

ALS

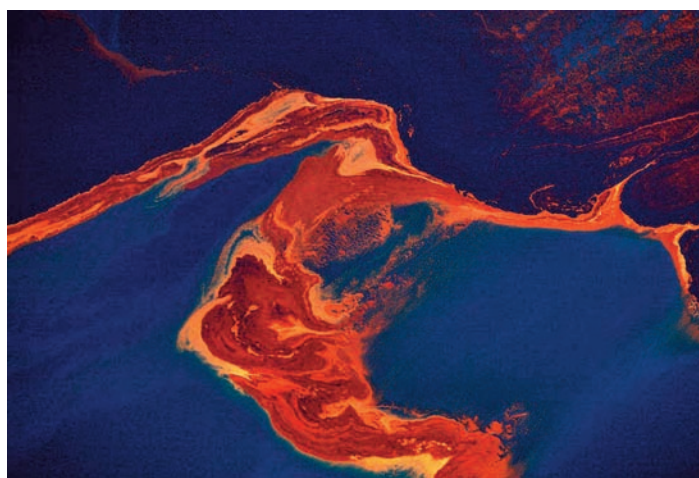
SCIENCE HIGHLIGHT

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Molecular Measurements of the Deep-Sea Oil Plume in the Gulf of Mexico

Assessing the environmental and public health impacts of the Deepwater Horizon oil spill is difficult owing to the extreme depth of the blowout and the large volumes of oil released. One strategy for remediation of the plume is to use the intrinsic bioremediation potential of deep-sea microorganisms to degrade the oil. This strategy depends on a number of environmental factors, including a favorable response of indigenous microorganisms to an increased concentration of hydrocarbons and/or dispersant. To study the effects of the spill, researchers collected deep-water samples from across the Gulf of Mexico and analyzed their physical, chemical, and microbiological properties using a variety of techniques, including synchrotron radiation Fourier-transform infrared (SR-FTIR) spectroscopy at ALS Beamline 1.4.3. The studies suggest that the plume did indeed stimulate indigenous deep-sea bacteria that are closely related to known petroleum degraders.

Seventeen deep-water sam-



The oil slick in the Gulf of Mexico as seen from a helicopter (photo by Rick Loomis, *Los Angeles Times*, May 6, 2010).

ples were taken between May 25 and June 2, 2010, from across the Gulf of Mexico. The data indicated the presence of an oil plume from 1099 to 1219 m deep at distances of up to 10 km from the wellhead with a southwest current. The average temperature within the plume was 4.7°C and the pressure was 1136 dB (1 atm = 191 dB). At most plume locations, a slight decrease in oxygen concentration was detected, indicative of microbial respiration and oxygen consumption, as would be expected if the hydrocarbons were being catabolized. Molecular biochemistry analyses revealed that the dispersed oil plume affected microbial cell densities and composition. Cell densities inside the plume were almost two times higher than outside the plume.

Genetic analysis of in-plume samples revealed that, while cell densities were higher, taxonomic diversity was lower. The enriched bacteria were restricted to a few members of the γ -Proteobacteria subphylum, including psychrophilic and psychrotolerant (cold-water thriving or tolerant) species. Further cloning and sequencing revealed that the samples were dominated by the order *Oceanospirillales*, with the observed sequences falling into one of two distinct *Oceanospirillales* groups. One of these groups is largely composed of known psychrophilic hydrocarbon degraders and microorganisms from hydrocarbon-dominated environments, including *Oleispira antarctica*, *Thalassolituus oleivorans* (psychrophiles), and *Oleiphilus messi-*

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nensis, all of which are known hydrocarbon degraders. Microscopic examination of cells col-



