Kill•Spill

Integrated Biotechnological Solutions for Combating Marine Oil Spills

Deliverable D6.4

Report on microbial degradation performance of small-scale boom prototypes

This project is supported by the European Union under the Food, Agriculture and Fisheries and Biotechnology theme of the 7th Framework Programme for Research and Technological Development under GA no. 312139

Work package	WP6 Development of multifunctional remediation agents for oil spills
Deliverable no	D6.4
Deliverable title	Report on microbial degradation performance of small-scale boom prototypes
Due date:	Month 36 (2015-12-31)
Actual submission date:	Month 43 (2016-08-11)
Start date of project:	2013-01-01
Deliverable Lead Beneficiary (Organisation name)	CSIC
Participant(s) (Partner short names)	IAMC-CNR, CSIC
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Dissemination Level: (PUblic, Restricted to other Programmes Participants, REstricted to a group specified by the consortium, COnfidential only for members of the consortium)	PU
Deliverable Status:	Final

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1 About this Deliverable

According to the Project activity foreseen in WP6 "Development of multifunctional remediation agents for oil spills" (Sub-task 6.4: Improved Biodegrading Boom for small oil spills), different types of booms constructed in this WP had to be tested to optimize the bioremediation process. Initially, project implies the use of absorbent material as the base of bio-booms by contemporary use of bacteria and other chemicals (nutrients and/or biosurfactants) for the recovery of contaminated matrices.

In addition to this activity, and especially due to lack of alternative boom prototypes sent in time to IAMC-CNR, we tried to use the immobilized bacteria encapsulated in the beads of chitosan. To our knowledge, this approach could be considered as a simplest solution to achieve the goals of Sub-task 6.4 by production of a boom with the most promising combination of sorbent and bioremediation agents. To obtain maximally efficient biodegradation, we used various combinations of hydrocarbonoclastic bacteria, surfactants and nutrients.

2 Introduction

To evaluate the different strategies for the bioremediation and to define the most promising scenario of environmental recovery, IAMC-CNR studied the possibility of application as bioremediation enhancing agent the chitosan beads containing immobilized bacteria, slow releasing fertilizers and bio-surfactant.

As less toxic technology of immobilization, we use the encapsulation approach, which can produce semi-permeable particles of different shape with diameters ranging from few nanometers to a few millimeters can be realized by incorporating the biological components (cells) in the matrices.

As it described elsewhere, this approach seems more beneficial than direct release of hydrocarbon-degrading microorganisms in the environment (Bayat et al., 2015; Dellagnezze et al., 2016). This may be due to plain protective effect of the matrices. In fact, encapsulation is often referred to as an alternative way to protect organisms from adverse environmental conditions. The objective of encapsulation, therefore, is the realization of a micro-environment in which the microorganisms will survive during the period of storage (shelf-life) up to the moment of release and specific usage.

During this activity we were oriented on development of encapsulation technology, which will be further tested through trials both at microscale (microcosms) and mesoscale (mesocosms).

Up to date the activity was concentrated on:

- Encapsulation of optimized and most efficient bacterial consortia in chitosan beads (BCCB);
- Test of biodegradation activity of BCCB at microscale (microscosms);
- Addition of biosurfactant in chitosan beads during BCCB encapsulation and production of modified BCCB (mBCCB)
- Test of biodegradation activity of BCCB at microscale (microscosms).

Report on the application of bacteria/consortia encapsulated in chitosan beads for recovery of oil polluted seawater in microcosm (test on biodegradation activity) and mesocosm experiments is given below.

