Omics of oil biodegradation

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Omics studies (metagenomics, transcriptomics, metabolomics, proteomics) for marine oil biodegradation research increased rapidly after the 2010 Deepwater Horizon (DWH) accident in the Gulf of Mexico. Since then, it has been demonstrated how omics techniques can be used to model and better understand pre-spill environments, monitoring during a spill and post-spill. Data that encompass everything from the ecosystem to the molecular level are needed for understanding the complicated process of petroleum biodegradation in marine environments. Consequently, using omics for monitoring oil in the ocean will help in developing more robust systems models and would make responses to spills much more defensible in terms of risks to the environment and people. Omics is enabling for a Systems Biology approach to oil spills which allows a search for hidden interactions and attributes at different trophic levels because ‘the whole is greater than the sum of its parts’.

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Introduction

Metagenomics, transcriptomics, proteomics, and metabolomics have played a significant role in environmental monitoring and bioremediation efforts in recent years. This trend was catalyzed by the marine oil biodegradation research after the 2010 Deepwater Horizon (DWH) spill in the Gulf of Mexico. Physical clean-up efforts were hindered by technical or logistical difficulties caused by the depth at which this spill occurred. DWH is the deepest oil well blowout (~1500 m below the surface) that has ever occurred. The uncertainty surrounding the fate of oil in deep sea environments presented a unique research opportunity. The first studies demonstrated faster-than-expected hydrocarbon biodegradation rates in deep waters [1]. Based on field and lab studies, hydrocarbon composition changes were observed with distance from the DWH well blow out which helped to reveal a variety of hydrocarbon-degrading microorganisms [1]. The indigenous microorganisms were adapted to natural seeps of crude oil in that marine environment [2]. Rapid oil biodegradation was facilitated by water-soluble constituents in the crude oil and through the injection of subsea dispersant [3]. The microbial community composition and dominant taxa changed with the degradation of certain components of the crude oil thus changing the petroleum composition through time. The more easily degraded components were consumed first by microbes that could grow faster during the initial environmental conditions, followed by microbes that could degrade more difficult to metabolize components and/or daughter products created by the initial biodegradation [4].

Oil spills have severe effects on the ambient area around the release site [⁵]. These incidents may occur during oil and gas exploration or production-related activities, including accidents during storage or transport [⁵]. Oil biodegradation by microorganisms can be effective in reducing some of the immediate risks associated with oil spills while having minimal adverse effects on the environment [⁵]. Temperature, oxygen concentration, and available nutrients can influence the rate and extent of oil bioremediation [⁶]. The selection of the most appropriate response option(s) following an oil spill typically involves the consideration of many factors and trade-offs, for example, urgency related to nearness and sensitivity of risk factors and long-term effects on the environment.

Marine microbial communities are a vital component of global carbon cycling, and numerous studies have shown that populations of known oil-degrading bacteria are ubiquitous in marine environments. Omics technologies will prove useful in establishing baseline communities and activities for example, during natural exposure from underwater oil seeps. Understanding the microbial community composition and diversity aids in the prediction of...
petroleum biodegradation by microbial communities in situ and is therefore an important component of oil spill response decision-making process. Successive blooms of taxonomically distinct indigenous microbial populations as the oil weathers and labile components are sequentially degraded, leaving less-readily degraded components to feed subsequent blooms of microbes is critical to understanding the best alternatives for long-term spill response.

**Microbial communities across marine basins**
The presence of marine petroleum hydrocarbon pollution has both natural and anthropogenic sources. Anthropogenically, the growing demands for fossil fuel and its derivative products over the last millennia have resulted in increased extraction and exploration which in turn increased offshore drilling, pipelines, and shipping spills globally. This creates potentially hazardous situations as it increases the risk of large-scale marine pollution events worldwide. Natural seeps provide points of entry for hydrocarbons as oil and gas are slowly released from cracks or fissures on the ocean floor over time. Global estimates suggest that 47% of oil released into marine environments comes from these natural oil seeps [5**]. Primarily, seeps have attracted attention as an indicator of a subsurface accumulation of petroleum, but, we can also use them to identify potential hydrocarbon degrading bacteria and their catalytic mechanisms [7]. The microbiota residing in the waters near these natural seeps are primed for hydrocarbon degradation through repeated exposure, forming a memory response to this contaminant [8]. Valentine et al. [9] found that the memory response of these oil degrading microbes led to a rapid increase in their abundance and accelerated oil biodegradation during the Deepwater Horizon (DWH) Spill [10*].

