Microbial and additive formulations for enhanced bioremediation and biotreatments

by the

BIOSURFACTANTS producer strain *Rhodococcus sp.* HFO180-S2B (réf. Collection Madep N° TB-4058)

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KILL SPILL

Integrated Biotechnological Solutions for Combating Marine Oil Spills

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Rhodococcus sp. **HFO180-S2B (Madep)**

Biosurfactants and their applications

- Biosurfactants can be defined as **surface-active biomolecules produced by microorganisms** with a wide-range of applications.

- When compared to chemical or synthetic surfactants, **biosurfactants gained several advantages** including their biodegradability and ecological biocompatibility.

- Due to their unique functional properties, biosurfactants were **used in several industries** including :
  - ✓ organic chemicals,
  - ✓ petroleum and petrochemicals,
  - ✓ mining and metallurgy (mainly bioleaching),
  - ✓ agrochemicals and fertilizers,
  - ✓ foods and beverages,
  - ✓ cosmetics and pharmaceuticals,
  - ✓ and many others….

- They can be used as emulsifiers as well as **de-emulsifiers, wetting agents, foaming agents, spreading agents, functional food ingredients and detergents**.

- The biosurfactants can be used in **environmental cleanup** by biodegradation and detoxification of industrial effluents and in **bioremediation** of contaminated soil.

Rhodococcus sp. **HFO180-S2B (Madep)**

Colonies of the strain on solid medium

- The strain Rhodococcus erythropolis HFO-S2B (MADEP) has excellent abilities to solubilize (by biosurfactants production) and to degrade HFO-180 and DANSK crude oils.

- Colonies of HFO-S2B on TSA (Tryptone Soya Agar, after 3-4 days)

ACTYGEA

MADEP SA

Rhodococcus sp. **HFO180-S2B (Madep)**

Pysiological properties

Physiological parameters tested	Results
Growth temperature	5°C to 37°C (optimum 20-30°C)
PH growth	5 to 9 (optimum 7 to 8,2)
Anaerobic growth	No
Growth on basal mineral medium + 33 g NaCl + 1-20g / l simple organic compounds (lactate, dextrose, glycerol)	Good
Growth and degradation of various types of vegetable oils (colza or rapeseed, soybean, olive, coconut, linseed, sunflower, nut, peanut, grape seed, sesame, safflower).	Good
Degradation and solubilization of heating oil and dielectric oil.	Good
Solubilisation of PAH's (chrysene, benzo-a-pyrene, benzo-a-anthracene) and PCB (Aroclor 1260)	Good
Binding capacity (biofilm formation) on Kaldnes K1 Media	Good

