



Oil Biodegradation in Deep Marine Basins

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Abstract

Nine important hydrocarbon (oil) basins where offshore petroleum leases have been licensed are compared. These nine basins (Gulf of Mexico, Eastern Mediterranean's Nile Deep-Sea Fan, Central Mediterranean and the Sirte Basin, North Sea, Caspian Sea, Angola, Trinidad and Tobago, Great Australian Bight, and Brazil's Amazonian Deep-Sea Basin) are geographically separated and are impacted by very different water masses. The geochemical parameters of these basins are quite distinct, for example, salinities ranging from 39 psu in

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the Eastern Mediterranean to 12 psu in the Caspian. Additionally, parameters like temperatures of the bottom water are also very distinct, with the temperature in the deep water of the Eastern Mediterranean being between 12 °C and 14 °C and the temperature of the deep water in the North Sea being −2 °C. Each basin represents a unique ecosystem in which distinct microbes may thrive. These distinct environmental parameters may act to constrain the extent of hydrocarbon degradation in these basins. Another potential constraint on hydrocarbon degradation is the extent of natural hydrocarbon seeps in the area. Though many basins have similar if not 16S rRNA identical strains of oil-degrading bacteria, *Colwellia psychrerythraea* from different basins showed that a mixture of natural selection and neutral evolution has contributed to the divergence of these. Most if not all deep ocean basin microbial communities are dominated by *Thaumarchaeota* below 200 m. These microaerophilic, ammonium oxidizer, psychrophiles are very adapted to an oligotrophic lifestyle, and though many in this group will degrade oil, they are rapidly outcompeted by other bacteria in oil or high hydrocarbon intrusions, thus the virtual “canary in the coal mine.” Cometary biodegradation of oil is well documented but could be an important natural attenuation mechanism for oil in deep marine basins with episodic methane seeps. Microbial community structure can also predict concentrations of oil in deep basins. Many other synergistic effects require more research in environmental systems biology in deep marine basins.

1 Introduction

Deep marine basins have been exposed to oil and oil analogs for millions of years. Indeed, as hydrocarbons fall through the water column to the ocean floor, the easiest to degrade components are biodegraded, leaving the more recalcitrant components like polyaromatic hydrocarbons and asphaltenes. Since much of this hydrocarbon originates as phytoplankton from surface waters, it is not surprising that in deep marine basins that microorganisms have adapted to degrading these recalcitrant hydrocarbons as carbon and energy sources in these oligotrophic environments. Indeed, many deep basins also have natural oil seeps from oil reservoirs that are more than 6000 m in the subsurface. At these depths, the marine phytoplankton that originally made up the oil strata underwent extreme pressures and temperature for the diagenesis of the oils in these reservoirs. Thus, oil like other hydrocarbons reaching deep marine basins is natural and is expected to have microorganisms capable of degrading it, over the millennia of adaptation that would have occurred (Hazen et al. 2016). Oil in subsurface reservoirs is 100–400 million years old (Tissot and Welte 1984). Oil-degrading taxa are identified in all three domains of the tree of life (Fig. 1).

In deep marine basins, oil is dominated by natural seeps, 600,000 tonnes/year of petroleum worldwide from 1990 to 1999 averages (NAS 2003). Anthropogenic sources during this same period accounted for 678,000 tonnes/year or 53% (Fig.

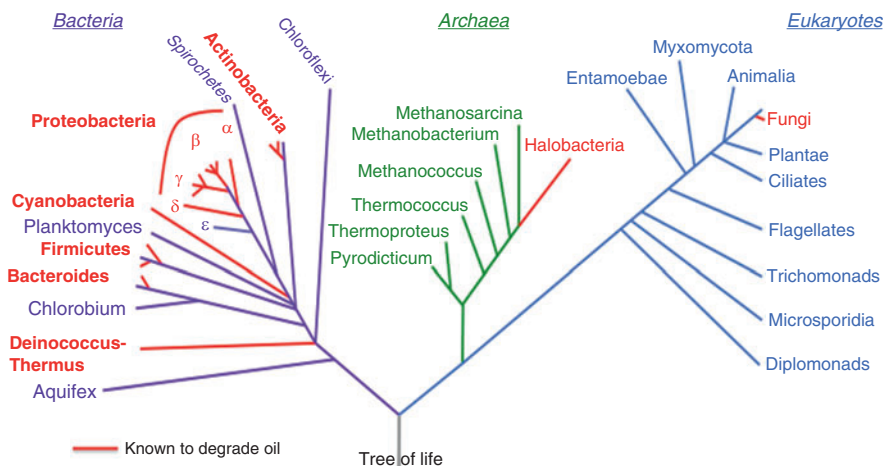
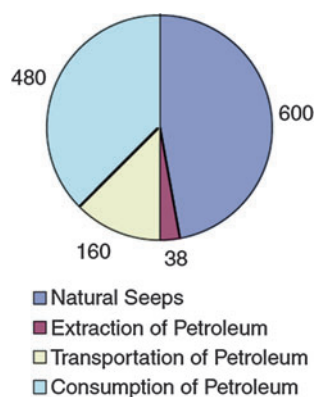


Fig. 1 Oil-degrading microbial taxa. Oil-degrading microbial phyla, highlighted in red, have been identified from all three domains of life. (After Hazen et al. 2016)

Fig. 2 Relative contribution of average, annual releases (1990–1999) of petroleum hydrocarbons (in kilotons) from natural seeps and activities associated with the extraction, transportation, and consumption of crude oil or refined products to the marine environment. (After NAS 2003)



2) (NAS 2003). Oil can come into the deep basin from the surface (spills), natural seeps, and leaks from production wells in the basin (Fig. 3). Recent studies on the *Deepwater Horizon* (DWH) oil spill have resulted in many new studies in deep water which previously had received little attention (Hazen et al. 2016).

As sources of new oil reservoirs on land became scarcer over the last 20 years, offshore oil production had dramatically increased. Though the production costs were much greater than wells on land, since the oil reservoirs being tapped were deeper, the value of the product was higher due to its lighter nature and higher value as fuel. However, the DWH blowout in deep water created a cautionary reevaluation and more risk assessment studies. In addition, unconventional oil production and shale gas production increased by more than 702% since 2007. This in turn has caused many oil companies to abandon offshore oil production in deep marine basins, including leases and exploration. “Global oil discoveries fell to a record low in 2016 as companies continued to cut spending and conventional oil projects