The following deep-sea basins have received some attention as their drilling and exploration activities have increased the need for appropriate spill response measures: 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. These basins are found in different geographic locations which are impacted by water masses that have different temperatures, salinities, dissolved oxygen (DO) concentrations and limiting nutrients from land surfaces and diagenesis of hydrocarbon deposits in the deep [8]. Geochemical gradients exist within these basins ranging from warmer, well oxygenated surface waters to cold temperatures, darkness, high pressures, and possible anaerobic conditions at deeper depths. Distinct communities of hydrocarbon degraders thrive in the deep ocean and surface waters with varying metabolic capabilities [1,3*,4*,8,11]. Environmental factors in these basins influence the microbial community composition and the abundance of potential hydrocarbon degrading microorganisms [3*]. The effect of temperature, depth, and the physical properties of the native oil have an impact on the microbial community response and their degradative capabilities [12,13].

Though seawaters generally have high microbial abundance and diversity, hydrocarbon contamination will support the proliferation of a set of specialized obligate hydrocarbon utilizers known as obligate hydrocarbonoclastic bacteria (OHCB) [7,12]. After the DWH spill, the microbial community responded in different ways based on depth. Though surface and deep waters were both dominated by Gammaproteobacteria, the genera belonging to this class differed at these depths. Oceanospirillaceae, Pseudoalteromonas, Pseudomonas, Vibrio, Acinetobacter, and Alteromonas were dominant in the warm surface waters, whereas the prominent organisms in the colder, deeper waters were Oleispira, Colwellia, and Cyclolastics [3*,6*,11,12]. This is consistent with other reports of probable hydrocarbon degrading genera found in other deep sea oil basins [13]. The Oceanospirillales family, are frequently associated with hydrocarbon pollution and biodegradation of aliphatic hydrocarbons and are ubiquitous across marine basins, with variation in specific genera amongst basins [3*,9]. Oleispira and Colwellia have been previously isolated from Arctic environments with hydrocarbon pollution and are psychrophilic microorganisms. These microbes have adapted to the limitations on biodegradation imposed by lower temperatures to produce cold-adapted enzymes [3*,14].

**Omics role in pre-spill response planning**
The Spill Impact Mitigation Assessment (SIMA) model utilizes natural in situ biodegradation as a baseline response and for comparison of efficiency and hazards related to other remediation techniques [15**]. It is considered as the starting point of ‘no intervention’ for the SIMA model (https://www.ipiec.org/resources/awareness-briefing/guidelines-on-implementing-spill-impact-mitigation-assessment-sima/ IPIECA, 2018). Natural degradation of oil is dominated by bacterial biodegradation, as such the in-depth knowledge provided by omics is crucial for decision making and targeted response plans [8]. Omics data is a main component used in the Structured Learning in Microbial Ecology (SLiME) model to predict biodegradation rates [16]. Having a basic understanding and local microbial sampling done before contamination to establish a baseline community leads to more informed predictions of hydrocarbon transport and natural transformation rates [17*].

SIMA utilizes the Oil Spill Contingency and Response (OSCAR) model which take some level of microbial data into account but could be greatly enhanced by site-specific bacterial community data (Table 1). The abundance and diversity of potential hydrocarbon degrading bacteria have been shown to vary significantly by both location and water depth [3*,6*,18] making site-specific
Table 1