Rhodococcus sp. **HFO180-S2B (Madep)**

Strong solubilization of PAH`s, PCB and dieletric oil

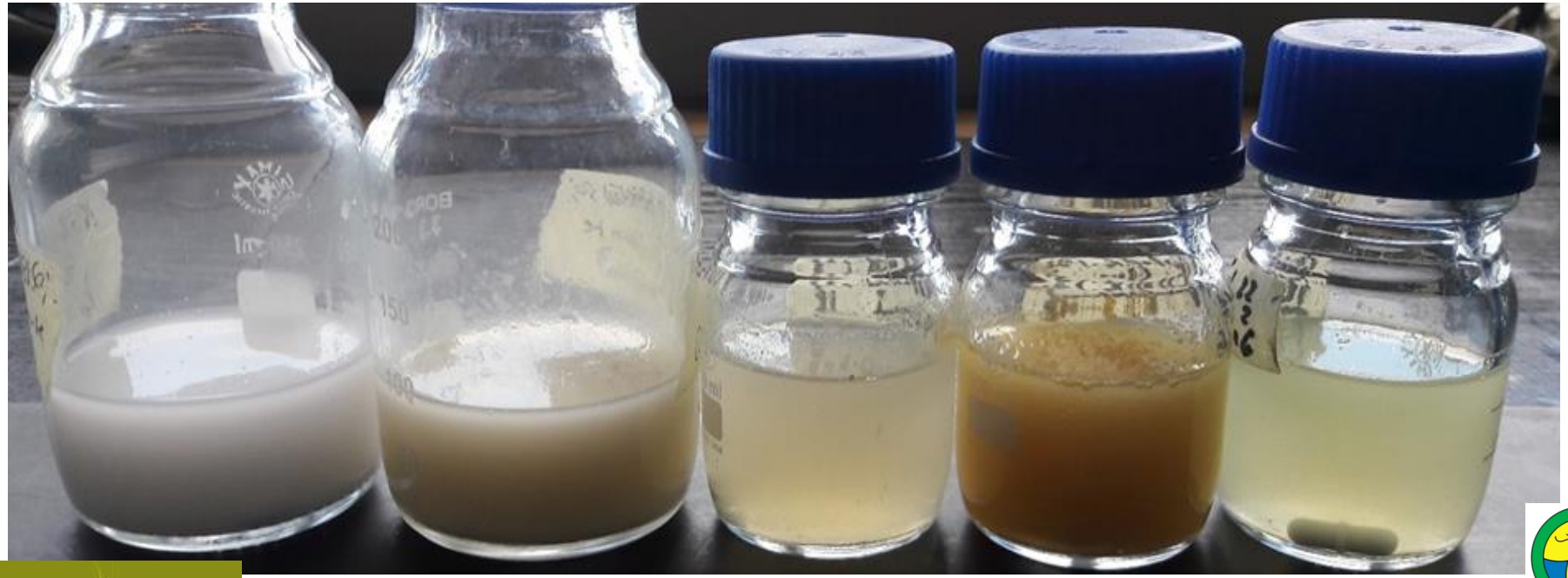
Biomass inoculated at startup : 10-20 millions CFU HFO-S2B/ml.

(Cells taken from a petri dish, Typtone Soya Agar medium).

Medium and pollutants : Mineral basal medium with 1 g/l of PCB or Aroclor 1260, chrysene, benzo(a)pyrene, benzo(a)anthracene, and 5 g/l of dielectric oil.

Incubation : Under magnetic stirring 250 rpm, at 20°C during 14 days for PCB, chrysene, benzo(a)pyrene, benzo(a)anthracene, and 5 days for dielectric oil.

PCB (Aroclor 1260) **Dieletric oil** **Chrysene** **Benzo(a)pyrene** **Benzo(a)anthracene**



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Rhodococcus sp. **HFO180-S2B (Madep)**

