A novel approach for assessing the biodegradability of constituents in petroleum substances

Difficulty in generating biodegradation data on all petroleum substance constituents

Petroleum substances (PS) are derived from crude oil and contain hundreds to thousands of individual chemicals or constituents. They are considered UVCBs, i.e. substances of unknown or variable composition, complex reaction products or biological materials. PS must undergo environmental assessment as part of the REACH registration process to be marketed in the EU (European Union). This assessment includes information on the biodegradability of the chemical constituents in a substance, which determines whether a substance is considered persistent in the environment.

Regulatory-accepted biodegradation tests are typically performed on single chemicals in multiple environmental media, and the half-lives are compared against regulatory criteria for persistence determination.[1] However, the complex nature of PS UVCBs makes it challenging to evaluate the biodegradation of all the constituents. Performing a biodegradation test on the whole PS is possible; however, unless detailed analytical techniques are applied, the result would generally be the average biodegradation rate of all of the constituents. In such a case, a very persistent constituent could possibly be overlooked, which is not ideal from a regulatory perspective. Still, there are so many constituents in most PS UVCBs that testing them individually or even testing representative constituents would be time- and resource-intensive, especially as most of the constituents are not commercially available. However, as explained below, combining analytical techniques such as two-dimensional gas chromatography (GCxGC) with biodegradation testing on whole petroleum substances can generate persistence screening data for the constituents of PS more efficiently than relying on single constituent biodegradation testing.

Petroleum substance constituents can be separated using GCxGC

Petroleum substances that are refined or processed from crude oil are composed of hundreds to thousands of hydrocarbon constituents. Petroleum analytical chemists use GCxGC to separate the different constituents, which are then gauged by an appropriate detector for petroleum molecules, such as a flame ionization detector (FID).[2] The GCxGC technique separates constituents based on polarity in the first dimension and volatility in the second dimension; a conceptual diagram of the output is shown in Figure 1 on page 24. For PS, like the vacuum and hydrotreated gas oil (VHGO) and straight run gas oil (SRGO) investigated in this work, there are thousands of peaks on the chromatogram, indicating the presence of thousands of constituents.

Concawe is undertaking a novel project with SINTEF and other researchers to explore the use of analytical techniques to generate biodegradation data on multiple petroleum substance constituents simultaneously.
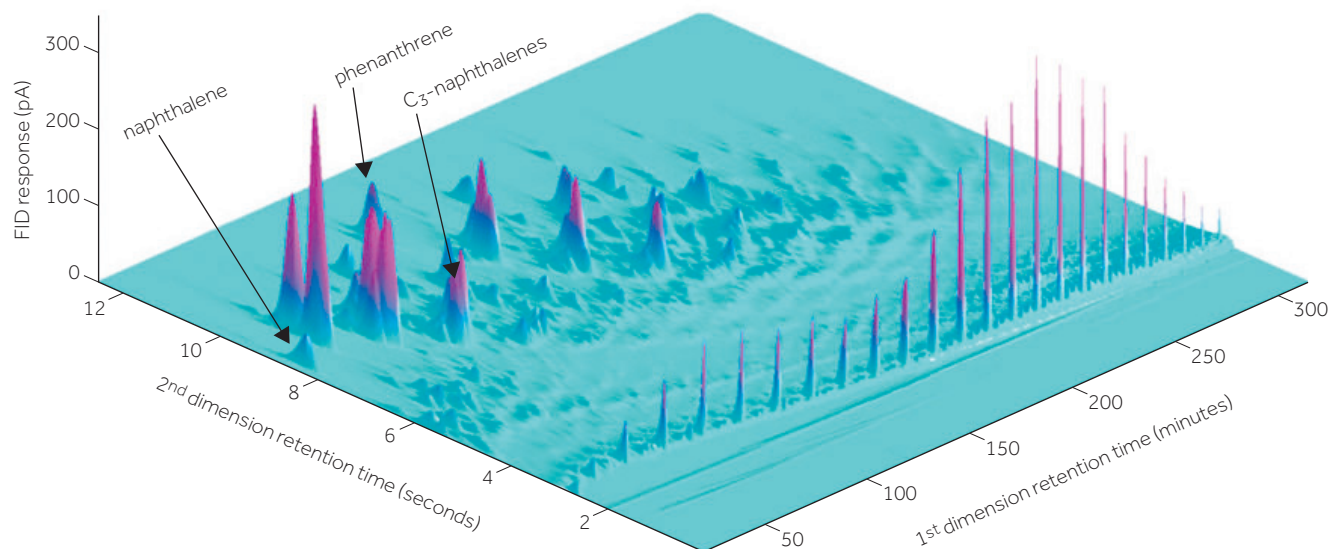
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Figure 1: A visual diagram of a GCxGC-FID chromatogram[3]