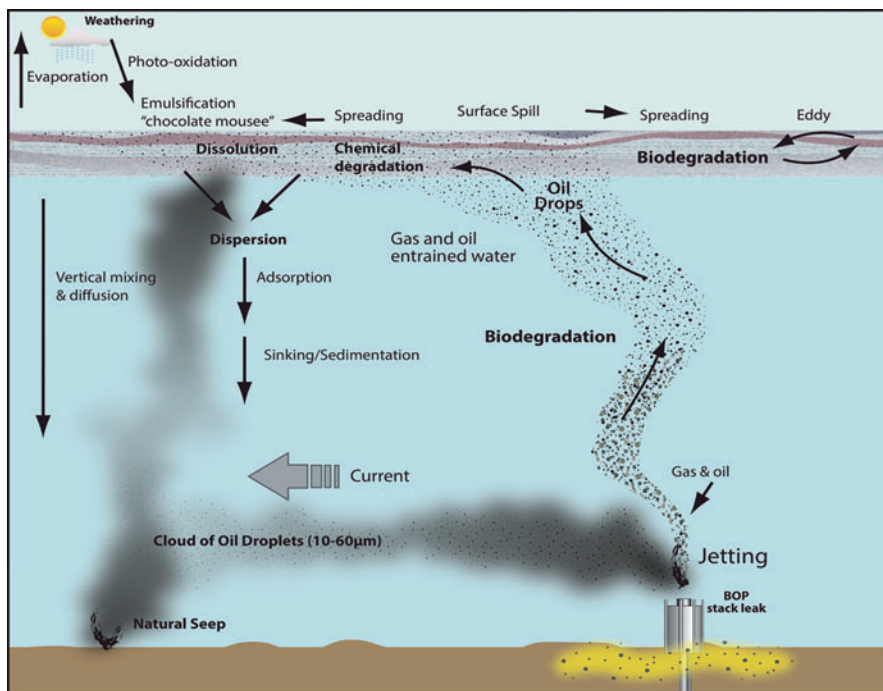


Fig. 3 Fate of oil spills in deep marine basins (*BOP* BlowOut Prevention device)

sanctioned were at the lowest level in more than 70 years,” according to the International Energy Agency (IAE 2017). “The offshore sector, which accounts for almost a third of crude oil production and is a crucial component of future global supplies, has been particularly hard hit by the industry’s slowdown. In 2016, only 13% of all conventional resources sanctioned were offshore, compared with more than 40% on average between 2000 and 2015” (IAE 2017).

Nine important hydrocarbon (oil) basins where offshore petroleum leases have been licensed is compared (Fig. 4).

2 Deep Marine Bacterial Oil Degraders

Deep marine basins in the water column and sediments have members of all of the major oil-degrading phyla found in other environments (Fig. 1). In the *Gulf of Mexico* water from the 1100 m plume during the *Deepwater Horizon* spill contained 16 subfamilies in the gamma-proteobacteria that were significantly enriched in the plume out of 951 subfamilies detected in 62 bacteria phyla (Fig. 5) (Hazen et al. 2010). Sediment at 1500 m below the deepwater plume was dominated by *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, and *Actinobacteria* (Kimes et al. 2013; Mason et al. 2014), whereas typically deep-sea sediments are dominated



Fig. 4 Nine important hydrocarbon (oil) basins where offshore petroleum leases have been licensed

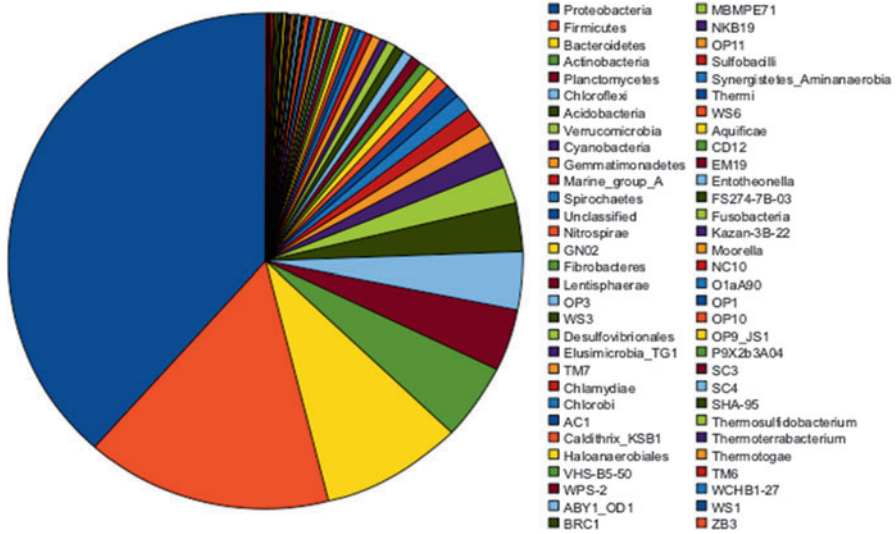


Fig. 5 Deepwater microbial communities during *Deepwater Horizon* oil spill (951 subfamilies were detected in 62 bacterial phyla. Only 16 subfamilies in Gammaproteobacteria significantly enriched in plume). (After Hazen et al. 2010)

by *Euryarchaeota*, *Proteobacteria*, *Firmicutes*, and *Chloroflexi* (Biddle et al. 2008, 2011).

The *Caspian Sea* has many natural seeps and oil production throughout the basin. It is estimated that the Caspian receives between 70 and 90 tonnes of petroleum each year (Chicherina et al. 2004). Total petroleum levels in the Caspian range between 0.067 and 2 mg/L (Korshenko and Gul 2005). The highest levels of petroleum hydrocarbons were found in the Southern Caspian. These concentrations range from 0.17 to 0.07 mg/L. Caspian Sea water from 200–600 m is dominated by *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Alphaproteobacteria*, *Actinobacteria*, and *Thaumarchaeota*. Fungi capable of oil degradation have also been isolated from the Caspian Sea water column (Salmanov 2006; Lein et al. 2010). Studies on the Caspian Sea indicate that the surface of deepwater sediments with low oxygen levels was dominated by *Gammaproteobacteria*; however, surface sediments with bottom waters under hypoxic conditions were dominated by *Deltaproteobacteria*. The ammonia-oxidizing *Thaumarchaeota* was dominant in all surface sediments (Mahmoudi et al. 2015).

Eastern Mediterranean's Nile Deep-Sea Fan has numerous natural hydrocarbon seeps (Heijs et al. 2008; Mastalerz et al. 2009; Omeregic et al. 2009; Felden et al. 2013). Deep water from 400 to 1200 m is dominated by SAR406, *Gammaproteobacteria*, *Deltaproteobacteria*, *Alphaproteobacteria*, *Actinobacteria*, *Chloroflexi*, and *Thaumarchaeota* (Techtmann et al. 2015). The microbial community in the deep water was significantly correlated with inorganic phosphate, silicate, nitrate, and depth. Deep sediments were dominated by *Actinobacteria*, *Bacilli*, *Chloroflexi*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*,

and *Epsilonproteobacteria* and the archaea *Methanosarcinales*, *Thermoplasmatales*, *Halobacteriales*, and *Crenarchaea* (Heijs et al. 2008). Sulfate reduction, aerobic and anaerobic methanotrophy, aerobic sulfide oxidation, and aerobic and anaerobic heterotrophy were the dominant metabolic processes in the deep sediment (Heijs et al. 2008).

