Optimization of washing conditions with biogenic mobilizing agents for marine fuel-contaminated beach sands

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A R T I C L E   I N F O

A B S T R A C T

Washing is a rapid and effective treatment to remediate contaminated sands impacted by oil spills, although synthetic additives used to increase extraction efficiency may cause additional pollution issues due to their intrinsic toxicity and very often low biodegradability. In this study, different biogenic mobilizing agents (soybean lecithins, cyclodextrins, cholic acids, plant-derived cleaners, rhamnolipids and sophorolipids) were tested in the washing of beach sands artificially contaminated with the Intermediate Fuel Oil IFO-180. Among these, a de-oiled soybean lecithin (SL-1), hydroxypropyl-β-cyclodextrins (HPB-CD) and sophorolipids (SR) achieved hydrocarbon removals close to those attained with the synthetic surfactant Triton™ X-100 (TX) in preliminary washing tests carried out at constant mixing rate, water/sand ratio and IFO-180 contamination level using agents concentrations close to their critical micelle concentration (0.1% and 1% w/v for microbial and non-microbial agents, respectively). The effects of agent concentration, water/sand ratio, mixing rate and IFO-180 contamination on hydrocarbons removal were modelled using face-centred central composite design and ANOVA. Optimal washing parameters for sand contamination levels in the range 0.5–20 g/kg were identified with response surface methodology. While HPB-CD and SR performed equally to TX only at low sand contaminations, SL-1 attained hydrocarbon removal higher or equal to that of TX at any IFO-180 contamination and at lower application rates. SL-1 also outperformed TX when minimizing the water/sand ratio, *i.e.*, the volume of water used. Considering its lower toxicity, higher biodegradability and higher hydrocarbon removal efficiencies, SL-1 is an effective and environmentally sustainable alternative to synthetic surfactants in washing treatments for marine fuel-contaminated sands.

Introduction

Oil spills are frequent in marine environments, mainly due to accidental releases that occur during transportation over the sea, maintenance of drill sites and pipelines and catastrophic spill events [1]. The vulnerability of coastal environments to oil spill is long well known and severely affects all layers of the resident trophic chain [2]. Oil spills pollute natural environments with a mixture of aliphatic and aromatic hydrocarbons and asphaltenes particularly toxic and recalcitrant to bioremediation. Remediation technologies from oil spills can be physical, chemical or biological [3]; among these, *ex-situ* soil washing is one of the fastest intervention technologies to remove fuel components and reclaim the natural soil, sediment or sand. Water alone, however, is ineffective in removing fuel components, thus additives are required to increase solubility of such compounds [3,4]. Among these additives,

surface-active agents (surfactants) are particularly useful: these molecules contain both hydrophilic and hydrophobic moieties which allow them to change the properties of the medium (*i.e.* density, surface tension, solvent properties, etc.) and to form aggregates in the water medium after reaching a threshold concentration, termed the critical micelle concentration (CMC) [5]. Most of these agents, however, do pose severe environmental issues, due to their resistance to biodegradation and/or direct or indirect toxicity [6]; consequently, water used in the washing process and the reclaimed sands would require further treatment to remove both contaminant and surfactants [7].

Biogenic pollutant mobilizing agents constitute the major alternative for an environmentally friendly washing treatment, mainly attributed to their high biodegradability and lower toxicity [4,8]. They may be of plant, animal or microbial origin and act on hydrophobic pollutants as surfactants, *i.e.*, through micelle formation, or enhanced

dissolution in aqueous media without forming micelles (*e.g.* cyclodextrins). Some of these compounds have already shown removal efficiencies superior or comparable to synthetic surfactants such as Tween80, Triton X-100 or SDS [9]. Lecithins are phospholipids, typically of plant origin, used in the food and pharmaceutical industry and are also known as effective biostimulants for hydrocarbon biodegradation [10] as well as oil dispersants [11]. Cyclodextrins are cyclic oligosaccharides of glucopyranose having a hydrophobic cavity and are produced by fungal enzymatic conversion of starch; they are mainly used in pharmaceutical formulations to deliver drugs, but are known to interact with and thus solubilize polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs) and other organic hydrocarbons, forming inclusion complexes with the pollutants [7,12]. Plant extracts contain biogenic mobilizing agents such as triglycerides, saponin, natural gums and various polysaccharides [13] and are often present in cleaning products or used, more recently, as washing additives [14,15]. Bovine bile and other cholic acids of animal origin, on the other hand, are less common mobilizing agents for soil washing, but they actually improve the mobility of PAHs and PCBs in historically contaminated soils [16]. Microbial surfactants constitute the most diverse category of biogenic pollutant mobilizing agents, and include sophorolipids, rhamnolipids, surfactins and high molecular weight polymers such as emulsan or alasan [9,17,18]. There is currently a vast effort to promote the use of these compounds in remediation processes [4,5,7,19–21].

Previous washing studies utilizing biogenic pollutant mobilizing agents focused on soils contaminated with aromatic hydrocarbons or diesel fuel oil. Coastal environments, however, and particularly beach sands, are characterized by a high salinity, which changes the ionic strength of the water phase used, thus also modifying the behavior of surfactants and making them generally less effective [1,22]. On the other hand, marine fuels, the main source of pollution from sea spills, accidents or sunken ships, contain much higher percentages of residual oil compared to crude oils or diesel, which constitute a major issue in their washing processes. In particular, intermediate fuel oils for marine uses (IFOs) typically consist of 25–35% w/w of saturates (alkanes, cicloalkanes and waxes), 40–60% w/w aromatics (mostly PAHs) and 15–25% w/w polar low-volatile compounds (asphaltenes and resins) [23]. The washing efficiencies of biogenic mobilizing agents from a matrix having such high intrinsic salinity contaminated by heavier marine fuels might thus differ significantly from those reported on soils contaminated by lighter fuels.

Multiple factors may influence the washing process, the most important being the mobilizing agent-to-pollutant ratio, which is furthermore dependent on the nature of both chemicals, the agent concentration in the water washing solution and the water-to-sand/soil ratio. In the case of surfactants, agent concentration may be particularly important, since only at concentrations equal to or above the CMC will micelle formation occur. High water-to-sand ratios facilitate emulsion formation by reducing viscosity and increasing interfacial contact of the crude oil and surfactant. However, they produce a larger amount of contaminated water effluent per unit of sand washed, which must be further treated. Time is also a relevant parameter in the process that may vary together with mixing rate. Biogenic mobilizing agents are usually more expensive than synthetic traditional counterparts, thus process designs must also consider lowering costs through manipulation of process parameters. The effect of the above parameters on the washing efficiency may vary with the agent used. To perform a comparative evaluation of different agents, optimal washing conditions for each should thus be determined; however, such parameters cannot be considered individually, as they affect the efficiency synergistically. Statistical approaches might reduce the number of assays to screen multiple parameters at once. Response Surface Methodology (RSM) is a statistical technique which associates functional relationships between a determined, associated set of variables and an output of interest, often supported by the Taguchi design of experiments. This methodology has

been previously implemented to optimize significant process parameters in washing of fuel-contaminated soil [24].

