

# RESEARCH

## Biodegradation of Fuel Oil Under Laboratory and Arctic Marine Conditions

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The aerobic biodegradation of the components of a fuel oil under Arctic summer conditions follows a pattern that is indistinguishable from that exhibited under temperate conditions. Straight chain alkanes and small aromatics are degraded first, followed by branched alkanes and larger and alkylated aromatics. We present data on the biodegradation of heptadecane as a representative *n*-alkane, pristane as a representative *iso*-alkane, and naphthalene, phenanthrene, and chrysene and their alkylated forms as representative two-, three- and four-ring aromatic hydrocarbons. In particular, the pattern of degradation of the alkylated aromatics allows the identification of biodegradation in samples collected from the field and the estimation of the extent of biodegradation that occurred in the In-Situ Treatment of Oiled Sediment Shorelines Field Trials.

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### Introduction

Oil entering the marine environment, whether from seeps or anthropogenic sources, is subject to a variety of natural processes. Most of these, such as dispersion, dissolution, evaporation and burial, change the physical state or location of the oil, but do not remove it from the environment. Only combustion, photooxidation, and biodegradation convert hydrocarbons to carbon dioxide and water. Combustion at sea requires containment and ignition (Buist *et al.*, 1999), and can be quite effective under favorable conditions. Photooxidation can degrade polycyclic aromatic hydrocarbons (Garrett *et al.*, 1998), but the majority of any oil is eventually removed by biodegradation (National Research Council, 2002; Prince, 2002). Most marine environments have relatively low levels of the nitrogen and phosphorus nutrients that are essential for bacterial growth on hydrocarbons, and so biodegradation

of oil stranded on shorelines is typically limited by their supply. The application of fertilizers (bioremediation) to partially alleviate this limitation has proven to be an environmentally-acceptable and cost-effective way of stimulating biodegradation in temperate climates (Atlas, 1977; Prince, 1993; Lee *et al.*, 1995; Swannell *et al.*, 1996; Venosa *et al.*, 1996; Prince & Bragg, 1997; Lee & Merlin, 1999). There have been strong indications that similar results can be expected in the Arctic (Sendstad *et al.*, 1982, 1984; Sveum, 1987a,b; Sveum & Ladousse, 1989) and the Antarctic (Delille *et al.*, 1998).

To further address the potential that bioremediation will be effective on Arctic marine shorelines, a multinational collaborative project entitled In-Situ Treatment Of Oiled Shoreline Sediments took place on Spitsbergen (78°N, 17°E) (Sergy *et al.*, 2003; Prince *et al.*, 2003). Clear proof that oil loss from a shoreline is due to biodegradation rather than physical movement can come from monitoring the chemical composition of the residual oil, because biodegradation shows a clear preference for some hydrocarbons over others. This preferential degradation is the subject of

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this paper. Laboratory studies were carried out with the same oil that was used in the Spitsbergen trials and microbial inocula from the shorelines that had been oiled and treated as part of the study. Experiments were also carried out with a weathered Alaska North Slope crude oil at low temperatures to determine whether or not temperature has any effect on the pattern and overall end-point of biodegradation.

In temperate climates the aerobic biodegradation of crude oil follows a well-defined pattern. Straight chain alkanes and small aromatics are degraded first, followed by branched alkanes and larger and alkylated aromatics. The aromatic compounds exhibit a particularly diagnostic pattern. Smaller aromatics are degraded before larger ones (e.g., naphthalene more rapidly than phenanthrene, phenanthrene more rapidly than chrysene), and increasing alkylation slows biodegradation.

A preliminary account of this work has been presented (Garrett *et al.*, 1999).

## Experimental Methods

The laboratory experiments used saline Bushnell-Haas medium (Bushnell & Haas, 1941) with trace elements and vitamins added (see Prince *et al.*, 1994) to ensure that nitrogen and phosphorus nutrients were not limiting microbial growth. The progress of biodegradation was monitored over three months, analyzing the residual oil at various times by gas chromatography coupled with mass spectrometry (GC/MS, see Garrett *et al.*, 1998). Eight flasks were used for the experiments with sediment from Spitsbergen and the

IF-30 fuel oil used in the field experiment, and duplicates were completely extracted after 1, 3, 6 and 12 weeks (see Garrett *et al.*, 1998). These laboratory experiments were carried out at room temperature since most marine Arctic microorganisms are thought to be mesophilic (e.g., Sagemann *et al.*, 1998), and this allowed extensive biodegradation in a reasonable time frame. A separate series of experiments, in duplicate, compared the progress of biodegradation of an artificially-weathered Alaska North Slope oil (Prince *et al.*, 1994) by organisms from Prince William Sound, Alaska (see Prince *et al.*, 1994) at 6 and 20 °C after 90 days.

