The Prestige Oil Spill. 2. Enhanced Biodegradation of a Heavy Fuel Oil under Field Conditions by the Use of an Oleophilic Fertilizer

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A field bioremediation assay using the oleophilic fertilizer S200 was carried out 10 months after the Prestige heavy fuel-oil spill on a beach of the Cantabrian coast (North Spain). The field survey showed that S200 significantly enhanced the biodegradation rate, particularly of high molecular weight n-alkanes, alkylcyclohexanes, and benzenes, and also observed within the C1-phenanthrenes and dibenzothiophenes. Through the analysis of some target aliphatic and aromatic hydrocarbons a number of chemical indicators for assessing the efficiency of field bioremediation as well as identifying the source of highly weathered samples collected in the area after the spill are defined.

Introduction

The oil tanker Prestige sprang a leak off Finisterre Cape (Galicia, NW Spain) on November 13, 2002. Six days later the ship broke into two pieces and sank 240 km west off Galicia. In all, about 60 000 t of a Russian heavy fuel oil (type M-100) were spilled, affecting a large part of the Galician coast and much of the Cantabrian coast and the southern Bay of Biscay.

Although mechanical extraction is the primary recommended procedure to clean up oiled coastal areas (4) it is not feasible everywhere, and other techniques have been developed to this end. Among them is bioremediation based on the supply of nitrogen and phosphorus fertilizers to the contaminated site to enhance the natural oil biodegradation process (2, 3). This was considered a promising procedure for the treatment of oiled shorelines, and since the large-scale operation after the Exxon Valdez oil spill in Alaska in 1989 (4, 5), there has been substantial progress in applications all over the world, from temperate to polar climates (6), so that bioremediation is now recognized as an appropriate oil spill response tool (7).

Among various agents used for surface bioremediation, oleophilic products are considered particularly suitable, since nutrients remain in the oil phase, as was demonstrated with the application of the oleophilic fertilizer Inipol EAP22 after the Exxon Valdez oil spill (4). Nevertheless, in some cases the results in terms of efficiency have been limited by the incidence of physical processes causing oil loss or even the poorly designed field trials (8, 9).

In the present study, we describe a field bioremediation assay carried out after the Prestige oil spill on a beach of the Cantabrian coast (North Spain) using the oleophilic fertilizer S200. Although this has been applied to the bioremediation of light oil spills, as far as we know this is the first time that it has been applied to a heavy residual product. The quantitative assessment of the microbial biodegradation was carried out by the chemical characterization of the oil at molecular level and the results were compared with those previously obtained in vitro (10). Information from laboratory tests will not necessarily parallel the actual behavior of the oil in the environment, since this accounts for numerous variables. However, laboratory tests may provide useful information for assessing the potential biodegradability of the spilled product and allow understanding of the degradation pathways and prediction of the long-term weathering of the spilled oil.

Materials and Methods

Chemicals. A fuel-oil sample (density 0.995 g cm−3; viscosity 30000 cSt at 15 °C, S 2.28%), containing 22% aliphatics, 50% aromatics, and 28% resins and asphaltethenes, was obtained from the Prestige cargo tanks. The oleophilic fertilizer S200 was kindly supplied by IEP Europe (Madrid, Spain). This fertilizer, containing 7.9% N and 0.6% P, consisted of a microemulsion of a saturated solution of urea (nitrogen source) in oleic acid as a carrier, an oleophilic phosphate ester (phosphorous source and surfactant), and a viscosity reducer.

Dichloromethane and n-hexane were obtained from Merck (SupraSolv grade) (Darmstadt, Germany). Solid-phase cartridges (SPE) of cyanopropyl—silica (SiO2/CN, 1.0:0.5 g, 6 mL) were obtained from Interchim (Montluçon, France). Aliphatic hydrocarbon standards (n-alkanes) were obtained from Fluka Chemie (Buchs, Switzerland) and the 16 EPA PAHs standard solution (10 ng·L−1 in cyclohexane) was purchased from Dr. Ehrenstorfer-Schäfers (Augsburg, Germany).

Experimental Design. The experiment was conducted on a beach known as Virgen del Mar, near Santander, at the north coast of Spain (3° 52′ 53″ W; 43° 28′ 35″ N). The beach was mainly composed of large and medium cobblestones overlying a mixed sand and gravel base which were covered by a thin layer of fuel. The experiment spanned a period from September 2003 to May 2004. The ambient temperature registered through the experiment ranged from 3.8 to 22.5 °C (av 13.8 °C). The average humidity was 53%.

