

Self-healing capacity of deep-sea ecosystems affected by petroleum hydrocarbons

Understanding microbial oil degradation at hydrocarbon seeps is key to sustainable bioremediation protocols

Alberto Scoma^{1,2}, Michail M Yakimov^{3,4}, Daniele Daffonchio⁵ & Nico Boon¹

Crude oil or petroleum is a naturally occurring liquid that was formed in geological sediments from organic material under high hydrostatic pressure (HP). It has enormous economic importance as the basis for fuels, plastics, and a huge range of chemicals; the global economy consumes about 30 billion barrels of oil, or 4.8 cubic kilometers, each year. Much of this annual production comes from offshore oil-fields and/or is shipped across the world, which poses a considerable risk of accidents and oil spills that contaminate the marine environment. The Exxon Valdez (1989), Prestige (2002), or Deepwater Horizon (DWH) offshore platform (2010) disasters affected huge areas of the oceans and adjacent shores with devastating impact on the fauna and flora.

There is therefore huge interest in technologies to clean up spilled oil at shores and in the deep seas. This in turn has triggered interest in natural biodegradation processes by microorganisms that are able to break down oil and thereby remove it from the environment. These oil-consuming archaea and bacteria thrive around natural marine hydrocarbon seepages, such as the spectacular hydrothermal black smokers or the cold hydrocarbon seeps that were first discovered in the Gulf of Mexico and in subduction zones of the Pacific Ocean [1]. Recent research showed that these hydrocarbon

springs at depths of 200–3,500 m below surface level (bsl) fuel fantastic and highly diverse deep-sea ecosystems, spanning from asphalt and oil seeps, hypersaline lakes, and gas chimneys, to mud volcanoes and pock-marks.

Natural oil spills and oil weathering

The amount of oil that is naturally entering the oceans at these sites is estimated to be more than 700 million liters per year [2]. In contrast, anthropogenic oil spills into marine environments are estimated to amount to more than 120 million liters per year [2]. The main causes are routine operations—drilling, manufacturing, storing, transporting, and managing waste—or leaking pipelines and sunken vessels. Together, anthropogenic and natural oil spills would cover the world's oceans with a 25-molecule-thick layer. The fact that the oceans are not destroyed by this oil pollution is testament to the efficiency of specialized hydrocarbon-degrading microorganisms, which are physiologically adapted to and routinely make a meal of crude oil. The study of these microbial oil eaters could therefore yield highly valuable knowledge not only about how nature cleans up oil contamination but also to inspire novel remediation measures to deal with anthropogenic oil spills.

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Petroleum is a complex mixture of gaseous, liquid, and solid hydrocarbons that undergo several physicochemical processes—comprehensively referred to as weathering—once it spills into ocean waters (Fig 1). Within few hours, it is subjected to spreading, evaporation, dissolution, dispersion, and emulsification; (photo)oxidation, sinking, and biodegradation start after about 1 day, the latter of which can potentially last for years. Spreading and evaporation, particularly low-viscosity oil with a higher content of volatile compounds, are enhanced by the action of wind, waves, and warm temperatures. Waves and water turbulence also induce dispersion by breaking the oil slick on the sea surface into droplets: smaller droplets remain floating in the upper water column and larger ones

1 Center for Microbial Ecology and Technology (CMET), Ghent University (UGent), Ghent, Belgium. E-mails: alberto.scoma@ugent.be; alberto.scoma@bios.au.dk

2 Center for Geomicrobiology, Department of Bioscience, Aarhus University, Aarhus, Denmark

3 Institute for Coastal Marine Environment (IAMC), National Council of Research (CNR), Messina, Italy

4 Immanuel Kant Baltic Federal University, Kaliningrad, Russia

5 Biological and Environmental Sciences and Engineering Division, King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia