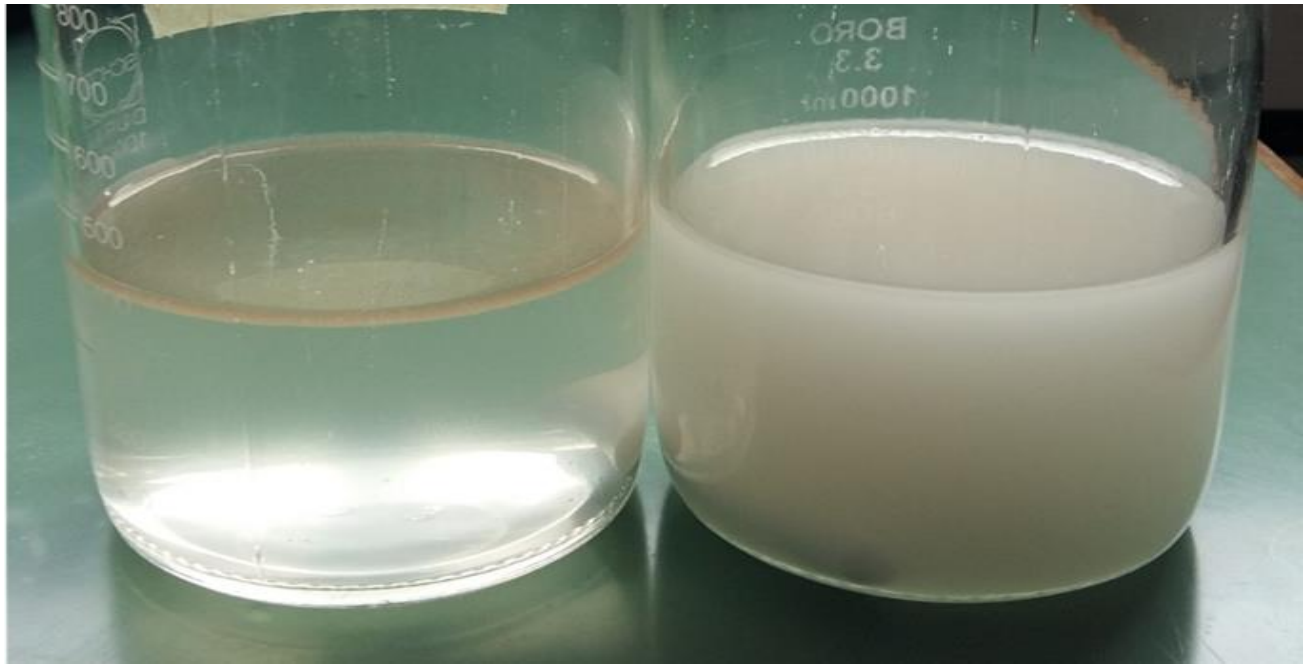
Very speed solubilization of heating oil

Biomass inoculated at startup : 100-120 millions CFU strain HFO-S2B/ml (Cells taken from a petri dish, Typtone Soya Agar medium).

Conditions + polluant : Basal mineral medium + 10 g/l heating oil.

Incubation : Under magnetic stirring 750 rpm, at 20°C during 4 hours.

WITHOUT strain HFO-S2B **WITH strain HFO-S2B**



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Rhodococcus sp. **HFO180-S2B (Madep)**

Very speed washing heating oil from soil

Stirring time (min) 0,5 Kg soil + 1 l tap water	Residual HC in washed soil (Index C10-C40, ppm) WITHOUT strain HFO-S2B	Residual HC in washed soil (Index C10-C40, ppm) WITH strain HFO-S2B
0	3'150 (0 %)	**3'150 (0 %)**
5	3'030 (6 %)	**1'020 (69 %)**
15	3'010 (7 %)	**602 (82 %)**
30	2'980 (8 %)	**555 (83 %)**
60	2'955 (8 %)	**490 (85 %)**

- In brackets the % oil washed.
- HC = total hydrocarbons (heating oil).
- Tests carried out on thin part of soils (sieved to 0.2 mm).
- Biomass used : culture of *Rhodococcus* fournished by the partner ACTY produced under biosurfactants conditions/medium (Ref. Actygea Lot # Acty HFO2B-351/001, at 1.2 x 10^9 CFU/ml).
- Biomass added to soils : 4 x 10^7 CFU HFO-S2B/g soil.

ACTYGEA

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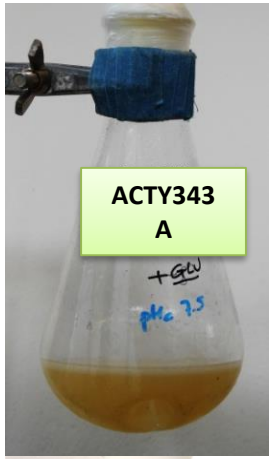
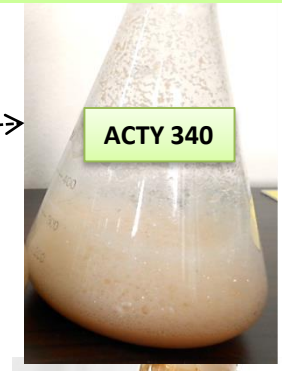
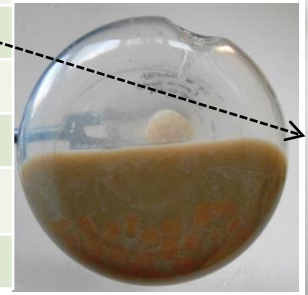
Rhodococcus sp. HFO180-S2B (Madep)

