# **Geochemistry and Microbiology** in **Seep Prospecting**

Studies offshore Nova Scotia have shown that combining geochemistry and microbial genomics can effectively de-risk offshore deepwater oil and gas exploration.

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Hydrocarbon seep detection is often the first step in indicating possible prospectivity in frontier offshore basins. Hydrocarbon seeps can be detected indirectly by satellite imagery and, if available, by seismic evidence. Since these are indirect methods, they are usually followed up with surface core sampling with geochemical analysis to provide stronger evidence of actual occurrence. Geochemical data can be then used to differentiate between biogenic and thermogenic hydrocarbons. In practice, detecting subsurface petroleum seepage from analysis of cores is difficult. This is because the geochemical expression of the seepage area is very limited, and the geochemical character of a shallow sediment core sample is a usually a mixture of different inputs, including migrated hydrocarbons and recent and ancient organic matter deposited with the sediments.

Microbiological approaches have been used to complement geochemical data in detecting petroleum seepage for de-

Map of offshore Nova Scotia. Highlighted locations are site 2016-41, which shows the presence of thermogenic gas hydrate and strong indications of petrogenic liquid hydrocarbons, and sites 2016-48 and 49 (very close to each other), which show the presence of biogenic gas hydrates. The sites by a star are additional 2016 sites where there is support from both geochemical and microbiological data for hydrocarbon seepage from the subsurface. Yellow dots indicate 2015 sites and green dots are 2016 sites.



risking exploration (e.g. Hubert and Judd, 2010). One method is to examine sediments for bacteria that actively metabolize hydrocarbons in the seabed. This is most often done using traditional growth-based screening and more recently, as in the study described here, using genomic tools like PCR assays to target functional genes, such as methane- and alkane-monooxygenases for aerobic or anaerobic hydrocarbon degrading microbial populations. The advent of these genomics tools is critical since growth-based screens are typically failing to capture a large majority (i.e. >99%) of the microbial diversity in natural samples (Amann et al., 1995); by sequencing DNA directly this 'uncultured' majority is included in the analytical signal. Here we describe how combining geochemical and microbiological data is increasing confidence that there are hydrocarbons migrating to the surface, close to deepwater prospects offshore Nova Scotia.

# Offshore Nova Scotia

The shallow water Scotian Shelf (<200m water depth) is relatively well explored, with 25 discoveries and production until recently from the Sable Island area. In contrast, the deepwater Scotian Slope, which extends from the shelf break at 200m to almost 4.000m water depth, is poorly explored. Just 13 locations have been drilled over an area of 80,000 km<sup>2</sup>, with only four of these in more than 2,000m water depth. There have been only minor discoveries or shows in shallower wells reported to date. Although drilling has not been very successful, oil and gas seeps have been reported using indirect methods. However, there has been no definitive proof to link these seep reports to the presence of working petroleum systems on the Scotian Slope.

The principal objective of offshore piston-coring expeditions has been to identify evidence for an oil-prone source rock on the Scotian Slope from geochemical analyses of sediment samples in close proximity to surface expression of petroleum seepage. Prospective sites were evaluated on





the basis of available seismic reflection data, interpretations of sea-surface hydrocarbon slick occurrences imaged in satellite data, and near real-time assessment of seabed and water column anomalies using multibeam echo sounder and high resolution seismic reflection systems. It should be noted that even when the sampling vessel is on location, hitting a target feature with a piston core is very difficult, with the majority of cores not managing to sample the seabed close to the target. Piston coring in 2,500–3,000m water depth can vary as much as 500m laterally from the target as a consequence of the wireline deviating 5° from vertical, which is not uncommon when working on the open ocean. This is critical, as Adams and Dahdah (2011) noted that sediment cores collected 15 to 25m (49–92 ft) away from a real target might not show a thermogenic geochemical signature in the resulting sediment core.

Three expeditions took place between 2015 and 2018. In 2015 and 2016, a total of 70 piston cores were taken from different locations (Campbell and MacDonald, 2016, Campbell, 2019). In 2018 an autonomous underwater vehicle revisited interesting sites, where additional gravity coring was performed (Campbell and Normandeau, 2019).

Data Obtained from Cores Recovered cores were variable in depth and up to 10m long. They were immediately sampled near the base for headspace gas analysis and multiple additional depths were sampled from each core for geochemical and microbiological analysis. Geochemical analyses were performed by Applied Petroleum Technology (APT) and microbiological assays were performed by the Geomicrobiology Group in the Department of Biological Sciences at the University of Calgary. Gas samples were analyzed for composition and isotopes. Sediment samples were evaluated for their Total Organic Carbon (TOC) content, extracted and the total extract (EOM) analyzed by gas chromatography (GC). A subset of extracts was selected for more detailed gas chromatographymass spectrometry analysis based on the appearance of their EOM-GCs. Geochemistry methods and data can be found in Fowler and Webb (2015, 2017, 2018). Bacterial community composition was determined on triplicate sediment samples throughout the entire depth of the cores through 16S rRNA gene amplicon sequencing using the method of Dong et al. (2017).