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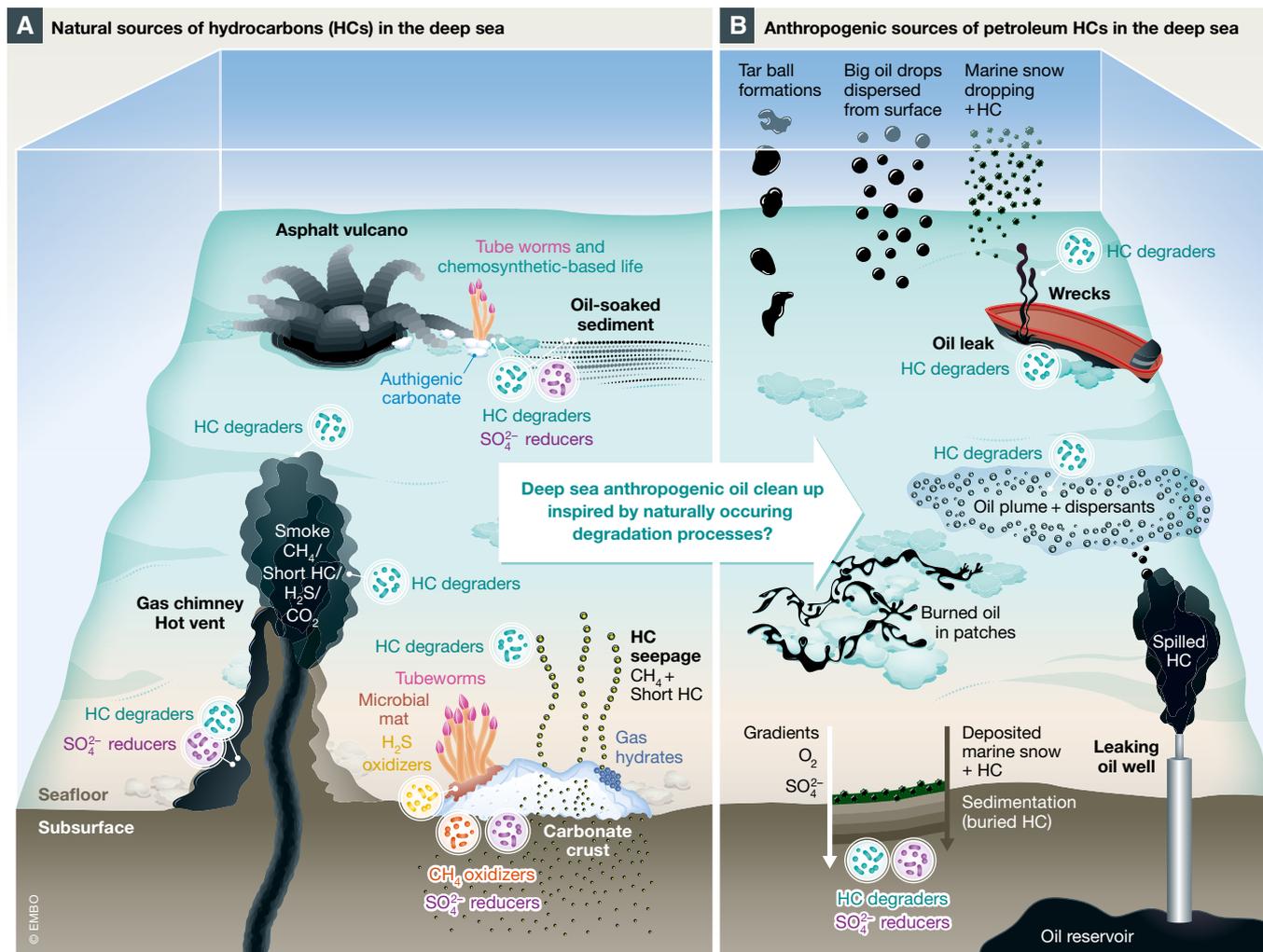


Figure 1. Natural and anthropogenic sources and pathways of petroleum hydrocarbons in the deep sea.

rise back to the sea surface. These oil droplets have a greater surface-to-volume ratio than oil slick, which facilitates other weathering processes, such as dissolution, sedimentation, and biodegradation.

Emulsification occurs if seawater droplets are suspended in oil and creates the so-called highly viscous chocolate mousse, which binds a significant volume of water, delays weathering processes, and presents a major challenge for cleaning up owing to its stickiness. Dissolution affects the water-soluble fraction of light hydrocarbons. Oxidation, favored by sunlight, is a slower process, which, alongside other chemical modifications, promotes tar formation in thick layers of highly viscous oil. Weathered oil rarely sinks to the ocean bottom, but some sinking occurs through the so-called marine snow: algal flocks that naturally form in the

upper water column and that attach to petroleum hydrocarbons. Most of the weathered oil, however, continues to float on the surface until it ends on shorelines. It is also here where oil spills have their most visible effects along with the sea birds and marine mammals that are washed ashore coated in a glossy black oil slick.