The **Central Mediterranean and the Sirte Basin** were largely the same for both water and sediment microbial communities as the Eastern Mediterranean. However, the microbial community structure in the water column was driven by dissolved oxygen, temperature, and salinity (Techtmann et al. 2017). The levels of hydrocarbon-degrading bacteria in coastal waters at locations in the Mediterranean have been determined to be $10\text{--}10^2$ cells/ml of water (Youssef et al. 2010). In many locations, the addition of oil enriches a robust community of oil-degrading microbes (Moursy and El-Abagy 1982; Santas et al. 1999; Zrafi-Nouira et al. 2009; Ibraheem 2010; Youssef et al. 2010; Chekroud et al. 2011; Farag and Soliman 2011). Cyanobacteria have been found to be a part of this community (Ibraheem 2010). The authors suggest that their data supports either the ability of these cyanobacteria to degrade hydrocarbons or a mutualism between these cyanobacteria and aerobic hydrocarbon-degrading bacteria. This community also contains hydrocarbon-degrading fungi from the genus *Candida* (Farag and Soliman 2011).

The **Great Australian Bight** (GAB) has only a few natural seeps but is of interest for oil production (Logan et al. 2010). Over the years, there have been a number of studies aimed at identifying the presence of hydrocarbon seeps within Australia's margins (Logan et al. 2010). To date, the only naturally occurring hydrocarbon seeps identified are located in Northern Australia's carbonate-rich shelf in the Timor Sea (Rollet et al. 2006; Wasmund et al. 2009; Logan et al. 2010). In Southern Australia, the presence of naturally occurring bitumen asphaltites within the GAB suggests that a naturally occurring seep may be present off of Australia's southern margin. However, there is no direct evidence and despite surveys of the area, no natural seeps within the GAB have been recorded (Struckmeyer et al. 2002; Logan et al. 2010). The GAB is very oligotrophic and one of the deepest basins being considered for petroleum exploration. In the water column, the *Thaumarchaeota* are the dominant microorganism below 185 m in depth (Techtmann et al. 2017). Other groups found are *Prochlorococcus*, *Synechococcus*, SAR11, *Rhodobacteriales*, *Oceanospirillales*, *Alteromonadales*, and *Bacteroidetes* (Wilkins et al. 2013). Microbial diversity in sediments near a methane seep has revealed novel aerobic methanotroph diversity (Wasmund et al. 2009). Within hydrocarbon seeps in the Timor Sea, phylogenetic analysis revealed the presence of sequences affiliated with *Gammaproteobacteria*, *Deltaproteobacteria*, *Alphaproteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Firmicutes*, and *Nitrospira*. Additionally, sequences associated with aerobic methanotrophs were identified, while sequences related to methanotrophic Archaea were found to be absent. Analysis of genes within the porewater revealed the absence of the methanogenic functional gene, methyl coenzyme M reductase, thus providing further evidence for the lack of methanotrophic assemblages at the seeps (Wasmund et al. 2009).

The *Angola Basin* (OSA) is a deepwater upwelling basin with a significant number of natural oil seeps (Berger et al. 1998). *Crenarchaeota* are the most dominant group of microbes in OSA below 400 m and are common in low-nutrient deep ocean environments worldwide. The *Crenarchaeota* are generally microaerophiles that are known to be ammonium oxidizers, which gives them a competitive advantage in low-nutrient environments. The microbial community in the OSA is driven by total organic carbon, dissolved oxygen, salinity, and temperature (Hazen et al. 2016). Other microbes in the water included *SAR406*, *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, *Euryarchaeota*, and *Thaumarchaeota*. The dominant sediment organisms are *Gammaproteobacteria*, particularly psychrophilic *Enterobacteriaceae*, *Alteromonadaceae*, *Oceanospirillaceae*, and *Legionellaceae* (Schauer et al. 2010). Other proteobacteria, *Chloroflexi* and *Planctomyces*, were also found in the sediment.

In the *North Sea* temperature, salinity, and availability of nutrients are some of the drivers that can dictate the microbial community structure. Often in marine systems, movement of waters by currents and tides and stratification help to determine these factors. In the North Sea, the loosely defined regions of the shallow continental shelf of the southern and central North Sea, the Norwegian Trench, and the Faroe-Shetland Channel have distinct current systems and vertical stratification. Natural hydrocarbon seeps in the North Sea as well as anthropogenic inputs of hydrocarbons contribute the overall hydrocarbon load of the Sea. Riverine inputs carry with it anthropogenic hydrocarbons and are estimated to contribute between 40 and 80 kilotons of petroleum hydrocarbon per year into the North Sea (Bedborough et al. 1987). Another significant source of anthropogenic hydrocarbons is the offshore oil and gas industry. This industry has had a major presence in the North Sea since the 1960s. Some studies (Bedborough et al. 1987) estimate that the offshore petroleum industry contributes around 23 kilotons of petroleum per year to the North Sea. The phytoplanktonic communities of the North Sea have been the subjects of a large number of studies (Dale et al. 1999; Riegman and Kraay 2001; Kuipers et al. 2003; Loder et al. 2012). Another study examining the long-term shifts in community structure examined the microbial community of the Helgoland Roads during August over the course of a half-century (Vezzulli et al. 2012). As in other studies, this report finds that *Alpha-* and *Gammaproteobacteria* dominate the community over the course of these decades. *Oceanospirillaceae*, *Halomonadaceae*, and *Alteromonadales* increase over time. The most significant increase that was observed is in the *Vibrio* spp. This increase in *Vibrios* is strongly correlated with sea surface temperature. Therefore, the authors conclude that the warming of sea surface temperatures has resulted in drastic shifts in the microbial communities of the North Sea. The bacterial and archaeal communities of the North Sea are key players in various geochemical cycles within the sea. In the Tommeliten seep, no methanotrophs were found. However, other organisms were found that might serve as functional analogs for the degradation of complex organic matter. Sequences for a relative of *Desulfitobacterium anili* were found at the Tommeliten site. This organism has previously been shown to oxidize various hydrocarbons such

as naphthalene and xylene (Widdel et al. 2007). In addition, a 16 s rDNA clone for a member of the *Oceanospirillales* was recovered from the Tommeliten site.

