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Bioremediation of oil polluted marine sediments: A bio-engineering treatment

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Summary. The fate of hydrocarbon pollutants and the development of oil-degrading indigenous marine bacteria in contaminated sediments are strongly influenced by abiotic factors such as temperature, low oxygen levels, and nutrient availability. In this work, the effects of different biodegradation processes (bioremediation) on oil-polluted anoxic sediments were analyzed. In particular, as a potential bioremediation strategy for polluted sediments, we applied a prototype of the "Modular Slurry System" (MSS), allowing containment of the sediments and their physical-chemical treatment (by air insufflations, temperature regulation, and the use of a slow-release fertilizer). Untreated polluted sediments served as the blank in a non-controlled experiment. During the experimental period (30 days), bacterial density and biochemical oxygen demand were measured and functional genes were identified by screening. Quantitative measurements of pollutants and an eco-toxicological analysis (mortality of *Corophium orientale*) were carried out at the beginning and end of the experiments. The results demonstrated the high biodegradative capability achieved with the proposed technology and its strong reduction of pollutant concentrations and thus toxicity. [**Int Microbiol** 2015; 18(2):127-134]

Keywords: bioremediation · biostimulation · chronically polluted sediments · oil-degrading bacteria · *Corophium orientale* (Crustacea, Amphipoda)

Introduction

The fate of hydrocarbon pollutants in marine environment is largely determined by abiotic factors (e.g., temperature, pH, dissolved oxygen and nutrient concentration), which influence the processes of oil weathering and degradation [25]. At an early stage, light fractions of oil are naturally removed; mostly by evaporation, then by photo-oxidation and geochemical reactions. Heavy fractions are dispersed or dissolved by waves but a portion settles at the sea bottom, where local chemical-physical conditions tend to limit biodegradation. In many aquatic systems the bottom layer of the water column contains a higher concentration of pollutants than the upper layer, mainly because of the settling process and the lower rate of biodegradation. Consequently, sediments become a sink for pollutants that may later travel back up through the water column by re-suspension, causing a deterioration of water quality.

Among the many environmental challenges, the management and remediation of contaminated marine sediments is perhaps the most difficult one, but it must be dealt wit, because of the significant threat to the quality of aquatic and

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terrestrial ecosystems. An appreciation of the relationships between contaminated marine sediments and ecosystem quality has driven the search for technologies aimed at sediment remediation [3,24].

Sediment management techniques can be classified as ex situ and in situ. In the former, the contaminant is removed from the environment and treated elsewhere. In situ technology, however, has several advantages over ex situ approaches because it does not require the removal and transport of the contaminants and therefore minimizes their potential risks outside the contaminated environment. In situ remediation processes used in the field can be physical (including capping or solidification/stabilization, containment with barriers) or biological (including bioaugmentation and biostimulation). Of these, biological techniques have become more important over the last decade, mainly because of their low environmental impact, generally lower costs, and their ability to degrade organic contaminants and thus restore the sediments [30].

Although the positive results achieved using bioremediation strategies have been reported [1,27], their success depends on the presence of specialized hydrocarbon-degrading microbial consortia and suitable environmental conditions. In marine sediments, the low temperature, unavailability of principal nutrients (e.g., nitrogen, phosphorus, iron), and the low oxygen concentration are limiting factors for the development of a microbial population capable of biodegradation [5]. These limitations have hindered the application of bioremediation methods (bio-stimulation/bio-augmentation) in marine anoxic environments. Instead, the use of aerobic bioremediation methods in the recovery of polluted sediments has relied on historical observations that microorganisms use oxygen-incorporating enzymes to initiate their attack on hydrocarbons [27]. Given these difficulties, the development of an in situ confined moving system would offer a promising technical solution.

In the present study, a modular system aimed at optimizing the biodegradation process was implemented and tested. The system was designed to operate directly in the field but retains the advantages of controlled methods that do not impact the surrounding environment. The effects of the addition of air and/or slow-released fertilizer, temperature regulation, and the addition of oil sorbents on the efficiency of the system were evaluated. The abundance of the indigenous microbial communities in both the untreated (control) and treated sediments was monitored for 30 days after the contamination event. We also measured microbial activity (biochemical oxygen demand, BOD), screened for functional genes, analyzed petroleum hydrocarbon content, and carried out eco-toxicological bioassays using the organism *Corophium orientale*.

Materials and methods

Experimental mesocosms. Five different experiments were carried out in rectangular tanks of 144-liter capacity (120 cm long, 30 cm deep, 40 cm wide). The tanks were supplied with a continuous flow (l liter/min) of seawater drawn from Messina harbor (38º11′42.58″ N, 15º34′25.19″ E) and filtered through a 200-μm nylon mesh to remove large metazoans and detritus. Sandy sediments were also collected from the harbor and artificially polluted with 11 g of crude oil (Kashagan Fresh Oil, ENI Technology, Italy)/kg to simulate an oil spill. Prior to its use in sediment contamination, the oil was weatherized in a 2-day incubation at 30 ± 1ºC. The temperature inside the mesocosms was maintained at about 17 ± 1ºC throughout the experimental period.