The aims of this work were: (i) to screen a consistent number of biogenic mobilizing agents of different origins in order to preliminarily assess their potential efficiency in washing treatment of beach sand contaminated with a marine fuel (IFO-180) and (ii) to optimize washing conditions with the most promising biogenic mobilizing agents using RSM, with or without the additional constraint of minimizing water consumption (*i.e.*, the production of a water effluent requiring additional treatment steps), in order to compare their maximal hydrocarbon removal efficiencies.

Materials and methods

Sand contamination

Sand was collected from the shore of Ravenna (Italy) and artificially contaminated with an intermediate fuel oil 180 (IFO-180), a mixture of 98% residual oil and 2% distillate oil obtained from the heavy and medium fractions of crude oil containing 27.1% saturates (n-C_{10} to n-C_{35} alkanes), 41.7% aromatics (2 to 5-ring PAHs), 27.0% resins, and 4.2% asphaltenes [25]. IFO-180 was dissolved in a mixture of hexane and dichloromethane (1:20) at 40 g/l and this solution used to contaminate beach sand homogeneously at different IFO-180 final concentrations in the range 0.5 to 20 g/kg. In particular, the sand was suspended in the IFO-180 solution and thoroughly mixed under a fume hood until complete evaporation of the solvent, followed by further incubation for 10 days under fume hood and periodic mixing to complete IFO-180 weathering.

Mobilizing agents tested

The following mobilizing agents were used. (i) Synthetic surfactant Triton™ X-100 (TX) (Sigma Aldrich Italia, Milano, Italy) as benchmark for washing tests; (ii) soy lecithin products, namely SOLEC™ F (SL-1) and TEXTROL™ F10 (SL-2) (Solae Italia s.r.l., Cernusco sul naviglio (MI), Italy); (iii) hydroxypropyl-*β*-cyclodextrin (HPB-CD) and randomly methylated *β*-cyclodextrin (RAMEB) (Cargill S.r.l, Milano, Italy); (iv) commercial cleaning products based on plant extracts, namely SC1000 and SuperSolv Safety Solvent (SSS), (BIOBASED Europe, Glegarnock, UK); (v) bovine bile acids (BB) (Industria Chimica Emiliana s.r.l., Reggio Emilia, Italy); and (vi) microbial surfactants, namely rhamnolipids (RL) from *P. aeruginosa* and sophorolipids (SR) from *C. bombicola* ATCC 22214.

For rhamnolipids production, actively growing *P. aeruginosa* PAO1 cultures were used as inoculum for batch flasks containing mineral salt medium with 2% (v/v) glycerol or oleic acid as carbon source in batch conditions at 37 °C. Rhamnolipids were solvent-extracted according to Smyth et al. [26]. For sophorolipids production, active cells of *C. bombicola* ATCC 22214 were used to inoculate a bioreactor containing glucose yeast extract and urea medium and operated in fed-batch conditions at 28 °C, feeding glucose and rapeseed oil. Crude extract mixture was obtained as the settled product from fed-batch cultivations operated without the use of antifoam, according to Shah et al. [27]. CMC values were determined for each biogenic agent via measurement of IFT with the pendant drop method, using a DSA30 drop shape analyser (KRÜSS GmbH, Hamburg, Germany), according to Berry et al. [28]. The main characteristics of the biogenic agents used are reported in Table 1.

Washing tests

All washing tests were performed at room temperature (RT) using 25–50 g of contaminated beach sand and 25–500 ml of a surfactant/mobilizing agent solution in distilled water in sealed 1 l Erlenmeyer baffled flask on rotary shakers. Different surfactant concentrations,

Table 1

Main characteristics of the biogenic agents used. N/A: not applicable.

Biogenic Agent	Main characteristics and active component(s)	CMC
SOLEC™ F (SL-1)	Deoiled soy lechitin (97% w/w), HLB 7, phosphatidylcholine	14–16 g/l
TEXTROL™ F10 (SL-2)	Soy lechitin for zootechnic and industrial use, HLB 4, phosphatidylcholine	12–14 g/l
hydroxypropyl-β-cyclodextrin (HPB-CD)	Technical grade	N/A
randomly methylated β-cyclodextrin (RAMEB)	Technical grade	N/A
SC1000	Proprietary ternary non-ionic surfactant compound of biobased ingredients. Includes non-ionic surfactant, tall-oil fatty acids.	1.2 g/l
Supersolv Safety Solvent (SSS)	Proprietary blend of biobased ingredients. Includes ethoxylated alcohol surfactant, Tall-oil fatty acids, surfactant.	1.1 g/l
Bovine bile acids (BB)	Cholic acid (5–8% w/w), deoxycholic acid (5–8% w/w), chenodeoxycholic acid (2–4% w/w)	6–10 g/l
Rhamnolipids (RL)	Mixture of a mono-rhamnolipid ($C_{26}H_{48}O_9$, MW: 504) and a dirhamnolipid ($C_{32}H_{58}O_{13}$, MW: 650) at approximately 1:2 ratio, 20% (w/w)	$\approx$ 50 mg/l
Sophorolipids (SR)	C_{18} acidic and C_{18} lactonic mixture of sophorolipids congeners at approximately 1:2 ratio, 40% (w/v)	$\approx$ 60 mg/l

Table 2

description of the parameters and the levels tested in the optimization of washing with SL-1, HPB-CD, SR and TX. Ranges for surfactant concentrations were different for i) SL-1, HPB-CD, TX and ii) SR, because of the lower CMC for SR.

Level	A	B	C	D
	Agent concentration (% w/v)	Water/sand ratio (v/w)	Mixing rate (rpm)	IFO-180 concentration (g/kg)
−1	0.1[i]–0.01[ii]	1	80	0.5
0	2.55[i]–0.11[ii]	5.5	150	10.25
+1	5[i]–0.2[ii]	10	220	20

water/sand ratios (v/w), mixing rates (rpm) and IFO-180 contaminations (g/kg) were used in the preliminary washing tests and in the central composite design tests as described below.

