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Evaluation of biodiesel as bioremediation agent for the treatment of the shore affected by the heavy oil spill of the Prestige

P. Fernández-Álvarez [a], J. Vila [b], J.M. Garrido [a,*], M. Grifoll [b], G. Feijoo [a], J.M. Lema [a]

[a] *Department of Chemical Engineering, School of Engineering, Avda. Lope Gómez de Marzoa, University of Santiago de Compostela, E-15782 Santiago de Compostela, Spain*
[b] *Department of Microbiology, Faculty of Biology, University of Barcelona, Diagonal 645, E-08028 Barcelona, Spain*

Abstract

The efficiency of different bioremediation products (nutrients, microorganisms and biodiesel) was tested using tiles located in both the supra-littoral and intertidal zones of a beach that was affected by the heavy oil spill of the Prestige. Neither nutrients nor microorganisms meant an improvement with respect to the natural processes. The addition of biodiesel improved the appearance of the treated tiles and apparently accelerated the degradation of the aliphatic and aromatic fractions of the residual fuel oil. Nevertheless, PAHs degradation was similar and very high in all the treatments (80–85% after 60 days). On the other hand, the evolution with time of the amount of vanadium was similar to that of $17\alpha(H),21\beta(H)$-hopane, so it was concluded that vanadium could also be used to estimate the extent of oil degradation in the field. These results also suggested that the residual fuel oil mineralization was very low throughout 1 year in all the treatments. Moreover, the increase of the oxygen content of the residual oil from around 1% till 4–8% indicated that the partial oxidation of hydrocarbons took place, and that the hydrocarbon oxidation products accumulated in the polar fractions. In general, the results pointed out that bioremediation techniques were not suitable for the recovery of shores affected by heavy oil spills.
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Keywords: Prestige oil spill; Bioremediation; Biodiesel; Vanadium

1. Introduction

On 13 November 2002, the single-hulled oil tanker Prestige, carrying 77,000 metric tonnes of M-100 Russian fuel oil (equivalent to the ASTM no. 6 heavy fuel oil), sprang a leak due to a heavy impact during a storm in front of Cape Finisterre (A Coruña, NW Spain). On 19 November 2002 the vessel finally broke in two and spilt 63,000 tonnes of fuel oil that affected 1900 km of shoreline in Northwest and North Spain and West France [1]. In general, an effective physical clean-up was achieved in sand beaches due to the poor penetration of the water-in-oil emulsion in the sediments. However, the fuel oil remained firmly stuck to rocks and pebbles so high pressure hot water flushing was applied for their efficient clean-up. Unfortunately, this technique was labor intensive and time consuming, consumed energy and a huge amount of wastes were produced.

Opposite to the conventional physical clean-up techniques bioremediation might be an environmentally acceptable and cost-effective alternative for achieving the complete removal of fuel oil from rocks or sand. Several shoreline studies have been carried out to assess the effectiveness of bioremediation on oil spills in the field, both after a real spill incident and intentionally releasing oil into the environment [2–5]. Nevertheless, bioremediation amendments were usually tested on sediments whereas few trials were performed with oil polluted rocky substrates [6,7], which were the target of our study since rocks were seriously affected by the Prestige oil spill. Since the water-soluble amendments are easily drained away when applied to a rocky surface, oleophilic fertilizers could be an alternative because they are able to adhere to oil and provide the oil–water interface, where biodegradation mainly occurs [8], with nutrients. Several formulations like Inipol EAP 22 (used after the *Exxon Valdez* accident), S200 (used on a beach affected by the Prestige oil spill), and natural products such as fish and meat meals have been tested in field experiments. Nevertheless, variable results regarding their effectiveness have been obtained [9–11].

* Corresponding author. Tel.: +34 981 563100x16778; fax: +34 981 528050.
E-mail address: equenlla@usc.es (J.M. Garrido).

Biodiesel (vegetable oil methyl esters) has recently been proposed as an alternative oleophilic agent, since it is cheap (similar price to fossil diesel), non-toxic and readily biodegradable [12]. It has been used for the removal of crude or fuel oil from shorelines or affected sediments [13,14] and for the stimulation of crude oil degradation when applied to contaminated sand in laboratory tests [15]. Furthermore, it has been stated that the addition of biodiesel to soil containing coal tar involved the increased degradation of 2 and 3 rings PAHs, which was ascribed to tar solubilisation and dispersion thereby increasing the PAH bioavailability [16]. Only one report describes a mesocosm experiment that shows the potential use of biodiesel as a cleaning agent on a rocky substrate (cobbles and gravel), and the authors concluded that field trials were needed [17].