3 Microcosms experiments: test of biodegradation activity

3.1 Immobilisation of bacteria in chitosan beads and microcosms experiments

In order to enhance the effectiveness of microorganisms in natural environments, the microbial immobilization in biological matrices is an alternative technique that has been evaluated (Gentry et al., 2004, Le-Tien et al., 2004 and Siripattanakul and Khan, 2010). Immobilization or encapsulation process can provide a physical support for biofilm formation and slow release of microbial cells in the surrounding medium, resulting in an increased capacity to support stressful environmental conditions, consequently enabling more efficient biodegradation process when compared to those performed by free cells (Chen et al., 2007). In addition, encapsulation may allow the control of nutrient flow, lowering the concentration of toxic compounds in the microenvironment of the cells and minimizing cell membrane damage, besides protecting from predation and competition; thereby mimicking a miniature bioreactor in the environment (Tyagi et al., 2011). The choice of a proper matrix can influence the success of the microbial response and some natural polymers have been evaluated elsewhere, like alginate, agarose and agar (Vassileva et al., 2003 and Ahamad & Kunhi, 2011). Chitosan is a polymer obtained from deacetylation reactions of chitin, a polysaccharide present in crustaceans, as shrimps and lobsters. Due to some intrinsic features, this material presents some advantages when compared with synthetic matrix, such as biodegradability, non-toxicity and availability in the natural environment (Angelim et al., 2013). Entrapment process enables a high concentration of microorganisms (up to 10^8 cells in one gram of beads) in chitosan beads even when used in continuous bioreactors, with no significant decrease of cells when compared with free cell condition (Hsieh et al., 2008).

3.2 Experimental set-up

Preliminary test for evaluate the degradation capability of the strains and/or bacterial consortia after encapsulation different microcosm experimentation were carried out. Each microcosm contained 100 mL of mineral minimum medium ONR7a, 5 g of beads of specialized hydrocarbons-degrading bacterial consortium (PSO and PSM; each 10^8 cell in one gram of beads) and petroleum hydrocarbons as the sole source of carbon and energy (0.1% of Arabian Light Crude Oil or 300 ppm of mixture of polycyclic aromatic hydrocarbons [PAH; 100 ppm phenanthrene, 100 ppm pyrene and 100 ppm benzo(α)pyrene] in accordance with the metabolic demands of the consortia in the study).

Abiotic controls were prepared with culture medium and the substrates tested. At the beginning of the experimental period (T_0) and after 7 (T_7) and 14 (T_{14}) days, samples were taken from each microcosm for assessing total bacterial abundance (DAPI direct counts) and the degradation of hydrocarbons. The quantitative analysis of hydrocarbons was assessed by analysis by GC-MS and all data were expressed as a percentage of oil or PAHs remaining.

3.3 Results

The microbial consortium PSO on the seventh day of incubation showed the rates of degradation of 74%, which arrived at a rate of 94% on the fourteenth day of incubation (Figure 1). Remarkably, only heaviest fraction of crude oil ($>C_{35}$) has remained in the PSO microcosm (Figure 2). The biodegradation capacity of the microbial consortium PSM was evaluated with the mixture of polycyclic aromatic hydrocarbons. On the seventh day of incubation no statistically significant changes in the mixture of polyaromatic hydrocarbons was detected between control and PSM consortium. Nonetheless, second week of experimentation was characterized by initiation of PAH degradation and 40% of added compounds (mainly phenanthrene; see Figure 2) was consumed by PSM consortium.