Pairing a whole PS biodegradation test with GCxGC analysis can give biodegradation information on PS constituents

To assess the biodegradability of the numerous constituents in PS, Concawe is working with several scientific research contractors (SINTEF, Jonas Gros and Oleolytics) to combine the biodegradation testing methodology with the capabilities of the GCxGC analytical technique plus data analysis, as summarised in Figure 2 on page 25. The objective of this study is to generate biodegradation information for the constituents in PS during whole petroleum substance biodegradation testing.

For simplicity, related constituents, which would come out close together on the chromatogram, can be grouped into 'hydrocarbon blocks'.[4] Samples of the test medium are taken at different times over the course of the biodegradation test (64 days) and analysed by GCxGC-FID to see which peaks and blocks are present and at what concentration.

Figure 2: Flow diagram for the SINTEF biodegradation peak tracking project
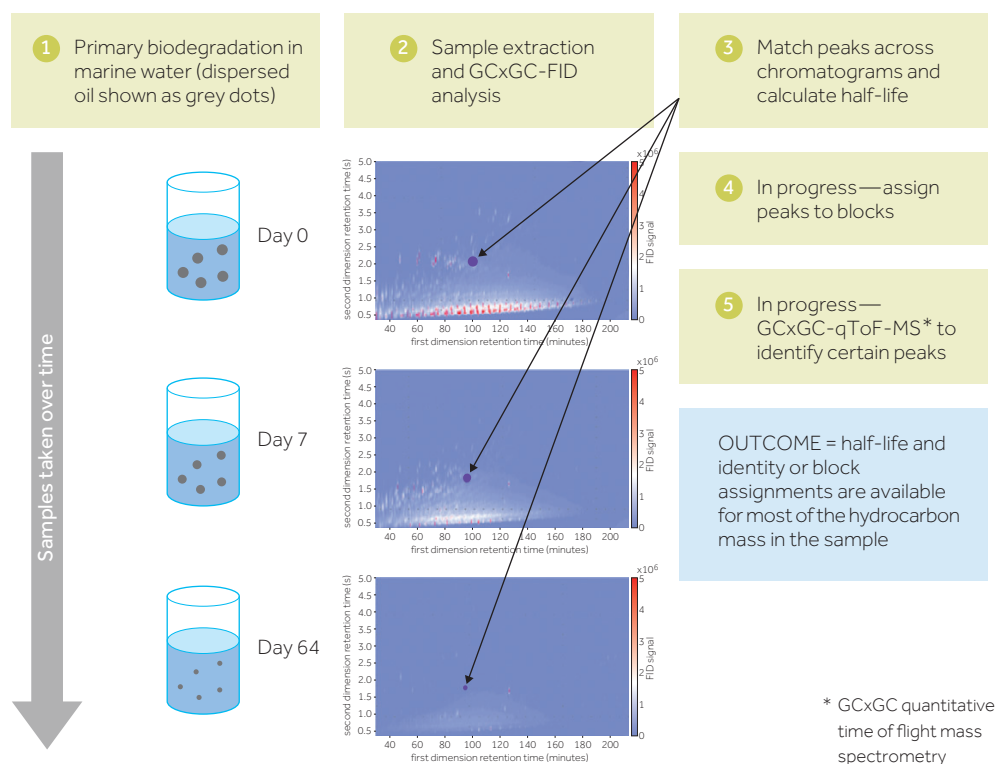
By tracking the peaks and blocks over time, as shown in Figure 2, it is possible to generate primary biodegradation half-life data for all of the trackable peaks and blocks. Only *primary* biodegradation half-lives are obtained and not complete mineralisation, as the GCxGC-FID is mostly capable of detecting PS but not its degradation products. This limits the regulatory application of these primary biodegradation half-lives; they can be used as screening data rather than full persistence assessment data. However, this process can identify constituents that show a long primary biodegradation half-life and which may need further assessment.

So far in the project, SINTEF Ocean has performed biodegradation testing similar to an OECD 306 Biodegradability in Seawater protocol,[5] which is a regulatory-accepted biodegradation screening test. SINTEF used natural seawater as the microbial inoculum in rotated closed bottles containing VHGO and SRGO at 13°C for a 64-day biodegradation test.

