Trials of bioremediation on a beach affected by the heavy oil spill of the Prestige

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Abstract

The objective of this study was to assess the efficiency of several bioremediation products in accelerating the in situ biodegradation of the heavy fuel oil spill of the Prestige. Trials of bioremediation were conducted in sand, rocks and granite tiles on the beach of Sorrizo (A Coruña, NW Spain) that was polluted by the spill. Neither the added microorganisms nor the nutrients significantly enhanced the degradation rate of the fuel oil in rocks, granite tiles or sand. PAH degradation up to 80% was determined in sand and tiles. In tiles the oxygen content of the residual oil increased from 1.6% up to 8% in 90 days, which could be explained by the accumulation of products coming from the partial oxidation of the hydrocarbons. Eighteen months after the spill, the rocks of the beach were still coated by a black layer of weathered fuel oil. For this reason an oleophilic product, sunflower biodiesel was tested on a rock. The application of biodiesel accelerated the gradually clean-up of the polluted surface and could also accelerate the degradation of the residual oil.
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Keywords: Bioremediation; Fuel oil; Prestige oil spill; Biodiesel; Partial oxidation

1. Introduction

Petroleum products are the main goods of the worldwide maritime transport since in 2002 they involved 40% of the cargo shipped in the world. Despite the technological progress accidental oil spills still take place and are one of the major causes of ocean pollution, producing ecological disasters of great public concern. Within the European Atlantic, the English Channel and the Galician coast in NW Spain are the most affected areas since most of the European marine traffic passes through them. In particular, Galicia is at the top of maritime tragedies of the last 25 years and the most recent example is the oil spill of the Prestige [1]. The Prestige carried 77,000 metric tonnes of M-100 Russian fuel oil, equivalent to no. 6 heavy fuel oil (specific gravity of 0.993 at 15 °C, viscosity of 30,000 cSt at 15 °C and sulphur content of 2.58%). On 13th November 2002, this 26-year-old single-hulled oil tanker sprang a leak due to a heavy impact during a storm in front of Cape Finisterre (A Coruña, NW Spain).

The ship was towed along 243 miles in bad weather conditions and on November 19, 2002 the vessel finally broke in two and sank to a depth of 3800 m. Sixty three thousand tonnes of fuel oil were spilled into the ocean and gradually came ashore between November 2002 and May 2003. Whereas most major oil spills occur close to the coast [2], the Prestige broke up 130 miles distance offshore and therefore the spill affected 1900 km of shoreline in Northwest and North Spain and West France. Furthermore, the oil formed a viscous water-in-oil emulsion known as "chocolate mousse" which caused almost a two-fold increase of the quantity of pollutant since the water content in the emulsions reached 45% [3].

During the clean-up of the shore most of the oil was removed manually mainly by volunteers. Heavy earthmoving equipment, including bulldozers and front-end loaders, were also used in sand and gravel beaches, despite these methods were not recommended since they might spread the pollutants on the site. In general, an effective clean-up was achieved in sand beaches due to the poor penetration of the water-in-oil emulsion in the sediments due to its high viscosity, 100,000 cSt at 15 °C. During the first stages, the heaviest accumulations of oil from the rocky shore were recovered manually, but at the end a layer of fuel

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oil remained firmly stuck to the surface of rocks and pebbles. The removal of this layer was difficult and time consuming, but in many cases natural processes dealt with it quickly and effectively, especially where wave action and tidal water movements were strong. Nevertheless, in special circumstances such as beaches heavily used by the public, final 'polishing' to a very high standard may be justified [4]. Moreover, due to the bad weather conditions during the arrival of the fuel oil ashore and to the fact that the accident took place in a spring tide period, the pollution affected seriously to the supra-littoral rocks which were not usually exposed to the action of the sea. For an efficient clean-up of these oil residues, high pressure hot water flushing was used in more than $1,100,000 \, m^2$ of coast in Spain. However, this technique was labour intensive and time consuming, consumed energy and a huge amount of wastes were produced.

Bioremediation might be an environmentally acceptable and cost-effective alternative for achieving the complete removal of fuel oil that could not be efficiently recovered either from rocks or sand by physical means. There are two main approaches to this technology: bioaugmentation, in which oil-degrading bacteria are added, and biostimulation, in which nutrients are added. The success of oil spill bioremediation depends on the ability to establish and maintain conditions that favour enhanced oil biodegradation rates, such as the levels of nutrients and microorganisms. Furthermore, this technology is based on the premise that a large percentage of oil components are readily biodegradable [5]. In principle, the biodegradability of fuel oil of the Prestige could be low due to its chemical composition, since it was comprised of 22.9% saturated hydrocarbons, 52.7% aromatic hydrocarbons, 12% resins and 12.4% asphaltenes [3]. Oudot [6] reported only 11% biodegradability of the no. 6 fuel oil of the Erika wreck in laboratory experiments over 80 days. In contrast, preliminary biodegradability tests performed in our laboratory with the different biostimulation and bioaugmentation amendments applied to the beach of Sorrizo, showed a potential high degradability of the fuel TPHs (total petroleum hydrocarbons), with biodegradation percentages that ranged between 6% and 45% after only 7 days. In addition, enrichment cultures with autochthonous microorganisms and Prestige fuel oil as the sole

carbon and energy source degraded about 90% of the fuel TPHs in 2 weeks.

One of the main objectives of this study was to assess the efficiency of bioremediation strategies for the treatment of the beach of Sorrizo (A Coruña, NW Spain) that was seriously affected by the heavy oil spill of the Prestige. Physical removal methods had been used in a preliminary clean-up of the beach before starting up the study in April 2003. After that, several bioaugmentation and biostimulation products were periodically applied throughout the beach in order to study their effect on the biodegradation rates in the field. Furthermore, an oleophilic agent, sunflower biodiesel, was assayed on a polluted rock to study if it accelerated its clean-up, improving its aesthetical appearance, and if it enhanced the degradation rate of the oil.