Two plots 10 m apart were set parallel to the shoreline, each one covering an area of 12 m² (2 × 6 m) in the upper intertidal zone. In the one closer to the sea, only occasionally covered by water, a biostimulation treatment based on the addition of the oleophilic fertilizer S200 was carried out. S200 was diluted with seawater and applied using a backpack sprayer to thinly coat the oiled cobblestones, to come up with a proportion of C/N/P of 120:10:1, approximately, according to the prescribed dosage by IEP Europe. Two applications were performed (15.8 g N m⁻² and 1.37 g P m⁻²), at the beginning of the experiment and after 20 days. The other plot was kept as a control.

Each plot was divided into 9 sectors and fuel samples were collected in each one of them at 0, 30, 60, and 220 days.
by scraping the cobblestones with a scalpel. Samples were placed in glass jars and kept cool (4 °C) until analysis.

**Chemical Analysis.** One gram of each individual oil residue was dissolved in 5.0 mL of dichloromethane, phase-separated, and percolated through 2 g of anhydrous sodium sulfate. One mL of the eluate was carefully evaporated until dryness to determine the fuel-oil content of the sample. An aliquot (5–10 mg) was solvent-exchanged to hexane and then fractionated in a previously conditioned cyanopropyl–silica solid-phase cartridge (6.0 mL of hexane), as reported elsewhere (11). The aliphatic and aromatic fractions were obtained by eluting with 4.0 mL of hexane (F1) and 5.0 mL of hexane–dichloromethane (1:1) (FII), respectively, were concentrated to a final volume of 500 μL, and spiked with 25 μL of triphenylamine (8 μg g⁻¹ in hexane) as an internal standard.

Both fractions were then analyzed by GC-MS on a TRACE-MS Thermo Finningan TRACE-GC 2000 gas chromatograph (Dreieich, Germany) fitted with a HP 5MS (30 m × 0.25 mm i.d. × 0.25 μm film) capillary column (J&W Scientific, Folsom, CA) as described elsewhere (10).

**Assessment of Fuel-Oil Biodegradation.** The biodegradation process was monitored by following the changes in the chemical composition of the oil using the 17α(H),21β-(H)-hopane (m/z = 191) as an internal conservative molecular marker (12). The distributions of n-alkanes relative to hopane were determined by using, respectively, the fragment ions at m/z 85 and 191. The distributions of PAHs were also related to hopane by using the molecular ion of the former and the internal standard triphenylamine in the first and second fractions. The first-order biodegradation rate constants and half-lives (t₁/₂) for both n-alkanes and parent and alkylated PAHs, and the corresponding 95% confidence intervals, were calculated by nonlinear regression of the relative concentrations (n = 9) of each compound along time in the control and fertilized plots.

A nonparametric test (Mann–Whitney U test) was performed using the SPSS 12.0 software (SPSS Inc., Chicago, IL) to assess the statistical significance in the biodegradation indexes between plots, at a given sampling period and throughout the experiment. We considered that two sets of samples (n = 9) were statistically different when the
significance value was lower than 0.05 ($p < 0.05$). In this respect, no significant differences were found between the two plots at the beginning of the experiment (day 0).

**Results and Discussion**

**Biodegradation of the Prestige Heavy Fuel-Oil Aliphatic Fraction.** $n$-**Alkanes.** The samples collected at the beginning of the experiment (10 months after the spill) were severely depleted in the lower $n$-alkane fraction ($< n$-C20) as a result of weathering, but at the end (220 days later) the whole series of $n$-alkanes had experienced an important depletion in both plots with respect to the polycyclic hopane, which should be attributed to biodegradation. The occurrence of biodegradation in the control plot can be due to the presence of endogenous degrading microorganisms, especially in chronically hydrocarbon polluted sites (13) and also evidenced after the Prestige oil spill (14). The degradation followed the typical pattern in the marine environment, with a decreasing rate with the increase of the carbon number (15). However, in the fertilized plot the biodegradation was significantly enhanced at the $n$-C25–C35 range (Mann–Whitney $U$ test, $p < 0.05$), especially during the first 60 days (Figure 1). The respective half-lives of these $n$-alkanes and their 95% confidence intervals are summarized in Table 1, those decreasing in the range of 1.6- to 3.2-fold (average 2.4) in the fertilized plot. The possible role of the formulation surfactant in increasing the solubility and therefore the bioavailability of heavy oil components to microorganisms is to be explored but this idea has been applied in several events (16, 17).