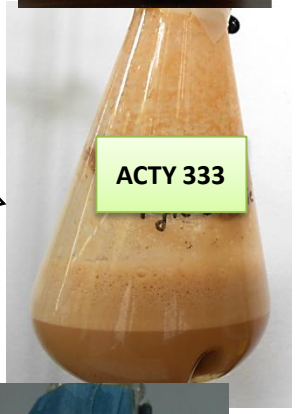
Growth in different production media

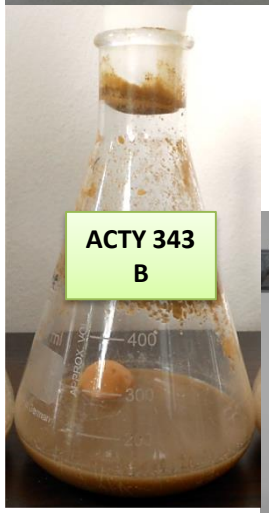
Medium	Vegetable Oil	Source C		Ref
ACTY 340	(20 g/l)	Glu	Gly	ACTYMEDAT
ACTY 333	(100 g/l)		Gly	ACTYMEDAT
ACTY MP1968	(100 g/l)		Gly	ACTYMEDAT
ACTY 343/A	(100 g/l)	Glu		ACTYMEDAT
ACTY 343/B	(100 g/l)		Gly	ACTYMEDAT
ACTY 345	(100 g/l)	Glu		ACTYMEDAT
MMB	(100 g/l)	Glu		MADEP (SA)
UCHb	(100 g/l)			Uchida (1989)
Rapp	(20 g/l)			Rapp (1979)

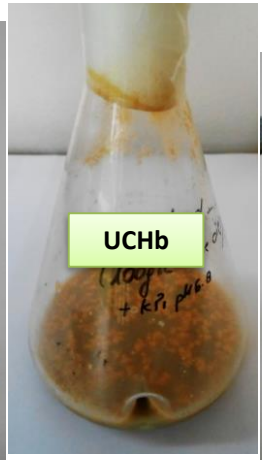












ACTYGEA

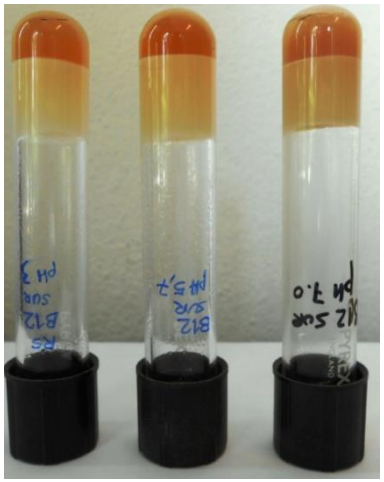
MADEP SA

Rhodococcus sp. HFO180-S2B (Madep)