2. Materials and methods

2.1. Experimental site and set-up

This field study was undertaken on the beach of Sorrizo (A Coruña, NW Spain) (Fig. 1) at approximately $43°18'$ N, $8°34'$ W, which was seriously affected by the oil spill of the Prestige. In this zone of the Atlantic Ocean there are two tides per day, with a tidal range between 2 m (neap tides) and 4 m (spring tides). Sorrizo is a little sheltered bay with a great variety of polluted environments (sand, pebbles, rocks) and that shows a great heterogeneity despite its little size. The beach was divided into seven zones that, between April and October 2003, received different treatments (Fig. 1): zone 1, the water-soluble commercial fertilizer Nitrophoska® Suprem (20% N, of which 9.5% is $N–NO_3$ and 10.5% $N–NH_4$, 5% $P–P_2O_5$, 10% K; Compo, Germany); zone 2, no treatment (control zone); zone 3, the commercial bioaugmentation product B350 (aerobic and facultative anaerobic hydrocarbon-degrading bacteria and micronutrients; Bio-Systems Co., USA); zone 4, rocks were treated with the commercial bioaugmentation product L1800 (aerobic and facultative anaerobic hydrocarbon-degrading bacteria, micronutrients and a biodegradable surfactant; Bio-Systems Co., USA), whereas in sand and pebbles B350 was applied (Bio-Systems
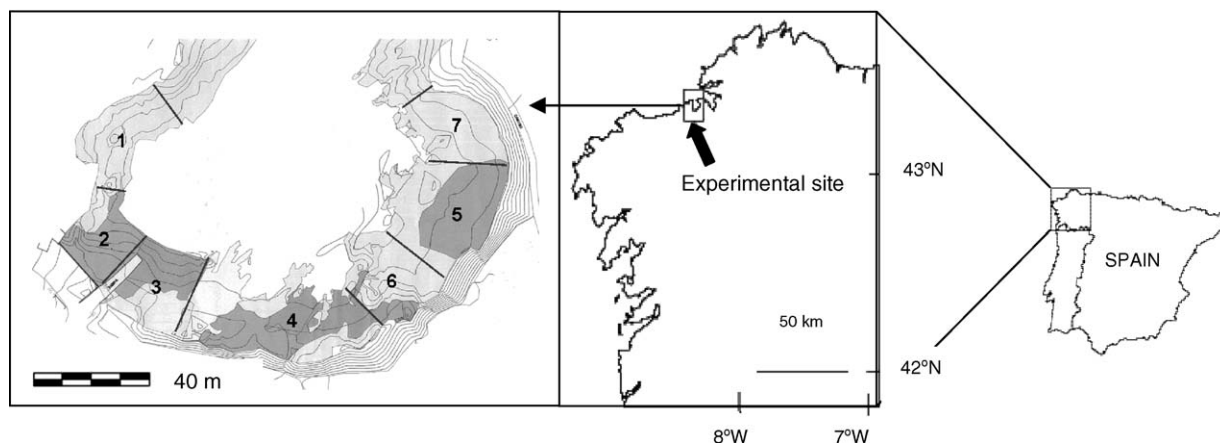

Fig. 1. Location of the beach of Sorrizo (A Coruña, NW Spain) and schematic diagram indicating the seven zones in which the different treatments were applied. The elevation change between any two adjacent contour lines in the topography map is 1 m.

Co., USA); zone 5, an authochtonous microbial culture obtained by enrichment of fuel-degrading populations from the beach of Corrubedo (A Coruña, NW Spain), also affected by the spill; zone 6, no treatment (control zone). The treatment applied in zone 7 is specified below. The fertilizer Nitrophoska® Suprem, used in zone 1, was also applied in zones 3–5 together with the bioaugmentation products. The bioaugmentation products were applied once or twice a month at the doses recommended by the manufacturer, whereas the dose of Nitrophoska in each application was of 400 kg/ha in zones 1, 3 and 4, and 80 kg/ha in zone 5. Water dilutions of the fertilizer, B350 and the autochthonous culture were spread by irrigation, whereas L1800 was applied to rocks by high pressure water at 40 °C.

The temperature and the dissolved oxygen concentration were measured in situ in the interstitial pore water of all the zones. Samples of interstitial pore water and seawater were monthly collected to monitor levels of nutrients, concentration of dissolved PAHs, and changes in microbial populations. Samples of sand and oil from rocks were also periodically taken for residual oil analyses. Interstitial pore water samples were collected from a single point at each experimental zone at a depth of 20–40 cm, the exact site being re-sampled throughout the study. For the analysis of the residual oil in sand, for each data point four subsamples of approximately 250–300 g were collected from each zone at a depth of approximately 20–40 cm. The sampling points were placed in staggered rows. Once in the laboratory the subsamples were combined to produce a composited sample, a portion of which (200 g) was dried with anhydrous sodium sulphate and solvent extracted. For the analysis of residual oil in rocks for each data point a single sample was collected from one rock at each experimental zone. The sample was obtained by mechanically scrapping part of the oil layer (approximately 200 mg).