Sampling, solvent extraction and chemical analysis

For each sample, an aliquot of the homogeneous sand suspension was withdrawn and allowed to settle; after removal of the water phase, sand was air-dried overnight. Hydrocarbon extraction was performed from 5 g of sand with 5 ml of hexane:acetone (1:1). Normal decane (n-C_{10}) was used as internal standard, since it was not present in weathered, IFO-180-contaminated sand samples. Samples were sonicated at 50 kHz for 5 m, shaken horizontally overnight at 150 rpm, RT, and sonicated again as previously stated. After centrifugation at 5000 rpm for 10 m, organic extracts were analysed by GC-FID, using an HP-5 capillary column (30 m × 0.25 mm × 0.32 μm) under conditions adapted from the literature [29] as follows: injection volume 5 μl, splitless mode; injector temperature 270 °C; N_2 gas carrier, pressure 19 psi, column flow 2.1 ml/min; oven at 40 °C for 3′, heated at 20 °C/min until 190 °C and then at 60 °C/min until 325 °C; detector temperature 320 °C. Total petroleum hydrocarbons (HC) were quantified using standard calibration curves of IFO-180, 0.1 to 20 g/l, using n-C_{10} as internal standard to evaluate recovery; n-alkanes were quantified against 7-points calibration curves of standard n-C_{10} to n-C_{40} mixture (Ultra-Scientific Italia s.r.l., Bologna, Italy) in the range 0.01 to 50 mg/l each.

Preliminary washing test

A preliminary washing test aimed at the selection of the most promising biogenic mobilizing agents for the following statistical optimization step was carried out with 50 g of sand contaminated with IFO-180 at 5 g/kg, 350 ml of water (*i.e.*, water/sand ratio 7:1 v/w), mixing at 150 rpm for 48 h. To compare the potential efficiency of different agents under the same washing conditions (*e.g.*, mixing rate, sand/water ratio, sand contamination level), the agents' concentrations in the water phase were chosen considering the CMC values of the surfactants used, above which surface tension values do not fall further for each

compound (Table 1). In particular, for surfactants having CMC values in the range 1–15 g/l (SL-1, SL-2, SC1000, SSS, BB) a 1% (w/v) concentration was selected, whereas for those having CMC values slightly lower than 0.1 g/l (RL, SR), *i.e.* at least one order of magnitude lower than other surfactants, a 0.1% (w/v) concentration was selected. For TX, whose CMC value is approximately 0.6 g/l, and cyclodextrins, that do not form micelles, the higher concentration (*i.e.*, 1% w/v) was used.

Central composite design

For the optimization procedures, the statistical design of experiment (DoE) was based on Face-Centred Central Composite Design (CCF) for the above 4 parameters (except surfactant concentration in the case of the surfactant-free control) using 2 levels plus the central point, and 1 response (total hydrocarbons removal% after 24 h) (Table 2). The experimental design, including single replicates for each factorial and axial point and 6 replicates for the central one, resulted in a total of 30 washing tests for each agent (20 tests for the surfactant-free control) (Table S1). Sampling was performed after 24 h of mixing. ANOVA was applied to obtain the best fitting model from the data describing the effect of the investigated parameters on hydrocarbons removal by each agent. The second order model quality was assessed by the squared correlation factor (R^2–preferably greater than 0.8) and the ANOVA was carried out using Fisher test (with 95% confidence level) applied to the model (significant with p-value < 0.05), parameters and lack of fit (not significant with p-value > 0.05). The standard deviation of the model is derived from the 6 replicate central points at 95% of confidence. Models were validated by performing additional washing tests using random points within the experimental domain and comparing the observed removal efficiencies with those predicted by the model.

Optimization

Optimal values for the washing of beach sand contaminated at different IFO-180 concentrations (D parameter) were identified for each surfactant/mobilizing agent. Two optimization criteria were used: (i) maximum HC removal after 24 h (%) when all other washing parameters (A – surfactant concentration, B – water/sand ratio, C – mixing rate) were allowed to fall within the defined range (−1 to +1 level); and (ii) HC removal after 24 h (%) when surfactant concentration and mixing range (parameters A and C) were allowed to fall within the defined range while minimizing the water/sand ratio (parameter B).

To further optimize SL-1 over time (E parameter), DoE was used by fixing A, C and D parameters in corresponding optimal values for SL-1 washing (see Results and discussion), and varying parameters B and E in range using 2 levels plus the central point, and 1 response (HC removal%) (Table 5). The full factorial design 2^2 augmented with 4 axial points ($\alpha = 1$) and 5 replicates for the central point, resulted in a total of 13 washing tests (Table S8). The second order model quality was assessed by the correlation factor and the ANOVA was carried out using

Fisher test as described above. Design-Expert® Software was used for the experimental design, ANOVA and identification of optimal washing parameters.

Results and discussion

Preliminary screening of the agents

In order to identify the most promising biogenic mobilizing agents for the subsequent statistical optimization step, a preliminary washing test of sand contaminated with IFO-180 at 5 g/kg was carried out using selected water/sand ratio (equal to 7), mixing rate (150 rpm) and concentration of mobilizing agents (10% for non-microbial and 1% for microbial, according to their CMC values). While impacted sites in industrial areas very often exhibit aged contamination, typically resulting in strong binding/adsorption to soil particles of pollutant molecules which are usually more difficult to solubilize, remediation activities of shorelines are often started more promptly after the contamination event, thus addressing weathered contaminants less tightly associated to the matrix. In order to simulate a few days' old coastal spill, tests were conducted on beach sand freshly contaminated with IFO-180 marine fuel after weathering for 10 days.

Triton™ X-100 (TX) removed total HC up to $71.0 \pm 3.1\%$ and $82.7 \pm 0.5\%$ and n-alkanes up to $56.4 \pm 4.7\%$ and $73.9 \pm 1.4\%$ after 4 h and 48 h of treatment, respectively (Fig. 1).