A previous field study was carried out by the authors on the beach of Sorrizo (A Coruña, NW Spain), affected by the oil spill of the Prestige, in order to assess the efficiency of several water-soluble bioremediation agents on the site and on granite tiles placed on the beach (April 2003–September 2004) [18]. The work on granite tiles had focused on the supra-littoral ones, since the accident had taken place during a spring tide period and under bad weather conditions, so the fuel arriving ashore had reached the supra-littoral rocky areas. It was found that neither microorganisms nor nutrients significantly enhanced the degradation rate of the fuel oil. Furthermore, several months after the beginning of the study the weathered fuel oil remained firmly stuck to the surface of rocks, what meant a great environmental and social problem. Therefore, an assay was carried out applying sunflower biodiesel on a polluted rock and it was observed that this product improved its appearance. Moreover, it seemed that the incorporation of biodiesel into the layer of residual fuel oil could have accelerated its degradation.

The main matters of concern regarding the pollution caused by the Prestige fuel oil spill were the black layer of oil that remained firmly stuck to the rocky surfaces, and the presence of compounds such as PAHs. Thus, the main objective of this new study was to check the effect of biodiesel on the appearance of a heavy oil polluted rocky surface and on the degradation of this heavy oil by using tiles located in both supra-littoral and intertidal zone of the beach of Sorrizo. The other aim was to assess the effectiveness of the water-soluble amendments previously tested on the beach, in the intertidal zone. Finally, vanadium was evaluated as an alternative internal marker to $17\alpha(H),21\beta(H)$-hopane in order to estimate the degradation of the components of the oil in environmental samples.

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. The average temperature throughout the *in situ* experiment (April 2004–March 2005) was around $14.1°C$, typical of the Atlantic climate. In this zone of the Atlantic Ocean there are two tides per
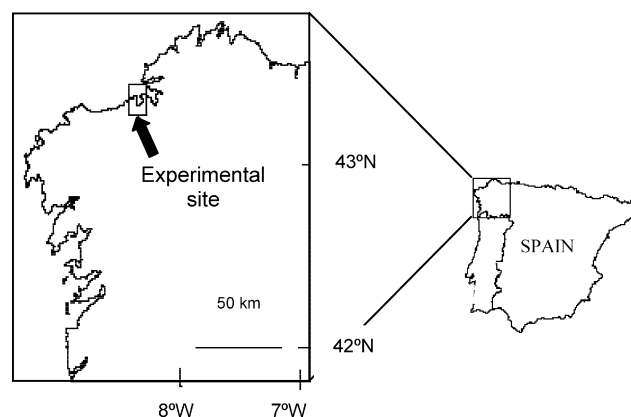

Fig. 1. Location of the beach of Sorrizo (A Coruña, NW Spain).

day, with a tidal range between 2 m (neap tides) and 4 m (spring tides).

The experimental unit was a granite tile of $15\,cm \times 15\,cm \times 1\,cm$, since this is the most usual rocky substrate in the Galician coastline, and groups of nine tiles were gathered on wooden boards to form an experimental panel [19]. The oil used for this experiment was slightly weathered heavy fuel oil from the Prestige which had been collected from the sea, and which was found to be comprised of 24.1% saturated hydrocarbons, 38.3% aromatic hydrocarbons, 6.5% resins and 31.1% asphaltenes. Approximately 13 g of this water-in-oil emulsion of the Prestige were applied to each tile using a paintbrush.

The assayed products included some of the bioremediation formulations previously tested in this site. They were the water-soluble commercial fertilizer Nitrophoska$^®$ Suprem (NPK) (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); the commercial bioaugmentation product B350 (aerobic and facultatively anaerobic hydrocarbon-degrading bacteria and micronutrients; Bio-Systems Co., USA); an autochtonous microbial inoculum (UB), obtained by enrichment of fuel-degrading populations from the beach of Corrubedo (A Coruña, NW Spain), also affected by the spill; and the oleophilic agent sunflower biodiesel. Two of the panels were located in the supra-littoral zone (SL): untreated control (SL CT) and biodiesel + UB + NPK (SL BD); and five of them were placed in the intertidal zone (IT): untreated control (IT CT), NPK (IT NPK), B350 + NPK (IT B350), UB + NPK (IT UB) and biodiesel (IT BD). These intertidal panels (Fig. 2) remained immersed in seawater approximately half of the time since they were located in the middle of the tidal range. The bioremediation amendments were monthly applied with a hand sprayer from April 2004 to February 2005, except for the biodiesel, which was only applied until July 2004 (four applications in the first 90 days). For the commercial products the doses used were those recommended by the manufacturers. When needed, $100\,g/m^2$ of biodiesel, and $0.05\,L/m^2$ of the indigenous microbial consortia UB, were added. The relatively low dose of applied biodiesel avoided that the mixture residual oil–biodiesel was drained away. The pure biodiesel (B100) was produced at the laboratory through the transesterification of

Fig. 2. Photograph of the experimental panels with tiles located in the intertidal zone of the beach.

the triglycerides of sunflower oil with methanol and sodium hydroxide.