Human interventions to deal with oil spills

Various technologies are available to deal with oil spills and for remediation. Booms and other physical barriers on the sea surface prevent oil from spreading and help to recover it with a skimmer or a sorbent. The latter is also being used to remove petroleum from shorelines. In the case of offshore oil spills, burning increases sedimentation, as the most volatile fraction is combusted and

the remaining denser residues sink to the seafloor. Chemical dispersants, a mixture of surfactants and solvents, enhance dispersion and the formation of oil droplets. As the loss of the most soluble fraction makes these oil droplets denser over time, dispersion acts in a similar manner as *in situ* burning, and contaminates a vast area of the seafloor.

The vast majority of oil spills concern surface waters. Some natural weathering processes, such as dissolution, dispersion, or sinking, and remediation technologies increase underwater contamination. Accidental large-scale deep-sea oil contamination is a relatively new problem. Until recently, the main source of pollution was seepage from shipwrecks. There are about 9,000 such wrecks worldwide that carry 3,000–23,000 millions of liters of oil [3]. Many wrecks lie in shallow coastal waters—tens of

meters bsl—but others sank to the deep-sea bottom, which complicates recovery of both the wreck and the oil. The Prestige, for instance, sank 160 km off the coast of North-West of Spain at 3,500 m bsl. In these cases, remediation technologies consist in removing or re-floating the wreck, provided it is sufficiently intact. Alternatively, tapping and pumping the oil out of the wreck remains the best option, and such technologies have been successfully tested even at deep-sea locations.

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The 2010 DWH oil spill in the Gulf of Mexico represents a new threat for the deep-sea ocean bottom. The explosion of a well rig at about 1,500 m bsl caused the largest marine oil spill in human history: it released 795 millions of liters of oil that formed multiple oil plumes at 1,000–1,200 m bsl [2]. The primary response to a leaking oil well is capping the well head, but technical difficulties prolonged the release of oil for 83 days. Although it is a rare yet remarkable case of deep-sea oil contamination, the risk for similar spills in the future remains high, given continued efforts to access deep-sea oil reservoirs and the present lack of deep-sea remediation techniques. At larger depths, light hydrocarbons (C2 to C5) are predominantly present in the liquid oil phase owe to the high HP and low temperature, and may act as solvents for longer hydrocarbons, thereby increasing dispersion. Spilled oil from the deep-sea bottom eventually reached the sea surface where it was subjected to enhanced dissolution, as it went through the whole water column.

During the DWH spill, up to seven million liters of the chemical dispersant COREXIT were sprayed at the sea surface or injected directly at the leaking well. Notwithstanding, the oil eventually covered an ocean area of about 20,000 km² [2]. Finally, 41 millions of liters of oil on the surface were burned [4], but the potential biodegradation (if any) of the leftover residues on the seafloor remains unclear. The appropriateness and effectiveness of applying chemical dispersants to combat the DWH spill were highly debated [4]. While some suggested a microbial community shift in the oil plume, there is

however no definite evidence that the dispersants had any stimulatory effect on increased microbial digestion of hydrocarbons [2]. This is particularly important as biodegradation remains the only solution to remove spilled oil from deep-sea environments.

Microbial oil degraders at surface and shallow waters, and in sediments

Of particular importance for bioremediation are microorganisms—known as hydrocarbonoclastic—that almost entirely depend on hydrocarbons as a source of carbon and energy [5]. In general, biodegradation of fossil oil, including the most recalcitrant petroleum fractions, relies on the availability of electron acceptors. Depending on the terminal electron acceptor, hydrocarbonoclastic bacteria can oxidize and break down petroleum constituents using oxygen, nitrate, ferric iron, or sulfate. When oxygen is available, aerobic processes that rely on various oxygen-incorporating enzymes, such as mono- and dioxygenases, are generally more rapid and efficient. Anaerobic breakdown of hydrocarbons start with the activation of carbon–hydrogen bonds through carboxylation, methylation, oxygen-independent hydroxylation, and (sub) terminal carbon addition to fumarate by glycol radical-bearing synthases [6]. As hydrocarbons have low chemical reactivity, marine sediments that are dominated by anoxic conditions pose biochemical challenges for microbial degradation.