Comparable removal of both total HC ($68.8 \pm 1.6\%$ and $82.9 \pm 0.4\%$) and n-alkanes ($51.2 \pm 3.4\%$ and $75.2 \pm 0.7\%$) were obtained with the higher Hydrophile-Lipophile Balance (HLB) soy lecithin SOLEC™ F (SL-1) after the same treatment times, whereas remarkably lower removals of both HC and n-alkanes were attained with Textrol™ F10 (SL-2) (Fig. 1). The different HLB might explain the different efficiencies between the two soybean lecithin products, as also shown in a similar previous report [30]. Since the two soy lechitins products have a very similar CMC but differ in HLB and oil content (being SL-1 product de-oiled), the lower efficiency of SL-2 product might also be due the presence of higher amounts of oily components, that may be concurrently emulsified by the surface-active molecule, thus reducing the availability of free lecithins for the washing process. SL-1 seems a promising candidate biogenic mobilizing agent to replace chemical equivalents. Indeed, soy lecithins have been already shown to increase PAH bioavailability and biodegradation in historically contaminated soils treated in solid-phase and slurry-phase reactors [31]; however, in washing tests carried out on light crude oil-contaminated laboratory soils, they exhibited very limited removal efficiencies, which

were remarkably lower than those attained with microbial mobilizing agents [32,33].

Cyclodextrins were less effective than TX and SL-1 both in terms of total HC and n-alkane removal (Fig. 1). Marked differences were observed in the removal efficiencies of the 2 cyclodextrins. In particular, both HPB-CD and RAMEB-CD removed 34–38% of n-alkanes after 48 h, whereas much higher removal of total HC was obtained with HPB-CD ($60.9 \pm 2.0\%$) than with RAMEB-CD ($37.0 \pm 4.7\%$) (Fig. 1). Since the non-alkane fraction of IFO-180 HC contains a wide range of compounds, $i.e.$ aromatics, resins and asphaltenes, the higher removal yields obtained with HPB-CD may be due to a more effective interaction of this cyclodextrin with one or more classes of these components. Although the interaction of different cyclodextrins with several pesticides has been modelled [34], no information on the specific interaction of both cyclodextrins with the IFO-180 components is available. Nevertheless, HC removals obtained with the best performing cyclodextrin (HPB-CD) were approximately 73% of those obtained with TX and SL-1 for total HC and approximately 52% of those obtained with TX and SL-1 for n-alkanes, making HPB-CD a suitable candidate for further comparison through process parameter optimization.

Plant-derived cleaning products and BB achieved HC removal efficiencies which were remarkably lower than that of TX, whereas n-alkane removal efficiencies were high, especially for SSS which removed up to $62.3 \pm 1.2\%$ in 48 h. Therefore, due to the lower efficiencies obtained compared to TX and other biogenic agents, these plant-derived solvents and BB were discarded from the subsequent optimization processes.

Among the two microbial surfactants, SR allowed higher removal of both total HC (up to $63.2 \pm 2.9\%$) and n-alkanes (up to $60.8 \pm 2.0\%$) than RL ($45.6 \pm 3.1\%$ and $38.2 \pm 0.0\%$ for total HC and n-alkanes, respectively). Although both microbial surfactants were applied at concentrations well above their CMC values, which were very similar, the lower HLB of SR (10–13 vs 23) [21,35] may explain their higher hydrocarbon removal efficiency.

Studies on surfactant-enhanced washing of oil-contaminated soil report wide percentages of total HC removal of up to 80% [36–38], which can be further increased up to 97% using statistical optimization of washing parameters [24,39]. Such a process, however, is markedly affected by the oil contamination ($i.e.$ composition and physico-chemical characteristics of the oil, concentration and aging of contamination, etc.) and the soil characteristics, thus making results hardly comparable between different studies, even when carried out under similar process conditions ($e.g.$, surfactant concentration, water/soil ratio, mixing rate) [4,40]. Interestingly, in the work of Urum et al. [33],
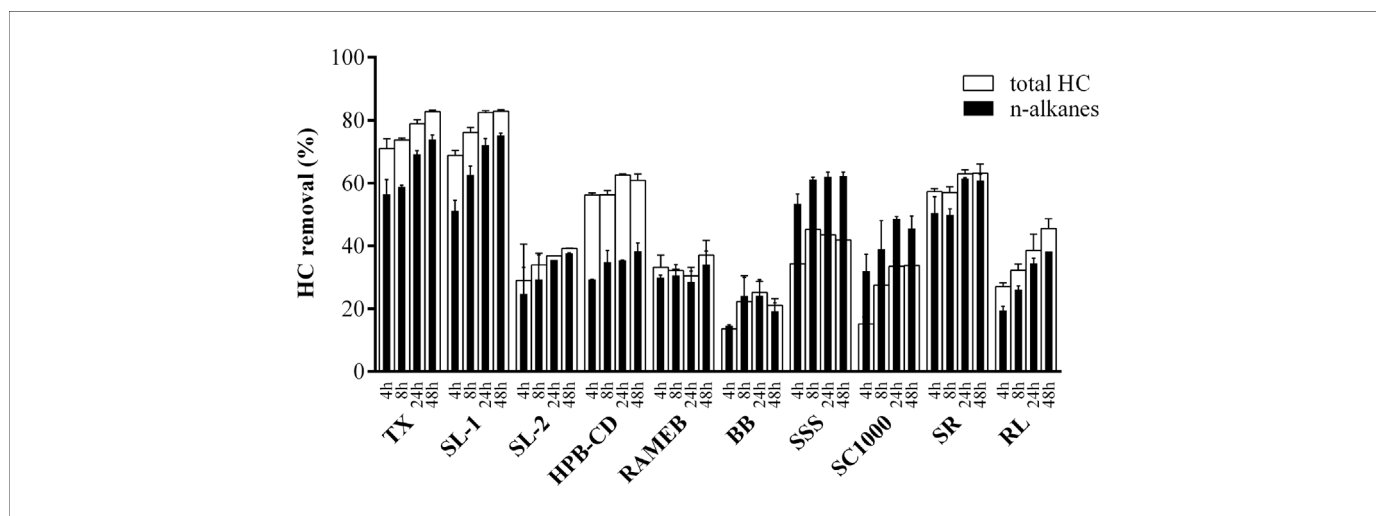

Fig. 1. Total hydrocarbon removal (white bars) and n-alkane removal (black bars) for each biogenic agent in the preliminary washing screening tests. Each bar represents the average of two replicates for each time point (4, 8, 24 and 48 h).

lecithin was considered unfit for soil washing as its removal percentages were well below 50% at RT, whereas on the fuel-contaminated sand under the washing conditions used in these preliminary tests, only SL-1 exhibited very high hydrocarbon removal comparable to that of TX. Similarly, previous studies reported a similar efficiency for HPB-CD and RAMEB-CD in phenantrene-contaminated soil washing (70%) [12], while HPB-CD outperformed RAMEB-CD in this preliminary sand washing test. Other studies reported a much higher hydrocarbon removal efficiency (70%) of RL on highly oil-contaminated weathered soils, compared to that observed in this preliminary test on IFO-180 fuel-contaminated sand [19,32,41].