The tiles were sampled at days 0, 30, 60, 90, 150, 240, and 360 to monitor the extent of degradation of the residual oil until March 2005. Furthermore, a periodic photographic monitoring was carried out in order to assess the improvement in the appearance.

2.2. Laboratory analytical techniques

2.2.1. Extraction and fractionation of the residual fuel oil: gravimetric analysis

Prior to extraction each granite tile was divided in two parts. The residual oil from both portions was recovered by ultrasonic extraction with dichloromethane–acetone (1:1, v/v). The extracts were dried over anhydrous sodium sulphate and concentrated to dryness under vacuum, and then they were gravimetrically analyzed to determine the total solvent extractable material (TSEM). Then, the residue was dissolved in *n*-hexane and fractionated by alumina column chromatography (US EPA method 3611B [20]). The fractions were concentrated to dryness under a nitrogen stream and weighed in order to determine the ratio of saturates, aromatics, resins and asphaltenes respect to the total weight. The sum of the mass of saturates and aromatics was defined as the gravimetrically determined total petroleum hydrocarbons (GTPH).

2.2.2. Gas chromatography/mass spectrometry (GC/MS) analysis

The analytical procedures and instrumentation for these analyses have been described elsewhere [18]. The MS was operated in the selected ion monitoring (SIM) mode to detect and quantify the 16 PAHs included in the EPA's list of priority pollutants and the 17α(H),21β(H)-hopane, used as a conservative internal biomarker [21]. To estimate the remaining percentage of each PAH at each sampling time its concentration was normalized respect to that of 17α(H),21β(H)-hopane and divided by the normalized concentration of the PAH in the fuel oil before any

treatment, as it is shown in the following equation:

$$\%\text{Remaining} = \left[\frac{C_t/H_t}{C_0/H_0} \right] \times 100$$

where C_t and H_t are the concentrations of the target analyte (PAH) and hopane at time t, respectively, and C_0 and H_0 are the concentrations of the same components at the beginning of the experiment ($t = 0$).

2.2.3. Elemental analysis

The analyses to determine the oxygen content of dried extracts of fuel oil were conducted in an EA 1108 elemental analyzer by Carlo Erba.

2.2.4. Inductively coupled plasma optical emission spectrometry (ICP-OES)

The vanadium content of the residual oil was determined by ICP-OES in a Perkin-Elmer (Wellesley, MA) Optima 4300 DV apparatus. An aliquot of the extracted residual oil was taken to dryness and dissolved into 15 mL of tetralin (1,2,3,4-tetrahydronaphthalene), since this solvent was found to be suitable for the determination of trace elements in petroliferous materials [22]. Each sample was analyzed in triplicate at several wavelengths (270.093, 290.880, 292.402, 309.310, 310.230 and 311.071 nm).

3. Results and discussion

3.1. Evolution of the appearance

Fig. 3 shows the initial appearance of the tiles (15 April 2004) and the aspect of the control supra-littoral tiles and the biodiesel-treated tiles at the end of the experiment (31 March 2005). No significant changes were observed throughout the study in the appearance of the supra-littoral control, and the same happened with the intertidal control and those tiles treated with nutrients and/or microorganisms. With regard to the supra-littoral biodiesel-treated tiles, some 'cracks' arose on the fuel oil from the first weeks. But doubtless, the greatest evolution took place after December 2004, 5 months after the last application, when the fraction of oil-covered surface noticeably diminished. This positive evolution continued until the end of the experiment. The intertidal biodiesel-treated tiles already showed a highly cracked fuel oil surface during the first experimental months, and at the end of the study the fraction of oil-covered surface was also significantly lower than the initial after a constant evolution throughout 12 months.

Thus, it was clear that the application of biodiesel caused an improvement of the appearance of the tiles polluted with slightly weathered heavy fuel oil from the Prestige and located on the beach. This observation had been already made in our previous study [18] where the percentage of surface coated with oil of an intertidal rock had appreciably decreased 22 months after the first application of biodiesel. In that case the rock was polluted with highly weathered oil, since the experiment had begun more than a year after the oil spill.

