenzene and total xylenes (BTEX) (Mason *et al.* 2012).

Oil Plume Degradation Patterns Reveal Evidence of Indigenous Hydrocarbon-Degrading Bacteria

Between 1000 and 1200 meters below the ocean surface, dissolved oxygen levels around the oil plumes decreased, indicating that heterotrophic bacteria were respiring as they metabolized the hydrocarbons present in the oil plume (Hazen *et al.* 2010, Valentine *et al.* 2010). Valentine *et al.*

(2010) calculated that up to 70% of the decrease in dissolved oxygen concentration around the oil plumes could be attributed to the bacterial respiration of the natural gases ethane and propane and further reasoned that ethane and propane consumption by bacteria could account for up to 2/3 of the bacterial community's productivity during the initial time period after the plume had formed at the well-head.

Followed over time, the degradation patterns inside oil plumes provide evidence for the order in which bacteria consumed and hence degraded hydrocarbons. The ratio of C26 to C15 alkanes increased with the age of the oil plume, suggesting that light chain hydrocarbons were preferentially consumed during the early time period following the plume's formation (Hazen *et al.* 2010). Hazen *et al.* (2010) calculated the degradation half-life of these light chain hydrocarbons to be 1.2 to 6.1 days. Interestingly, Valentine *et al.* (2010) observed that the ratio of methane to ethane and butane actually increased with the age of the plume, suggesting that despite methane's simplicity, it actually degraded later rather than earlier during the time period after the plume had formed. Further evidence for the preferential degradation of simple and light chain hydrocarbons comes from the compositional analysis of oil that reached coastal beaches as early as June (2 months after the spill). Oil deposited on the coastal beaches was highly degraded and weathered and consisted mainly of higher molecular weight straight chained, branched, and aromatic hydrocarbons (Kostka *et al.* 2011).

*16s rRNA Gene Sequences Reveal Oil Plume Bacteria belong to the Class
Gammaproteobactiera*

Due to the influx of hydrocarbons, bacterial cell count within the plumes increased as much as two-fold compared to uncontaminated sites at the same depth (Hazen *et al.* 2010); however, overall species diversity decreased within the plumes compared to uncontaminated sites at the same depth (Mason *et al.* 2012). Mason *et al.* (2012) analyzed the DNA sequence of the 16s rRNA gene to determine phylogenetic composition of the bacteria in the oil plumes. Plume samples taken from late May (about a month after the spill) indicated an increase in relative abundance of bacteria belonging to the order Oceanospirillales. In addition, bacteria from the order Oceanospirillales accounted for 80-90% of the DNA sequences in oil plumes samples (Mason *et al.* 2010), whereas it accounted for only 3% (Mason *et al.* 2012) and 5% (Hazen *et al.* 2010) of the DNA sequences in uncontaminated site samples. Samples taken

from late June (about two months after the spill), however, indicated an increase in relative abundance of bacteria belonging to the genera *Cycolclasticus* and *Colwellia* (Valentine *et al.* 2010). Bacteria from the order Oceanospirillales and from the genera *Cycolclasticus* and *Colwellia* all belong to the class of bacteria known as Gammaproteobacteria, which contains many known hydrocarbon-degrading bacteria. The change in relative abundance of different Gammaproteobacteria between sampling times provides evidence of community succession.

Analysis of bacterial composition of oiled beach sands affected by the *Deepwater Horizon* spill also showed an increased abundance of Gammaproteobacteria including the genera *Alcanivorax*, *Marinobacter*, and *Acinetobacter* (Kostka *et al.* 2011). The 16s rRNA gene sequence identified in oil plume samples corresponding to the order Oceanospirillales had an 88% sequence identity to the 16s rRNA gene of the species *Alcanivorax borkumensis*, found in oil beach sands (Mason *et al.* 2012). This finding suggests the close genetic relationship between the Gammaproteobacteria of oiled beach sands and of oil plumes and the possibility of similar responses to influxes of hydrocarbons. Kostka *et al.* (2011) demonstrated that the genus *Alcanivorax* utilized a narrow range of carbon substrates consisting mainly of straight-chained and branched hydrocarbons, while the genera *Marinobacter* and *Acinetobacter* were able to utilize a broader range of hydrocarbons including PAHs. These findings from oiled beach sands indicate that there is niche specialization of carbon substrate metabolism among the bacteria of oiled beaches (Kostka *et al.* 2011). If this concept of niche specialization based on carbon substrate metabolism is extended to the bacteria found in oil plumes (which is reasonable given their close genetic relationship), it could provide further evidence for the preferential degradation of hydrocarbons and help to explain the successional pattern found among bacteria in the oil plumes.