Sediment characterization. The main physical-chemical variables (pH, temperature, redox) were measured using a multiparameter probe (Waterproof CyberScan PCD 650, Eutech Instruments, the Netherlands). Sediment mineralogy was analyzed by X-ray powder diffraction (PW 14 1373, Philips, Holland). Sediment water content was calculated as the difference between the wet and dry weights of the sediment and expressed as a percentage. Grain size was determined by the sieving technique. Total organic matter was measured as previously described [11]. The concentrations of NO_3, NO_2^+, NH_4^+, and PO_4^{3-} were estimated using a QuAAtr AutoAnalyzer (Seal). After their collection and artificial contamination, the sediments (26 kg) were left in stagnant water for 10 months (T_{300}) to immerse them in anoxic conditions.

Biodegradation system. The system designed to produce biostimulating conditions consisted of cylinders 30 cm long and 15 cm in diameter. Each cylinder had a glass cap with four holes for the introduction of: (i) the aeration system; (ii) the thermostat; (iii) the oil sorbent; and (iv) the slow-release fertilizer. The cap could be removed for seawater and sediment sampling. The aeration system consisted of five tubes (28 cm long, 4 mm diameter) externally connected to an air pump (Turbolence 2000 AirPump, Tetra). The cylinders were filled with 3 l of natural seawater and 1.5 kg of polluted sediments and placed inside the experimental mesocosms to promote biostimulation (Fig. 1).

Experimental conditions. Experiments. The five different experimental conditions evaluated in this study are described in Table 1. The control (SED) experiments consisted of polluted sediment without air, fertilizer, sorbent, and/or temperature regulation. The four other experiments examined the effect of the continuous addition of air (A), slow-release fertilizer (F), and both air and fertilizer as well as either the oil-sorbent (SED+A+F+S) or a temperature control (SED+A+F+T). All experiments were performed in duplicate. **Slow-release fertilizer.** At the beginning (T_0) of the experiment, 25 mg of a slow-release fertilizer (Miracle-Gro NPK 18:9:11; ScottsMiracle-Grow, Marysville, OH, USA) was added to the systems SED+A+F, SED+A+F+S, and SED+A+F+T. **Oil sorbent.** Five g of oil-absorbent material (X-Oils,Hellmann-tech, Lehrte, Gemany) was added to the SED+F+S mesocosms. The sorbent was confined within the cylinder throughout the experiment. **Temperature.** In the SED+F+T experiment, the temperature within the biostimulation cylinder was controlled and maintained at 28 ± 1°C , throughout the experiment. This was higher than the temperature of the surrounding environment within the mesocosm (17 ± 1°C). **Sampling strategy and variables tested.** Microbial dynamics were monitored by using a sterile corer to collect sediment samples from each bioremediation system at regular intervals (0, 5, 10, 15, 20, 25 and 30 days). Total DAPI (4′,6-diamidino-2-phenylindole; Sigma-Aldrich, Milan, Italy) counts, MPN (most probable number), BOD, and TERHC (total extracted and resolved hydrocarbons and their derivates) composition were determined. The expression of functional genes was screened as described below. Ecotoxicological tests (mortality of *Corophium orientale*) were carried out at the beginning (T_0) and end (T_{30}) of the experiments.

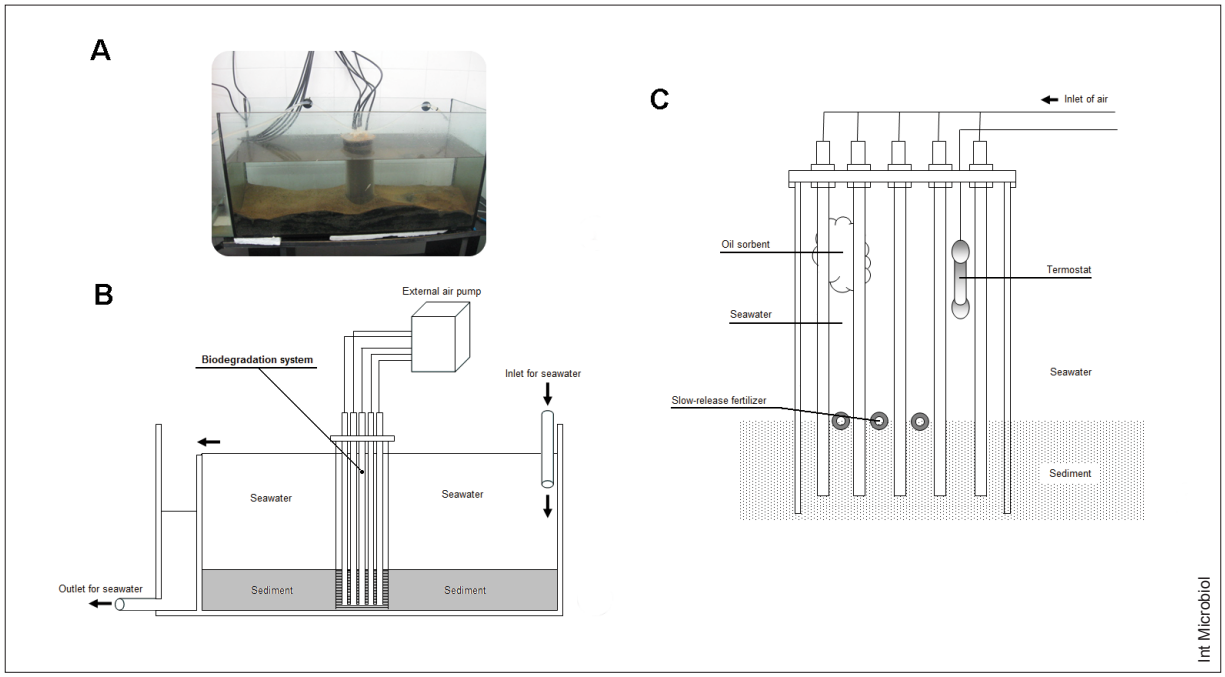

Fig. 1. Diagram of the system used in this study (**A**), and engineering and hydraulic schematization of the system and mesocosm used in this study (**B**, **C**).