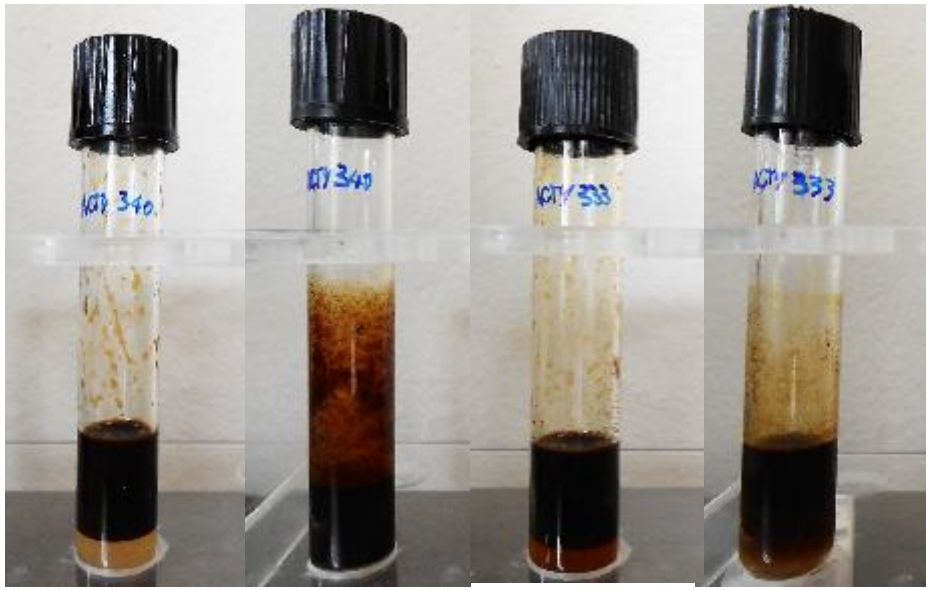
Applications : Emulsification of Solvents and Oils

Medium	EI_{24} (%) * hexadecane	EI_{24} (%) * Crude oil
ACTY 340	61	75 +/- 1.4
ACTY 333	63	76 +/- 0.6
ACTY MP1968	68	97 +/- 4.9

EI_{24}(%) in Hexadecane



EI_{24}(%) in danish crude oil



ACTYGEA

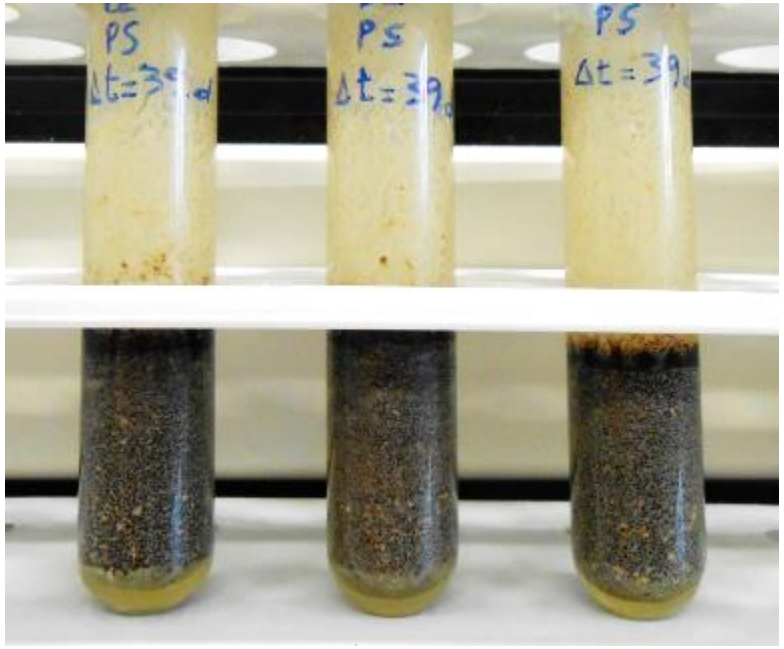
MADEP SA

Rhodococcus sp. HFO180-S2B (Madep)

Applications : Emulsification of Solvents and Oils

EI_{24}(%) in Danish crude oil

Stability of emulsion POST sterilization (39 days/20°C)