## Results and Discussion

### *Biodegradation of IF-30 fuel oil by microorganisms from Spitsbergen*

Figure 1 presents the total ion chromatograms of the initial oil, and oils extracted after 1, 3, 6 and 12 weeks of incubation in the laboratory at room temperature. The resolved peaks in the initial oil are *n*-alkanes and *iso*-alkanes. The *n*-alkanes were completely degraded within 1 week under these conditions, and the *iso*-alkanes were essentially completely degraded within 3 weeks (see Fig. 2).

Aromatic hydrocarbons are not seen as resolved peaks in the total ion scans of Fig. 1, but they are readily resolved by selective ion monitoring. Since IF-30 is a moderately heavy fuel oil, it contains negligible amounts of one-ring aromatic compounds, but it contains significant amounts of two, three and four ring aromatics. Figure 2 shows the progress of bio-

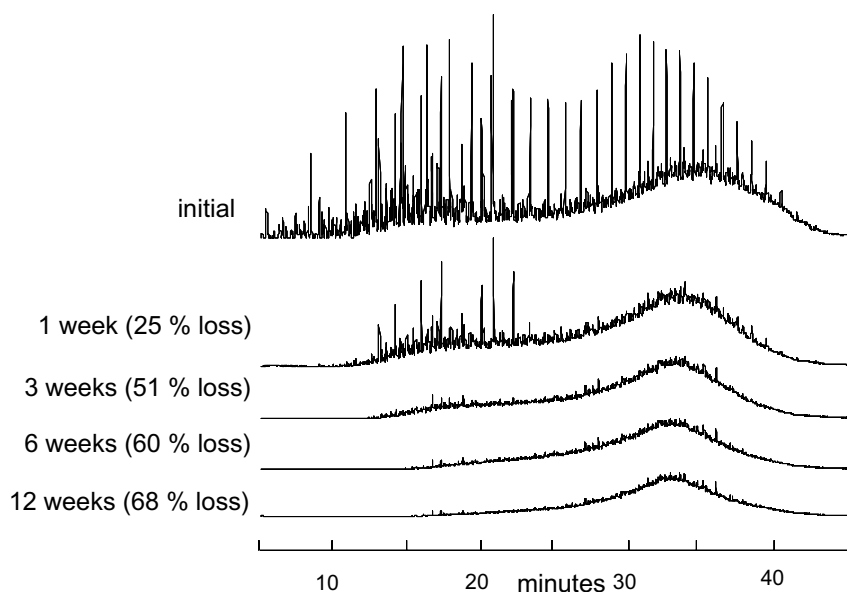
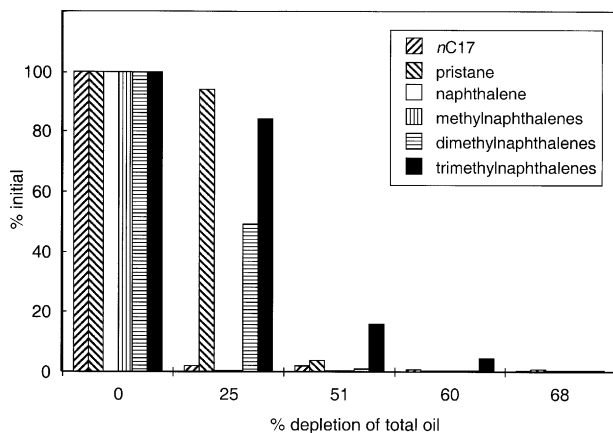
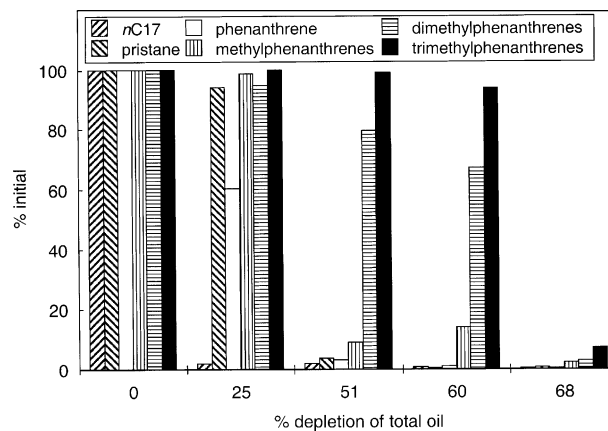


Fig. 1 Total ion chromatograms of initial oil, and oil extracted from cultures incubated for the indicated time.