Sulfate-reducing bacteria of the class Deltaproteobacteria are among the key players of petroleum degradation in oxygen-deprived marine ecosystems. Unfortunately, the overwhelming majority of them have not been yet cultivated; thus, little is known about their physiology. In contrast, we are now in a good position to cultivate and study the aerobic microbial degraders of aliphatic and aromatic hydrocarbons. These fall within the class of Gammaproteobacteria (e.g., *Alcanivorax*, *Arenibacter*, *Cycloclasticus*, *Marinobacter*, *Oleibacter*, *Oleiphilus*, *Oleispira*, *Polycyclovorans*, *Porticoccus*, and *Thalassolituus*), Alphaproteobacteria (e.g., *Novosphingobium*, *Roseobacter*, *Sphingomonas*, and *Thalassospira*), Actinobacteria (e.g., *Mycobacteria* and *Rhodococcus*), and Firmicutes (e.g., *Planomicrobium*). Physiological studies and genome sequencing have begun to reveal how and under which conditions they exploit hydrocarbons as a source of carbon and energy. For instance, genome analysis of the

unique marine hydrocarbon degrader *Cycloclasticus*, which is able to grow on a vast spectrum of polyaromatics—unsubstituted and substituted naphthalenes, phenanthrene, fluorene, and pyrene—indicates all these compounds are metabolized by a very limited set of hydrolytic enzymes with exceptionally broad specificity. By contrast, proteome analysis of the alkane-degrading marine bacterium *Alcanivorax*, a model microorganism for hydrocarbon degradation, suggested that depending on the molecular weight and structure degradation proceeds via several routes of terminal oxidation, involving alkane hydroxylases, monooxygenases, and P450 cytochromes, which gives this microorganism a potential ecological advantage over more specialized hydrocarbon degraders.

The impact of spilled oil on the deep-sea environment

The majority of the microbial life in the ocean takes place below the photic zone at 200–300 m bsl, depending on oceanic region and particulate matter concentration in the water. Further down, the absence of light, higher nutrient concentrations, different compositions of organic matter, lower density and different type of prokaryote biomass, lower temperature, and increasing HP distinguish the dark ocean from the photic zone [7]. Inorganic nutrient concentrations are higher owing to re-mineralization of sinking material. Particulate organic matter such as marine snow is heterogeneously distributed, and dissolved organic matter consists likely of multiple different compounds, each at very low concentrations, which limits their utilization. Prokaryote cell concentration and metabolic activities are lower and with a different diversity. Photosynthetic microorganisms are lacking and archaea become more abundant in the deep, equaling or even exceeding bacterial concentrations.

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Primary production in the dark ocean occurs primarily by autotrophic ammonium- and nitrate-oxidizing prokaryotes. In most regions, temperature is down to 0–4°C and HP increases 1 MPa every 100 m, which can

affect the physiological response of microorganisms already at moderate depths [8]. In most of the dark ocean waters, oxygen is the major electron acceptor, and aerobic metabolism dominates. Toward the sea bottom, the rapidly changing conditions to anoxia in both particulate matter and sediments favor the coexistence and vertical succession of different anaerobic metabolisms [7]. The unique environmental conditions of the deep sea together with the chemical nature of the pollutant are therefore major factors impacting the physiology of hydrocarbons degradation, microbial succession, and, ultimately, clean up.

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Anthropogenic spills occur under very different circumstances compared to natural oil seeps. In case of an accident, such as the Prestige or DWH disasters, the amount of spilled oil quickly exceeds the capacity of local marine communities to degrade petroleum, and influences microbial succession and degradation rates. In contrast, prolonged leaking of hydrocarbons from sunken vessels or *in situ* burning followed by oil droplets sinking to the sea bottom will likely stimulate different biodegradation processes and enrich specialized communities. The response of microbial communities will also differ based on whether the contamination occurs in pristine environments or in deep-sea areas that are already exposed to natural hydrocarbon seeps. As microbial communities around natural oil seeps are primed for hydrocarbon degradation, one could argue that it may fasten the response rate to an oil spill. However, the recent finding that the ubiquitous, fast oil-degrading *Alcanivorax* genus that rapidly blooms in surface waters could not withstand HPs equivalent to the deep-sea plumes formed after the DWH [8] highlights our lack of understanding of the main physiological requirements supporting microbial oil degradation underwater.