	BT POST-STER
EI24 (%)	**87,0 % +/- 1.51**

ACTYGEA

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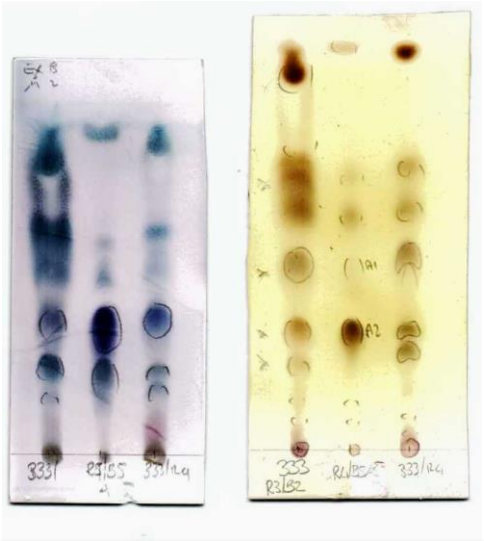
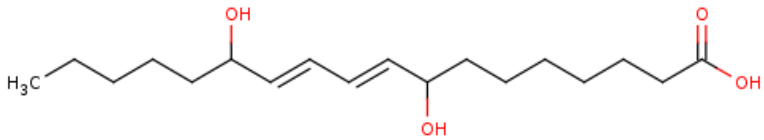
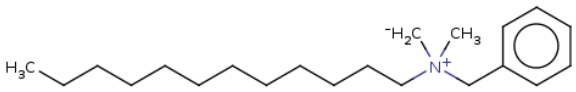
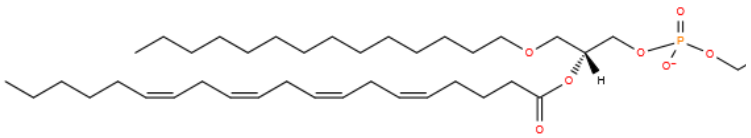
Applications: Production of surface active biomolecules – MS analysis (in collaboration with CSIC – Madrid)

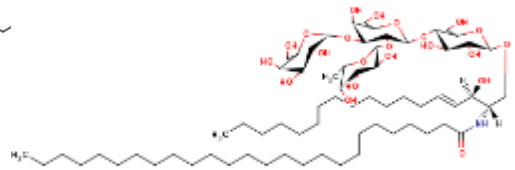


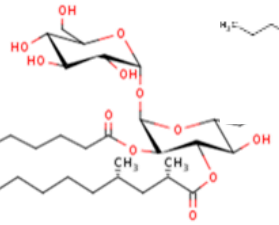
TLC ANALYSIS OF THE EXTRACT ORGANIC
VANILLIN REAGENT (LEFT) AND ORCINOL REAGENT (RIGHT)