Microbial Mitigation

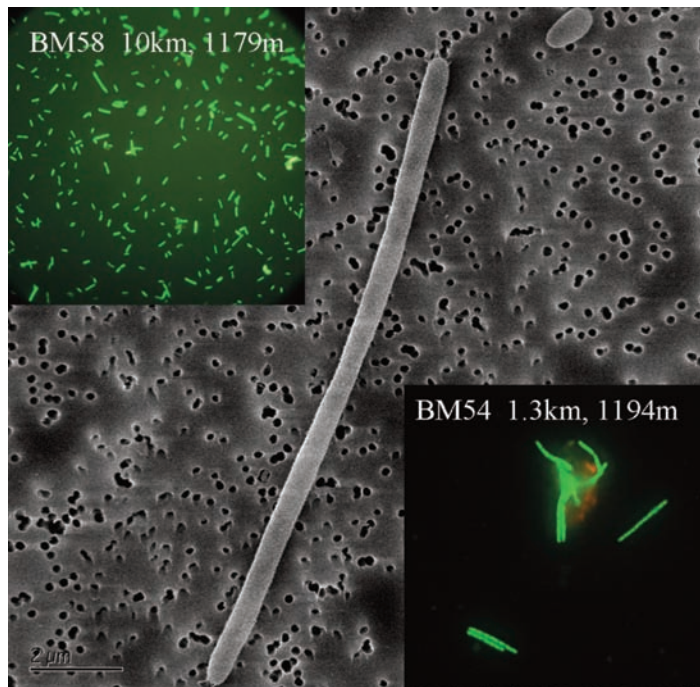
The Deepwater Horizon blowout in the Gulf of Mexico on April 20, 2010, resulted in the largest oil spill in the history of the United States. The biological effects and expected fate of the oil are unknown, partly due to the extreme depth and magnitude of this event and partly due to the primary initial mitigation strategy that injected unprecedented quantities of oil dispersant directly at the wellhead (1544 m below the sea surface). Indigenous deep-sea microorganisms that degrade oil could represent a significant natural attenuation mechanism; but this would depend on how native microorganisms respond to an increased concentration of hydrocarbons and/or dispersant at such extreme depths and temperatures ($\sim 4^{\circ}\text{C}$). A collaboration led by Berkeley Lab researchers here reports that the dispersed hydrocarbon plume stimulated the growth of a type of bacteria that thrives in cold temperatures and at great depths. Infrared spectroscopy at the ALS, with the ability to study microbial processes at the molecular level, provided key pieces of the puzzle.

lected within the plume also revealed that the dominant cell type exhibits a distinctive morphology typical of the *Oceanospirillales*.

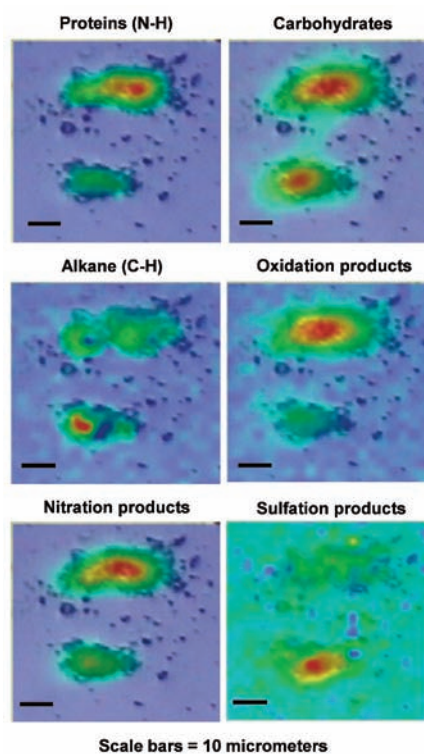
Using SR-FTIR data collected at infrared Beamline 1.4.3, the researchers found absorption features at ~ 1730 , ~ 1610 , and $\sim 1150\text{ cm}^{-1}$ that are associated with biomolecule-rich regions. The mid-infrared light (~ 2.5 - to 15.5 -m wavelength or ~ 4000 - to 650 cm^{-1} wavenumber) from the beamline is ideal for studying living bacteria individually or in small groups. This light is nondestructive and provides label-free, fingerprint-like spectra originating from the characteristic vibrational frequencies of various functional-group molecules. The absorption features from the samples are well described for the carbonyl ($\text{C}=\text{O}$), nitrogen oxide (NO_x), and sulfur oxide (SO_x) vibration modes, and they are character-

istic of oil degradation products as well as macromolecules of biological samples. These infrared spectra are not consistent with those typically found in marine macroaggregates. Most importantly, we do not observe them in the nonplume samples at the same depth.

The bioremediation potential of these microorganisms largely depends on how fast they degrade the oil. Based on the biodegradation rates from four data sets (two from the field and two from the laboratory), the researchers predicted that oil half-life is somewhere between 1.2 to 6.1 days. This is consistent with values reported in the literature for comparable temperature and field conditions. The frequent episodic oil leaks from natural seeps in this area suggest that the deep-sea microbial community may have adapted to fluctuating concentrations of hydrocarbons over long periods of time.



Scanning electron micrographs of dominant bacteria collected in the plume (1099 to 1219 m deep). The dominant cell type exhibits a distinctive morphology typical of the *Oceanospirillales* order of Proteobacteria. Insets: Acridine orange stains of samples at two distances from the source.



Infrared images showing the spatial distribution of various oil and microbial products.