Other series of long-chain $n$-alkyl derivatives eluting in the same fraction, namely $n$-alkylcyclohexanes ($m/z$ 82) and benzenes ($m/z$ 92) had also been extensively biodegraded after 60 days in the fertilized plot, whereas they were still present at the end (220 days) in the control plot, as shown in Figure 2. Besides the clear evidence of the differences in the profiles, the statistical significance test of the data on the concentrations of the individual components of the series along the experiment confirmed the effect of the fertilizer already after 60 days ($p = 0.001–0.027$). The presence of additional C1–C3 substituents in the alkylbenzene ring, including a C20 isoprenoid side chain, evidenced by the diagnostic ions 106, 120, and 134, respectively, delayed the degradation of the corresponding series, although at the end of the experiment biodegradation was also complete in the fertilized plot. It is interesting to notice that within the series of $n$-alkyltoluenes ($m/z$ 106) (Figure 3), the following degradation trend was observed in the early degradation stages: $m/z > o>$. Consequently, these patterns suggest that the degradation may proceed through both the aromatic ring and the side chain, which may involve the natural occurrence of either a variety of microbial communities with different

<table>
<thead>
<tr>
<th>$n$-alkane</th>
<th>$t_{1/2}$ (days)</th>
<th>95% confid. interval</th>
<th>% depletion</th>
<th>$t_{1/2}$ (days)</th>
<th>95% confid. interval</th>
<th>% depletion</th>
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<td>44–91</td>
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<td>70</td>
<td>63</td>
<td>49–88</td>
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<td>61</td>
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<td>118–197</td>
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<td>67</td>
<td>54–89</td>
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<td>130–206</td>
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<tr>
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<td>145–229</td>
<td>45</td>
<td>81</td>
<td>65–110</td>
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<td>166–265</td>
<td>38</td>
<td>88</td>
<td>71–115</td>
<td>80</td>
</tr>
<tr>
<td>C32</td>
<td>317</td>
<td>228–453</td>
<td>31</td>
<td>99</td>
<td>80–130</td>
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<tr>
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<td>230–478</td>
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<td>113</td>
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<td>C34</td>
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<td>46</td>
<td>131</td>
<td>107–168</td>
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<td>241</td>
<td>188–300</td>
<td>47</td>
<td>136</td>
<td>113–171</td>
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**FIGURE 2.** Ion chromatograms of alkylcyclohexanes ($m/z$ 82) and alkylbenzenes ($m/z$ 92) of oil samples collected along the experiment. Each profile is presented on a scale relative to the largest peak. The numbers indicate the carbon atoms of the side chain. Asterisks (*) indicate phytanylbenzene.
metabolic activities or bacterial strains exhibiting different metabolic pathways \((18, 19)\).

**Molecular Markers.** Molecular markers have been proposed for oil fingerprinting and weathering assessment, with the most commonly used being the polycyclic steranes and terpanes \((20)\). Relevant molecular indexes are listed in Table 2 and their evolution in the samples collected along the experiment is shown in Figure 4. The observed trends are consistent with those found in the laboratory experiments \((10)\) and indicate a fair extent of oil biodegradation. The most significant indicators are the depletion of the diasteranes \((27\text{dia})\) and C-27 sterane components \((27\beta)\) as well as the decrease of the 14\(\alpha\),(H),17\alpha\,(H)\) steranes \((29\alpha\alpha)\) versus the 14\(\beta\),(H),17\beta\,(H)\) isomers and of the 20R \((29\alpha\alpha\alpha)\) versus the 20S isomers, with the former two particularly enhanced in the fertilized plot according to the statistical evaluation of the data corresponding to both plots. These features can be used in assessing the efficiency of field bioremediation as well as in identifying the source of highly weathered samples collected in the area.

**Aromatic Fraction. Polycyclic Aromatic Hydrocarbons (PAHs).** The gas chromatogram of the aromatic fraction exhibited a predominance of alkylated hydrocarbons in the original oil. However, the environmental exposure of the beached oil significantly depleted these components, mainly by water washing and evaporation, so that only the higher homologues were considered. As shown in Figure 5, the degradation of PAHs was apparent in both plots for all parent 3–4 ring components and progressively decreased with increasing alkylation in the control plot, following the expected pattern of microbial PAH degradation \((21–23)\).