Dispersion methods, bacterial counting (DAPI), and MPN counts. Prior to dispersion of the samples, they were incubated for at least 15 min with Tween 80 (final concentration, 1 mg/l). An ultrasonic cleaner bath (Branson 1200 Ultrasonic Cleaner, Branson, USA) and the protocol described by Kuwae and Hosokawe (1999) were used to achieve bacterial dispersion from the sediments (10 min). After centrifugation (8 min, 8000 ×*g*) and collection of the water-Tween 80 phase, the samples were prepared as previously reported [5,35]. DAPI counts were obtained using formaldehyde (2% final concentration) fixed cells. The results are expressed as the number of cells/ml. At the same time, the hydrocarbon-degrading bacteria were enumerated using a miniaturized MPN method [4] with slight modifications [5].

Screening of functional genes. The presence/absence of functional genes involved in the degradation of hydrocarbons was examined to study the metabolic and functional efficiency of the microbial population that developed during the experiments. RNA (from 10 g of experimental sediments) was extracted from the cells using the MasterPure complete DNA&RNA purification kit (Epicenter, Biotechnologies, Madison, WI, USA) according to the manufacturer's protocol. The rRNA-crDNA heteroduplex was synthesized by reverse transcription as previously indicated [5]. PCR assays were done using specific primers created for the metabolic genes of the main members of hydrocarbonoclastic bacteria; specifically, for the alkane hydroxylase of *Alcanivorax* sp. (alkB1-R, 5′-GCTTAGGAACAACGGTTCAGG-3′; alkB1-F; 5′-AATTGGCCTATATCTCGTA-3′) [9] and *Thalassolituus* sp. (alkB-ThlR342, 5′-GGGCCATACAGACAAGCAA-3′; alkBThlF125, 5′-GACGTCGCCACACCTGCC-3′) [21] and for the aromatic ring-hydroxylating dioxygenase of *Cycloclasticus* sp. (PhnC-R, 5′-GCCCAATACCTTGGTTACCG-3′, PhnaC-F, 5′-CCCATCAGGCAACCCGACAG-3′) [16]. The crDNA products were used as the templates in a standard PCR. Total DNA from strains *Alcanivorax* sp., *Thalassolituus* sp., and *Cycloclasticus* sp. was extracted as described above and served as the positive controls in the PCR amplifications. The PCRs were performed by using Qiagen Taq Polymerase (QIAGEN, Valencia, CA, USA) according to the manufacturer's protocol.

Hydrocarbon determination and BOD measurement. The composition of TERHC was analyzed by high-resolution gas chromatography-mass spectrometry (GC-MS) at the beginning (T_0) and end (T_{30}) of the experiments and quantified according to previously described protocols [10].

Table 1. Set-up of experimentations carried out during the study

	Sediment	Air	Temperature	Oil-sorbent	Fertilizer	Code*
Experiment 1	✓	–	–	–	–	SED
Experiment 2	✓	✓	–	–	–	SED+A
Experiment 3	✓	✓	–	–	✓	SED+A+F
Experiment 4	✓	✓	–	✓	✓	SED+A+F+S
Experiment 5	✓	✓	✓	–	✓	SED+A+F+T

*SED, marine sediment; A, air addition; F, fertilizer addition; S, sorbent addition and T, temperature regulation.

TERHC were extracted from sediment samples (1 kg) using the 3550C EPA (US Environmental Protection Agency) procedure [28]. High-resolution GC-MS was carried out using a TurboMSAutoSystemXL GC (Perkin-Elmer, Foster City, CA, USA) as previously indicated [5]. BOD was measured using a BOD sensor (VELP Scientifica, Milan, Italy) according to the manufacturer's instructions.

***Corophium orientale* bioassay.** *Corophium orientale* was obtained from CIBM (Livorno, Italy) and used according to a previously described procedure [26]. Juveniles and young adults that passed through a 1000-µm sieve and were retained on a 710-µm mesh were selected for the ecotoxicology experiments, which were carried out in 2.5-liter glass flasks containing approximately 2 cm of sediments and 1000 ml of filtered seawater. The seawater was aerated and kept at a constant temperature (16 ± 2°C). The flasks were illuminated for 12 h daily using a lamp containing six light tubes (36W, 120 cm). Each flask was inoculated with 100 randomly selected amphipods. No food was added to either the test or the control chambers. After 10 days, the surviving organisms were counted. Missing amphipods were assumed to have died. The sensitivity of the populations was estimated as the fraction of dead organisms compared to the initial number of added amphipods. All the experiments were performed twice.