The coast of **Trinidad and Tobago** is very complex environment where many water masses interact. This location is increasingly becoming an important region for oil and gas production. The complex environment of this region makes it an interesting site to examine the microbial communities present in the waters and sediments and their potential to degrade hydrocarbons. Some of the microbiological work in the region has involved examining the microbes that colonize the unique gradients associated with the riverine inputs into the Atlantic from the Orinoco and the Amazon Rivers. One such paper examines the levels and turnover times of methane (CH₄) and carbon monoxide (CO) in the waters of the Caribbean Sea surrounding Trinidad and Tobago (Jones and Amador 1993). In rivers methane is produced microbially by methanogenic archaea, and CO is produced primarily by the photooxidation of organic matter. CO is subsequently consumed through microbial oxidation of CO. An interesting trend was seen in the Gulf of Paria, where the CO and CH₄ concentrations reached a local maximum. This is most likely due to effects on the flow of riverine waters into the gulf. This work confirms that the waters around Trinidad and Tobago are highly influenced by inputs of both nutrients and organic matter from the Orinoco River. A large number of mud volcanoes have been discovered near Trinidad and Tobago in the Barbados Prism (Biju-Duval et al. 1982; Brown and Westbrook 1987, 1988; Brown 1990; Griboulard et al. 1991; Deville et al. 2003). These mud volcanoes are rather randomly distributed along the continental slope (Deville et al. 2006, 2010). These mud volcanoes have been shown to exude both methane and higher hydrocarbons (Le Pichon et al. 1990a, b; Henry et al. 1996). The microbiology of deep sediments adjacent to Trinidad and Tobago was investigated by Guezennec and Fiala-Medioni (1996). They used phospholipid ester-linked fatty acids (PLFA) to examine the bacterial abundance and diversity of a mud volcano in the Barbados trench near Trinidad and Tobago. Prior to this work, white and reddish mats were observed near these seep sites indicative of some bacterial colonization (Le Pichon et al. 1990a). PLFA analysis estimated the cell numbers in these mud volcanoes to be 1.5×10^9 cell/g of sediment. These cell numbers are similar to others reported in nearby sediments of the Venezuelan Basin (5×10^8 cells/g). The lipid profiles of sites near the white and reddish mats suggested the presence of sulfur-oxidizing bacteria. Further, lipid characteristics of both type I and type II methanotrophs were common at all sites sampled. This would follow the high levels of methane present at these sites. The authors conclude that type I methanotrophs are more abundant than type II in all of the sediments. Other lipids commonly found in both sulfate-reducing bacteria as well as alkane-degrading bacteria were found in these sediments. Archaeal lipids potentially belonging to methanogens were also found in these sediments. While this study does shed some light on the groups of bacteria present in these mud volcanoes, further work needs to be done to characterize these communities. For example, one of the groups of lipids found at high levels could either be contributed from sulfate-reducing bacteria or alkane-degrading bacteria.

The *Amazonian Deep-Sea Basin* brings distinct hydrographic and geochemical features to the waters around Brazil and has a large effect on the microbial communities present in these waters. In particular the role that river inputs have on these waters drastically affects the microbial communities present. The complex water masses in the deep water also harbor unique niches for microbes to flourish. Amazonian Deep-Sea Basin water contained oil degraders in the bacteria (*Alteromonadaceae*, *Colwelliaceae*, and *Alcanivoracaceae*), archaea (e.g., *Halobacteriaceae*, *Desulfurococcaceae*, and *Methanobacteriaceae*), and eukaryotic microbes (e.g., *Microsporidia*, *Ascomycota*, and *Basidiomycota*) (Campeao et al. 2017). The sediments off the coast of Brazil are relatively unexplored in terms of their microbial diversity. Despite the active oil and gas industry, which has characterized many of the seafloor and sub-seafloor features, the microbial community of the seafloor is relatively unknown. Microbes have been shown to be present in relatively high numbers (Cragg et al. 1997). Work associated with the Ocean Drilling Program reported bacterial cell numbers around 10^9 cells/g of sediment in the surface sediments and decreasing to 10^6 cells/g in the deeper sediments. Due to the oligotrophic nature of the open ocean water, oxygen is able to penetrate fairly deeply into the sediments of this region (Wenzhofer et al. 2001). One study characterized the hydrocarbon-degrading community associated with sediments impacted by a catastrophic oil spill in the Guanabara Bay (Brito et al. 2006). Thirty-two bacterial strains were isolated from oil-enriched mesocosms of Guanabara Bay sediment. The majority of these strains were *Alpha*- and *Gammaproteobacteria*. Many of these strains were related to *Marinobacter* spp. and *Alcanivorax* spp. The *Alphaproteobacteria* were shown to be able to degrade many of the branched chain hydrocarbons. Another study investigated various methods to stimulate hydrocarbon degradation in coastal sediments (Silva et al. 2009). In these sediments, hydrocarbon degraders were shown to be a significant proportion of the heterotrophic bacterial population. The addition of fertilizer and biosurfactant helped to stimulate the removal of hydrocarbons from this system.

3 *Colwellia psychrerythraea* in Deep Marine Basins

Colwellia psychrerythraea are often found in cold, oil-contaminated marine environments both in the deep, near shore, and sediment. Recent in-depth genomic and phenotypic studies of identical isolates from distant basins suggest that even when they show the same 16S rRNA identity, they show differential salt tolerance and distinct carbon source utilization (Techtmann et al. 2016). Differences in genomic content were also shown to encode for different functional capacity. Large segments of the genome appear to be acquired by horizontal gene transfer. Some of these genes confer increased functionality and selective advantage; however, the majority of differences do not appear to be related to adaptation to different environmental lifestyles. This suggests that a mixture of natural selection and neutral evolution has contributed to the divergence of these organisms and the great genetic and

phenotypic diversity present within this species. This observation may well be the norm for oil degraders rather than the exception.

4 *Thaumarchaeota* in Deep Marine Basins (Canary in the Coal Mine)

The *Thaumarchaeota* dominate the microbial community in the water column in nearly all deep basins that have been studied. This is predominantly because these psychrophilic, microaerophilic, ammonia-oxidizers are very adapted to the oligotrophic environment that dominates the depths of these basins. Some *Thaumarchaeota* have also been reported to degrade oil at low concentrations. Recent studies of four of these basins demonstrated that there were significant differences in the abundance and diversity of *Thaumarchaeotes* between these four basins and that their distribution showed biogeographic patterning (Techtmann et al. 2017). These studies have also demonstrated that oil and other hydrocarbons will cause the *Thaumarchaeotes* to disappear in the water column and sediments since they cannot compete with other oil degraders. Thus, disappearance of *Thaumarchaeotes* in deep marine basin water columns could be a good indicator of oil and/or hydrocarbon presence, i.e., “canary in the coal mine.”