The biodegradation achieved in the untreated plot could be due to the presence of autochthonous microbial populations enriched by seawater nutrients \((24, 25)\). However, a significant difference observed in the fertilized plot was that by the end (220 days) an enhancement of the biodegradation of the more alkylated components had occurred, as confirmed by the statistical test of the two populations which gave significance values \((p)\) ranging from 0.0001 to 0.0078 for the studied compounds. The percentages of biodegradation and the kinetics of depletion of the main parent and alkylated PAHs are shown in Table 3, where their half-lives decreased in the range of 1.8- to 3.6-fold (average 2.4) in the fertilized plot. Thus, while the C3-chrysenes underwent 21% degradation in the untreated plot, in the fertilized plot it increased

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**FIGURE 3.** Ion chromatograms of alkyltoluenes \((m/z 106)\) of oil samples collected along the experiment. Each profile is presented on a scale relative to the largest peak. The numbers indicate the carbon atoms of the side chain. Asterisks (*) indicate 1-methyl-3-phytanylbenezene. (A) Evolution of the expanded section of the profile with increasing biodegradation.

**FIGURE 4.** Molecular marker fingerprinting of samples collected during the experiment compared with the original fuel and the effect of the fertilizer S200, assessed by the Mann–Whitney U-test \((p < 0.05)\). Index definitions are indicated in Table 2.
up to 79%. In turn, Maki et al. (26) in a bioremediation experiment carried out on the Japanese coast affected by the Nokhodka heavy-oil spill did not find any significant enhancement of biodegradation for any of the analyzed PAHs when using exogenous fertilization with hydrophilic nutrients, slowly releasing nitrogen and phosphorus. As in the case of the aliphatic fraction, the question of whether this enhancement is due to an increase in the bioavailability of the heavier components favored by the presence of the surfactant component in the fertilizer formulation is still open.

The addition of S200 also increased the biodegradation rate during the first 60 days. Röling et al. (27) described a similar effect in a field-scale evaluation of bioremediation with hydrophilic fertilizers of an oil-contaminated beach mudflat, but unlike the present work, the degradation was basically limited to the alkylnaphtalenes, as the alkylphenanthrenes and dibenzothiophenes were not significantly biodegraded in any of the treated plots for almost 1 year.

TABLE 2. Diagnostic Ratios Used as Source and Weathering Indicators for the Prestige Oil Samples

<table>
<thead>
<tr>
<th>m/z</th>
<th>diagnostic index</th>
<th>definition</th>
<th>structures</th>
</tr>
</thead>
</table>
| 191  | %27Ts            | 100*Ts/(Tm + Ts) | Ts: 18α(H) – 22,29,30-trisnorbornane
| 191  | %29αβ            | 100*29αβ/[29αβ + 30αβ] | Tm: 17α(H) – 22,29,30-trisnorbornane
| 191  | %32αβ/S          | 100*32αβ/[32αβ + 32αβ] | 28αβ: 17α(H), 21β(H) – 30-norhopane
| 217  | %27dia           | 100*27d/(R + S) | 30αβ: 17α(H), 21β(H) – hopane
| 217  | %29αS            | 100*29αS/[29αS + 29αR] | 32αβ: 17α(H), 21β(H) – bisnorhopane (22S and 22R)
| 217  | %29β/(R + S)     | 100*29β/[29β + 29β] | 27β: 4-ethyl-14α(H), 17α(H) – cholestane (20S and 20R)
| 218  | %27ββ            | 100*27ββ/[27ββ + 27ββ] | 27ββ: 4-ethyl-14β(H), 17β(H) – cholestane (20R and 20S)
| 218  | %28ββ            | 100*28ββ/[28ββ + 28ββ] | 27ββ: 4-ethyl-14β(H), 17β(H) – cholestane (20R and 20S)
| 218  | %29ββ            | 100*29ββ/[29ββ + 29ββ] | 27ββ: 4-ethyl-14β(H), 17β(H) – cholestane (20R and 20S)
| 231  | %26TA            | 100*26TA/(26TA + 28TA) | aromatized cholestane (20S) and 24-ethylcholostane (20S)
| 206/212 | D2/P2        | 100*2D2/(2D2 + 2P2) | dimethylbenzothiophenes (D2) and phenanthrenes (P2)
| 220/226 | D3/P3        | 100*2D3/(2D3 + 2P3) | trimethylbenzothiophenes (D3) and phenanthrenes (P3)
| 212/256 | D2/C2        | 100*2D2/(2D2 + 2C2) | dimethylbenzothiophenes (D2) and chrysenes (C2)
| 226/270 | D3/C3        | 100*2D3/(2D3 + 2C3) | trimethylbenzothiophenes (D3) and chrysenes (C3)
| 256/230 | C2/P2+2       | 100*2C2/(2P2 + 2C2) | dimethylchrysenes (C2) and pyrenes (Py2)
| 270/244 | C3/P3        | 100*2C3/(2C3 + 2P3) | trimethylchrysenes (C3) and pyrenes (Py3)