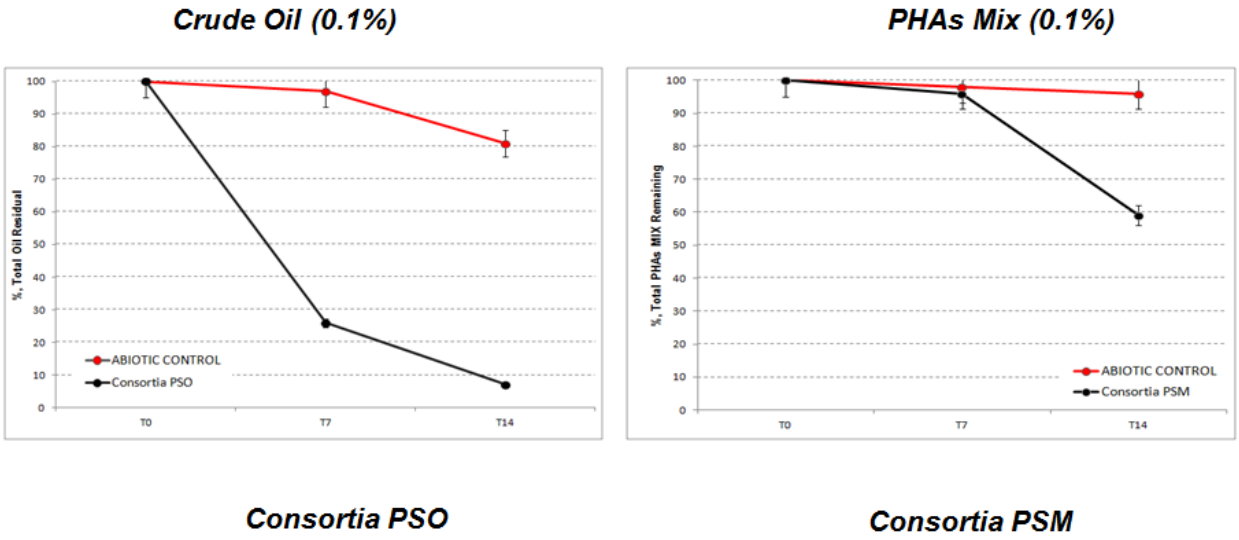

Figure 1 **Percentage (%) of total oil/PHAs remaining detected during two weeks in microcosm experiments.**

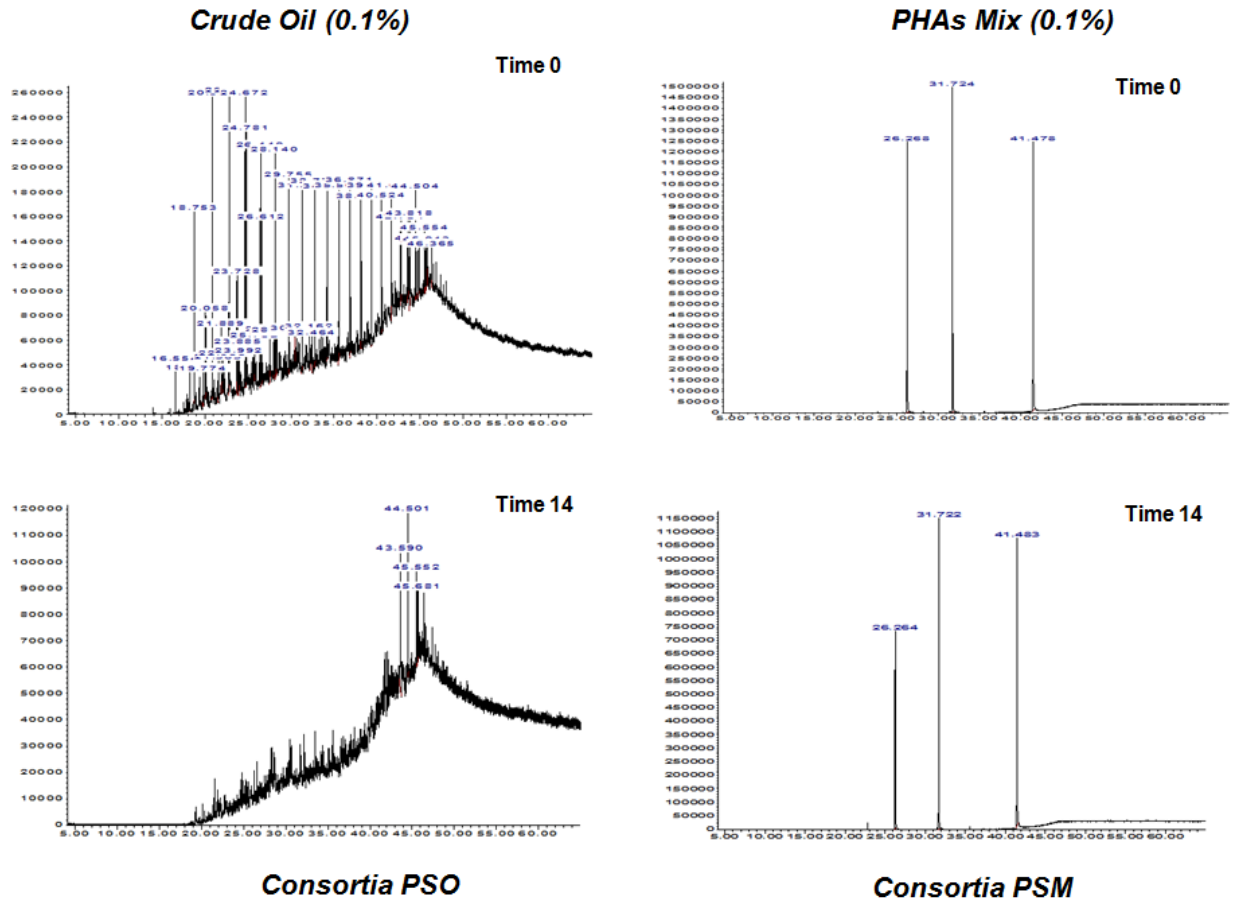

Figure 2 **GC-FID chromatogram of degradation of oil and PHAs in microcosms experiment.**