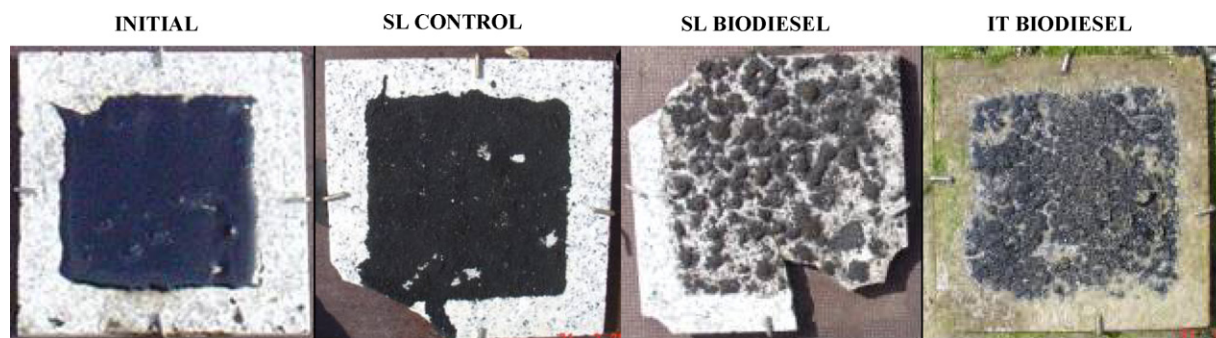**INITIAL** **SL CONTROL** **SL BIODIESEL** **IT BIODIESEL**

Fig. 3. Photographs of the tiles at the beginning of the study (15 April 2004) and 1-year later (31 March 2005). SL: supra-littoral, IT: intertidal.

For the most heavily oiled rocky shores the oil remains for a long time in the form of asphalt "pavement" which is rather difficult to remove [23]. Thus, a possible application of the biodiesel could be the removal of highly weathered heavy fuel oil stuck on rocks, which requires a lot of time, is very expensive and is very aggressive to the environment when performed by high pressure hot water flushing. In a separated assay carried out on the beach of Sorrizo this oleophilic agent was applied to a rock coated with highly weathered oil, and once the methyl esters had incorporated into the oily phase the rock was washed with pressurized ambient temperature seawater for releasing the mixture fuel oil–biodiesel. It was observed that the time invested in cleaning up the rock with only freshwater was twice as long as with biodiesel having been previously applied to the surface. In this way the shoreline clean-up costs and the ecological impact of this aggressive technique could be reduced.

3.2. Loss of GTPH

Table 1 summarizes the percentages of depletion of the amount of GTPHs in the control and biodiesel-treated tiles. Unfortunately, some non-consistent results were obtained since it was not possible to carry out a proper monitoring of the residual amount of fuel oil because of the successive storms which affected the zone. They caused the movement of the experimental panels resulting in an accidental loss of residual oil by the breakage of the tiles and/or the friction of the surface of oil with the sand and the materials pulled to the shore by the sea. In general, there was a significant reduction in the content of GTPHs in both control and treated tiles, which was therefore attributed to natural processes. The percentage of reduction of GTPHs was lower in the intertidal control (70% at the end of

the experiment), whereas the percentages achieved in the supra-littoral control (around 90%) were very similar to those found in the biodiesel-treated tiles. The results from the intertidal tiles treated with nutrients and/or microorganisms (data not shown) were very similar to those observed for the intertidal control, so these amendments did not enhance the removal of GTPHs. Nevertheless, the reduction in GTPH did not involve a decrease of the area coated with oil in the supra-littoral control tiles or an improvement of the appearance of the rock. The fact that the depletion of GTPHs did not significantly change the aspect of the oil was consistent with the asphaltene-range hydrocarbons being responsible for the tar-like appearance of the layer of oil.

The reduction of GTPHs could be due to either a washing effect or a stimulation of the degradation processes. In order to distinguish between washing and degradation the ratio of GTPH to TSEM was determined (Fig. 4). In the literature it is assumed that the more weathered is a sample of oil, the smaller this ratio is [24], so this gravimetric index was used to obtain a first quantification of the degradation of saturates and aromatics. After 90 days the concentration of GTPH in the supra-littoral tiles decreased from 0.62 to 0.40 following a similar trend in both the control and biodiesel-treated tiles (35% degradation). Afterwards, in the control tiles the ratio remained practically constant until the end of the experiment, whereas in the biodiesel-treated ones the concentration still decreased until 0.30 at 150 days (52% degradation), and then it remained constant. In the intertidal tiles the results were very similar for all the treatments, except for the biodiesel-treated tiles. In general, the concentration of GTPH decreased from 0.62 to 0.50–0.55 (19% degradation), whereas in the biodiesel-treated tiles this ratio decreased until approximately 0.35 in 150 days (44% degradation), and then it kept practically constant.

In general, the decrease of the GTPH to TSEM ratio took place during the first 90–150 days of the experiment for all the treatments. Furthermore, the natural attenuation processes seemed to be more important in the supra-littoral zone since at the end of the experiment the values of the ratio were 0.35 and 0.45 in the supra-littoral and intertidal control tiles, respectively. It seemed that the application of biodiesel caused a decrease in the concentration of GTPH, which was practically the same in the supra-littoral and intertidal zones. In order to check how the mass of added biodiesel could have influenced the determination of the GTPH to TSEM ratio in the biodiesel-treated tiles, a fractionation of the oleophilic agent was carried out.