To study the effect of the treatments on contaminated rocks in a more controlled way, in June 2003 an additional experiment was set up using granite tiles (15 cm × 15 cm × 1 cm). One side of the tiles was coated with a thin layer of fresh fuel oil and groups of tiles were gathered on a wooden board to form an experimental panel [7]. This fuel oil was slightly weathered fuel oil from the Prestige which had been collected from the sea (17.7% saturates, 47.6% aromatics, 13.7% resins, 21% asphaltenes). The panels were located in both supra-littoral and intertidal zones of the beach and the bioremediation agents were monthly applied from June 2003 to January 2004 with a hand sprayer with the same doses used on the beach. All the formulations used in the beach were applied to the tiles in the supra-littoral zone, whereas tiles located in the intertidal zone were only treated with the product B350 and the authchtonous microbial culture. Control tiles were located in both zones of the beach in order to compare the treated ones with natural attenuation. The study of these two areas responded to the fact that not only the intertidal zone was affected by the oil spill, but also the supra-littoral zone. Single granite tiles receiving each of the treatments were periodically collected to monitor the extent of degradation of the residual oil.

From March 2004 on, an experiment was started in a new zone of the beach, zone 7 (Fig. 1) that remained untreated.

Between March and May 2004 one face of an intertidal polluted rock located in this zone was treated three times with sunflower biodiesel using a hand sprayer (at days 0, 30 and 60 of the experiment). Pure biodiesel (B100) was produced through the transesterification of the triglycerides of the sunflower biodiesel with methanol and sodium hydroxide. Its approximate elemental composition was 77% C, 12% H and 11% O. Together with the chemical analysis of selected oil samples a photographic monitoring of the treated face and of one of the untreated faces of the rock was carried out in order to assess the improvement in the appearance of the polluted surface. The digital photographs were converted to monochrome images with 256 gradations of grey (level of darkness between 0 and 255) with image processing software (Adobe® Photoshop® CS2, version 9.0). Then, the pixels were classified into two groups according to the darkness of they grey colours (black pixels in group 1 and white pixels in group 2) [8]. The black pixels represented the areas which were coated by the residual oil whereas the white pixels indicated those areas which were residual oil free. Finally, the histogram command of the software allowed to know the percentages of coverage of the two groups of pixels, which were used to calculate the percentage of the rock surface coated with oil.

2.2. Laboratory analytical techniques

2.2.1. Interstitial pore water and seawater analyses

The dissolved oxygen concentration in the interstitial pore water from the different zones was occasionally determined in the field with the oxygen electrode Oxi 340i (WTW, Germany). The concentrations of nitrate, ammonium and orthophosphate in seawater and interstitial pore water were quantified using spectrophotometric methods [9]. The microbiological analyses were carried out using the most probable number (MPN) technique which allowed the separate enumeration of total aerobic heterotrophic microorganisms, as well as aliphatic and aromatic hydrocarbon degrading microbial populations [10]. The concentration of the 16 PAHs included in the US-EPA list of priority pollutants was also determined in water by GC/MS. The samples were collected towards a 250 mL glass bottle containing 20 mL of isopropanol. In the laboratory the PAHs were extracted with dichloromethane using a separatory funnel and then the extraction solvent was exchanged to isooctane. Finally, the extract volume was adjusted to 1 mL. The determination was carried out in a HP-5890 gases chromatograph with a HP-5972 mass selective detector.

2.2.2. Residual oil analyses

Residual oil from sand samples (previously combined with anhydrous sodium sulphate) and tiles was recovered by ultrasonic extraction with dichloromethane–acetone (1:1 v/v), while samples from rocks were directly dissolved in the same solvent mixture. The extracts were dried over anhydrous sodium sulphate and concentrated to dryness under vacuum. After gravimetric analysis, the residue was dissolved in *n*-hexane and fractionated by alumina column chromatography (US EPA method 3611B [11]). The saturated hydrocarbons were eluted with *n*-hexane, the aromatic compounds with dichloromethane and the

resins with methanol, whereas the asphaltenes remained in the column. Each fraction was concentrated to dryness under a nitrogen stream and weighed in order to determine the percentage of saturates, aromatics, resins and asphaltenes respect to the total weight (SARA analysis). As resins and asphaltenes are considered recalcitrant to the biological treatments while saturates and aromatics are considered degradable [5], an estimation of the extent of oil degradation with regard to the original spilled fuel oil was obtained by using the proportion of saturates and aromatics respect to the polar fractions [12].

For GC/FID and GC/MS analyses the saturated and aromatic fractions were redissolved in dichloromethane and combined to obtain the total GC-detectable TPH content. Prior to analysis *o*-terphenyl (0.01 mg/mL) (Sigma–Aldrich) was added as internal standard. GC/FID analysis was performed with a TRACE GC2000 (Thermo) gas chromatograph equipped with a flame ionization detector (FID). GC/MS analysis where performed in a Hewlett Packard HP5890 Series II gas chromatograph coupled to a HP5989 mass spectrometer (MS). For both GC/FID and GC/MS analyses the gas chromatographs were equipped with a HP-5 capillary column (30 m × 0.25 mm i.d.) with 0.25 μm film thickness and helium used as the carrier gas at a flow rate of 1.1 mL/min. The column temperature was held at 50 °C for 1 min and then programmed to 320 °C at 5 °C/min. This final temperature was held for 10 min. Injector, transfer line and analyzer temperatures were set at 290, 290 and 320 °C, respectively. The samples of 1 μL were injected in splitless mode. The MS was operated in the selected ion monitoring (SIM) mode to detect and quantify the 16 PAHs included in the US-EPA list of priority pollutants, the internal standard *o*-terphenyl, and the $17\alpha(H),21\beta(H)$-hopane used as a conservative internal biomarker [13]. The ions selected to detect all this products were those reported elsewhere [14]. The $17\alpha(H),21\beta(H)$-hopane was purchased from Chiron AS (Trondheim, Norway), while the 16 PAHs standard solution used for quantification was purchased from Dr. Ehrenstorfer (Ausburg, Germany). To estimate the percentages of recuperation of each PAH its concentration was normalized respect to that of $17\alpha(H),21\beta(H)$-hopane. The percentages of PAH degradation were then calculated for the sum of those ratios respect to that obtained for a sample of undegraded fuel from the Prestige used as reference.