| Hydrocarbon class | Enzyme                              | Action on hydrocarbons                                                                 | References |
|-------------------|-------------------------------------|----------------------------------------------------------------------------------------|------------|
| Aliphatics        | Alkane 1-monoxygenase               | C5–C16 alkanes, alkyl benzenes, cycloalkanes                                           | [24,25,26] |
|                   | Alcohol dehydrogenase               | Oxidation of hydrocarbon intermediates to fatty acids                                  | [24,27]    |
|                   | Soluble/particulate methane         | C1–C8 alkanes, C1–C5 halogenated alkanes, alkenes, cycloalkanes                        | [24,25,26] |
|                   | Eukaryotic cytochrome P450 hydroxylases | C10–C16 alkanes, Fatty acids                                                        | [28]       |
|                   | Bacterial cytochrome P450 hydroxylases | C5–C16 alkanes, cycloalkanes, C15–C30 alkanes                                             | [24]       |
|                   | Dioxygenases                        | C10–C30 alkanes                                                                         | [24]       |
|                   | Alkyl-succinate and arylalkyl-succinate synthases | n-alkanes                                                                      | [29]       |
| Aromatics         | Naphthalene 1,2-dioxygenase         | Addition of molecular oxygen to low molecular weight aromatic hydrocarbons to form alcohol intermediate | [30,31]    |
|                   | Benzene dioxygenase                 |                                                                                       |            |
|                   | Toluene dioxygenase                 |                                                                                       |            |
|                   | Ring-cleaving dioxygenases (intradiol and extradiol dioxygenases) | Ring cleavage of catecholic compounds (main intermediate in the biodegradation of aromatic hydrocarbons) | [32,33]    |
|                   | Eukaryotic cytochrome P450 hydroxylases | Transformation of aromatic hydrocarbons to trans-dihydrodiol | [32,33] |
|                   | Ethylbenzene dehydrogenase          | Hydroxylation of aromatic hydrocarbons                                                 | [24,29]    |
|                   | Alkyl-succinate and arylalkyl-succinate synthases | Fumarate addition to aromatic hydrocarbons, alkyl-aromatic hydrocarbons | [24,29] |

Omics data crucial to better model fate of hydrocarbons. Though potential microbial hydrocarbon degraders are widespread, variation in environmental pressures will result in differences in these microbes, even among the same groups of bacteria. Thus rates of degradation and microbial response cannot be treated as constant across basins and depths [8]. During DWH we found that the biodegradation rates of Macondo oil varied from total petroleum half-life’s of 1.2–6.1 days [1], while the TPH half-life’s in Eastern Mediterranean, Great Australian Bight, Central Mediterranean, Angolan Coast and Caspian varied from 11 to 21 days for indigenous oil.

Omics data can inform the model if the local microbial community contains any known hydrocarbon degraders, via genomics; if the bacteria present are expressing oil degrading genes, via transcriptomics; and further down to proteins, metabolites, and so on, (Table 2). Omics samples should not stop at genomics (DNA analysis); while knowing which potential degraders are present is important, it does not fully account for cometabolic degradation and biosurfactant producers [8]. Omics also provides the benefit of not needing laboratory cultivation, as many of the potential taxa are not successfully cultured [19]. Microcosm studies are subject to bottle effects (changes in nutrient composition in enclosed systems over time result in shifts of the microbial community that cause variations from what was present in the in situ environment at the time of collection) which limits their use in mimicking in situ degradation [15**]. Lack of culturing ability and communities subject to bottle effects necessitates in situ data. A systems biology approach to planning for potential incidents is ideal in analyzing the effects of hydrocarbon pollution at the molecular, cellular, and community level to provide insight on the innate degradative capabilities that can be employed for bioremediation. Modeling from a systems biology approach in conjunction with nutrient data should help elucidate limiting

Table 2

| Biodegradation actions       | Omics tracking/analysis                                                                 |
|-----------------------------|----------------------------------------------------------------------------------------|
| Progression of hydrocarbon degradation | Genomic community analysis is used to identify community and dominant taxa shifts through progression of hydrocarbon degradation [1,8,19,34] |
| Microbially produced biosurfactants | Functional gene analysis can show which bacteria are producing biosurfactants, and metagenomic analysis can show which organisms have the potential to create biosurfactants [8] |
| Memory response             | Identification of genes/organisms consistently associated with hydrocarbon degradation for use as biomarkers in the detection of hydrocarbon contamination [8,35] |
| Co-metabolic degradation    | Identification of unique or unusual degradation pathways with real-time analysis of community metabolic state [13,33] |
factors in the hydrocarbon degradation pathways to better plan for nutrient amendments and dispersants [16,20]. Models like SLiME [16] can be used with rapid sequencing techniques to provide results in one day that predict how a oil plume will change and how fast it will degrade. After amendments and engineering controls have been applied to ameliorate the extremes of the oil spill, samples can be taken monthly to monitor hydrocarbon concentrations, increasing time between samples as it proceeds until contamination is below detectable limits for critical risk receptor concerns.