Table 1

Percentages of depletion of the amount of GTPHs in the control and biodiesel-treated tiles

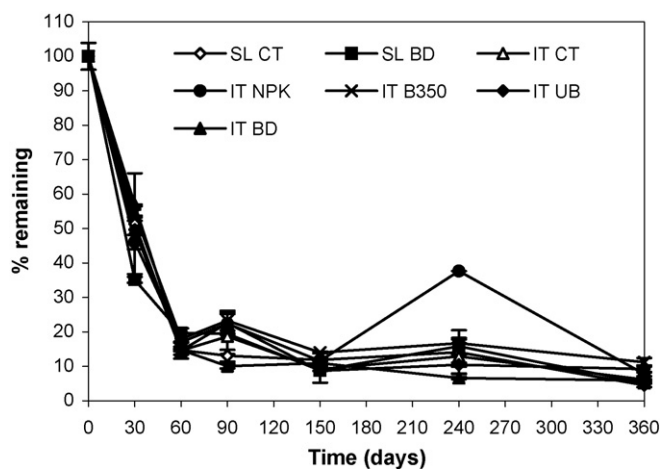| Experimental panel	Time (days)						
	0	30	60	90	150	240	360
SL control	0	51	65	84	90	75	87
SL biodiesel	0	68	69	70	78	83	89
IT control	0	41	10[a]	66	63	47	68
IT biodiesel	0	12[a]	69	83	70	74	86

[a] Non-consistent results.

Fig. 5. Remaining percentages of the sum of the 16 EPA priority pollutant PAHs for the different treatments.

and physical losses [2,21]. Fig. 5 shows the evolution of the percentages of the hopane-normalized values for the sum of the 16 PAHs at each sampling time with respect to those obtained at day 0 (remaining percentages) in the supra-littoral and inter-tidal tiles. The results show the dramatic degradation of PAHs in all tiles during the first 60 days (remaining percentages of 15–20%). From this moment until the end of the experiment the percentages of remaining PAHs decreased more gradually reaching values of 5–10% at day 360. No significant differences among the bioremediation treatments or the different locations on the beach were observed, this indicating that the obtained disappearance patterns might be those of natural processes.

A study of the evolution of the disappearance patterns for individual PAHs showed, as previously observed for the sum of the 16 target PAHs, that there were not significant differences between treatments and controls (data not shown), but differ-ent loss rates were observed depending on the PAHs molecular weight. Fig. 6 shows the evolution of the remaining percent-ages of PAHs according to number of rings in the supra-littoral control tiles as an example. The extent of degradation of PAHs declined with increasing benzene ring number in all the controls

Fig. 4. Ratio of GTPH to TSEM in supra-littoral (a) and intertidal tiles (b).

Approximately 80% of the biodiesel eluted in the aromatic frac-tion, whereas the remaining 20% eluted with the methanol or remained in the column (polar compounds). Thus, the increase of mass affected mainly the aromatics, so this could lead to underestimate the GTPH to TSEM ratio. Nevertheless, it must be taken into account that biodiesel oxidation could occur under some conditions [25,26]. It was supposed that a fraction of the products of oxidation of biodiesel could have eluted together with the polar fraction of the oil, affecting the determination of the GTPH/TSEM ratio. However, no literature on the oxidation of biodiesel in the environment (air, sunlight, water and so on) was found.

3.3. Loss of total target PAHs

To determine the possible effect of the treatments on the degradation of the 16 PAHs included in the US-EPA list of priority pollutants, the TPHs of the residual fuel (total petroleum hydrocarbons, i.e. saturates and aromatics) were ana-lyzed by GC/MS. All analyte concentrations were normalized to $17\alpha(H),21\beta(H)$-hopane concentration, used as the conservative internal biomarker, to distinguish between degradation losses
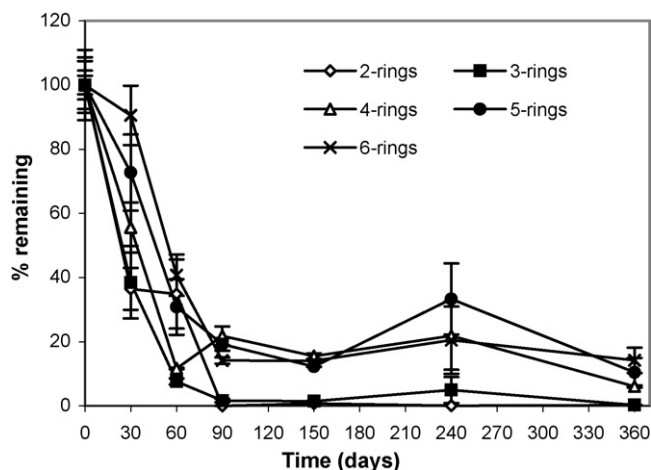