SL-1 was the most promising biogenic mobilizing agent selected for the second phase of the study, aiming to optimize washing conditions and to identify the maximum HC removal attainable. Since HPB-CD and SR exhibited HC removals which were slightly lower than TX, these agents were also included to determine if process optimization could increase HC removal efficiencies up to or beyond those of TX.

Response surface analyses with selected agents

Optimization of HC removal efficiency with the selected biogenic mobilizing agent (SL-1, HPB-CD and SR) were carried out using four parameters in the ranges described in Table 2; the same optimization procedure was tested on TX as benchmark synthetic surfactant and water as surfactant-free control (see Table S1 for complete description of each run and results).

The model which best described surfactant-free washing (blank) was a second order polynomial function (Table 2) in which IFO-180 concentration and the quadratic term of IFO-180 concentration are the main significant parameters affecting the washing efficiency, along with the water/sand ratio and the interaction between water/sand ratio and IFO-180 concentration (Tale S2). According to the model, hydrocarbon removal increases at increasing water/sand ratio and mixing rate and drastically decreases at increasing IFO-180 concentrations (Tables Table 3and S2). While the model is highly significant ($p < 0.0001$), its R^2 is only slightly above the threshold limit set, meaning that this model could explain only 84.8% of the variability.

In contrast, TX washing efficiency was found to be a cube root function of a polynomial of all parameters (Table 3), with very high coefficients for TX concentration, water/sand ratio and IFO-180 concentration, as well as the interaction between mixing rate and IFO-180 concentration (Table S3). According to the model, however, the most impacting effect is exerted by IFO-180 concentration (Figs. Figure 2a and S1). Lack-of-Fit (LoF) value higher than the significance threshold implies that the HC removal prediction (%) can be considered valid only within the experimental domain (Table S3), but with 92.57% of variability explained and $p < 0.0001$ (Table 3).

The function describing hydrocarbon removal in washing with SL-1 was a second order polynomial of all factors, of which the most significant are water/sand ratio and mixing rate, resulting in higher removal percentages for high values of these parameters (Tables Table 3 and S4, Figs. Figure 2b and S2). This is consistent with other studies

which showed that the most influential factors to impact oil-contaminated weathered soil washing with soy lecithin was the volume of washing solution/soil mass ratio [41]; here, however, predicted total hydrocarbon removal efficiencies are remarkably higher which is most likely due the different nature of the contaminated matrix and composition of the contaminant. The model is highly significant ($p < 0.0001$), but can only explain 80% of the variability.

According to the second order polynomial model describing the washing efficiency with HPB-CD, the washing efficiency is mainly affected by mobilizing agent concentration and the mixing rate, along with the interaction between mixing rate and IFO-180 concentration. The model is significant ($p = 0.0003$) although a $R^2 < 0.8$ implies a lower accuracy in predicted HC removal (%) than the others (Tables Table 3 and S5, Figs. Figure 2c and S3). Interestingly, according to the model, higher hydrocarbon removal is obtained at decreasing mixing rates; this may be due to the different mechanisms of cyclodextrins in the solubilisation of hydrophobic molecules, which is based on the formation of complexes between the hydrophobic molecule and the hydrophobic cavity of a single cyclodextrin molecule, rather than the formation of micelles [34]. The possibility of using lower mixing rates may potentially allow reduction in energy costs of the washing process.

Finally, the function describing the HC removal efficiency of SR was found to be a second order polynomial according to which response is significantly impacted by mixing rate and IFO-180 concentration, along with the quadratic term of the surfactant concentration. In particular, higher hydrocarbon removal is favoured by increasing mixing rates and decreasing IFO-180 concentrations, along with increasing water/sand ratios and surfactant concentration (Tables Table 3 and S6, Figs. Figure 2d and S4).

To validate these empirical models, additional washings were performed by choosing random combinations of the above parameters in the experimental domain, different from the design points; the observed removal efficiencies were compared with those predicted by the model. Fig. 3 shows that the observed hydrocarbon removal did not differ significantly from the predicted values within 95% level of confidence, thus confirming that all the models for the selected mobilizing agents were reliable within the experimental domain.

Optimization of washing conditions

The empirical models described previously were applied to predict the maximum HC removal efficiency attainable with each agent under optimized washing conditions. Two scenarios were considered: (i) maximum total HC removal when all parameters are allowed to fall within their range with no constrains and (ii) maximum total HC removal when parameter B (water/sand ratio), *i.e.*, the volume of water effluent to be further treated, is minimized (B = 1).

While maximum hydrocarbon removal in the range 30–49%, depending on IFO-180 concentration, can be obtained with surfactant-free water, maximum removal from 66% to 86% can be achieved at increasing IFO-180 concentrations and high water/sand ratio with TX (Table 4). Significantly higher maximum removal (about 97%) can be

Table 3
Final equations in terms of coded factors of response surface reduced quadratic models describing the washing of IFO-180-contaminated beach sands with TX, SL, SR and HPB-CD. Y = Hydrocarbon removal% at 24 h.