The elemental analysis to determine the oxygen content of the extract of fuel oil brought to constant weight was conducted in an EA 1108 elemental analyzer by Carlo Erba.

3. Results and discussion

3.1. Interstitial pore water and seawater

The level of nutrients was found to be similar in all the zones of the beach, regardless of the application of bioremediation products. In general, between April 2003 and September 2004 the concentration of nitrate in the interstitial water samples ranged between 0.25 and 1 mg N–NO$_3$/L, the concentration of ammonia between 0.15 and 0.4 mg N–NH$_4$/L and the concentration of orthophosphate between 0.05 and 0.15 mg P–PO$_4$/L.

The only effect of the addition of nutrients was the appearance of peaks of concentration (maximum 4.5 mg/L of inorganic N and 0.3 mg/L of P) during a few days after the application, but these high values decreased quickly towards the background levels. This could be easily explained since the tides washed away the water-soluble amendments applied in the intertidal zone. However, the levels of nutrients were already relatively high because of the runoffs coming from the agricultural lands close to the beach. It is thought that concentrations approaching 1–2 mg of inorganic nitrogen/L of interstitial pore water should support near optimum hydrocarbon biodegradation activity [15] so the nutrient concentration in the sandy area of Sorrizo could be considered to be appropriate for biodegradation processes.

Microbial analysis carried out between April and September 2003 showed the presence of both aliphatic, 10^3–10^5 MPN/mL, and aromatic-degrading microbial populations, also 10^3–10^5 MPN/mL, in the interstitial pore water of every zone. In general, there were no differences in the MPN levels attributable to the addition of the products between the control, the bioaugmentation- and the biostimulation-treated zones. The percentages of aliphatic and aromatic degraders with regard to heterotrophic microorganisms reached values of 10–40% and 5–20%, respectively, these values confirming that an important fraction of the microorganisms present in the beach were able to degrade hydrocarbons. These results can be explained by the sporadic exposure of this zone to hydrocarbons and to the fact that the number of degraders might be increased by many orders of magnitude after being exposed to oil.

Analyses of the interstitial pore water and seawater samples carried out between April 2003 and February 2004 showed the presence of practically all the 16 PAHs included in the US-EPA list of priority pollutants, whose sum of concentrations gradually decreased with time (Table 1). They were detected in seawater and in zones 1, 2 and 5 until 8 months after the accident and until 11 months after in zones 3 and 4. These differences in the persistence of PAHs were attributed to the heterogeneity of the beach, zones 3 and 4 presenting initial values 10 times higher that the rest. None of the treatments seemed to accelerate the disappearance rate of the PAHs from the interstitial water. The

Table 1

Concentration of the sum of the16 US-EPA priority pollutant PAHs in seawater and interstitial water samples

Date	Concentration of PAHs (μg/L)					
	Seawater	Zone 1	Zone 2	Zone 3	Zone 4	Zone 5
14/04/03	0.107	–	0.041	0.055	7.149	–
30/04/03	0.161	0.049	0.127	2.054	3.426	0.15
28/05/03	0.048	0.287	0.04	0.105	0.737	0.33
18/06/03	0.162	0.214	0.328	0.262	1.35	0.323
21/07/03	0.085	0.075	0.103	0.202	0.45	0.072
25/08/03	ND	ND	ND	0.04	0.015	ND
24/09/03	ND	ND	ND	0.07	0.015	ND
24/10/03	ND	ND	ND	0.031	0.055	ND
10/12/03	ND	ND	ND	ND	ND	ND
12/02/04	ND	ND	ND	ND	ND	ND

ND: not detected.

most persistent compounds were phenanthrene and anthracene that were detected until October 2003, 11 months after the arrival ashore of the first black tide.

3.2. Sand

The TPH content of sand samples collected during the study from all zones ranged between 0.02 and 0.6 g/kg of dry sand, with fluctuations not attributable to the effect of the treatments. In fact, the highest values were found in zones 3 and 4, which was in agreement with the highest values and persistence of PAHs in the interstitial pore water of these zones. These low values were consequence of the poor penetration of the fuel oil into the sand due to the high viscosity of the water-in-oil emulsion (100,000 cSt at 15 °C), and to the preliminary physical clean up that was carried out on the beach before the beginning of the study. Another evidence of the low degree of pollution in sand was the concentration of 16 PAHs included in the US-EPA list of priority pollutants, which was in general lower than 3 mg/kg of dry sand.

In addition to its low concentration, the residual oil extracted from sand samples before starting up the treatments already presented a high extent of degradation. From the gravimetric analysis of the SARA fractions it was estimated that at least a degradation of 85% saturates and 75% aromatics had occurred, whereas the hopane normalized data showed between 20% and 80% of degradation for the target PAHs, depending on the sample. This high extent of degradation could be also observed by comparing the chromatograms of the saturated fraction from the original fuel oil emulsion, and from a sand sample obtained before the application of the bioremediation products (Fig. 2). The latter showed a depletion of practically all the resolved peaks of the chromatogram, including *n*-alkanes (from C_{13}–C_{32}) and some branched alkanes (i.e. pristane and phytane, generally used as internal biomarkers due to its high resistance to biodegradation). These high values of degradation only 5 months after the oil spill (April 2003) can be attributed to the appropriate environmental conditions for biodegradation processes, such as the aforementioned concentration of nutrients and presence of hydrocarbon-degrading microorganisms. Furthermore, the concentration of oxygen in the interstitial pore water was usually higher than 2 mg/L in all zones, and seawater temperatures ranged between 10 and 17 °C, which were near the optimal conditions of 15–20 °C referred by Jordan and Payne [16] for oil biodegradation in marine environments.