Fig. 6. Remaining percentages of the sum of the 2-, 3-, 4-, 5- and 6-ring PAHs in the supra-littoral control tiles.

and treatments tiles. As expected naphthalene (2 rings) was readily depleted, reaching nearly negligible levels after only 60 days of treatment. 3-ring PAHs were depleted to a lesser extent and were detected until the end of the experiment, even though at 90 days the percentages were only about 5–10% with respect to time 0. The highest molecular weight PAHs with 4, 5 and 6 benzenic rings showed differences in their degradation only during the first 30 days, reaching all of them remaining percentages of 20–35% after 90 days that remained constant until the end of the *in situ* experiment. Therefore, the loss patterns of the 2- and 3-ring PAHs were alike, and the same happened with the 4-, 5- and 6-ring PAHs. It is known that PAHs with 2–4-rings are biodegradable under aerobic conditions but the heaviest PAHs can only be biodegraded through co-metabolism [8]. But besides biodegradation, photochemical reactions can also cause the loss of PAHs. The experimental tiles located on the beach were exposed to sunlight which in the presence of oxygen has energy enough to transform some complex and/or recalcitrant oil compounds, such as the high molecular weight aromatic compounds, into more polar compounds that migrate to resins and asphaltenes [27]. These processes could explain the high extent of depletion of the PAHs with 5 and 6 rings.

PAHs degradation is a parameter commonly used for the assessment of oil biodegradation efficacy. The extent of degradation showed in Fig. 5 (PAHs) is much higher than that observed in Fig. 4 (GTPHs) but it must be stressed that the 16 PAHs included in the US-EPA list of priority pollutants mean only the 0.14% of the mass of the Prestige fuel oil, so the degradation rates of PAHs might not be representative of other hydrocarbons of the fuel oil.

3.4. Vanadium as alternative internal marker

$17\alpha(H),21\beta(H)$-hopane is considered to be an excellent internal marker to evaluate the extent of oil degradation since it is much more resistant to microbial biodegradation than most hydrocarbon components. However it is a trace component, so the estimation of the extent of degradation based on the amount of this marker might involve a certain error, mainly for the assessment of degradation of significant fractions of oil, such as saturates and aromatics. On the other hand, vanadium is one of the most abundant metals in crude oils; in fact the content of vanadium in the Prestige fuel oil was $170 \pm 5 \, \mu g/g$ [28]. Moreover, the majority of the V content of a crude oil is associated with porphyrins, which are stable [29], so this metal has been proposed as an alternative internal marker to $17\alpha(H),21\beta(H)$-hopane [30]. Another of the advantages of vanadium is that its determination is cheaper and requires less sophisticated equipment than the hopane.

Fig. 7 shows the evolution of the concentration of vanadium in fuel oil in the supra-littoral and intertidal control and biodiesel-treated tiles. The analyses of vanadium were not carried out for the tiles treated with water-soluble bioremediation amendments since they did not seem to improve the natural degradation processes at all. The content of vanadium ranged around 180 and 200 $\mu g/g$ in the supra-littoral and in the intertidal control, respectively, and it could be considered that it remained practi-
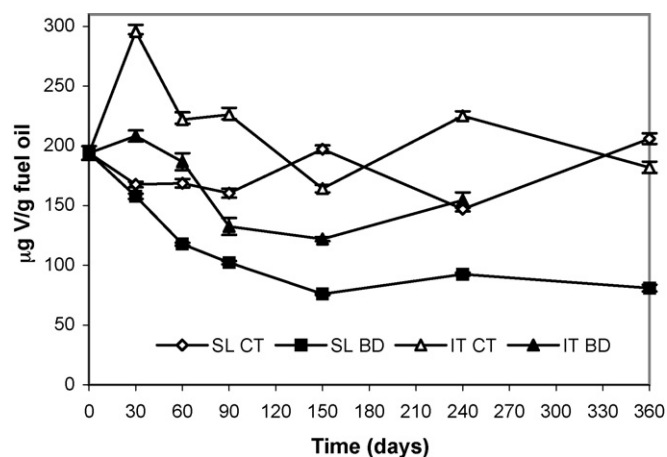

Fig. 7. Concentration of vanadium in the fuel oil of the control and biodiesel-treated tiles. The concentration of vanadium for IT BD after 360 days was not available.

cally constant with time. On the other hand, the concentration of vanadium decreased with time in the biodiesel-treated tiles, especially in the supra-littoral ones in which it was up to 80 $\mu g/g$.