| Mobilizing Agent	Final equation	F-value	*p*-value	R^2
Water (blank)	$Y = 41.97 + 5.32B + 2.47C - 19.91D + 8.24BD - 27.61D^2$	15.62	**< 0.0001**	**0.848**
TX (control)	$Y^3 = 518892 + 54204.9A + 47025.9B + 22324.3C + 86753.4D + 47250.8AD + 33548.5BD + 58667CD - 345968D^2$	32.72	**< 0.0001**	**0.9257**
SL-1	$Y = 86.32 + 2.58A + 7.32B + 4.98C + 2.24D - 2.13AC - 1.61AD - 20.17A^2$	14.26	**< 0.0001**	0.7999
HPB-CD	$Y = 64.14 + 5.02A + 1.09B - 5.95C - 3.27D + 6.38CD - 11.98A^2 + 8.23D^2$	6.63	**0.0003**	0.6783
SR	$Y = 59.36 + 5.11A + 5.84B + 10.00C - 26.53D + 2.86AB + 3.04AD + 3.40CD - 15.25A^2 - 17.10D^2$	14.26	**< 0.0001**	**0.8652**
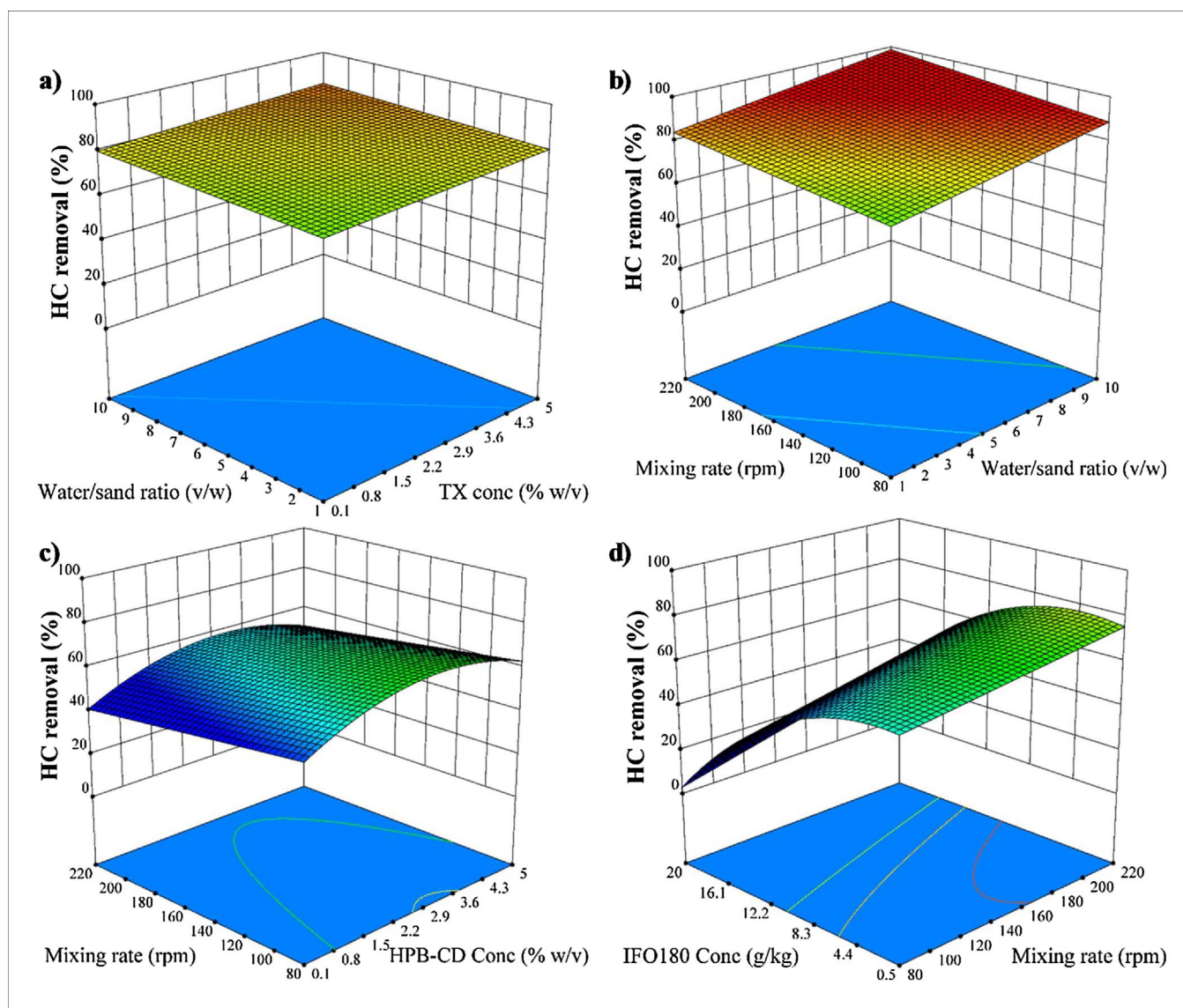
Fig. 2. Response surfaces for the most significant parameters for each agent: TX (a) SL-1 (b), HBP-CD (c) and SR (d). The contour plot for each parameters pair are reported separately in the Supporting Material (Figs. S1–S4).

achieved with SL-1, regardless of IFO-180 concentration and using large amounts of water (high water/sand ratio) (Table 4). Moreover, the model predicts higher maximum removal efficiencies for SL-1 than TX using lower surfactant concentrations at each IFO-180 contamination level. These HC removal values are extremely high compared to washing efficiencies reported previously for lecithins. A hydrocarbon removal lower that 15% was obtained on a weathered light crude oil-contaminated soil [33] and a similar, limited efficiency was obtained on another crude oil-contaminated soil after optimization of several washing parameters [39]. In our study, however, a much longer washing time (24 h *vs* 5–20 min) and a lecithin concentration above the CMC (2.5% vs 0.004–0.5%), were applied, which might explain the remarkably higher removal efficiency obtained. These HC removal values are also higher than those attained after 24 h washing treatments of oil-contaminated soils with different biosurfactants, such as rhamono-lipids and surfactin, applied at concentrations close to their CMC, [19], and comparable to those obtained with Brij35 [24].

Maximum predicted hydrocarbon removal ranges from 76 to 85% for HPB-CD and from 58 to 81% for SR, both depending on the IFO-180 concentration (Table 4). Cyclodextrins have been mainly investigated in the washing of PAH-contaminated soils and shown often to achieve

removal efficiencies in the range 30–70% for naphthalene, depending on the soil type, cyclodextrin type and concentration, and lower removal for higher molecular weight PAHs [12]. Sophorolipds have been shown to enhance PCB soil washing, attaining 30% PCBs removal [42]. The hydrocarbon removal that can be obtained with HPB-CD and SR under the optimized conditions identified here is thus comparable or higher than those reported for these surfactants on soils contaminated with different hydrocarbon mixtures. Since hydrocarbon removal comparable to those obtained with TX can be achieved with these biogenic mobilizing agents at low IFO-180 concentrations, they may represent an environmentally friendly alternative especially in case of washing processes treating low-contaminated sand.