Functional Composition of the Bacterial Community

Metagenome and metatranscriptome analysis of the bacterial community provided further support for the hypothesis that hydrocarbon degrading bacteria were able to respond dynamically and in a successional pattern to the influx of hydrocarbons.

Using metagenome analysis and oil plume samples taken from late May (about a month after the spill), Hazen *et al.* (2010) identified 1652 genes in the bacteria community involved in hydrocarbon degradation. Mason *et al.* (2012), who also used oil plume samples taken from late May,

identified the complete set of genes involved in the pathway of n-alkane degradation and the nearly complete set of genes involved in the pathway of cyclohexane degradation. Furthermore, these genes were found in high abundance. Genes for the degradation of aromatic compounds were less abundant, indicating that bacteria capable of degrading more complex compounds were not abundant in the community during early sampling after the initial formation of the plume (Mason *et al.* 2012).

The metatranscriptome analysis of oil plume samples taken from late May revealed that the order *Oceanospirillales* was not only the most abundant but also the most active; of the total RNA transcripts present at the time of sampling, 46-69% belonged to the order *Oceanospirillales* (Mason *et al.* 2012). Transcripts of genes involved in the degradation of PAHs and BTEXs were either not present or present below the detection threshold, indicating that during the initial time period following the plume's formation bacteria were not actively degrading the more recalcitrant and complex hydrocarbons (Mason *et al.* 2012). Transcripts of genes involved in the degradation of methane were found, albeit at low levels, accounting for less than 1% of the total transcripts in the sample (Mason *et al.* 2012). Although transcripts for methane degrading genes were detected at very low levels, they did nevertheless slightly increase in abundance in the distal plume samples, indicating that methane degradation was delayed (Mason *et al.* 2012). This supports the findings of Valentine *et al.* (2010), which indicated methane was not degraded during the early time period after the plume had formed but was instead degraded much later.

In addition to being able to metabolize hydrocarbons, bacteria must have other key characteristics to adapt quickly and efficiently to an influx of hydrocarbons. Mason *et al.* (2012) suggest that these characteristics include a means of motility, a mechanism for the avoidance of virus predation, and a way to uptake nutrients. Using the draft genome of the order *Oceanospirillales*, Mason *et al.* (2012) identified genes for each of these characteristics, suggesting that bacteria of the order *Oceanospirillales* may be successful colonizers under high influxes of hydrocarbons. This finding provides evidence to better explain the relatively fast growth and high relative abundance of bacteria belonging to the order *Oceanospirillales* during the initial time period following the formation of the plume.

Effects of the Chemical Dispersant COREXIT on Bacteria Communities

In an attempt to reduce the formation of oil slicks on the ocean surface as operating crews worked to stop the spill at the well-head and to prevent birds, mammals, and invertebrates from being coated in oil, 1.8 million gallons of the chemical dispersant COREXIT was applied both at the well-head and to the ocean surface. Toxicity tests used by the EPA to approve the use of COREXIT consider the effect of COREXIT only on invertebrates and adult fish populations and do not consider its effect on the bacterial community (Hamdan and Fulmer 2011). In fact very little is actually known about how COREXIT affects the bacterial community and much of the current research on the topic is in disagreement.