Sasaki et al. found from their laboratory assays that the concentration of vanadium relative to the weight of crude oil increased as oil mineralization took place, whereas it was practically constant in a control [30]. Nevertheless, in our case there are two additional processes which could cause a decrease of the concentration of V: the addition of a mass of biodiesel and the partial oxidation reactions. The former means the increase of the TSEM, decreasing in this manner the ratio of V to residual oil. The effect of this factor could be easily observed in Fig. 7, since the concentration of V decreased until 150 days in the biodiesel-treated tiles, which was the last sampling made after a biodiesel addition. On the other hand, the hydrocarbons of fuel oil were subjected to partial oxidations to more polar products, resulting in the incorporation of oxygen into the residual oil and in the decrease of the ratio of V to residual oil again. As an example, Oudot determined gravimetrically 11% degradation of the no. 6 fuel oil of the Erika and reported 17% increase in the mass of the polar fraction in laboratory assays [31]. Thus, although a certain extent of mineralization had taken place, this could not mean an increase of the vanadium content. Table 2 shows this increase of the oxygen percentage related to partial oxidations in both control tiles with time, but especially in the supra-littoral ones. The oxygen content increased more in the biodiesel-treated ones, but this was partly due to the fact that the added biodiesel had up to 10% of oxygen, opposite to the 1% of the Prestige fuel oil.

Table 2
Oxygen percentages in the fuel in the control and biodiesel-treated tiles

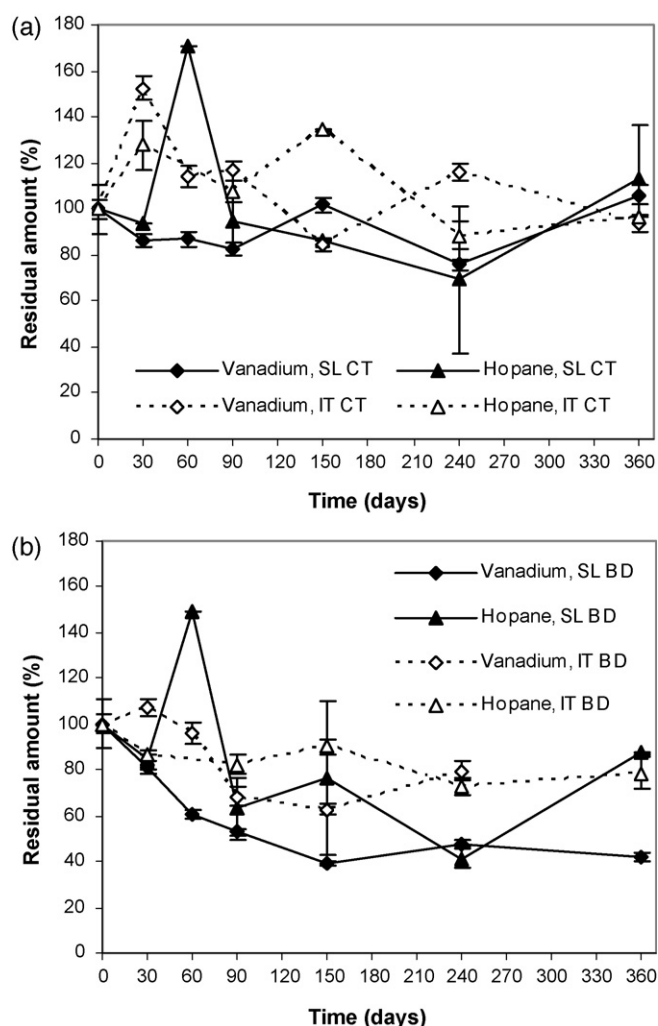| Experimental panel	Time (days)						
	0	30	60	90	150	240	360
SL control	1.06	2.21	3.78	5.00	5.16	4.25	4.48
SL biodiesel	1.06	3.14	6.91	8.48	9.37	9.76	8.03
IT control	1.06	2.12	2.95	4.08	3.69	3.00	3.99
IT biodiesel	1.06	2.86	5.70	10.11	7.24	9.83	6.63

Fig. 8. Residual percentages of vanadium and 17α(H),21β(H)-hopane in the control (a) and biodiesel-treated tiles (b). The concentration of vanadium for IT BD after 360 days was not available.

Furthermore, the increase of the oxygen content was higher in the supra-littoral control tiles than in the intertidal ones. This fact could explain the differences between both controls in the GTPH to TSEM ratios in Fig. 4.