**Fig. 2** Biodegradation of *n*-heptadecane, pristane (both measured as  $m/z = 57$ ), naphthalene ( $m/z = 128$ ), methyl-phenanthrenes ( $m/z = 142$ ), dimethyl- and ethyl-phenanthrenes ( $m/z = 156$ ) and trimethyl-, methyl-ethyl- and propyl-phenanthrenes ( $m/z = 170$ ), based on hopane as a conserved internal marker.

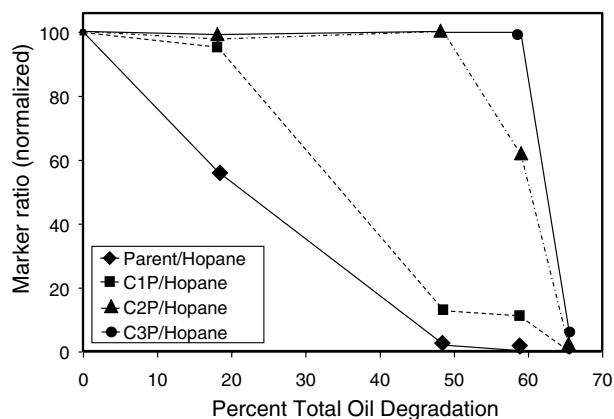


**Fig. 3** Biodegradation of *n*-heptadecane, pristane (both measured as  $m/z = 57$ ), phenanthrene ( $m/z = 178$ ), methyl-phenanthrenes ( $m/z = 192$ ), dimethyl- and ethyl-phenanthrenes ( $m/z = 206$ ) and trimethyl-, methyl-ethyl- and propyl-phenanthrenes ( $m/z = 220$ ), based on hopane as a conserved internal marker.

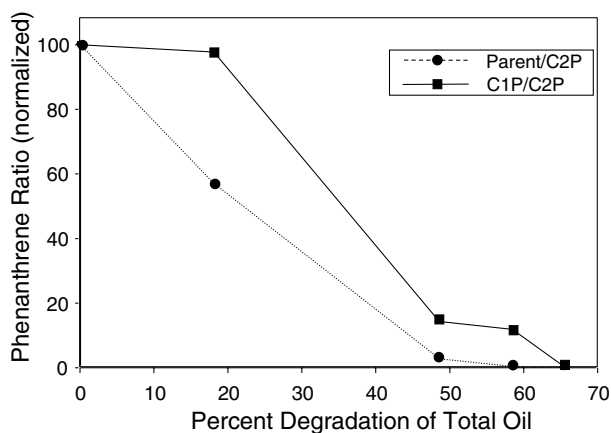
degradation of naphthalene (a two-ring aromatic) and its alkylated derivatives, together with the biodegradation of heptadecane (*n*C17) as a representative *n*-alkane and pristane (2,6,10,14-tetramethylpentadecane) as a representative *iso*-alkane. Heptadecane, naphthalene and the methylphenanthrenes were essentially completely degraded in the first week (during which 25% of the total detectable hydrocarbon was consumed), and pristane and the more alkylated naphthalenes were degraded soon thereafter. All the individual resolved *n*-alkanes and *iso*-alkanes exhibited similar behavior in our experiments, and no significant preferences were evident for any isomers of the alkyl-aromatics studied. The pattern of degradation of alkylated aromatics is shown more clearly in Fig. 3, which examines phenanthrenes. As expected (e.g., Elmendorf *et al.*, 1994; Douglas *et al.*, 1996), the parent compound is degraded most readily, followed by the methyl-phenanthrenes, dimethyl- and ethyl-phenanthrenes and trimethyl-, methyl-ethyl- and propyl-phenanthrenes. Quantification of biodegradation was achieved using hopane as a conserved internal marker that remained unchanged during the experiment (Prince *et al.*, 1994). A very similar pattern of degradation was seen for dibenzothiophene and its alkyl-substituted forms, in concert with our earlier findings (Douglas *et al.*, 1996).