5 Cometabolic Biodegradation of Oil

The aerobic cometabolic biodegraders are dependent upon oxygenases, e.g., methane monooxygenase, toluene dioxygenase, toluene monooxygenase, and ammonia monooxygenase. These enzymes are extremely strong oxidizers, e.g., methane monooxygenase is known to degrade over 1000 different compounds. However, like any bioremediation process, the proper biogeochemical conditions are necessary to maximize and maintain biodegradation, e.g., maintaining oxygen levels or other terminal electron acceptors that the cometabolic biodegrader is dependent (Hazen 1997; Hazen et al. 2016), and *Chapter in this book on Cometabolic Bioremediation*. In addition, cometabolic biostimulation may require pulsing of electron donor or electron acceptor to reduce competitive inhibition between the substrate the microbe can use and the contaminant. Pulsing of methane was found to significantly improve biodegradation of TCE rates by methanotrophs (Hazen 2010). Indeed, during the *Deepwater Horizon* (DWH) leak (Hazen et al. 2010), there was evidence that in the Gulf of Mexico where episodic releases of methane have occurred for millions of years from natural seeps, this pulsing of methane may be degrading oil and other organics via cometabolic biodegradation. The methane oxidizers bloomed during the DWH leaked above 400 m once the well was capped (Reddy et al. 2012; Redmond and Valentine 2012; Dubinsky et al. 2013). This suggests that intrinsic cometabolic bioremediation or cometabolic natural attenuation may be a serious phenomenon in the ocean (Stackhouse et al. 2017). Methanotrophs, methane-oxidizing bacteria, oxidize methane via a series of enzymes that are unique to this group. The primary

enzyme in this oxidation chain is methane monooxygenase. Methane monooxygenase is an extremely powerful oxidizer, thus giving it the capability of oxidizing a wide variety of normally recalcitrant compounds including oil (Cardy et al. 1991). See *Cometabolic Bioremediation* in this book.

6 Biogeochemistry and Oil Biodegradation

Following the *Deepwater Horizon* oil spill, a rapid enrichment of hydrocarbon-degrading microorganisms was observed in the water column, and surface and subsurface plume waters were dominated by known hydrocarbon-degrading bacteria (King et al. 2015). Perhaps because substrate availability was limited as the surface slicks coalesced into thick emulsified oil with much reduced surface area for microbial colonization, (Edwards et al. 2011) no increase in microbial biomass in the surface slick and that microorganisms exhibited enzymatic signs of phosphate stress consistent with previous observations that the Gulf of Mexico is limited by phosphate availability. Other studies (Hazen et al. 2010) found that microbial cell density in the deepwater oil plume was significantly higher than waters outside of the plume, $>5.5 \times 10^4$ cells/ml in the oil plume and $<2.7 \times 10^4$ cells/mL outside of the plume, but the increase was not as dramatic as might have been expected. This is likely due to the low concentrations of oil in the deepwater plume (<1 ppm). We note that the Gulf of Mexico has similar levels of nitrogen and phosphorus to several other deep marine basins undergoing active oil and gas exploration, but many of these other areas, such as the Eastern Mediterranean Sea, have a much lower phosphorus levels (Table 1). If a spill were to occur in this environment, the rates and extent of biodegradation may be initially much slower than in the Gulf of Mexico. On the other hand, as dispersed oil continues to dilute as it gets further from a spill site, it is likely that eventually the oil-to-available nutrient ratio will drop to a value where biodegradation can proceed, so if there are no immediate risk receptors, long-term biodegradation processes could remediate the spill. During DWH we also saw the total microbial biomass decrease quite slowly, since once the easy to degrade material was depleted and some of the oil degraders began to die, they became nutrients for organisms adapted to degrade their components (Dubinsky et al. 2013). This resulted in a temporal succession in the microbial community structure that was present so that microbes that could not degrade oil benefitted from the dead biomass as a resource (Fig. 6). This undoubtedly goes on in both spill scenarios and when there are natural episodic seeps of oil.

7 Environmental Systems and Synergistic Effects in Deep Marine Oil Biodegradation

Oil biodegradation in deep marine basins is best approached using multiple scales using environmental systems biology (Hazen et al. 2016; Hazen and Saylor 2016). Synergistic effects (Table 2) can result from combinations of oil droplet size,

Table 1 Physical/chemical comparison of deep basin with oil biodegradation

Nutrient	Eastern Med	Central Med	Great Australian Bight	Caspian	North Sea	Angola Basin	Brazil Basin	Gulf of Mexico
Nitrate ($\mu\text{g}/\text{kg}$)	178.4	160.0	144.7	127.8	569.6	20	25	208
Phosphate ($\mu\text{g}/\text{kg}$)	39.7	29.3	153.5	56.9	33.5	1.6	1.1	205
Ammonia ($\mu\text{g}/\text{kg}$)	28.0	30.0	125	739.6	12.5	10.2	ND	78
Iron ($\mu\text{g}/\text{kg}$)	6.5	ND	5.8	4.7	ND	ND	ND	56
Sulfate ($\mu\text{g}/\text{kg}$)	4.7	ND	1.6	1.9	ND	ND	ND	ND
Salinity (psu)	38.9	38.6	34.8	11.3	33.7	36	37	35
Temp ($^{\circ}\text{C}$)	13.8	13.7	2.5	6.8	3	4	5	4.8
Dissolved oxygen (mg/L)	6.4	5.5	5.0	0.5	ND	3.4	ND	4.9

ND no data

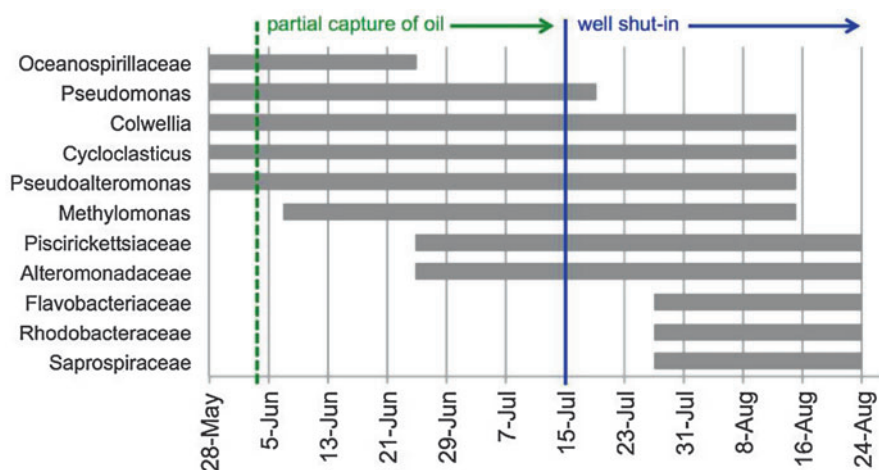


Fig. 6 Succession of the microbial community in the deep water of the Gulf of Mexico during the various phases of the response to the *Deepwater Horizon* oil spill. For each bar relative abundance was enriched over background in any sample (>2 -fold mean nonplume intensity). (After Dubinsky et al. 2013)

currents, oil type, degree of biodegradation, dissolution, cometabolic biodegradation, mineral fines, biosurfactants, temperature, gyres, and pressure (Hazen et al. 2016). Indeed, during the DWH spill, surface currents were to the NE, whereas the plume at 1100 m was moving to the SW; in addition there was a gyre at 1100 m that was causing recirculation from the well head to 15 km SW (Valentine et al. 2012). The droplet size was also demonstrated to greatly influence the biodegradation rate, whether caused by the jetting at the well head or the dispersant injected at the well