TABLE 3. Biodegradation Half-Lives (t1/2) of Target PAHs and the Corresponding 95% Confidence Intervals (n = 9) in Control and Fertilized Plots, and Percentages of Degradation at 220 days of the Experiment

<table>
<thead>
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<th>PAH</th>
<th>Control plot</th>
<th>Fertilized plot</th>
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<tr>
<td></td>
<td>t1/2 (days)</td>
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<tr>
<td>P</td>
<td>64</td>
<td>52–82</td>
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<tr>
<td>P1</td>
<td>97</td>
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<tr>
<td>P2</td>
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<td>121–302</td>
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<tr>
<td>C3</td>
<td>317</td>
<td>141–464</td>
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*a Not available.

FIGURE 5. Relative distribution of PAHs with respect to 30αβ-hopane, in control and fertilized plots: P, phenanthrenes; D, dibenzothiophenes; Fl/Py, fluoranthenes/pyrenes; BA, benz[a]anthracene; C, chrysenes; BbF/BkF, benzo[a] and benzo[k]fluoranthene; BeP, benzo[ep]pyrene; BaP, benzo[ap]pyrene; Pe, perylene. Each data point is the mean of nine independent replicates.
The C2- and C3-alkylphenanthrene, chrysene and dibenzothiophene ratios have been proposed for source recognition and weathering assessment of spilled oils (28). The corresponding values found along the experiment are shown in Figure 6 which exhibit trends similar to those found in a laboratory study (10). The D2/P2 and D3/P3 source ratios were rather conservative, according to the similar behavior of both series (see Table 3), whereas the weathering ratios D2/C2 and D3/C3 decreased considerably in time with a major influence of the oleophilic fertilizer. Conversely, the ratios of C2- and C3-alkylchrysenes and pyrenes (C2/Py2 and C3/Py3) increased as a result of the higher degradation of the pyrene derivatives.

Within the degradation of these alkyl series it has been found that some isomers are more easily degraded than others (29–31) so that their relative losses may provide additional evidence of the oil degradation progress. Indicators of early degradation are found within the C1- and C2-phenanthrenes, dibenzothiophenes, pyrenes, and chrysenes. The general trend was the preferential degradation of isomers with
components. The complexity of the profiles of dimethyl derivatives, with many overlapping peaks due to the number of possible isomers, does not always allow determination of individual compound biodegradation, although several studies have pointed out that isomers with β-substituents (e.g., 2- and 3-methyl) or with α-β-positions occupied are more easily co-oxidized than those with adjacent methyl groups (21, 32). Within the C2-phenanthrene profiles (m/z 206) (Figure 7), the 1,7- and 2,7-dimethylphenanthrenes seem to be the most refractory to biodegradation, whereas the 3,6-, 2,6-, and 2,3-dimethylphenanthrenes exhibit a significant depletion in the degraded samples (Figure 7). Similar trends were observed in weathered samples from the Exxon Valdez oil spill (33) as well as in laboratory degradation studies (10, 34). The profile of the C2-dibenzoanthiophenes (m/z 212) shows three groups of isomers more resistant to biodegradation, namely 4,6-, 3,6-, and 1,4- + 1,6 + 1,8-dimethyl components, while the 2,4-, 2,6-, 3,7-, and 1,3-dimethyl isomers are significantly depleted, indicating the prevalence of the β methyl substitution in determining the biodegradation of alkyl aromatic components.

Molecular Markers. The aromatic fraction also contains a number of molecular markers used for oil fingerprinting among them the totally or partially aromatized steranes and triterpanes (35). The profiles of the series of triaromatic steroid hydrocarbons (m/z 231) exhibited a degradation pathway similar to that of their saturated counterparts, the steranes, consisting of the relative depletion of the lower components (C26 > C27 > C28), although with a lower influence of the fertilizer. This is reflected in the C26TA ratio indicated in Table 2 and Figure 6, which parallels the trends found in laboratory studies and field exploration of biodegraded oils (10, 36).

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Literature Cited
(31) Budzinski, H.; Raymond, N.; Nadalig, T.; Gilewicz, M.; Garrigues, P.; Bertrand, J. C.; Caumette, P. Aerobic biodegradation of


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