Results

Sediment composition. According to the geochemical analysis, the sediment used in the study had a low concentration of water (<15%) and was dominated by the silt-clay fraction (i.e., <60 µm, ~95%), mainly constituted by quartz, albite, and silicates. Further analysis showed that the sediment contained low concentrations of organic matter (total organic matter, ~34 mg/g) and heavy metals (As, Cd, Mn, Hg, Pg, Cu, Zn, and Fe, all <10 mg/kg).

Total bacterial counts (DAPI) and MPN. Figures 2 and 3 show the total bacterial counts (DAPI) and the MPN, respectively, for all of five experimental conditions.

Screening of functional genes. At the beginning of the experiments, genes involved in biodegradation processes were not expressed, as determined by amplification with primers specific for functional genes. This lack of expression persisted throughout the experimental period under the control condition (SED). After five days (T_5), genes encoding the alkane mono-oxygenase of *Alcanivorax* sp. were amplified in the SED+A+F+T experiment. In the remaining experiments (SED+A, SED+A+F, SED+A+F+S and SED+A+F+T), positive amplification results were obtained only for the expression of genes related to *Alcanivorax* genus and only on days 15 and 20, close to the end of the 30-day experimental period. No positive amplification signal corresponding to the alkane hydroxylase of *Thalassolituus* sp. and the di-oxygenease of *Cycloclasticus* sp. was detected in any of the experiments.

Hydrocarbons composition and biochemical oxygen demand. The GC-MS analysis of hydrocarbon degradation showed that more than half of the TERHCs were degraded in the SED+A system after 30 days of incubation. In both the SED+A+F and SED+A+F+S mesocosms, 56% of the TERHCs were degraded. The best degradation results (76%) were obtained in the SED+A+F+T mesocosm, where the in-

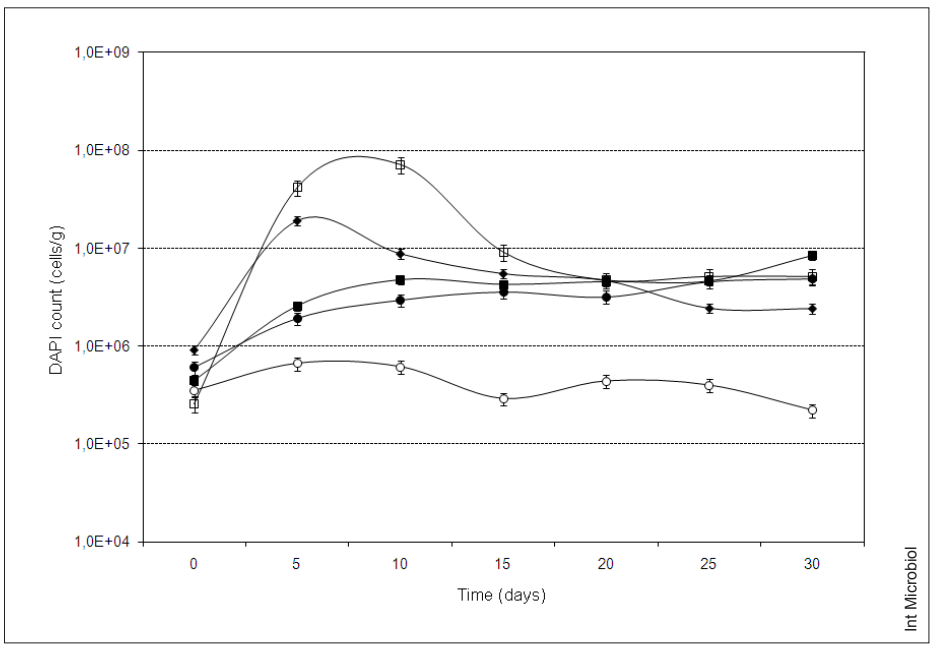

Fig. 2. Bacterial abundance determined by DAPI staining. Concentration (log cell/ml) of the cells observed during experimentations performed during this work. SED (empty circles), SED+A (filled circles), SED+A+F (filled squares), SED+A+F+S (filled diamonds) and SED+A+F+T (empty squares).