Table 2 Synergistic effects that impact biodegradation of oil. (After Hazen et al. 2016)

Factor working synergistically	Impact on biodegradation
Chemical dispersants + mineral fines	Individually each will promote dispersion of the oil. Combined, the formation and transfer of oil from the surface into the water column is enhanced
Autoinoculation + “memory response” of hydrocarbon degraders	Introduction of hydrocarbons to previously exposed water parcels leads to an increase in microbial abundance and accelerated hydrocarbon biodegradation
Oil droplet size + dispersion + biodegradation rates + dissolution	Enhances biodegradation, dissolution, and dispersion rates of oil hydrocarbons
Cometabolic biodegradation + dispersion + secondary electron donors	Enhances biodegradation, dissolution, and dispersion rates of oil hydrocarbons even when the oil itself cannot be a suitable electron donor
Biosurfactants from multiple microorganisms	Enhances bioavailability of poorly soluble compounds

head (Brakstad et al. 2014, 2015). Detailed microbiological models like Structured Learning in Microbial Ecology (SLiME) have also demonstrated that microbial community structure in deep marine basins can predict oil concentrations quite accurately (Smith et al. 2015). On board ship oil biodegradation simulations have also been shown to improve correlations with in situ collections.

8 Research Needs

To avoid and properly remediate disasters like the *Deepwater Horizon* spill, we need much more in-depth studies at an environmental systems biology level. This includes better models that take into account psychrophiles that can degrade oil and would not be predicted by simple Q10 formulas. More studies are also needed on dispersant usage with particular attention to realistic concentrations. On board ship studies and close attention to bottle effect, temperature and pressure have been found to critically effect conclusions of studies. The resources for doing these studies in deep basins are exceptionally high and need government commitments long term with a dynamic field test plan and experienced personnel for rapid deployment for any future spills.

References

- Bedborough DR, Blackman RAA, Law RJ (1987) A survey of inputs to the North-Sea resulting from oil and gas developments. *Philos Trans R Soc Lond Ser B-Biol Sci* 316:495–509
- Berger WH, Wefer G, Richter C, Lange CB, Giraudeau J, Hermelin O, Party SS (1998) The Angola-Benguela upwelling system: paleoceanographic synthesis of shipboard results from Leg 175. In: *Proceedings of the ocean drilling program, initial reports*. 175
- Biddle JF, Fitz-Gibbon S, Schuster SC, Brenchley JE, House CH (2008) Metagenomic signatures of the Peru margin seafloor biosphere show a genetically distinct environment. *Proc Natl Acad Sci USA* 105:10583–10588

- Biddle JF, White JR, Teske AP, House CH (2011) Metagenomics of the subsurface Brazos-Trinity Basin (IODP site 1320): comparison with other sediment and pyrosequenced metagenomes. *ISME J* 5:1038–1047
- Biju-Duval B, Le Quellec P, Mascle A, Renard V, Valery P (1982) Multibeam bathymetric survey and high resolution seismic investigations on the Barbados Ridge complex (Eastern Caribbean): a key to the knowledge and interpretation of an accretionary wedge. *Tectonophysics* 86: 275–304
- Brakstad OG, Daling PS, Faksness LG, Almas IK, Vang SH, Syslak L, Leirvik F (2014) Depletion and biodegradation of hydrocarbons in dispersions and emulsions of the Macondo 252 oil generated in an oil-on-seawater mesocosm flume basin. *Mar Pollut Bull* 84:125–134
- Brakstad OG, Nordtug T, Throne-Hoist M (2015) Biodegradation of dispersed Macondo oil in seawater at low temperature and different oil droplet sizes. *Mar Pollut Bull* 93:144–152
- Brito EMS, Guyoneaud R, Goñi-Urriza M, Ranchou-Peyruse A, Verbaere A, Crapez MAC, Wasserman JCA, Duran R (2006) Characterization of hydrocarbonoclastic bacterial communities from mangrove sediments in Guanabara Bay, Brazil. *Res Microbiol* 157:752–762
- Brown KM (1990) The nature and hydrogeologic significance of mud diapirs and diatremes for accretionary systems. *J Geophys Res* 95:8969–8982
- Brown KM, Westbrook GK (1987) The tectonic fabric of the Barbados Ridge accretionary complex. *Mar Pet Geol* 4:71–81
- Brown K, Westbrook GK (1988) Mud diapirism and subcretion in the Barbados Ridge accretionary complex: the role of fluids in accretionary processes. *Tectonics* 7:613–640
- Campeao ME, Reis L, Leomil L, de Oliveira L, Otsuki K, Gardinali P, Pelz O, Valle R, Thompson FL, Thompson CC (2017) The deep-sea microbial community from the Amazonian basin associated with oil degradation. *Front Microbiol* 8:13
- Cardy DLN, Laidler V, Salmond GPC, Murrell JC (1991) Molecular analysis of the methane monooxygenase (MMO) gene cluster of *Methylosinus trichosporium* OB3b. *Mol Microbiol* 5:335–342
- Chekroud Z, Gouda MK, Houhamdi M (2011) Biodegradation of crude oil in marine medium. *J Proteomics Bioinformatics* 4:231–237
- Chicherina OV, Leonov AV, Fashchuk DY (2004) Geographical and ecological characteristics of the Caspian Sea and modern tendencies in the evolution of its ecosystem. *Water Resour* 31:271–289
- Cragg BA, Law KM, Cramp A, Parkes RJ (1997) Bacterial profiles in Amazon Fan sediments, Sites 934 and 940. In: Flood RD, Piper DJW, Klaus A, Peterson LC (eds) Proceedings of ODP, scientific results. Ocean Drilling Program, College Station, pp 565–571
- Dale T, Rey F, Heimdal BR (1999) Seasonal development of phytoplankton at a high latitude oceanic site. *Sarsia* 84:419–435
- Deville E, Battani A, Griboulaud R, Guerlais S, Herbin JP, Houzay JP, Muller C, Prinzhofer A (2003) The origin and processes of mud volcanism: new insights from Trinidad. *Geol Soc Lond Spec Publ* 216:475–490
- Deville E, Guerlais S-H, Callec Y, Griboulaud R, Huyghe P, Lallemand S, Mascle A, Noble M, Schmitz J (2006) Liquefied vs stratified sediment mobilization processes: insight from the south of the Barbados accretionary prism. *Tectonophysics* 428:33–47
- Deville É, Guerlais S-H, Lallemand S, Schneider F (2010) Fluid dynamics and subsurface sediment mobilization processes: an overview from Southeast Caribbean. *Basin Res* 22:361–379
- Dubinsky EA, Conrad ME, Chakraborty R, Bill M, Borglin SE, Hollibaugh JT, Mason OU, Piceno YM, Reid FC, Stringfellow WT, Tom LM, Hazen TC, Andersen GL (2013) Succession of hydrocarbon-degrading bacteria in the aftermath of the Deepwater Horizon oil spill in the Gulf of Mexico. *Environ Sci Technol* 47:10860–10867
- Edwards BR, Reddy CM, Camilli R, Carmichael CA, Longnecker K, Van Mooy BAS (2011) Rapid microbial respiration of oil from the Deepwater horizon spill in offshore surface waters of the Gulf of Mexico. *Environ Res Lett* 6:9