During the 2016 expedition, gas hydrates were encountered for the

first time on the Scotian Slope, at three separate sites (Campbell, 2019). At sites 48 and 49 the methane in the hydrate had a mostly biogenic origin, whereas the gas composition and isotopes at site 41 indicated a thermogenic oil-associated gas, based both on its composition (i.e. its wetness) and isotopes (e.g.  $\delta^{13}$  methane values between -42.2 and -49.0%). Sediment samples from site 41 have EOM-GCs that show high amounts of lighter hydrocarbons over the  $nC_{15}$ - $nC_{20}$  alkane range (see figure above), including an unresolved complex mixture (UCM). Shallower samples show a larger UCM with n-alkanes in lower abundance relative to isoprenoids such as pristane and phytane, suggesting biodegradation is occurring in the shallow seabed. Samples from site 41 also show a higher relative abundance of thermally mature biomarkers compared to biologically inherited isomers which dominate most other samples, and also a higher concentration of diamondoids.

Site 41 appears to provide the best evidence to date for a mature oil-prone source rock on the deepwater Scotian Slope. This site was revisited in 2018, resulting in gravity core samples that confirmed this location is where petroleum seepage reaches the surface.

# **GEO Chemistry**

A seismic cross-line through the Tangier 3D Survey in the proximity of site 2016-41. It shows a salt diapir with a bright spot amplitude anomaly above it, indicating the possibility of reservoired hydrocarbons. Faults to surface are also present, representing potential for hydrocarbons to seep to the surface. This probably explains why petroleum seepage was detected in cores from sites 2016-41 and 2018-7, as they were taken in the closest proximity to the fault expression at surface. Other cores taken in 2015 and 2016, further from the surface expression of faulting, did not detect petroleum seepage.

Subsequent analysis of recently available 3D seismic data (BP's Tangier 3D), indicates that sites 2016-41 and 2018-7 were collected above a buried salt diapir with overlying seismic amplitude anomalies and crestal faults that likely act as conduits for migrating fluid, as shown on the seismic section above.

Geochemical indications of thermogenic gas and/or sediments with possible petrogenic hydrocarbons were observed at a number of other sites but, unlike at site 41, the data is ambiguous enough that varying degrees of uncertainty remain. To address this, geochemical results were compared with microbiological data. DNA sequencing of bacteria and archaea revealed that sites which showed geochemical anomalies for the presence of hydrocarbons had conspicuous microbial community profiles, with anomalies in certain groups of uncultured bacteria. The lack of cultured representatives for these bacteria means that they are poorly understood, since the only information about them comes from DNA sequencing and not culture-based physiology experiments. Therefore, the metabolic explanations for observed patterns are not straightforward, despite the striking distribution patterns in the seabed.



The microbial groups detected in the hydrocarbon-positive sediment samples are commonly observed in deeper sediments and were ubiquitous in the deeper (>1m) layers of the cores in this study. Depth profiles associated with a handful of microbial subspecies (i.e. differentiated by their gene relatedness) revealed patterns that are consistent with geochemical anomalies for hydrocarbons, suggesting that these bacteria (and their associated genomic marker sequences) can serve as biomarkers. As such, microbiology is offering an additional line of evidence in petroleum seep prospecting on the Scotian Slope. Studies in deepwater prospects in the Gulf of Mexico have shown similar patterns for closely related microorganisms (Hubert et al., 2018). Sites 48 and 49, where biogenic gas hydrates were encountered, show similar anomalies but with slight modifications, indicating that some bacteria do not distinguish between thermogenic and biogenic methane whereas others might, hinting at the possibility for bioassays that can identify migrated thermogenic hydrocarbons.

# **Increasing Certainty**

Sites with strong geochemical evidence for the presence of hydrocarbon seepage were mostly those with positive microbiological indications. Using data from two completely different techniques, we are confident that there is subsurface petroleum seepage close to four coring sites in the deepwater offshore Nova Scotia. These interpretations are supported by pore water sulphate concentrations that drop off rapidly with depth in these sediments, suggesting anaerobic hydrocarbondegrading populations are active at these sites.

From a petroleum systems perspective, our seep data supports the presence of one or more mature source rocks on the Scotian Slope, with at least one capable of generating a black oil in the vicinity of site 41.

## References Available Online

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