As discussed in the paper on the bioremediation field trials (Prince *et al.*, 2003), the oil applied to the shoreline was not the same for all the plots, and the concentrations of hopane in the oils on different parts of the plot were quite varied. Although the ratio of other hydrocarbons to hopane fell as biodegradation proceeded in the field, interpretation would have been potentially confounded if movement of oil on the shoreline were a significant phenomenon. Fortunately,

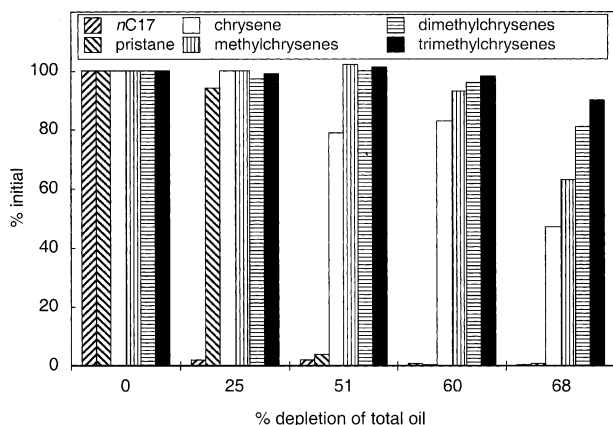
the ratio of phenanthrene to dimethyl- and ethyl-phenanthrenes was similar in all the oils, so the possibility of using changes in this ratio was investigated as a surrogate for monitoring biodegradation instead of ratios of hydrocarbons to hopane. Figure 4 replots the data of Fig. 3 as a function of percent total GC-detectable oil biodegradation, showing that the alkylated phenanthrenes potentially offer a suite of ratios that change at different times during the total biodegradation process. Figure 5 plots two ratios, the parent to dimethyl- and ethyl-phenanthrenes, and the ratio of methyl-phenanthrenes to dimethyl- and ethyl-phenanthrenes. It is clear that the two ratios provide a reasonably sensitive indicator of biodegradation up to 50% loss of total GC-detectable oil. As discussed in the accompanying paper (Prince *et al.*, 2003), we used the ratio of phenanthrene to dimethyl- and



**Fig. 4** Changes in the ratios of phenanthrene (Parent), methyl-phenanthrenes (C1P), dimethyl- and ethyl-phenanthrenes (C2P) and trimethyl-, methyl-ethyl- and propyl-phenanthrenes (C3P) to hopane plotted as a function of the biodegradation of total GC-detectable hydrocarbon.



**Fig. 5** Changes in the ratio of phenanthrene to the dimethyl- and ethyl-phenanthrenes (Parent/C2P) and methyl-phenanthrenes to the dimethyl- and ethyl-phenanthrenes (C1P/C2P) as a function of total oil degradation based on hopane as a conserved internal marker.



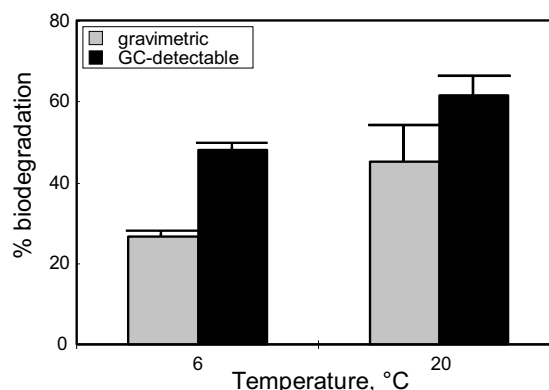
**Fig. 6** Biodegradation of *n*-heptadecane, pristane (both measured as  $m/z = 57$ ) chrysene, ( $m/z = 228$ ) methyl-chrysenes ( $m/z = 242$ ), dimethyl- and ethyl-chrysenes ( $m/z = 256$ ) and trimethyl, methyl-ethyl- and propyl-chrysenes ( $m/z = 270$ ) based on hopane as a conserved internal marker.

ethyl-phenanthrenes as an indicator of oil biodegradation in the field.

As expected, the biodegradation of chrysene (a four-ring aromatic hydrocarbon) lagged behind that of naphthalene, phenanthrene and dibenzothiophene. Nevertheless, once biodegradation began, it followed a very similar pattern (Fig. 6). This is noteworthy because organisms that can degrade four-ring aromatic compounds are not thought to be as common as those that can degrade smaller molecules (e.g., Kanaly & Harayama, 2000), yet they were clearly present on the oiled shorelines on Spitsbergen.