Lee and Levy [17] found that for low concentrations of a waxy crude oil the biodegradation proceeded rapidly in both an unfertilized control and a fertilized plot, and the obtained results in this study also indicated a fast degradation of fuel oil hydrocarbons in sand. In fact, 4–5 months after the arrival of the first black tide ashore, the percentages of degradation were high and remained within the same range in all the zones throughout the study (until September 2004). Due to this reason, it could not be concluded that any of the bioaugmentation or nutrient formulations have enhanced the biodegradation rates.
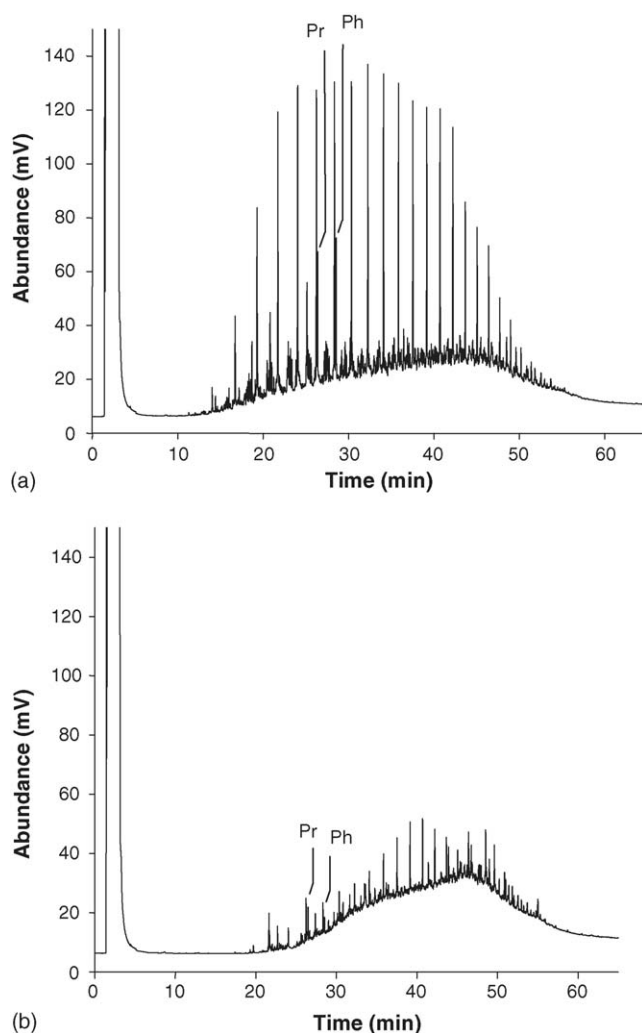

Fig. 2. GC/FID chromatograms of the aliphatic fractions of the water-in-oil emulsion of the Prestige (a) and of the residual fuel oil extracted from a sand sample collected from the beach in April 2003 before any treatment (b), Pr: pristane; Ph: phytane.

3.3. Rocks

Regarding the results obtained in rocks, those treated with a high pressure water solution of L1800 in zone 4 showed a partial detachment of the fuel oil stuck to the rocks, but the applications did not seem to enhance the extent of biodegradation of the remaining residual oil. The degradation percentages of the aromatic fractions from oil recovered from rocks located in zones 1–5 are shown in Fig. 3. The great variability of the results was primarily attributed to the spatial heterogeneity (for example the different thickness of the layer of oil) and secondly to the variability introduced by the sampling procedure. Unlike the sand, the percentages of degradation of oil from rocks increased with time, but due to the aforementioned high variability of the results, it could not be stated that there were significant differences in the degradation extent between the different zones. The percentages of degradation in zone 4 were similar to those found in the other zones despite the application of a product containing a surfactant (L1800) in this area. The percentages of degrada-
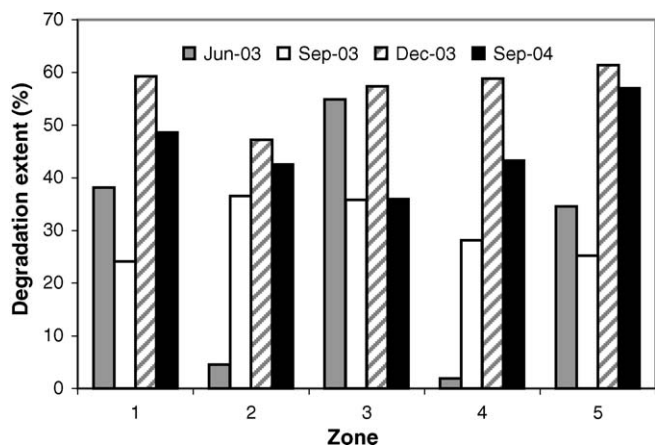
Fig. 3. Degradation percentages of aromatics from the fuel oil from rocks of zones 1–5 in four of the samplings.

tion at the end of the study were comparable to those observed in sand at the beginning of the study (70–80% for saturates and around 60% for aromatics). The accumulation of the recalcitrant compounds (resins and asphaltenes) might have limited a further degradation of the biodegradable fractions, which appeared to be "trapped" in the polar organic matrix suffering a reduction on their bioavailability. In fact, on the surface of the residual oil deposited on the rocks it was observed an asphalt-like layer of weathered oil that prevented the access of microorganisms, nutrients or oxygen to the fresher oil underneath.