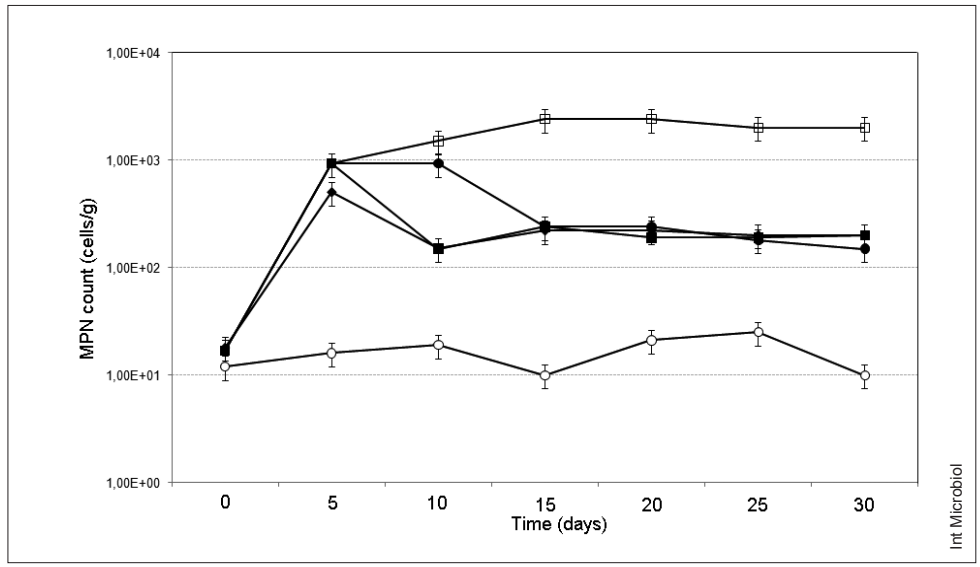

Fig. 3. Most Probable Number (MPN, log MPN/ml) during biostimulation experimentations. SED (empty circles), SED+A (filled circles), SED+A+F (filled squares), SED+A+F+S (filled diamonds) and SED+A+F+T (empty squares).

cubation temperature was higher. Minimum and maximum degradation of n-alkanes was achieved in the SED (5%) and SED+A+F+T (68%) experiments, respectively. In the SED+A, SED+A+F, and SED+A+F+S mesocosms, *n*-alkane (C_{10}–C_{32}) degradation was 47, 50, and 43%, respectively (Fig. 4). The BOD values measured at the beginning (T_0) and end (T_{30}) of the experimental period are reported in Fig. 5.

Assays on *Corophium orientale*. Based on the rates of *Corophium orientale* mortality, toxicity was highest in the SED sample, both at time zero and after 30 days of incubation. At the end of the experiments, toxicity in the SED+A, SED+A+F, and SED+A+F+S systems resulted in a mortality close to 50%. Toxicity in the sediments sampled in the SED+A+F+T and control mesocosms was very low, as at the end of the experiments 70% and 90% of the organisms were still alive.

Discussion

Recent efforts in environmental biotechnology have focused on the use of biological treatments for the restoration of polluted areas, with the best results obtained using bioremediation [1,27]. This emerging and promising technology can be used to clean up oil pollution in coastal and open seas, including areas that are chronically polluted (e.g., harbors, shipyards) [8,14,25]. However, hydrocarbon pollutants (crude oil) discharged in deep marine environments (sediments) are difficult to degrade [22,23]. Indeed, in natural polluted sediments, the rates of biodegradation are very low even after a long period of incubation [22,23].

In this study, we tested a methodology for the in situ recovery of anoxic marine sediments and evaluated the results of its laboratory-scale application. The proposed approach makes use of a simple device in which the limiting factors for biodegradation are controlled so as to enhance the development and selection of a bacterial microflora able to effectively degrade hydrocarbons. The proposed system was designed according to the two needs highlighted by Nikolopoulou and Kalogerakis [25]: (i) the development of low-cost oxygenation systems for aerobic bioremediation of contaminated anoxic sediments, and (ii) the development of novel bio-stimulant methods that are nontoxic to the marine environment. Our results demonstrated the importance of air injection in the development of bacterial biomass; indeed, the DAPI and MPN counts and the BOD values obtained in the experiments with air insufflation were higher than those in the control (untreated sediments). However, the introduction of nutrients and especially temperature regulation were also crucial in achieving high levels of biodegradation in the shortest possible time.

Studies have shown that bacterial degradation proceeds slowly at low temperature and that only certain bacterial genera can degrade aliphatic oil fractions [7,21]. In our system, the temperature increment may have favored the development of mesophilic bacteria, which are effective biodegraders. In the SED+A+F+T mesocosm, ~70% of the total oil and linear hydrocarbons were degraded. This level of degradation can be explained by an increase in microbial abundance/activity, as

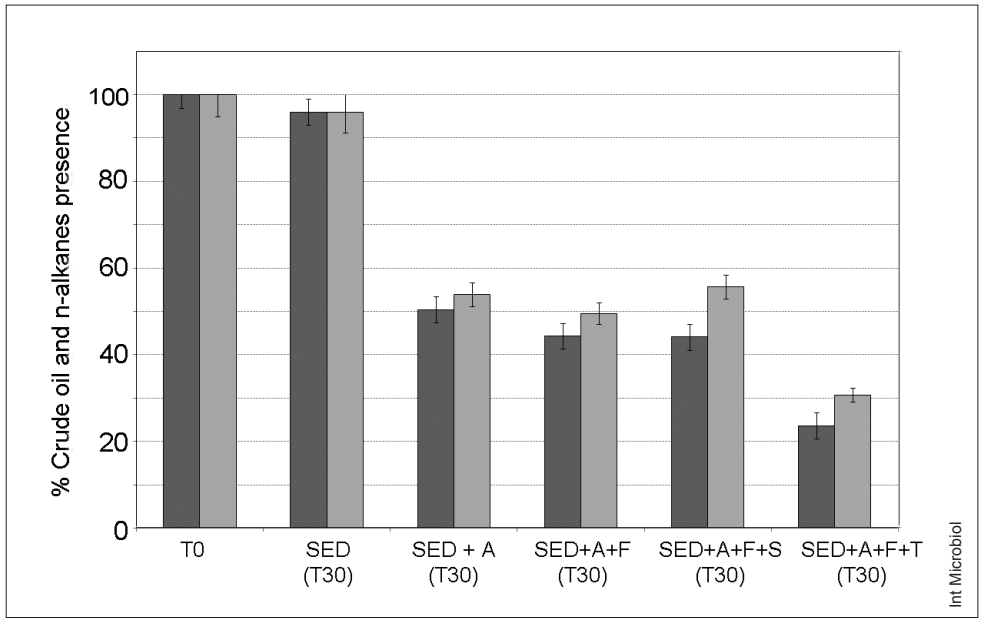

Fig. 4. Relative percentage of crude oil (dark grey bars) and *n*-alkanes (C_{10}-C_{32}; white grey bars) present in sediment at the beginning of experiment (T_0) and after 30 days of biostimulation experiment with addition of air (SED+A), slow-release fertilizer (SED+A+F), sorbent (SED+A+F+S) and temperature (SED+A+F+T).