When the amount of water used (water/sand ratio) is minimized, HC removal in the range 58–80%, 82–83%, 72–83% and 46–71% are predicted for TX, SL-1, HPB-CD and SR, respectively, using a water/sand ratio equal to 1 (Table 5). SL-1 is also the best performing biogenic mobilizing agent under these washing conditions, attaining removal efficiencies of total HC higher or equal to those of TX. HPB-CD and SR also perform better that TX at low IFO-180 concentrations, whereas lower efficiencies are obtained at higher sand contamination levels. To confirm these predictions, washing tests were performed using three
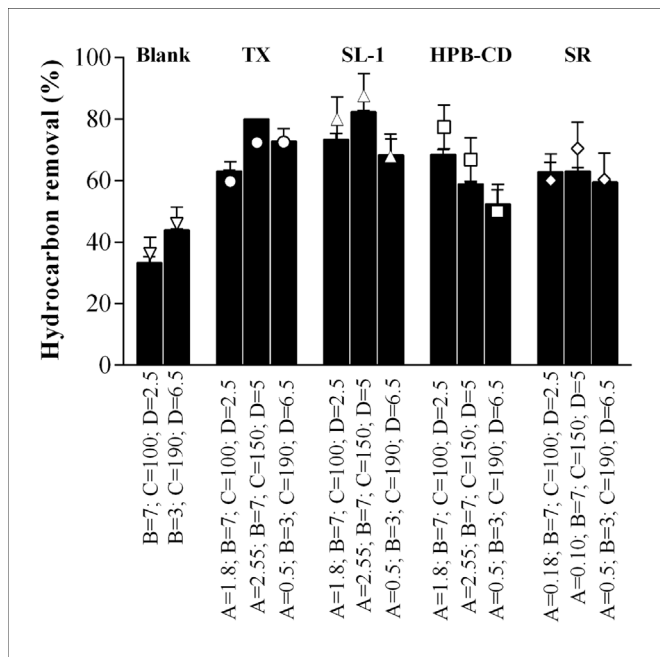
Fig. 3. Model validation tests. Bars represent observed HC removal percentages; symbols represent predicted values. Error associated to predicted removals is the model SD at level confidence of 95%; errors associated to observed removals are experimental errors.

Table 5

Predicted optimal washing conditions and corresponding maximum HC removal from sand contaminated at different IFO-180 concentrations using minimized water/sand ratio (B = 1) with no agents (blank) and with TX, SL, HPB-CD and SR. Errors associated to predicted removals are the models' SD at confidence level of 95%.

Mobilizing Agent	A	C	D	Maximum
	Agent concentration (% w/v)	Mixing rate (rpm)	IFO-180 concentration (g/kg)	HC removal (%)
Water	/	220	2.5	43.2 ± 5.4
	/	220	6.5	46.5 ± 5.4
TX	5	220	2.5	58.5 ± 6.4
	5	220	6.5	76.9 ± 6.4
	5	220	20	80.5 ± 6.4
SL-1	2.7	220	2.5	82.3 ± 7.3
	2.6	220	6.5	83.2 ± 7.3
	2.6	150	20	81.9 ± 7.3
HPB-CD	3.3	85	2.5	82.9 ± 7.1
	3.0	80	6.5	74.3 ± 7.1
	2.6	150	20	72.6 ± 7.1
SR	0.11	220	2.5	71.1 ± 8.6
	0.11	220	6.5	69.8 ± 8.6
	0.11	220	20	45.5 ± 8.6

different IFO-180 contamination values (D), while parameters A, B and C were defined by the empirical models (*i.e.*, with minimum water/sand ratio B = 1). The results confirmed the predicted efficiencies in all the cases studied, within 95% level of confidence (Fig. 4). Such removal efficiencies were obtained using a laboratory-contaminated beach sand weathered for 10-days, *i.e.* assuming a prompt response action after the contamination event. Lower HC removal might be obtained when washing actual contaminated beach sands, especially in case of longer aging of the contamination leading to a stronger adsorption of fuel hydrocarbons to the sand matrix. However, since the HC removal would be presumably affected for all agents, similar relative efficiencies among different agents could be expected. The empirical models allow one to obtain and compare the maximum HC removal efficiency of the selected agents (*i.e.* under their specific optimal washing conditions) at different sand contamination levels, and therefore represent a useful tool to select the most appropriate case-specific washing agent. Although they cannot be directly applied to determine the process

optimal conditions at full scale, due to differences in the mixing strategies used, they provide useful information on the main parameters that significantly affect the washing efficiency of each agent, that might streamline the process optimization at larger scale.

Overall, SL-1 achieved removal efficiencies comparable or higher than TX at all IFO-180 concentrations both using high or low water/sand ratios. While TX can be inhibiting to microbial communities and single microorganisms in the environment [43], lecithins are much less toxic [44] and have also been proposed as biocompatible biostimulation agents [45] or dispersants [11,46,47]. SL-1 can therefore be considered an effective environmentally friendly alternative to synthetic surfactants for washing sands contaminated by intermediate fuel oils. In addition, since higher or equal HC removal yields can be obtained under optimal washing conditions with an amount of SL-1 corresponding to approximately the half that of TX (2.5–2.7% w/v *vs* 5% w/v, respectively, applied at the same water/sand ratio of 10 or 1) (Tables 4 and 5), the use of a remarkably lower amount of product per kg of sand processed may contribute to balancing, at least partially, the higher cost of this biogenic agent (approximately €5/kg; Solae Italia s.r.l, personal communication).
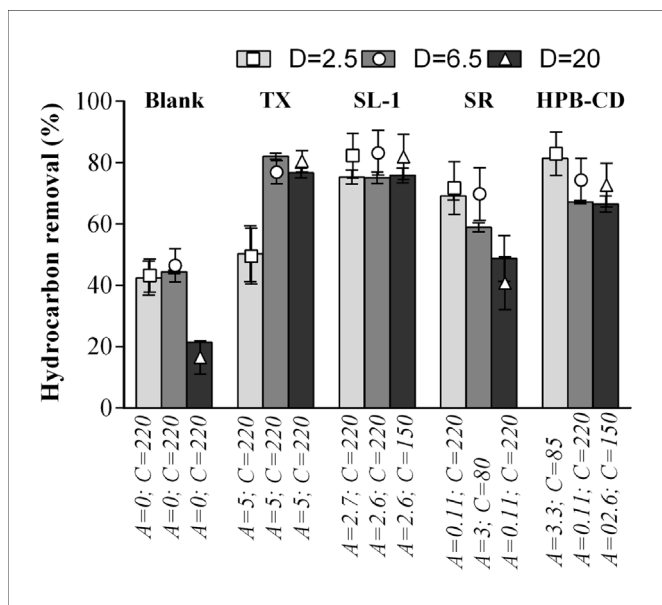
Table 4

Predicted optimal washing conditions and corresponding maximum HC removal from sand contaminated at different IFO-180 concentrations with no agents (blank), TX, SL, HPB-CD and SR. Errors associated to predicted removals are the models' SD at confidence level of 95%.