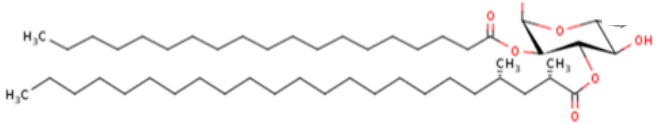


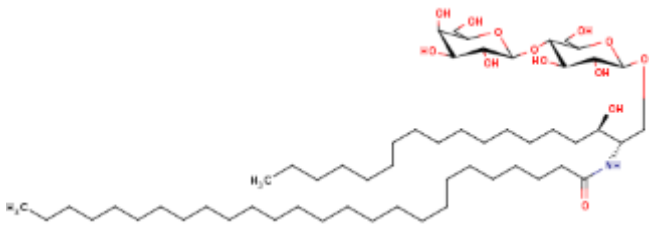


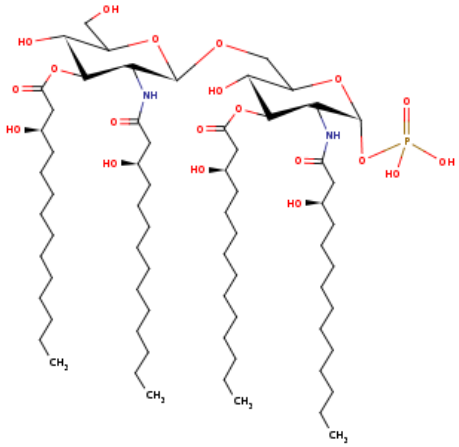


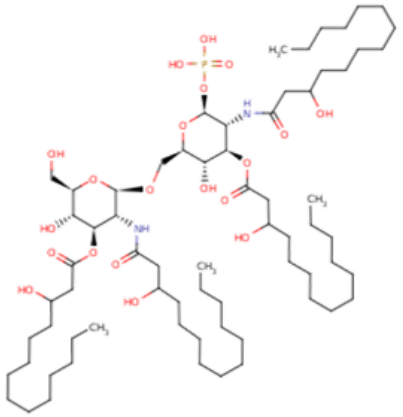










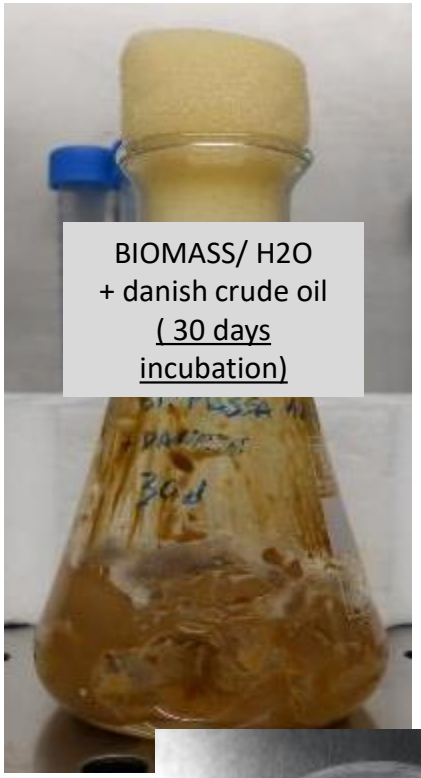


ACTYGEA

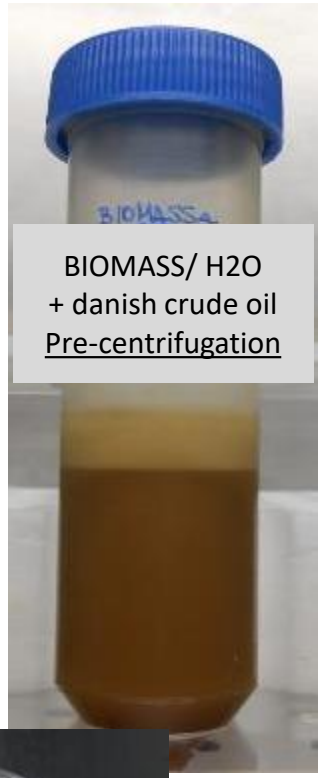
MADEP SA

Rhodococcus sp. HFO180-S2B (Madep)

Applications : Crude oil degradation



BIOMASS/ H2O
+ danish crude oil
(30 days incubation)



BIOMASS/ H2O
+ danish crude oil
Pre-centrifugation

BIOMASS/ H2O
+ danish crude oil
Post-centrifugation

Viability
of the strain
(at 30 days in crude oil)

Crude Extract
(CHCl3)