- Farang S, Soliman NA (2011) Biodegradation of crude petroleum oil and environmental pollutants by *Candida tropicalis* strain. *Braz Arch Biol Technol* 54:821–830
- Felden J, Lichtschlag A, Wenzhofer F, de Beer D, Feseker T, Ristova PP, de Lange G, Boetius A (2013) Limitations of microbial hydrocarbon degradation at the Amon mud volcano (Nile deep-sea fan). *Biogeosciences* 10:3269–3283
- Griboulard R, Bobier C, Faugères JC, Vernet G (1991) Clay diapiric structures within the strike-slip margin of the southern leg of the Barbados prism. *Tectonophysics* 192:383–400
- Guezennec J, Fiala-Medioni A (1996) Bacterial abundance and diversity in the Barbados trench determined by phospholipid analysis. *FEMS Microbiol Ecol* 19:83–93
- Hazen TC (1997) Bioremediation. In: Amy P, Haldeman D (eds) *Microbiology of the terrestrial subsurface*. CRC Press, Boca Raton, pp 247–266
- Hazen TC (2010) Cometabolic bioremediation. In: Timmis KN (ed) *Handbook of hydrocarbon microbiology: microbial interactions with hydrocarbons, oils, fats and related hydrophobic substrates and products*. Springer, Berlin
- Hazen TC, Saylor GS (2016) Environmental systems microbiology of contaminated environments. In: Yates M, Nakatsu C, Miller R, Pillai S (eds) *Manual of environmental microbiology*, 4th edn. ASM Press, Washington, DC, pp 5.1.6-1–5.1.6-10
- Hazen TC, Dubinsky EA, DeSantis TZ, Andersen GL, Piceno YM, Singh N, Jansson JK, Probst A, Borglin SE, Fortney JL, Stringfellow WT, Bill M, Conrad ME, Tom LM, Chavarria KL, Alusi TR, Lamendella R, Joyner DC, Spier C, Baelum J, Auer M, Zemla ML, Chakraborty R, Sonnenthal EL, D’Haeseleer P, Holman HYN, Osman S, Lu ZM, Van Nostrand JD, Deng Y, Zhou JZ, Mason OU (2010) Deep-sea oil plume enriches indigenous oil-degrading bacteria. *Science* 330:204–208
- Hazen TC, Prince RC, Mahmoudi N (2016) Marine oil biodegradation. *Environ Sci Technol* 50:2121–2129
- Heijs SK, Laverman AM, Forney LJ, Hardoim PR, van Elsas JD (2008) Comparison of deep-sea sediment microbial communities in the Eastern Mediterranean. *FEMS Microbiol Ecol* 64:362–377
- Henry P, Le Pichon X, Lallemand S, Lance S, Martin JB, Foucher J-P, Fiala-MÉdioni A, Rostek F, Guilhaumou N, Pranal V, Castrec M (1996) Fluid flow in and around a mud volcano field seaward of the Barbados accretionary wedge: results from Manon cruise. *J Geophys Res* 101:20297–20323
- IAE (2017) Global oil discoveries and new projects fell to historic lows in 2016
- Ibraheem IBM (2010) Biodegradability of hydrocarbons by cyanobacteria1. *J Phycol* 46:818–824
- Jones RD, Amador JA (1993) Methane and carbon monoxide production, oxidation, and turnover times in the Caribbean Sea as influenced by the Orinoco River. *J Geophys Res* 98(C2): 2353–2359
- Kimes NE, Callaghan AV, Aktas DF, Smith WL, Sunner J, Golding BT, Drozdowska M, Hazen TC, Suffita JM, Morris PJ (2013) Metagenomic analysis and metabolite profiling of deep-sea sediments from the Gulf of Mexico following the Deepwater Horizon oil spill. *Front Microbiol* 4:17
- King GM, Kostka JE, Hazen TC, Sobecky PA (2015) Microbial responses to the Deepwater Horizon oil spill: from coastal wetlands to the deep sea. *Annu Rev Mar Sci* 7(7):377–401
- Korshenko A, Gul AG (2005) Pollution of the Caspian Sea. In: Kostianoy AG, Kosarev AN (eds) *The Caspian environment*, vol 5P. Springer, Berlin/Heidelberg, pp 109–142
- Kuipers B, Witte H, van Noort G, Gonzalez S (2003) Grazing loss-rates in pico- and nanoplankton in the Faroe-Shetland channel and their different relations with prey density. *J Sea Res* 50:1–9
- Le Pichon X, Foucher J-P, BoulÉgue J, Henry P, Lallemand S, Benedetti M, Avedik F, Mariotti A (1990a) Mud volcano field seaward of the Barbados accretionary complex: a submersible survey. *J Geophys Res* 95:8931–8943
- Le Pichon X, Henry P, Lallemand S (1990b) Water flow in the Barbados accretionary complex. *J Geophys Res* 95:8945–8967