The DWH oil spill in the Gulf of Mexico was the first large scale spill where omics were implemented [4*,15**], and the data acquired opened new potential monitoring methods for the clean-up process. Genomic data regarding microbial community composition indicated a correlation between changes in the community structure and dominant taxa to the hydrocarbon composition of the oil as it changes during the degradation process [4*,15**,18,19]. Expanding on the knowledge gained from DWH, microbial omics data may be used as another stream of evidence in pre and post spill monitoring to identify: (1) best practices for spill response, (2) the presence of hydrocarbon contamination, and (3) how far have hydrocarbons been degraded. Building off the omics tracking data that began with DWH monitoring, the monitoring and omics data from future spills and contamination events can be fed back into upgraded models — creating a feedback loop for machine learning to build the best predictions and spill response plans that allow the models to be rapidly updated for a real time response for those in the field [15**].

Omics and bacterial data limitations

Microcosm studies are subject to bottle effects (changes in nutrient composition in enclosed systems over time result in shifts of the microbial community that cause variations from what was present in the in situ environment at the time of collection) which limits their use in mimicking in situ degradation [21]. Utilizing culture-independent methods from omics technologies has helped to broaden our knowledge of microbial species that were previously deemed as ‘unculturable’. This was seen during the DWH spill where the integration of molecular based and culture-independent techniques increased the capacity for real time assessment of oil biodegradation [13]. The use of powerful omics technologies and bioinformatics methods, including metatranscriptomics, proteomics, metabolomics, and metagenomics allowed scientists to comprehensively examine the microbial community structure, and their dynamic and metabolic capabilities associated with a major oil pollution event. It better guided our assessment of: who are they and what are they doing? Genomic data helped to identify trends in microbial succession and transcripts associated with hydrocarbon biodegradation. The combination of these approaches allowed researchers to investigate the metabolic pathways taken by the microbial community to degrade oil at different stages. This information may have otherwise been overlooked if species were only analyzed individually.

Though our knowledge has increased through these culture-independent techniques, there are still limitations in sampling efforts that can introduce bias into the data analysis. As previously mentioned, the ‘bottle effect’ can affect the microbial community during transport and storage of samples. Culture-independent methods also require large volumes of water to generate a sufficient cell mass for downstream analysis [22]. Filtration methods and materials may also introduce biases into what cells are retained versus destroyed based on their adherence. Biases can also be introduced through nucleic acid extraction kits and protocols. A major concern is the ability to lyse and extract the nucleic acids from a representative set of cells from the microbial community to capture the ecological diversity [22].

Molecular methods must be processed through various bioinformatics pipelines to be converted into a format that can be analyzed. Hence, these pipelines have the potential to introduce biases into our final conclusions. The databases chosen for analysis can also introduce further biases during gene calling and annotations since different databases have varying standards for gene classification and functional analysis [23*]. Awareness and anticipation of these biases will better prepare us to mitigate their effects on downstream analysis and conclusions. Some of these limitations can be overcome by using multiple techniques and pipelines to provide multiple lines of evidence for any conclusions made. The strength of the conclusions from this data is provided by a multi-omic analysis that not only allows us to assess the presence of hydrocarbon degrading species but also the genes, and biogeochemical pathways involved.

Conclusions

Since biodegradation is the ideal spill response, and the base line for hydrocarbon contaminant modeling, microbial data is essential for better preparation [15**]. Including multiple lines of omics research before and after contamination will allow for more robust models and response plans. Studying natural hydrocarbon seepages will also better our understanding of the microbial response without having to wait for another event. These seepages were key in the microbial response to Deepwater Horizon and providing the microbial communities with a memory response [15**]. Omics should be considered part of the baseline data taken when considering drill sites or during exploratory expeditions. Once active, each site should have personnel trained in properly collecting and storing microbial samples. Allowing for multiple lines of constant local omics data will improve current
modeling capabilities. Omics is a crucial step in biodegradation and cannot be ignored when preparing for spills.

Conflict of interest statement
Nothing declared.

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Papers of particular interest, published within the period of review, have been highlighted as:

● of special interest
 ● ● of outstanding interest

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