3.4. Granite tiles

The results reported above indicated that the concentration of residual oil in the sand was very low and that the extent of degradation of the pollutants was relatively high. Nevertheless, several months after the oil spill, the rocks remained coated with a layer of residual oil, which was an important environmental and social problem. The results from the analysis of the oil from rocks did not allow concluding if any of the products (nutrients, microorganisms or surfactants) enhanced the biodegradation rates due to the heterogeneity of environmental samples. Thus, a series of experiments with granite tiles coated with slightly degraded oil from the water-in-oil emulsion of the Prestige were started in June 2003 in order to overcome the high variability. As the accident took place during a spring tide period and under bad weather conditions, the fuel arriving ashore affected not only the intertidal rocky areas, but also the supra-littoral ones, so the study was conducted both in the supra-littoral and intertidal areas of the beach. Unfortunately, it was not possible to carry out a monitoring of the amount of residual fuel oil stuck on the tiles at each sampling time because of the successive storms that affected the beach, with the resulting loss of residual oil by the friction of the surface of oil with the sand and the materials pulled to the shore by the sea.

The results inferred from the SARA analysis indicated that in the supra-littoral zone the extent of the degradation was independent of the applied treatment (70% for saturates and aromatics after 30–60 days that kept until the end of the experiment)
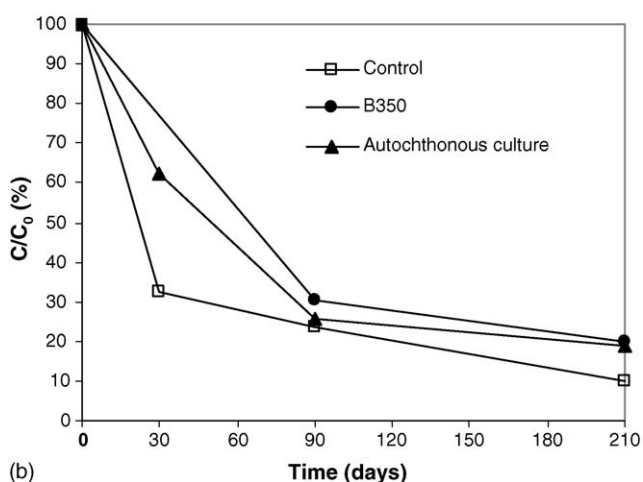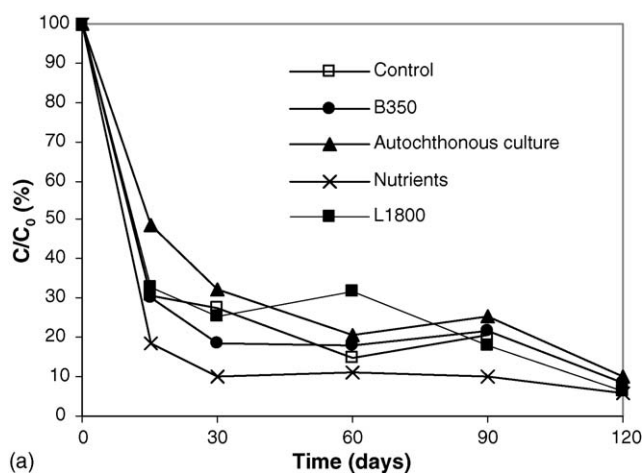

Fig. 4. Change in C/C_0 ratios with time in the supra-littoral tiles (a) and intertidal tiles (b). The C/C_0 (%) values are the percentage ratios of the hopane-normalized values of the sum of the 16 target PAHs at each sampling time to those at day 0.

whereas in the intertidal zone, the two assayed treatments accelerated fuel oil degradation during the 1st week, although after 210 days the percentage of degradation of aromatics was 75% in all cases. Fig. 4 shows the percentages of decrease of the hopane-normalized values for the 16 PAHs in the supra-littoral and intertidal tiles throughout the study. On the supra-littoral tiles the application of the bioaugmentation products did not enhance the degradation of PAHs respect to untreated controls, whereas the application of nutrients resulted in a better degradation percentage during a first experimental period. Surprisingly, in the intertidal tiles at the beginning of the treatments the best results were obtained in the control tiles, which was opposite to that deducted from the SARA analysis. However, the differences in the extent of degradation disappeared after 4 months, which probably reflected that the effect of the assayed treatments had not been significant with regard to the natural attenuation.

It was striking that the percentages of the target PAHs degradation were very high (more than 80% at the end of the study) and that the values reached in supra-littoral tiles were very similar to those of the intertidal tiles. The latter should be a more appropriate environment for biodegradation since they covered by the sea an important fraction of the day. The exposure of
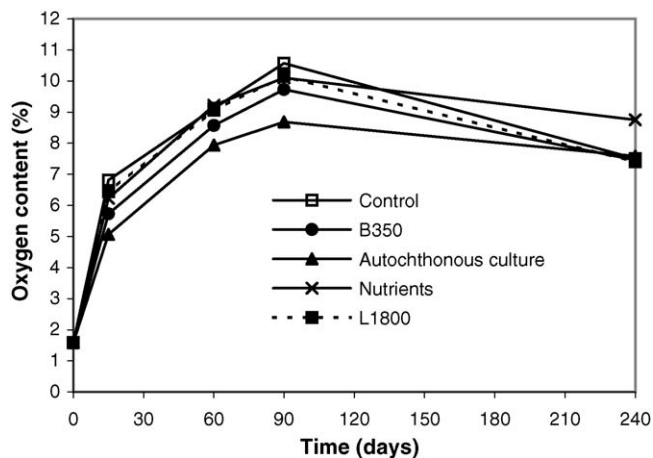
Fig. 5. Evolution with time of the oxygen percentage in the fuel from supra-littoral tiles.

rocks to sunlight was thought to have favoured photooxidation processes, that undoubtedly contributed to the high extent of degradation observed. In the presence of oxygen, sunlight has energy enough to transform some complex and/or recalcitrant oil compounds, such as the high molecular weight PAHs, into more polar compounds that migrate to the resin and/or asphaltene fraction. In these photo-chemical reactions the aromatic compounds are transformed into ones containing oxygen such as phenols, alcohols, ketones, aldehydes and carboxylic acids [18].