- Lein AY, Rusanov II, Klyuvitkin AA, Kravchishina MD, Zakharova EE, Veslopolova EF, Makkaveev PN, Ivanov MV (2010) Biogeochemical processes in the water column of the Caspian Sea in November 2008. *Dokl Earth Sci* 434:1381–1385
- Loder MGJ, Kraberg AC, Aberle N, Peters S, Wiltshire KH (2012) Dinoflagellates and ciliates at Helgoland Roads, North Sea. *Helgol Mar Res* 66:11–23
- Logan GA, Jones AT, Kennard JM, Ryan GJ, Rollet N (2010) Australian offshore natural hydrocarbon seepage studies, a review and re-evaluation. *Mar Pet Geol* 27:26–45
- Mahmoudi N, Robeson MS 2nd, Castro HF, Fortney JL, Techtmann SM, Joyner DC, Paradis CJ, Pfiffner SM, Hazen TC (2015) Microbial community composition and diversity in Caspian Sea sediments. *FEMS Microbiol Ecol* 91:1–11
- Mason OU, Scott NM, Gonzalez A, Robbins-Pianka A, Baelum J, Kimbrel J, Bouskill NJ, Prestat E, Borglin S, Joyner DC, Fortney JL, Jurelevicius D, Stringfellow WT, Alvarez-Cohen L, Hazen TC, Knight R, Gilbert JA, Jansson JK (2014) Metagenomics reveals sediment microbial community response to Deepwater Horizon oil spill. *ISME J* 8:1464–1475
- Mastalerz V, de Lange GJ, Dahlmann A (2009) Differential aerobic and anaerobic oxidation of hydrocarbon gases discharged at mud volcanoes in the Nile deep-sea fan. *Geochim Cosmochim Acta* 73:3849–3863
- Moursy AS, El-Abagy MM (1982) Microbial degradation of hydrocarbons in Ismailia canal water. *Environ Int* 7:423–427
- NAS (2003) Oil in the sea III: inputs, fates, and effects. The National Academies Press, Washington, DC
- Omeregio EO, Niemann H, Mastalerz V, de Lange GJ, Stadnitskaia A, Mascle J, Foucher JP, Boetius A (2009) Microbial methane oxidation and sulfate reduction at cold seeps of the deep Eastern Mediterranean Sea. *Mar Geol* 261:114–127
- Reddy CM, Arey JS, Seewald JS, Sylva SP, Lemkau KL, Nelson RK, Carmichael CA, McIntyre CP, Fenwick J, Ventura GT, Van Mooy BAS, Camilli R (2012) Composition and fate of gas and oil released to the water column during the Deepwater Horizon oil spill. *Proc Natl Acad Sci USA* 109:20229–20234
- Redmond MC, Valentine DL (2012) Natural gas and temperature structured a microbial community response to the Deepwater Horizon oil spill. *Proc Natl Acad Sci USA* 109:20292–20297
- Riegman R, Kraay GW (2001) Phytoplankton community structure derived from HPLC analysis of pigments in the Faroe-Shetland channel during summer 1999: the distribution of taxonomic groups in relation to physical/chemical conditions in the photic zone. *J Plankton Res* 23:191–205
- Rollet N, Logan GA, Kennard JM, O'Brien PE, Jones AT, Sexton M (2006) Characterisation and correlation of active hydrocarbon seepage using geophysical data sets: an example from the tropical, carbonate Yampi Shelf, Northwest Australia. *Mar Pet Geol* 23:145–164
- Salmanov MA (2006) Microbiological studies in the Deepwater area of the Southern Caspian Sea. *Microbiology* 75:206–212
- Santas R, Korda A, Tenente A, Buchholz K, Santas P (1999) Mesocosm assays of oil spill bioremediation with oleophilic fertilizers: Inipol, F1 or both? *Mar Pollut Bull* 38:44–48
- Schauer R, Bienhold C, Ramette A, Harder J (2010) Bacterial diversity and biogeography in deep-sea surface sediments of the South Atlantic Ocean. *ISME J* 4:159–170
- Silva A, Oliveira FS, Bernardes D, França F (2009) Bioremediation of marine sediments impacted by petroleum. *Appl Biochem Biotechnol* 153:58–66
- Smith MB, Rocha AM, Smillie CS, Olesen SW, Paradis C, Wu L, Campbell JH, Fortney JL, Mehlhorn TL, Lowe KA, Earles JE, Phillips J, Techtmann SM, Joyner DC, Elias DA, Bailey KL, Hurt RA Jr, Preheim SP, Sanders MC, Yang J, Mueller MA, Brooks S, Watson DB, Zhang P, He Z, Dubinsky EA, Adams PD, Arkin AP, Fields MW, Zhou J, Alm EJ, Hazen TC (2015) Natural bacterial communities serve as quantitative geochemical biosensors. *MBio* 6:e00326-00315

- Stackhouse B, Lau MCY, Vishnivetskaya T, Burton N, Wang R, Southworth A, Whyte L, Onstott TC (2017) Atmospheric CH₄ oxidation by Arctic permafrost and mineral cryosols as a function of water saturation and temperature. *Geobiology* 15:94–111
- Struckmeyer HIM, Williams AK, Cowley R, Totterdell JM, Lawrence G, O'Brien GW (2002) Evaluation of hydrocarbon seepage in the Great Australian Bight. *AAPEA J* 42:371
- Techtmann SM, Fortney JL, Ayers KA, Joyner DC, Linley TD, Pfiffner SM, Hazen TC (2015) The unique chemistry of Eastern Mediterranean water masses selects for distinct microbial communities by depth. *PLoS One* 10:e0120605
- Techtmann SM, Fitzgerald KS, Stelling SC, Joyner DC, Uttukar SM, Harris AP, Alshibli NK, Brown SD, Hazen TC (2016) *Colwellia psychrerythraea* strains from distant deep sea basins show adaptation to local conditions. *Front Environ Sci* 4:33
- Techtmann SM, Mahmoudi N, Whitt KT, Campa MF, Fortney JL, Joyner DC, Hazen TC (2017) Comparison of thaumarchaeotal populations from four deep sea basins. *FEMS Microbiol Ecol* 93:10
- Tissot BP, Welte DH (1984) *Petroleum formation and occurrence*. Springer, Berlin
- Valentine DL, Mezić I, Macesic S, Crnjarić-Zic N, Ivic S, Hogan PJ, Fonoberov VA, Loire S (2012) Dynamic autoinoculation and the microbial ecology of a deep water hydrocarbon irruption. *Proc Natl Acad Sci USA* 109:20286–20291
- Vezzulli L, Brettar I, Pezzati E, Reid PC, Colwell RR, Hofle MG, Pruzzo C (2012) Long-term effects of ocean warming on the prokaryotic community: evidence from the vibrios. *ISME J* 6:21–30
- Wasmund K, Kurtboke DI, Burns KA, Bourne DG (2009) Microbial diversity in sediments associated with a shallow methane seep in the tropical Timor Sea of Australia reveals a novel aerobic methanotroph diversity. *FEMS Microbiol Ecol* 68:142–151
- Wenzhofer F, Holby O, Kohls O (2001) Deep penetrating benthic oxygen profiles measured in situ by oxygen optodes. *Deep-Sea Res Part I-Oceanogr Res Pap* 48:1741–1755
- Widdel F, Musat F, Knittel K, Galushko A (2007) Anaerobic degradation of hydrocarbons with sulphate as electron acceptor. In: Barton LL, Hamilton WA (eds) *Sulphate-reducing bacteria: environmental and engineered systems*. Cambridge University Press, Cambridge, UK
- Wilkins D, Lauro FM, Williams TJ, Demaere MZ, Brown MV, Hoffman JM, Andrews-Pfannkoch C, McQuaid JB, Riddle MJ, Rintoul SR, Cavicchioli R (2013) Biogeographic partitioning of Southern Ocean microorganisms revealed by metagenomics. *Environ Microbiol* 15:1318–1333
- Youssef M, El-Taweel G, El-Naggar A, El-Hawary SE, El-Meleigy M, Ahmed S (2010) Hydrocarbon degrading bacteria as indicator of petroleum pollution in Ismailia Canal, Egypt. *World Appl Sci J* 8:1226–1233
- Zrafi-Nouira I, Guermazi S, Chouari R, Safi NMD, Pelletier E, Bakhrouf A, Saidane-Mosbahi D, Sghir A (2009) Molecular diversity analysis and bacterial population dynamics of an adapted seawater microbiota during the degradation of Tunisian zarzatine oil. *Biodegradation* 20:467–486