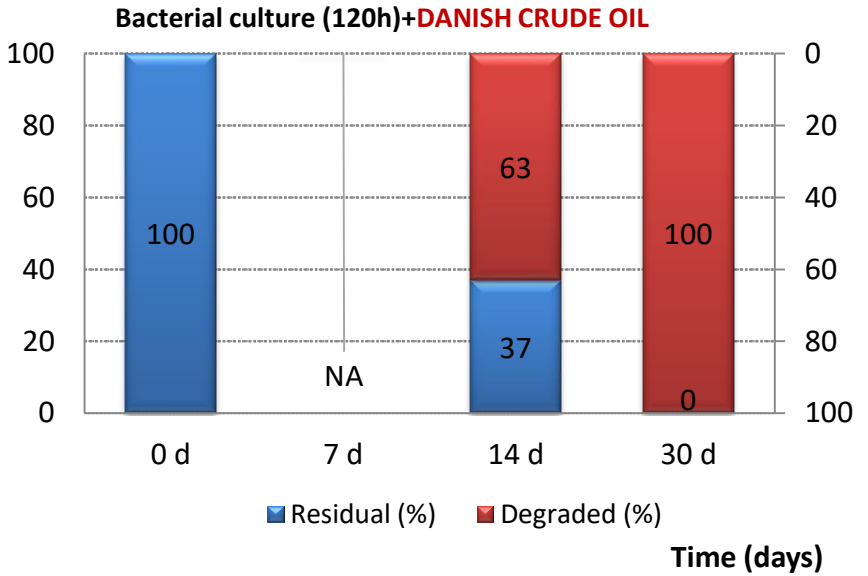
Crude oil residue
15%

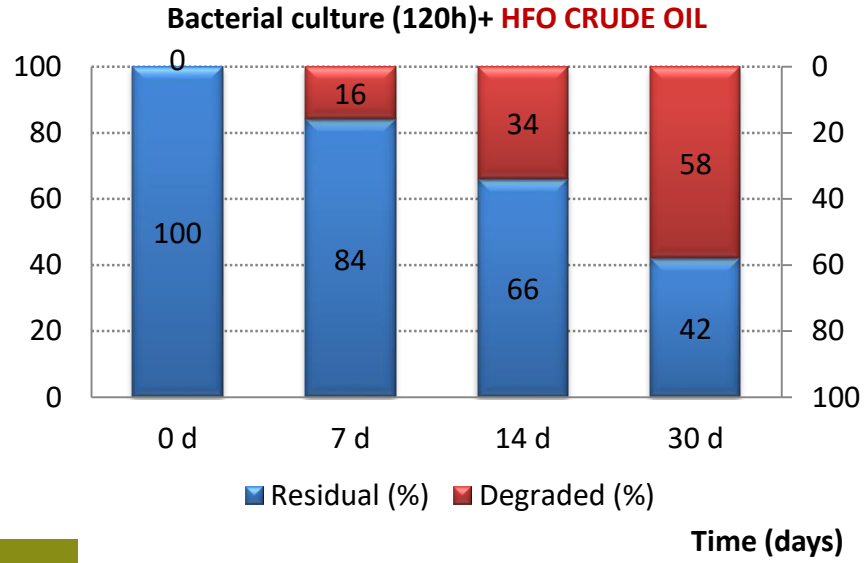
ACTYGEA

MADEP SA

Rhodococcus sp. HFO180-S2B (Madep)

Applications : Crude oil degradation





PARAMETERS:

MEDIUM: ACTY 333

AGE CULTURE: 120h

BIOMASS = BIOMASS 125 g/L

CRUDE OIL = 50 g/L

INCUBATION: 28°C/200 RPM

*LIGHT CRUDE OIL (DANISH)

**HEAVY CRUDE OIL (HFO)

ACTYGEA

MADEP SA

The goal of ACTYGEA and MADEP

- The goal of ACTYGEA and MADEP is to transfer the biosurfactant-capabilities (*) of the strain *Rhodococcus* sp. HFO180-S2B into **innovative formulations for environmental and industrial bioprocesses and applications.**

(*) These biosurfactants have both hydrophilic and hydrophobic regions.

Already available for :

- Industrial fermentation protocols to **produce large quantities** of the strain HFO180-S214B (ACTYGEA and MADEP).

- **Addition** of the strain HFO180-S2B to **contaminated sites** (soils, wastewater, leachates,...) for the **improvement of solubilisation, bioavailability and biodegradability of hydrophobic pollutants**, such as Crude Oils, PAHs, PCB, ... (MADEP and ACTYGEA).

Kill•Spill - Integrated Biotechnological Solutions for Combating Marine Oil Spills

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