evidenced by the increase in DAPI and MPN counts and by the increased BOD values.

These results are in accordance with those obtained by the screening of functional genes. In experiments carried out with air insufflations, nutrient addition, and temperature regulation, bacteria belonging to the *Alcanivorax* genus were present as early as day 5 of the experiment; under the other conditions, these bacteria were detected after day 15. The lack of expression of functional genes related to the other bacterial genera (e.g., *Thalassolituus* and/or *Cycloclasticus*) may have been due to the characteristics of the oil used in this study [21].

Although the experiments were conducted over 30 days, substantial differences in the abundance of the microbial population were detected only during the first 15 days. This suggests that specialization of the degrading population occurred within the first few hours, after the limiting factors characteristic of natural systems had been removed by the addition of an oleophilic slow-release fertilizer (Miracle-Gro NPK 18:9:11). However, the dissolution of these oleophilic nutrients depended on the mechanical-physical characteristics of the system, and the non-uniform dissolution resulted in their less than optimal availability to the microbial population. The addition of dissolved nutrients in the form of salts that are introduced into the experimental systems at defined concentrations may better promote the development of oil-degrading bacteria and of hydrocarbon-tolerant bacteria. Kasai et al. [16] examined the effects of slow-release fertilizers on oil biodegradation. They found that the addition of fertilizers promoted the degradation mainly of certain components of crude oil; thus, > 90% of n-alkanes (C_{15}–C_{30}) and > 60% of (alkyl)naphthalenes were degraded within 30 days whereas degradation of three-ring aromatics (phenanthrene, anthracene, fluorene and their alkyl substituted derivatives) was only 30%–40%. By contrast, in field experiments carried out on the sand and cobblestone beaches of Japan after the Nakhodka oil spill [18,19], alkanes were degraded to a lesser extent than naphthalene or fluorene and to the same extent as dibenzothiophene and phenanthrene.

Nutrient amendment to the hydrocarbon-contaminated sediments enhanced the biodegradation activity of the natural microbial community. Highly specialized marine hydrocarbonoclastic bacteria represent only a minor fraction of this community but play an important role in the biodegradation of petroleum hydrocarbons that accidentally enter the marine environment [33]. Our results were confirmed by the variation in BOD, which was higher in the microcosm containing the oleophilic slow-release fertilizer.

The addition of absorbent material (X-Oils, Hellmanntech) to the system was crucial in the early stages of the experiment, when air blown into the system caused the release of the oil present in the sediment into the water, as also shown

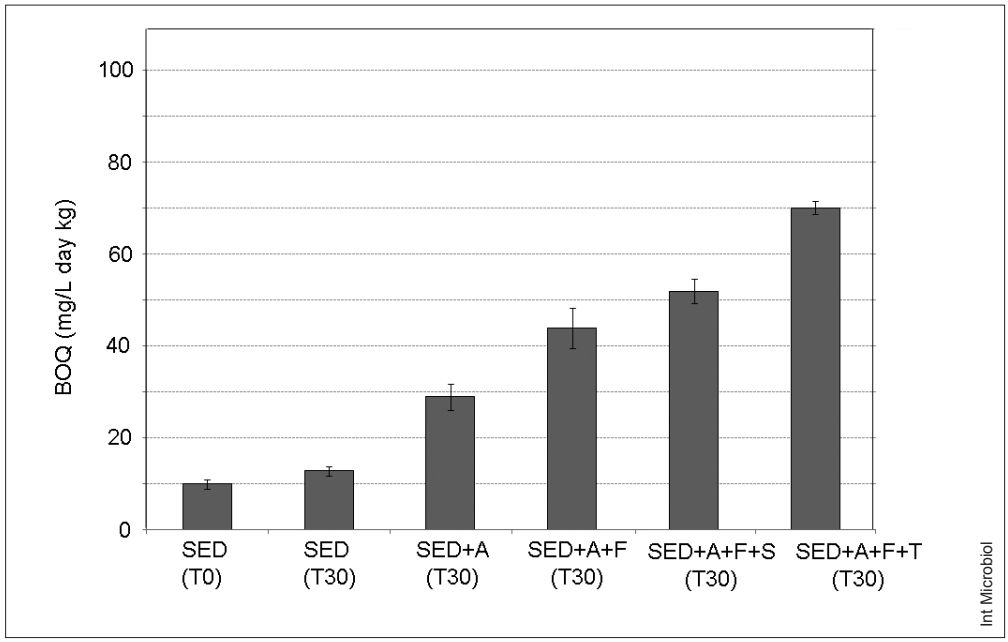

Fig. 5. Dynamic of oxygen consumption (biochemical oxygen demand, BOD, mg/l day kg) at the beginning (T_0) and end (30 days) of the experiments.