Hamdan and Fulmer (2011) concluded that the indigenous hydrocarbon degrading bacteria of the Gulf of Mexico are susceptible to COREXIT toxicity in a dose dependent manner, and thus the use of COREXIT may inhibit natural attenuation bioremediation. Indigenous bacteria found on oiled beaches affected by the *Deepwater Horizon* spill, including the genera *Vibrio*, *Acinetobacter*, and *Marinobacter*, experienced nearly complete cell death when exposed to a 1:100 dilution of COREXIT (Hamdan and Fulmer 2011). When exposed to a 1:1000 dilution of COREXIT, the genera *Acinetobacter* and *Marinobacter*, both of which degrade hydrocarbons present in oil, experienced a decrease in cell numbers and productivity, but the genus *Vibrio*, which does not degrade hydrocarbons present in oil, actually experienced an increase in cell number (Hamdan and Fulmer 2011). Bacteria of the genus *Vibrio* are able to metabolize surfactants and thus may have a selective advantage over hydrocarbon-degrading bacteria that cannot metabolize surfactants when exposure to dispersants occurs, which in turn may disrupt the natural attenuation process (Hamdan and Fulmer 2011).

In opposition to Hamdan and Fulmer (2011), Baelum *et al.* (2012) concluded that COREXIT did not inhibit the growth of indigenous bacteria in the Gulf of Mexico. Baelum *et al.* (2012) furthermore concluded that the application of COREXIT increased the initial degradation of the oil. The increase in the degradation rate was due to two reasons. First, in samples with oil and COREXIT, 60% of the oil was dissolved into solution, whereas in samples with oil alone, only 25% of the oil was dissolved. Thus the COREXIT increased the surface area of oil available and allowed bacteria to form more flocs—high bacteria

cell densities clustered around a high nutrient source. This ultimately led to a faster initial oil degradation rate. Second, COREXIT itself is composed of hydrocarbons, which bacteria can degrade and use as a carbon substrate. Bacteria that use COREXIT as an additional carbon substrate increase the overall productivity of the community and may stimulate the degradation of oil (Baelum *et al.* 2012). However, because Baelum *et al.* (2012) used incubation experiments carried out in the laboratory, the degree to which these findings can be extended to *in situ* conditions is uncertain.

Although Baelum *et al.* (2012) showed that bacteria could degrade oil in the presence of COREXIT, Ortmann *et al.* (2012) argued that in the presence of dispersant, the carbon that enters the food web via the metabolism of hydrocarbon-degrading bacteria is not transferred throughout the food web. In the absence of dispersant, however, the carbon that enters the food web via the metabolism of hydrocarbon degrading bacteria is transferred throughout the food web. In samples of oil alone, the biomass of hydrocarbon degrading bacteria (and thus the carbon they metabolized) was passed on to the next trophic level composed of eukaryotic ciliates. In samples of oil and dispersant, the biomass of hydrocarbon degrading bacteria (and thus the carbon they metabolized) was not transferred on to the next trophic level, and energy was lost from the food web (Ortmann *et al.* 2012). Thus, the use of COREXIT may decrease the overall productivity of the food web as a whole.

Conclusions

The *Deepwater Horizon* oil spill is unique among oil spills and has allowed scientists to better understand the *in situ* responses of indigenous bacteria of the Gulf of Mexico to a massive influx of hydrocarbons. Based on the research conducted by scientists in response to the oil spill, bioremediation using natural attenuation stands out as a leading candidate as a means to clean up the aftermath of the spill. Indigenous bacteria in the Gulf of Mexico may have evolved the ability to degrade hydrocarbons through recurrent exposure of oil leaks due to the presence of natural hydrocarbon seeps on the ocean floor. The indigenous hydrocarbon degrading bacteria display niche specialization in regards to their metabolism—each taxonomic bacterial group preferentially degrades a different class of hydrocarbons. The differential degradation of hydrocarbons generates a successional response in which different

bacterial taxonomic groups increase in abundance over time in relation to one another and in relation to the hydrocarbons available to use as substrates in metabolism. The impacts that the chemical dispersant, COREXIT, may have had on the bacteria community are still uncertain. Further research examining the bacteria community composition and its response to an influx of oil and dispersant should be conducted to aid in decisions regarding the mitigation of future spills. This is especially important for the Gulf because of the increasing demands for oil and the continued use of deep offshore drilling.

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