4 Mesocosm experiments

4.1 Experimental set-up

All experiments were carried out in circular tank of ~1,500 L capacity at IAMC-CNR facilities. The mesocosms were filled with ca. 1000 l of seawater taken directly from the harbor of Messina (38◦11 42.58N 15◦34 25.19E). Before using, seawater was filtered through a 200 mm nylon mesh to remove large metazoans and detritus. Seawater temperature (18 ± 2°C) was checked during the experimental period (30 days). Seawater was aerated and kept under agitation during all experimental period. In brief, mesocosm water was mixed by a pump (35 l h^{-1}), placed at the exit of each tank, that takes water from two opposite bottom corners and drives it below the surface. Both mesocosms (CONT and POOL) were supplemented with 70 ml (60 mg L^{-1}) of sterile Arabian Light Crude Oil to simulate a chronic pollution conditions (60 ppm). Mesocosm POOL was additionally supplemented with 250 g of chitosan beads containing 10^8 g^{-1} bacterial cells (Figure 3 and Figure 4).

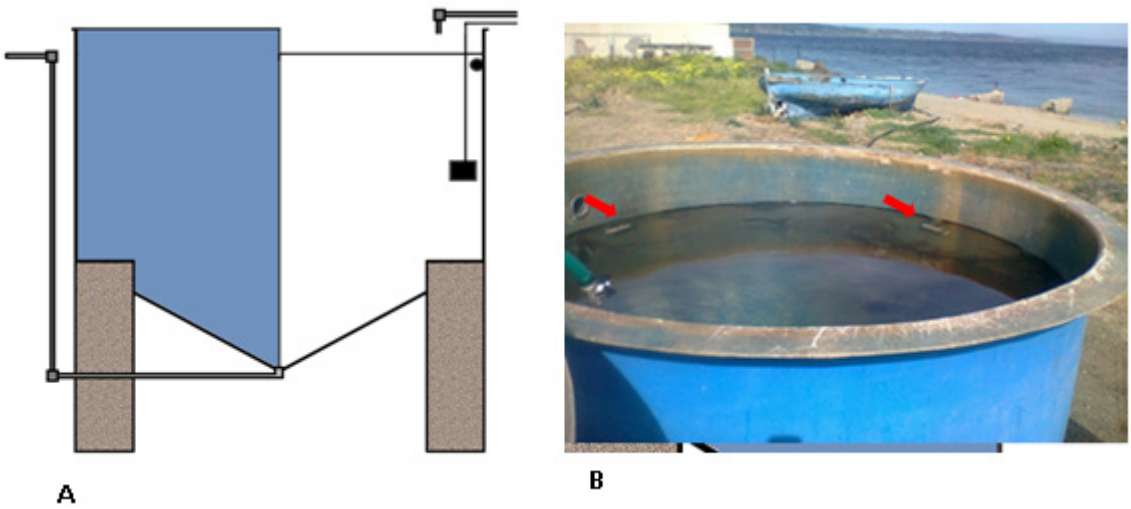

Figure 3 **Mesocosms tanks used for the experiments (A) Schematic representation of engineered and hydraulic system, (B) view of the system with traps of immobilised consortia**

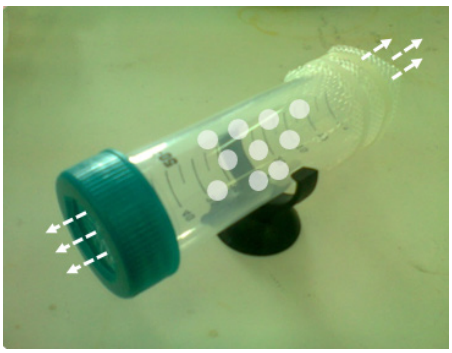

Figure 4 **Traps with chitosan beads used in this activity. Sucker was used to permit the adherence of this system on internal mesocosm wall; the white arrows indicate the possible area of emission of the bacterial cells released from the beads of chitosan.**