Mobilizing Agent	A	B	C	D	Maximum
	Agent concentration (% w/v)	Water/sand ratio (v/w)	Mixing rate (rpm)	IFO-180 concentration (g/kg)	HC removal (%)
Water	/	1	220	2.5	43.2 ± 5.4
	/	9.3	190	6.5	48.8 ± 5.4
	/	10	220	20	29.5 ± 5.4
TX	5	10	80	2.5	66.3 ± 5.0
	5	10	220	6.5	79.7 ± 5.0
	5	10	220	20	86.0 ± 5.0
SL-1	2.7	10	220	2.5	96.9 ± 7.3
	2.6	10	220	6.5	97.8 ± 7.3
	2.5	10	150	20	96.5 ± 7.3
HPB-CD	3.3	7.5	80	2.5	85.3 ± 7.1
	3	10	80	6.5	76.5 ± 7.1
	2.6	5.5	220	20	75.9 ± 7.1
SR	0.12	9.5	205	2.5	81.0 ± 8.6
	0.1	8.7	216	6.5	78.7 ± 8.6
	0.14	10	220	20	58.4 ± 8.6

Fig. 4. Observed (bars) and predicted (symbols) HC removal percentages under the optimal washing conditions for sand contaminated at different IFO-180 concentrations at the lowest water/sand ratio (B = 1). Error associated to predicted removals is the model SD at level confidence of 95%; errors associated to observed removals are experimental errors.

Table 6

Experimental design for the optimization of time and water/sand ratio in the washing with SL-1.

Level	B	E
	Water/sand ratio (v/w)	time (h)
−1	1	4
0	5.5	14
+1	10	24

Soy lecithin optimization over time

Given the high maximum hydrocarbon removal achievable with SL-1, optimal conditions (A = 2.6% w/v, C = 150 rpm and D = 20 g/kg) were used to design a new process optimization, using water/sand ratio (B) and time (E) parameters for a 2^2 full factorial design (Table 6). The best fitting, second order model obtained (Eq. (1) and Table S9), showed that the total HC removal is a function of both time and water/sand ratio at equal level of significance (Fig. 5a and Table S8).

$$Y = 83.37 + 7.43B + 8.88E + 1.28BE - 6.44B^2 - 5.09E^2 \qquad (1)$$

Equation 1: final equation in terms of coded factors for SL-1 washing over time (B: water/sand ratio; E: time).

Validation was performed using 9 h and 19 h time points, confirming the predictions within a 95% confidence level (Fig. 5b). The model suggests that high removal yields can be obtained within 14 h of washing, and that longer times do not lead to significantly higher removal efficiencies.

Conclusions

This study investigated the possibility of replacing synthetic surfactants with more environmentally acceptable products for washing coastal sands contaminated by marine fuels. Different surfactants/mobilizing agents of natural origin (plant, animal, microbial) were compared to Triton™ X-100 (TX) as benchmark product in preliminary washing tests, leading to the identification of the de-oiled soy lecithin product SOLEC™ F (SL-1), hydroxypropyl-β-cylodextrins (HPB-CD) and sophorolipids (SR) as the most promising agents. Statistical optimization of the most relevant washing parameters (agent concentration, water/sand ratio, mixing rate) revealed that SOLEC™ F can perform equally or better than TX under a wide range of sand contamination levels (0.5 to 20 g of IFO-180 fuel/kg of sand), both using a high water/sand ratio (10), which maximizes the hydrocarbon removal yield, and a low water/sand ratio equal to 1, which minimizes the volume of water used and of contaminated water effluents of the process needing further treatment. Moreover, a remarkably lower concentration of SOLEC™ F (approximately 50%) was required, at equal water/sand ratios, to achieve higher or the same hydrocarbon removal as TX; a higher price of the product might thus be at least partially balanced by its lower application rate, suggesting a possible overall economic
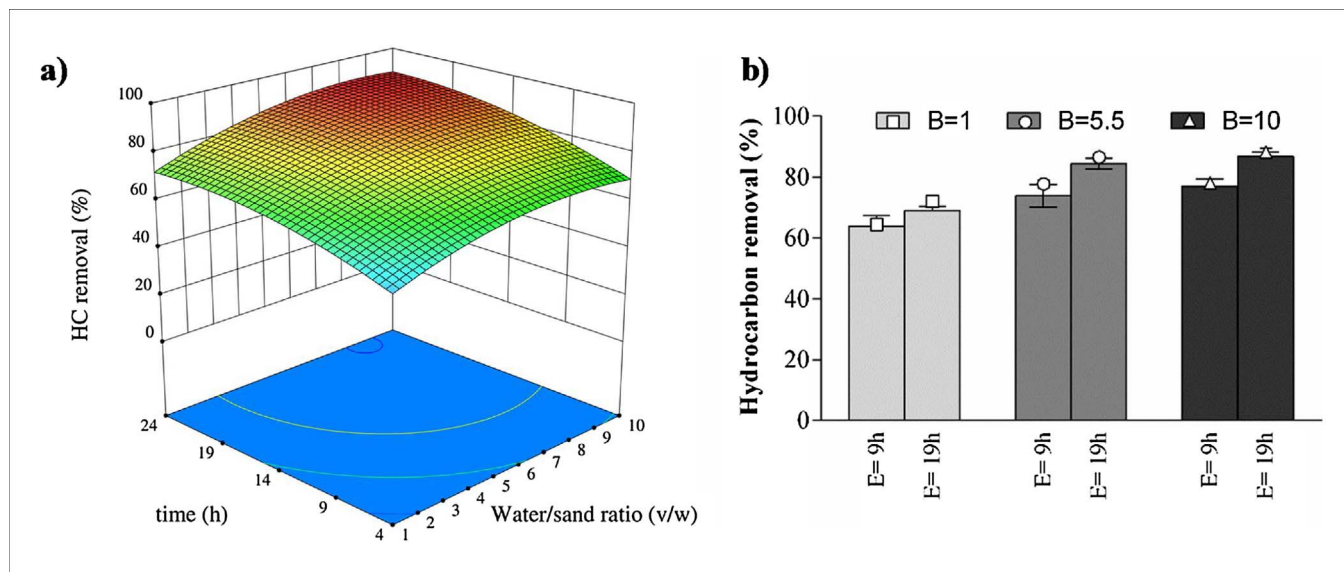

Fig. 5. (a) Response surface for the B and E parameter in washing with SL-1; (b) observed (bars) and predicted (symbols) HC removal percentages at E = 9 h and 19 h with B in range. Error associated to predicted removals is the model SD at level confidence of 95%; errors associated to observed removals are experimental errors.

competitiveness of the process based on de-oiled soy lecithin products. Overall, these data indicate that de-oiled soy lecithins are a promising, environmentally-friendly alternative to the use of synthetic mobilizing agents for the washing of beach sands contaminated by marine fuel hydrocarbons.

Conflict of interest

The authors declare that they have no conflict of interest.

Acknowledgements

Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at https://doi.org/10.1016/j.nbt.2017.12.007.

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