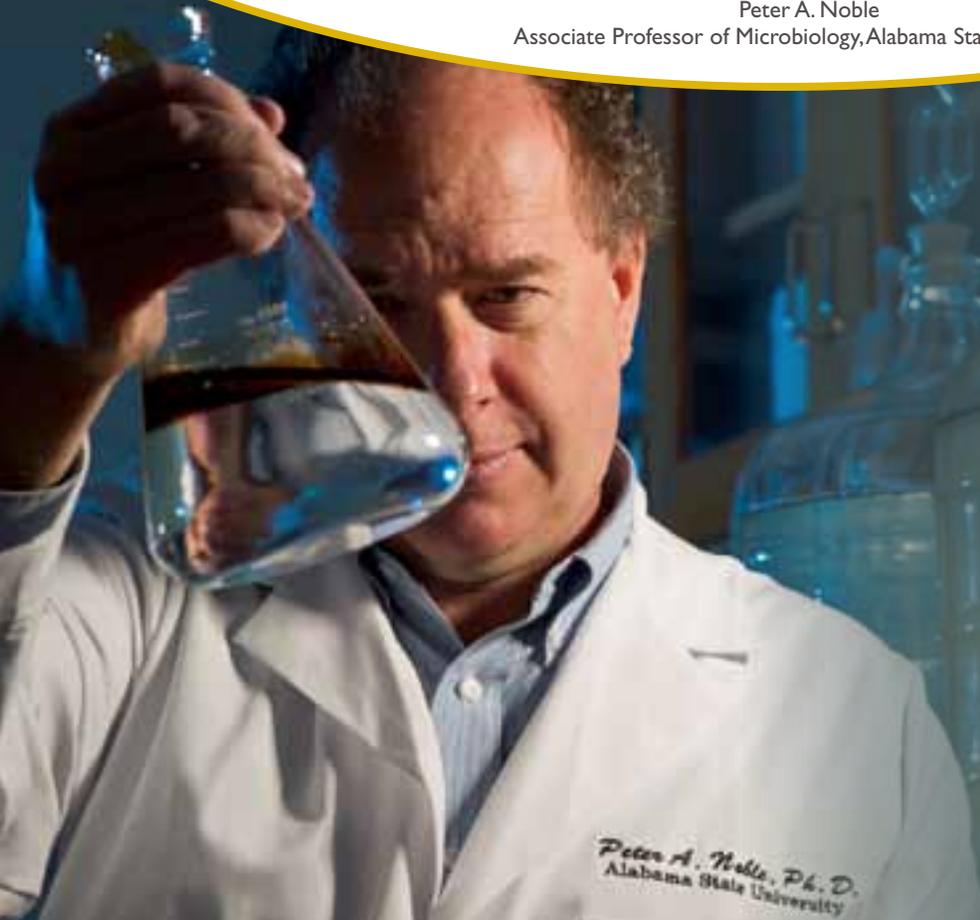


Metagenomic Analysis of Hydrocarbon Utilizing Microbes from the Gulf of Mexico

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The daily release of 40,000 to 100,000 barrels of oil from the Deepwater Horizon well site in 2010 lasted about three months, and resulted in massive swirling plumes of gaseous and liquid hydrocarbons that extended all the way from the ocean floor (~1500 m) to the air-water interface. A recent study suggests that microbial communities played an important role in the degradation of oil discharged from the Deepwater Horizon wellhead. Specifically, DNA sequences collected from the oil plume showed a predominance of one phylogenetic order of bacteria: the Oceanospirillales, and an increase in hydrocarbon degrading genes compared to control sites (Hazen et al. 2010).

A complicating factor in understanding the role of these microbes is the use of dispersants, which were injected into the oil at the wellhead and sprayed onto the oil at the air-water interface. The purpose of the dispersant was to lower the surface tension between the oil and the water so that large oil droplets become small oil droplets, increasing the higher surface to volume ratio. The higher ratio is presumed to make the oil more susceptible to microbial degradation. Unfortunately, little information exists on the interactions among the oil, the dispersant, and the microbes in the deep ocean under such high water pressure and low temperature. Intuitive reasoning suggests that oil should float to the air-water surface interface. However, under these conditions, long hydrocarbons (>50 carbon atoms) sink because of their density, medium-length hydrocarbons (>14 carbon atoms) crystallize into a waxy solid form, and methane gas forms a methane ice. *Whether the dispersants help the microbes degrade these different forms of hydrocarbons, especially under these environmental conditions, is not known.*

Although there is a paucity of information about hydrocarbon-utilizing microbes in the deep-ocean, here is what is known about microbes and oil, in general:

- (i) hydrocarbon-utilizing microorganisms are ubiquitous in the Gulf of Mexico because shipping vessels traveling up and down from the Mississippi River regularly release petroleum hydrocarbons,
- (ii) the physiology of specific hydrocarbon-utilizing microbes, such as *Alcanivorax borkumensis*, *Cycloclasticus pugetii*, *Oceanobacter kriegii*, and *Thalassolituus oleivorans* have been well studied,
- (iii) the microbial breakdown of carbon typically involves a significant division of labor, i.e., the breakdown of nutrients by one microbial population results in the production of waste products (metabolites) that are used by other microbial populations, and
- (iv) most marine microbes cannot be cultured in the laboratory.

Hence, while fundamental information exists on hydrocarbon-utilizing physiology of specific microbes

that can be cultured in the laboratory, little is known about metabolism and physiology of natural microbial communities microbes because the technology to investigate these microbes was not previously available.

Fortunately, Alabama State University has recently acquired high throughput technology to explore the response of natural microbial communities to hydrocarbons and dispersants in seawater and determine metabolic networks of hydrocarbon utilization through metagenomic analysis.

Metagenomics analysis involves the direct isolation of nucleic acids from environmental samples followed by high throughput DNA sequencing and data processing. This analysis is analogous to single microbial genome sequencing except, in metagenomic analysis, all the sequences recovered from an environmental sample are interrogated. Metagenomic analysis can be used to assess the impact of oil and dispersant on microbial communities through monitoring the change in DNA sequences and sequence diversities under different environmental conditions. The knowledge of the DNA sequences in a water sample will aid in the understanding of how metabolites are transferred and transformed in response to different hydrocarbons and dispersants. Moreover, an improved understanding of how microbial communities cope with oil and the dispersant in order to assess the potential of contaminated sites to recover from pollution, and increase the chances of bioaugmentation or biostimulation trials to succeed now and in the future. Lastly, metagenomics analysis has the potential to discover new genes and metabolic pathways that were previously not known due to limitations of the conventional methods. The assumption of the metagenomic analysis *is that the most abundant microbes will be highly represented in the resulting sequence data and that most of these sequences would not be detected by using conventional culturing or molecular approaches.*

Professors B.K. Robertson and P.A. Noble of Alabama State University have secured a Gulf Coast Marine Environmental Sciences Consortium (MESCC) initiative grant to investigate the microbial response to the Deepwater Horizon oil blowout. The objectives of the study are: to determine if oil and oil-plus-dispersant amended microcosms result in the predominance of one group of microbes and an increase in abundance

of hydrocarbon-degrading genes – as reported by field studies (e.g., Hazen et al. 2010) – and to demonstrate the utility of the metagenomic analysis to the study of microbial communities and their metabolic networks. The high throughput sequencing will be conducted using the National Science Foundation-funded Roche/454 sequencer. ■

LITERATURE CITED

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Water from Gulf of Mexico amended with oil and dispersant.