4.2 Results

Data obtained from GC-FID analysis show that after 30 days of experimentation, almost half of added crude oil was degraded compared to 15% of oil disappearance in control tank (Figure 5)

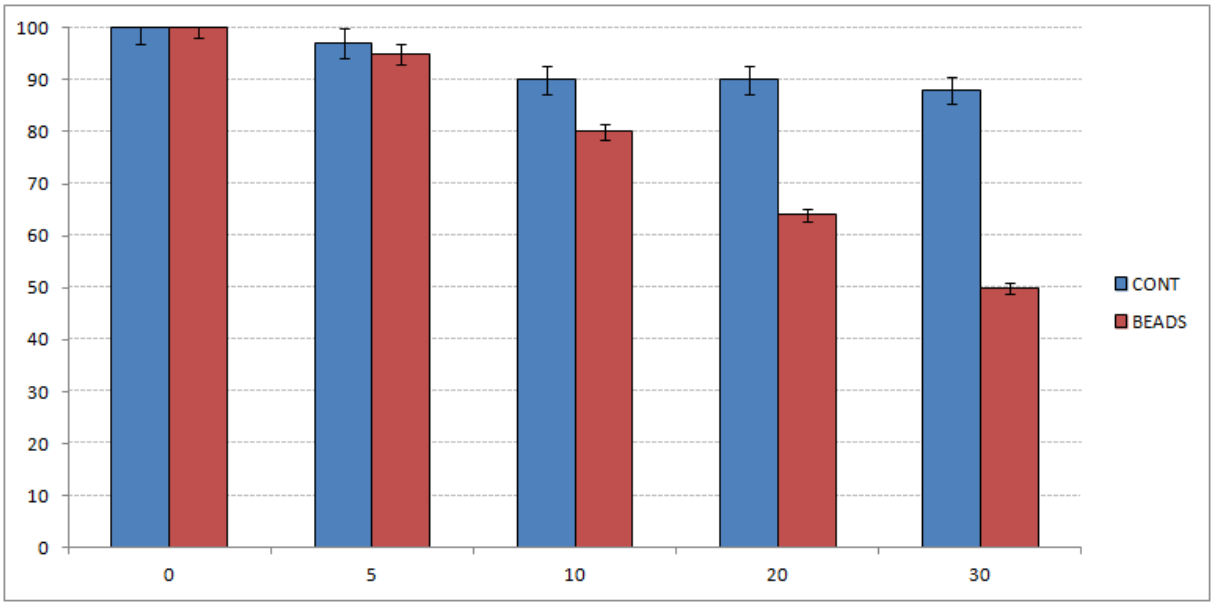

Figure 5 Percentage (%) of oil remaining in the control and in the treatment with beads

5 Conclusions

The results show the great potential of using bacterial cells encapsulated in chitosan. Although the rates of biodegradation obtained at the microscale (microcosms) (94%) significantly exceeded that, documented in mesocosms, half of crude oil released in 1,500 L tank was degraded during one month of experimentation. Taken together, both experiments demonstrated the applicability of chitosan encapsulation methodology.

We are aware, that some issues related to the viability of bacterial cells after encapsulation processes and their resilience (recovery of metabolic activity) after storage (shelf-life) still remain to clarify while we will prepare definitive formulation of chitosan encapsulation approach foreseen to be produced at the end of the Project.

Product	8 Bio-booms (IAMC-CNR)
Description	indigenous microbes on chitosan
Benchmark product/ technology	Bioaugmentation agents,
Status/stage of development	Formulation ready / 1 kg
Level of testing	Microcosms, mesocosm
M42 targets	Combine with other products
WP8 testing? when, where?	Mesocosms Messina
Pricing info (also for benchmark)	appr. 200-250€ /kg
Effectiveness	TPH and PAH reduction
Ecotox assessment	Yes WP7
Authorisation & Application	Not possible in Italian sea

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