in similr studies [12,13]. The reduction of contaminant petroleum in the sediment was accompanied by a reduction of the toxicity of the sediments. The sensitivity of *C. orientale*, an endemic Mediterranean amphipods species, to pollutants contaminating the water column (due to dredging activities) has been well demonstrated [2,26]. In fact, *C. orientale* is used as an indicator species in standardized eco-toxicological bioassays of harbor sediments [26]. We found that, after a reduction of the oil in the sediments, the toxicity to *C. orientale* was ~30%, which was much lower than that at the beginning of the experiment and in the untreated system (~ 95%; SED, T_0 and SED, T_{30}). Toxicity in the other mesocosms was also reduced (SED+A, 50%; SED+A+F, 55% and SED+A+F+S, 45%).

This study shows that, by stimulating the native microbial population, capping combined with in situ aeration is a feasible approach to the efficient recovery of petroleum-contaminated anaerobic marine sediments.

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Competing interests. None declared.

References

1. Beolchini F, Rocchetti L, Regoli L, Dell'Anno A (2010) Bioremediation of marine sediments contaminated by hydrocarbons: experimental analysis and kinetic modelling. J Haz Mat 182:403-407
2. Bigongiari N, Brida T, Carretti F, Pellegrini D (2004) Influence of Temperature on the Mortality and Sensitivity of *Corophium orientale*. Bull Environ Contam Toxicol 72:881-887
3. Bortone G, Arevalo E, Deibel I, Detzner H-D, Propris, LD, Elskens, F, Giordano A, Hakstege P, Hamer K, Harmsen J, et al (2004) Sediment and dredged material treatment synthesis of the SedNet work package 4, outcomes. J Soils Sed 4:225-232
4. Brown EJ, Braddock JF (1990) Sheen Screen, a miniaturized Most-Probable-Number method for enumeration of oil-degrading microorganisms. Appl Environ Microbiol 56:3895-3896
5. Cappello S, Caruso G, Zampino D, Monticelli LS, Maimone G, Denaro R, Tripodo B, Troussellier M, Yakimov MM, Giuliano L (2007) Microbial community dynamics during assays of harbour oil spill bioremediation: a microscale simulation study. J Appl Microb 102:184-194
6. Cappello S, Russo D, Santisi S, Calogero R, Gertler C, Crisafi F, De Domenico M, Yakimov MM (2012) Presence of hydrocarbon-degrading bacteria in the gills of mussel *Mytilus galloprovincialis* in a contaminated environment: a mesoscale simulation study. Chem Ecol 28:239-232
7. Coulon F, McKew BA, Osborn AM, McGenity TJ, Timmis KN (2007) Effects of temperature and biostimulation on oil-degrading microbial communities in temperate estuarine waters. Environ Microbiol 9:177-186
8. Da Silva MLB, Alvarez PJJ (2010) Bioaugmentation. In Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology, McGenity TJ, van der Meer JR, De Lorenzo V (eds)