The research on deep oil plumes and sediments after the DWH disaster unveiled some details on microorganisms' abilities to degrade petroleum. However, their

prevalence and activity are determined by the chemical nature of the polluting hydrocarbons and the actual environmental conditions, which may change during the course of the spill or be impacted by human intervention [2]. In the DWH oil plumes, the types of hydrocarbon were correlated with the succession of Oceanospirillales which dominated during the early spill phase when the concentration of *n*-alkanes was high, while *Colwellia*, *Cycloclasticus*, and others followed after aromatics remained. Psychrophilic *Colwellia* was likely advantaged by its capacity to grow on the components of dispersants' [2], and low temperature was also proposed as a factor for its enrichment. Methanotrophs were initially enriched feeding on methane released from the well, but their activity decreased after a few months despite the relatively high residual methane concentrations. Depletion of nutrients or oxygen that would limit methanotrophy was considered unlikely, although oxygen was massively consumed causing a persistent concentration anomaly in the deep plume.

In the DWH deep-sea sediments, microbial succession close to the well head was correlated with the enhanced deposition of hydrocarbons associated with marine snow, which triggered oxygen consumption at the seafloor and stimulated the growth of sulfate-reducing bacteria. Continued sedimentation has buried the spilled oil and created increasingly anaerobic conditions, where the most recalcitrant fraction could be subjected to biodegradation and assimilation although at a slower rate.

Natural, deep-sea hydrocarbon emissions define specialized microbial communities

The study of deep-sea niches around natural hydrocarbon emissions is providing additional insights on the long-term impact of oil underwater. The microbial diversity in hydrocarbon seeps is remarkably different from the surrounding environment, characterized by definite communities with selected biogeochemical functions [1]. Long-term exposure to hydrocarbons transforms these areas into hotspots for microbial biomass production. At hydrothermal vents and cold seeps, methane escapes the sub-seafloor together with small amounts of light petroleum hydrocarbons (up to C4). Sediments have limited oxygen concentrations and only

in the upper layers. Under the surface, methanotrophic archaea and sulfate-reducing bacteria mediate the anaerobic oxidation of methane coupled to sulfate reduction, which generates carbonate and hydrogen sulfide. The latter is consumed by other microorganisms through chemosynthetic processes, whereby carbon dioxide is fixed into microbial biomass using chemical energy.

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Several symbiotic animals, such as giant tube worms, mussels, snails, shrimps, and crabs, feed on the microbial biomass, overall constituting a fascinating ecosystem whose dynamics are not yet fully understood. Chemosynthetic-based life also occurs in the presence of heavy-oil emissions that form so-called asphalt volcanoes, first described at Chapopote Knoll in the southern Gulf of Mexico at 3,000 m bsl. Highly viscous oil, rich in asphaltenes, flows smoothly over the seafloor similar to a lava stream. As the initial density of the oil is lower than seawater, local bulges and whips form due to buoyancy. The oil then solidifies as it loses its soluble fraction, leaving solid brittle deposits [9]. Bacterial mats typically cover the asphalt surface and symbiotic organisms colonize their fissures. Contact with oxygenated and nutrient-rich seawater drives microbial degradation: in surficial layers, *n*-alkanes, isoprenoids, and other hydrocarbons lighter than C26 are degraded, contrary to asphalt layers deeper than 2 cm. In addition to oxygen, seawater supplies asphalt deposits with sulfate. This alternative electron acceptor supports further hydrocarbon degradation and methanotrophy [10]. Similar patterns are observed in oil-soaked sediments found close to asphalt volcanoes, with oil largely biodegraded in the upper layers (10 cm) where bacterial sulfate reducers are predominant together with methanotrophic archaea. However, depletion of sulfate as electron acceptor in deeper sediments hampers the hydrocarbon-degradation

Sidebar A: Further reading

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capabilities of the indigenous microbial community.

The existence of these ecological niches and their biogeochemical equilibria support the hypothesis that the capacity for self-healing from oil contamination is diverse and widespread in nature. Nonetheless, the biodegradation of persistent hydrocarbons takes an extremely long time and involves very complex ecosystems, the primary shaping factors of which are not yet completely understood. To successfully mimic such processes and design biological strategies to combat anthropogenic oil spills, we need to better understand the basic mechanisms that govern the biodegradation of specific petroleum fractions at *in situ* conditions. This knowledge could help us enormously to harness nature's powers to efficiently deal

with oil spills near shores or out in the oceans and alleviate the disastrous effects on both the environment and local communities.

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Conflict of interest

The authors declare that they have no conflict of interest.

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