In order to check the formation of partially oxidized compounds due to both photooxidation and biological activity, and their accumulation in the polar fractions, the percentage of oxygen of the oil from tiles was determined by elemental analysis. Fig. 5 summarizes the increase of the oxygen content in the residual oil from the supra-littoral tiles, and it shows that the percentage of oxygen increased from 1.6%, originally found in the oil of the Prestige, to approximately 8% in 90 days. However, the oxygen content in the intertidal tiles, which were exposed fewer hours to sunlight, also increased but to a lesser extent (until less than 5%).

The oxygen content of the residual fuel oil and of the saturate, aromatic and resin fractions was determined for samples of non-degraded and degraded oil from supra-littoral tiles, and the oxygen content of the asphaltenes was calculated using mass balances (Table 2). The percentage of oxygen increased with degradation in aromatics and resins but mainly in asphaltenes,

since in the latter the oxygen percentage increased from less than 1% to more than 9%. These results, together with those from the SARA analyses, showed that the polar oxygenated molecules coming from the partial degradation of TPHs migrated to the asphaltenes. In laboratory assays Oudot [6] determined gravimetrically 11% degradation of the no. 6 fuel oil of the Erika and reported 17% increase in the mass of the polar fraction that could be originated by the partial oxidation of hydrocarbons. Due to the reasons indicated above, the extent of mineralization of fuel oil gravimetrically determined should be corrected, taking into account the incorporation of oxygen into the residual fuel oil.

3.5. Biodiesel

As mentioned above, several months after the beginning of the study a black layer of weathered fuel oil remained firmly stuck to the surface of rocks and pebbles due to the accumulation of the components of the fuel oil recalcitrant to biodegradation, so the appearance of the beach hardly changed with time. The removal of these residues was difficult and time consuming, so the most efficient technique to clean these polluted surfaces was the high pressure hot water flushing. This technique was used in 1,100,000 m^2 of rocks and man made structures affected by the Prestige oil spill. Nevertheless, this technique is labour intensive and time consuming, consumes energy and a huge amount of wastes is produced. Ansell et al. [2] referred a productivity of 50 m^2/person-day for roughly cleaning rocks affected by the heavy oil spill of the oil tanker Volgoneft in Turkey. An alternative solution could be the use of the sunflower biodiesel.

This oleophilic agent was assayed in one of the faces of an intertidal rock located in an untreated zone of the beach (zone 7, Fig. 1), which was almost completely coated with fuel oil. The biodiesel was applied at a dose of 100 g/m^2, so the quantity added was small enough to avoid that either the biodiesel or the residual fuel oil drip. In fact, it was observed that the biodiesel rapidly incorporated into the layer of fuel oil. Another of the faces of the rock, also heavily polluted, remained untreated as a control. Fig. 6 shows the digital photographs of the biodiesel-treated and the untreated faces of the rock at the beginning (March 2004) and at the end of the experiment (January 2006). These photographs were processed with image processing software and expressed with two different groups of pixels to show the distribution of the oil in a clearer way. A gradual recovery of the aspect of the both faces of the rock was observed, especially in the biodiesel-treated face.

In order to check that the biodiesel accelerated the cleanup of the rock, the percentage of surface coated with oil was obtained from the digital photographs of both faces using an image processing software (Fig. 7). It was observed that despite the initial larger extent of oil coverage in the biodiesel-treated face (97% opposite 76% of the untreated face), after 22 months the percentage of surface coated with oil in the biodiesel-treated face was 16% whereas in the untreated face was 26%. This percentage decreased constantly in the biodiesel-treated face whereas in the untreated face it stopped decreasing after 15 months. It must also be stressed that until this moment, the percentage of coverage decreased faster in the face treated with

Table 2
Oxygen percentage of the four major fractions in non-degraded and degraded fuel oil. The non-degraded oil is that applied to granite tiles and the degraded oil was collected from a supra-littoral tile after 240 days on the beach

	Oxygen percentage (%)	
	Non-degraded fuel oil	Degraded fuel oil
Saturates	0.2334	0.1646
Aromatics	1.0947	4.1265
Resins	3.2533	7.2009
Asphaltenes	<1	9.1308
Residual oil	1.5882	7.4065

Table 3
SARA analysis of the residual fuel oil from the biodiesel-treated and untreated faces of the intertidal rock from zone 7