#### Biodegradation of crude oil at 6 and 20 °C

Figure 7 presents the results of an experiment using an inoculum from Prince William Sound, Alaska to degrade 1% artificially-weathered Alaska North Slope



**Fig. 7** Biodegradation of artificially-weathered Alaska North Slope crude oil at 6 and 20 °C by an inoculum from Prince William Sound, Alaska. The error bars represent the standard errors of the means of duplicate measurements.

crude oil in saline Bushnell–Haas medium. The cultures were incubated at 6 or 20 °C for 90 days. The artificially-weathered crude oil had lost all those molecules likely to evaporate under these conditions (essentially all molecules with fifteen or fewer carbons). It thus has the experimental advantage that it can be extracted from water with a suitable solvent, and evaporated to dryness with no significant loss of oil. In these experiments, oil was extracted from the cultures at the end of the experiment with methylene chloride. The oil was weighed to allow an estimate of total oil lost by biodegradation, and subjected to GC/MS analysis to allow determination of the percentage loss of hydrocarbons using hopane as a conserved internal marker (Prince *et al.*, 1994). The former measurement includes both hydrocarbons and polar molecules, such as the resins and asphaltenes, whereas the GC/MS measures only the hydrocarbons under our conditions. Only the hydrocarbons are thought to be significantly biodegradable (see Prince, 2002), so one would expect to see a greater measured biodegradation in the GC/MS assay. Biodegradation was extensive at both temperatures, whether assayed as loss of oil by weight after extraction, or loss of total GC-detectable hydrocarbon referred to hopane as a conserved internal marker. Using the latter measurement, 48% of the oil had been degraded in 90 days at 6 °C, 61.5% at 20 °C.

The pattern of degradation of *n*-alkanes, *iso*-alkanes and parent three-ring aromatics being degraded first, followed by the more alkylated aromatics, as discussed above, was seen at both temperatures.

#### Effect of nutrients, and relationship to field data

Note that all the experiments reported here had levels of biologically-available nitrogen, phosphorus, trace elements and vitamins that are believed to be

optimal for the growth of hydrocarbon-degrading microorganisms (Bushnell & Haas, 1941). Such levels probably would have unacceptable environmental implications in the field (USEPA, 1989) and bioremediation strategies aim to achieve only a small fraction of such concentrations. In the actual field trials (Prince, 2002), the addition of fertilizers soon after the oil was applied to the beach had the effect of approximately doubling the extent of biodegradation seen in the field. Fertilized plots had lost approximately half of the GC-detectable hydrocarbon after 195 ice-free days. Similar levels of biodegradation were achieved in about 21 days in the laboratory at 20 °C with adequate nutrients.

## Conclusions

The pattern of oil biodegradation by indigenous microorganisms from an Arctic shoreline is very similar to that exhibited by microorganisms from temperate marine environments (e.g., Elmendorf *et al.*, 1994; Douglas *et al.*, 1996). *n*-Alkanes are degraded before *iso*-alkanes, smaller aromatic molecules are degraded before larger ones, and alkylated polycyclic aromatic hydrocarbons are degraded more slowly than the parent compounds. These patterns are seen at both 6 °C and 20 °C, and verify the identification of biodegradation in samples collected from the field. They support the estimation of the extent of biodegradation that has occurred in an individual sample. In particular, changes in the ratio of phenanthrene to dimethyl- and ethyl-phenanthrenes are useful indicators of the extent of biodegradation of an intermediate fuel oil up to 50% removal of the total GC-detectable hydrocarbon.

Low temperatures clearly slow biodegradation, but the physical progress seems to follow that seen at warmer temperatures, albeit at a slower rate. In the last few years, several groups have reported on oil biodegradation at low temperatures (Margesin & Schinner, 1999; Gibb *et al.*, 2001; Eriksson *et al.*, 2001; Mohn *et al.*, 2001; Walworth *et al.*, 2001; Filler *et al.*, 2001), and cold-tolerant oil-degrading microorganisms have been isolated from Antarctic soils (Aislabie *et al.*, 2000; Baraniecki *et al.*, 2002). These papers emphasize that cold-tolerant organisms are very similar to those in temperate environments, and that their growth can be limited by biologically-available nitrogen and phosphate when hydrocarbons are present in significant quantities. The results of the field trials indicate that the functional diversity of biodegradation activity on Arctic Svalbard is very similar to that of temperate climates, such as Prince William Sound, Alaska, and coastal New Jersey. This biochemical diversity is matched by a taxonomic diversity that in-

dicates that the microorganisms present on oiled shorelines in Svalbard are quite similar to those that degrade hydrocarbons in warmer climates (Grossman *et al.*, 2000).

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