9. Della Torre C, Tornambè A, Cappello S, Mariottini M, Perra G, Giuliani E (2012) Modulation of CYP1A and genotoxic effects in European seabass *Dicentrarchus labrax* exposed to weathered oil: A mesocosm study. Mar Envir Res 76:48-55
10. Denaro R, D'Auria G, Di Marco G, Genovese M, Troussellier M, Yakimov MM, Giuliano L (2005) Assessing terminal restriction fragment length polymorphism suitability for the description of bacterial community structure and dynamics in hydrocarbon-polluted marine environments. Environ Microb 7:78-87
11. Fonti V, Beolchini F, Rocchetti L, Dell'Anno A (2014) Bioremediation of contaminated marine sediments can enhance metal mobility due to changes of bacterial diversity. Water Research 68:637-650
12. Gertler C, Timmis KN, Golyshin PN (2009) Microbial consortia in mesocosm bioremediation trial using oil sorbents, slow-release fertilizer and bioaugmentation. FEMS Microb Ecol 69:288-300
13. Gertler C, Timmis KN, Yakimov MM, Golyshin PN (2009) Populations of heavy fuel oil-degrading marine microbial community in presence of oil sorbent materials. J Appl Microb 107:590-605
14. Hazen TC (2010) Biostimulation In Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology, McGenity TJ, van der Meer JR, De Lorenzo V (eds), pp 4518-4527
15. Jiao N, Herndl GJ, Dennis A, Benner HR, Kattner G, Wilhelm SW, Kirchman DL, Weinbauer MG, Luo T, Chen F, Azam F (2010) Microbial production of recalcitrant dissolved organic matter: long-term carbon storage in the global ocean. Nature Rev Microbiol 8:593-599
16. Kasai Y, Kishira H, Sasaki T, Syutsubo K, Watanabe K, Harayama S (2002) Predominant growth of *Alcanivorax* strains in oil-contaminated and nutrient-supplemented sea water. Environ Microbiol 4:141-147
17. Kasai Y, Kishira H, Harayama S (2003) Bacteria belonging to the genus *Cycloclasticus* play a primary role in the degradation of aromatic hydrocarbons released in a marine environment. Appl Environ Microbiol 68:5625-5633
18. Lane DJ (1991) 16/23S rRNA sequencing. In: Stackerbrandt E, Goodfellow M (eds), Nucleic acid techniques in bacterial systematics. Wiley, New York, pp 115-175
19. Maki H, Hirayama N, Hiwatari T, Kohata K, Uchiyama H, Watanabe M, Yamasaki F, Furuki M (2003) Crude oil bioremediation field experiment in the Sea of Japan. Mar Poll Bull 47:74-77
20. Maki H, Utsumi M, Koshikawa H, Hiwatari T, Kohata K, Uchiyama H, Suzuki M, Noguchi T, Yamasaki T, Furuki M, Watanabe M (2002) Intrinsic biodegradation of heavy oil from Nakhodka and the effect of exogenous fertilization at a coastal area of the sea of Japan. Water Air and Soil Pollut 145:123-138
21. McKew BA, Coulon F, Osborn AM, Timmis KN, McGenity TJ (2007) Determining the identity and roles of oil-metabolizing marine bacteria from the Thames estuary, UK. Environ Microb 9:165-176
22. Miralles G, Grossi V, Acquaviva M, Duran R, Bertrand J, Cuny P (2007) Alkane biodegradation and dynamics of phylogenetic subgroups of sulfate-reducing bacteria in an anoxic coastal marine sediment artificially contaminated with oil. Chemosphere 68:1327-1334
23. Miralles G, Nerini D, Manté C, Acquaviva M, Doumenq P, Michotey V, Nazaret S, Bertrand J, Cuny P (2007) Effects of spilled oil on bacterial communities of Mediterranean coastal anoxic sediments chronically subjected to oil hydrocarbon contamination. Microb Ecol 54:646-661
24. Mulligan CN, Yong RN, Gibbs BF (2001) An evaluation of technologies for the heavy metal remediation of dredged sediments. J Haz Mat B 85:145-163
25. Nikolopoulou M, Kalogerakis N (2010) Biostimulation. Strategies for enhanced bioremediation of marine oil spills including chronic pollution. In Timmis KN (ed) Handbook of hydrocarbon and lipid microbiology, McGenity TJ, van der Meer JR, De Lorenzo V (eds), pp 2521-2529
26. Onorati F, Bigongiari N, Pellegrini D, Giuliani S (1999) The suitability of *Corophium orientale* (Crustacea, Amphipoda) in harbour sediment toxicity bioassessment. Aquat Ecosys Health Man 2: 465-473.
27. Rocchetti L, Beolchini F, Ciani M, Dell'Anno A (2011) Improvement of bioremediation performance for the degradation of petroleum hydrocarbons in contaminated sediments. Appl Environ Soil Sci, doi:10.1155/2011/319657
28. Rocchetti L, Beolchini F, Hallberg KB, Johnson B, Dell'Anno A (2012) Effects of prokaryotic diversity changes on hydrocarbon degradation rates and metal partitioning during bioremediation of contaminated anoxic marine sediments. Mar Poll Bull 64:1688-1698
29. Rodríguez-Blanco A, Vetion G, Escande M-L, Delille D, Ghiglione J-F (2010) *Gallaecimonas pentaromativorans* gen. nov., sp. nov., a bacterium carrying 16S rRNA gene heterogeneity and able to degrade high-molecular mass polycyclic aromatic hydrocarbons. Int J Syst Evol Microb 60:504-509
30. Rulkens WH, Bruning H (2005) Cleanup technologies for dredged fine sediments: review and future challenges. In: Olfenbuttel RF, White PJ (eds) Remediation of contaminated sediments: Finding achievable risk reduction solutions, C6-01
31. Schwieger F, Tebbe CC (1998) A new approach to utilize PCR-single-strand-conformation polymorphism for 16S rRNA gene-based microbial community analysis. Appl Environ Microbiol 64:4870-4876
32. Yakimov MM, Cappello S, Crisafi E, Tursi A, Corselli C, Scarfì S, Giuliano L (2006) Phylogenetic survey of metabolically active microbial communities associated with the deep-sea coral *Lophelia pertusa* from the Apulian Plateau, Central Mediterran Sea. Deep Sea Res, part I:62-75
33. Yakimov MM, Denaro R, Genovese M, Cappello S, D'Auria G, Chernikova TN, Timmis KT, Golyshin PN, Giuliano L (2005) Natural microbial diversity in superficial sediments of Milazzo Harbour (Sicily) and community successions during microcosm enrichment with various hydrocarbons. Environ Microbiol 7:1426-1441
34. Yakimov MM, Gentile G, Bruni V, Cappello S, D'Auria G, Golyshin PN, Giuliano L (2004) Crude oil-induced structural shift of coastal bacterial communities of Rod Bay (Terra Nova Bay, Ross Sea, Antarctica) and characterization of cultured cold-adapted hydrocarbonoclastic bacteria. FEMS Microbiol Ecol 49:419-432
35. Zampino D, Zaccone R, La Ferla R (2004) Determination of living and active bacterioplankton: a comparison of methods. Chem Ecol 20: 411-442
36. Zhao B, Wang H, Li R, Mao X (2010) *Talassospira xinhensis* sp. nov., a polycyclic aromatic hydrocarbon-degrading marine bacterium. Int J System Bacteriol 60:1125-1129