	Date	Saturates (%)	Aromatics (%)	Resins (%)	Asphaltenes (%)
Before the application	March 04	10	34	18	38
Untreated face	September 04	11	33	15	41
Biodiesel-treated face	September 04	9	24	19	48
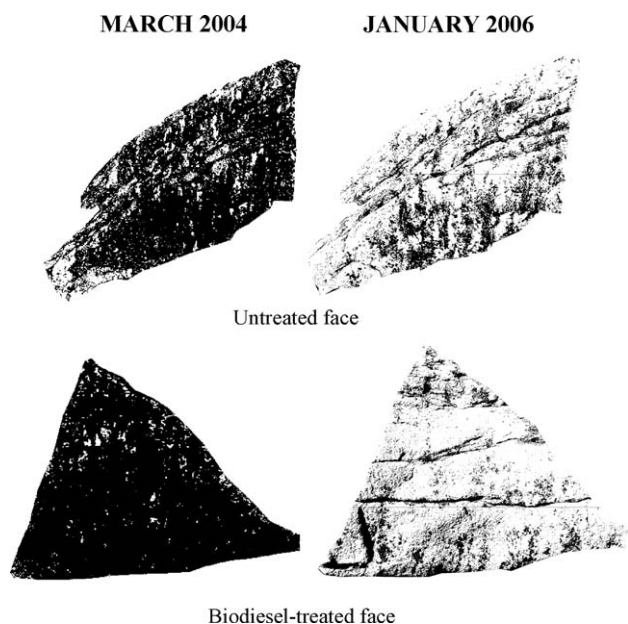
Fig. 6. Digital photographs expressed with two groups of pixels of the biodiesel-treated and the untreated faces of an intertidal rock taken in March 2004 and in January 2006. The black pixels represented the areas covered with residual fuel oil.

biodiesel, and since the data seemed to have a linear correlation they were fitted with a linear regression ($y = -0.123x + 96.1$ and $r^2 = 0.9856$, $y =$ percentage of surface covered with fuel oil and $x =$ time in days). Thus, it was estimated that it would take
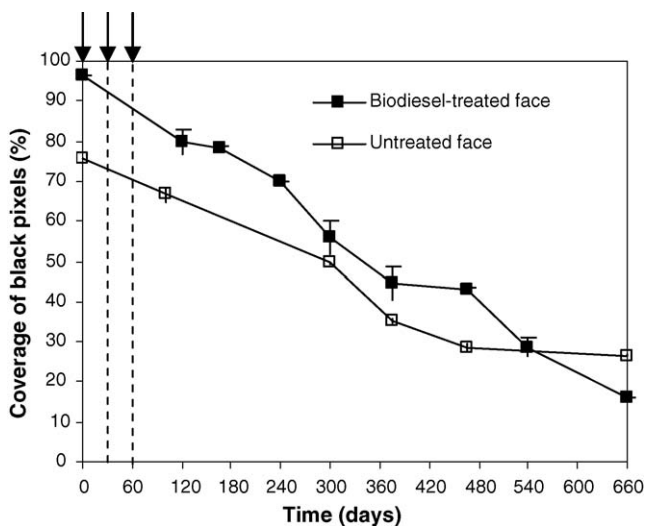

Fig. 7. Percentage of the surface covered with residual fuel oil of the biodiesel-treated and the untreated faces of an intertidal rock. The arrows indicate the dates of application of biodiesel (days 0, 30 and 60 of the experiment).

approximately 26 months for the biodiesel-treated face to be completely cleaned (May 2006). This figure could not be predicted for the untreated face since no changes were observed throughout the last 6 months of the experiment.

Furthermore, the SARA analysis could indicate a possible increase of the degradation of the oil stuck to the biodiesel-treated face in comparison to the untreated face of the rock (Table 3). Neither the percentage of saturates nor the percentage of aromatics of fuel oil from the untreated face decreased throughout the six first experimental months, whereas in the biodiesel-treated face the percentage of aromatics decreased from 33% to 24%.

The use of biodiesel as a solvent had been previously referred for the removal of crude or fuel oil from shorelines or affected sediments [19,20]. Mudge and Pereira [21] referred that the application of biodiesel to contaminated sand with crude oil in laboratory tests stimulated oil degradation. Moreover, Taylor and Jones [22] stated that the addition of biodiesel to soil containing coal tar involved in an increased degradation of coal tar PAH, which was ascribed to tar solubilisation and dispersion thereby increasing the PAH bioavailability. Biodiesel might act as a co-substrate and surfactant, which also accelerated the degradation of fuel oil in the rock.

Some of the characteristics that make feasible the application of biodiesel in the environment are that it is a cheap product, since it has a similar price to the fossil diesel fuel and that it is readily biodegradable and non-toxic [23]. Furthermore, oppositely to the use of hot water flushing this method does not produce wastes that have to be treated afterwards. In this way the high shoreline clean-up costs and the ecological impact of this aggressive technique could be avoided.

4. Conclusions

Neither bioaugmentation formulations nor biostimulation products accelerated the degradation of the residual fuel oil in the affected rocks and sand with regard to natural attenuation. The extent of degradation in sand at the beginning of the study was already very high and it remained practically constant throughout all the study. Hydrocarbon degradation percentages in rocks increased with time, but it could not be stated if the degradation was faster in any of the zones due of the spatial heterogeneity.

The experiments with granite tiles indicated that neither microorganisms nor nutrients stimulated degradation. All the tiles, including the controls, presented a high extent of target PAHs degradation (more than 80%), thus indicating the importance of the natural attenuation processes. The high extent of TPHs degradation did not correspond to a total mineralization of the depleted products, but to the formation of partially oxidized

compounds. Results of the oxygen analysis in the four SARA fractions of the fuel indicated that the oxygen content increased in aromatics and resins but mainly in asphaltenes, which could be explained by the accumulation of products coming from the TPHs partial oxidation.

Despite the high degradation extent, fuel oil pollution still seriously affected rocks which were covered by a black layer of residual fuel. The application of the vegetable biodiesel to a face of a rock gradually enhanced the aesthetical recovery of the rock with time with regard to a control face. The drip of neither the biodiesel nor the residual fuel oil was observed. The results indicated that the incorporation of biodiesel into the layer of fuel oil could accelerate its degradation. Thus, the application of biodiesel could be an alternative to the use of some aggressive physical methods, such as high pressure hot water washing, that was broadly used for cleaning rocks affected by this oil spill.

Acknowledgments

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