Fig. 8 shows the evolution of the changes in the amounts of vanadium and 17α(H),21β(H)-hopane relative to TSEM in the control and biodiesel-treated tiles. Regarding the control tiles, a certain correlation between the concentrations of vanadium and those of 17α(H),21β(H)-hopane was observed. Furthermore, the amount of both components remained practically constant, with values slightly higher in the intertidal tiles. In the biodiesel-treated tiles the correlation between V and hopane was less clear, and unlike the control tiles, the amount of both components decreased with time.

From the results it could be stated that the disappaerance of vanadium, if it took place, followed a similar pattern to that of the hopane. Thus, it could be possible to use vanadium as an internal marker to estimate the degradation of the components of the fuel oil in the field. Moreover, from Fig. 8a, it was observed that the expected increase in the concentration of both components

attributable to biodegradation must have been compensated with the decrease due to partial degradation processes, as Oudot had previously concluded. In addition, from Fig. 8b it was observed that after the 150 first days, when the amount of the two components decreased mainly due to the application of biodiesel, the amount of V and hopane also remained practically constant. Thus, this could be an indicator of the ineffectiveness of the biodiesel as a bioremediation agent for the fuel oil since the pattern during the last days of the experiment would be practically the same than that observed in the controls.

Thus, taking into account the obtained results it could be inferred that despite the measured GTPHs and PAHs degradation, fuel oil mineralization did not occur at a significant extent throughout the year of environmental assays in any of the treatments. This could be partly explained in the supra-littoral tiles which were often exposed to environmental conditions that made biodegradation processes difficult, especially in sunny days (absence of water, high temperatures reached at the black surface of the residual oil). However, the environmental conditions in the intertidal zone of the beach were more appropriate for biological degradation [18]. The background levels of nutrients in seawater were relatively high because of the runoffs coming from the agricultural lands close to the beach. Furthermore, microbial analysis showed the presence of both aliphatic and aromatic-degrading microbial populations in the interstitial water. And finally, seawater temperatures in this region range between 10 and 17 °C, which are near the optimal conditions of 15–20 °C for oil biodegradation in marine environments. Nevertheless, the presence of the recalcitrant compounds (resins and asphaltenes) in the fuel oil might have limited the observed degradation of the aliphatic and aromatic fractions, which could be "trapped" in the polar organic matrix suffering a reduction on their bioavailability.

A field bioremediation study has been also carried out on a beach of cobblestones affected by the Prestige oil spill using the oleophilic commercial fertilizer S200 [32]. The researchers performed two applications of 200 g/m², so the total dose was similar to that used in our experiment (four applications of 100 g/m²). The authors concluded that this agent enhanced the biodegradation rate of high molecular weight n-alkanes (C25–C35), alkylcyclohexanes, alkylbenzenes and alkylated PAHs. In particular, they observed a decrease of the depletion percentage of 15–20% in some of the target PAHs studied in the present work (fluoranthene, pyrene, benz[a]anthracene, chrysene) in the fertilized plot compared with the control plot. The study of the degradation was limited to specific compounds but they did not indicate if the enhanced depletion of them resulted in the decrease of the amount of weathered oil, the detachment of a significant fraction of the stuck fuel, or the improvement of the appearance of the beach.

4. Conclusions

The efficiency of different bioremediation products (nutrients, microorganisms and biodiesel) was tested using tiles located in both the supra-littoral and intertidal zones of a beach that was affected by the heavy oil spill of the Prestige. The

addition of the water-soluble amendments, both nutrients and microorganisms, did not mean any improvement with respect to the controls. On the other hand, the biodiesel improved the appearance of the treated tiles and apparently accelerated the degradation of the aliphatic and aromatic fractions of the residual fuel oil. Nevertheless, PAHs degradation was similar and very high in all the treatments (80–85% after 60 days). Despite the main objective of this work was the evaluation of biodiesel as bioremediation agent it was concluded that this oleophilic agent could be use as cleaning agent for the oiled rocky shores, since it shows quite a lot of advantages in comparison with high pressure flushing.

Furthermore, it was found that vanadium and $17\alpha(H)$, $21\beta(H)$-hopane could be used alike as internal marker for the estimation of biodegradation and/or weathering of heavy fuel oil under field conditions. The time-courses of the changes of the amount of these two markers suggested that the residual fuel oil mineralization was very low throughout 1 year in all the treatments. The increase of the oxygen content of the residual oil from around 1% till 4–8% indicated that the partial oxidation of hydrocarbons took place, and that the hydrocarbon oxidation products accumulated in the polar fractions.

The presence of recalcitrant compounds in the Prestige heavy fuel oil (resins and asphaltenes) might have limited the degradation extent of the saturated and aromatic fractions, which could be "trapped" in the polar organic matrix, suffering a reduction on their bioavailabiliby. Therefore, the obtained results pointed out that bioremediation techniques are not suitable for the recovery of shores affected by heavy oil spills.

Acknowledgements

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