Samples were taken weekly or bi-weekly, and hydrocarbon material in the samples was extracted from the water samples and analysed by GCxGC-FID. At the end of the experiment (day 64), 73–81% of the overall VHGO mass and 84–88% of the overall SRGO mass had been degraded. The GCxGC chromatograms from the different sampling time points were then aligned using an alignment algorithm,[6] so that a peak representing the same constituent is able to be compared over the different chromatograms over time (see Figure 2). The alignment algorithm only works on peaks that are unequivocally the same constituent; thus not all the peaks on the chromatogram can be tracked. A little over half the mass of the VHGO or SRGO is made up of trackable peaks in this exercise.

A half-life for the tracked peak is calculated using the data from the GCxGC-FID, where peak size on the chromatogram is reflective of the relative concentration of that constituent(s) versus a non-biodegraded control peak (e.g. steranes). The half-lives were calculated by fitting to an exponential decay curve.

Ongoing and future work

In the first phase of this work, the focus was on individual tracked peaks representing constituents comprising half of the total mass of the VHGO or SRGO. Future and ongoing work is now focusing on two open questions:

1. How will we assess the biodegradation potential of the remaining mass not represented in the peak assessment; and

2. How can the peaks that require further assessment be identified?

For the first question, while about half of the mass is not trackable as individual peaks, the remaining mass is still detectable on the chromatogram (Figure 3).

Figure 3: Half-lives for the tracked peaks overlaid on the chromatogram of the fresh, non-biodegraded oil for VHGO
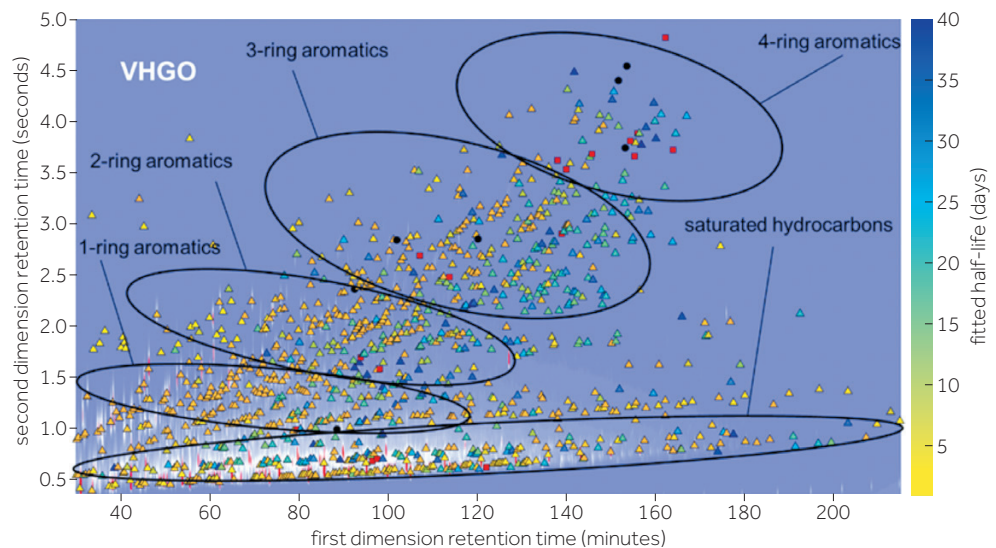
Notes:
The locations of general hydrocarbon classes are indicated on the chromatogram. Darker colours indicate longer half-lives (less biodegradable).

These non-tracked peaks can be grouped into hydrocarbon blocks, and a half-life for a block could be calculated. This is the same idea as the hydrocarbon blocking approach which has historically been used by Concawe to describe the composition of petroleum substances by grouping together similar constituents in a 'block'. This process is formalised in the Concawe PetroRisk tool,[1] which is widely used by the petrochemical industry to support REACH registrations. Concawe is now working with Oleolytics to determine a hydrocarbon blocking template to derive half-lives for the blocks.

For the second question, Concawe is also working with SINTEF-Ocean to perform GCxGC-quantitative time of flight mass spectrometry (GCxGC-qToF-MS) on the VHGO and SRGO samples to more confidently identify peaks. The use of the mass spectrometer detector will give information on the molecular structure of the constituents in certain peaks. The peak identification will be used to confirm which constituents need further assessment, and to verify that the hydrocarbon blocking approach is as accurate as possible.

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[1] https://www.concawe.eu/reach/petrorisk/