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Bioremediation techniques

Cedre Information Day

10th March 2015

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Research Department

After the incident, main responses

At Sea

Mechanical recovery

Dispersion

In Situ Burning

On the Shoreline

Initial clean-up

Manual cleaning

Sand screening

and Bioremediation

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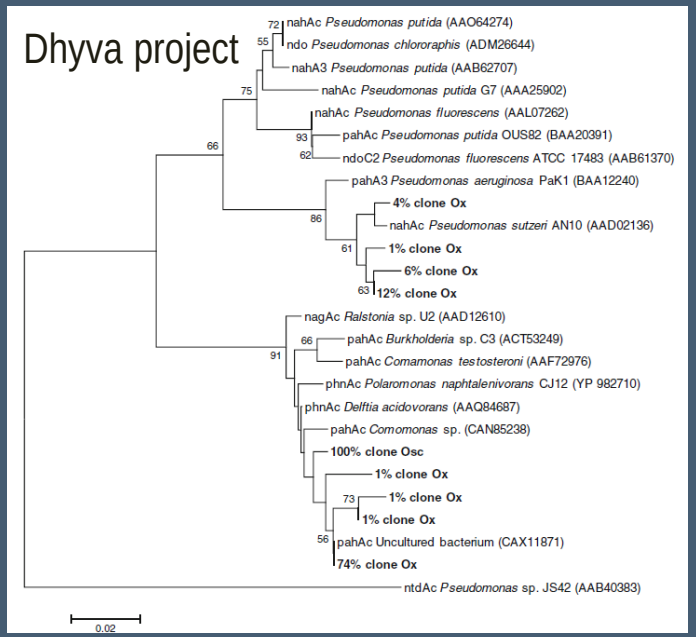
The bioremediation of a contaminated environment involves influencing environmental conditions to optimise the natural biodegradation of the contaminant.

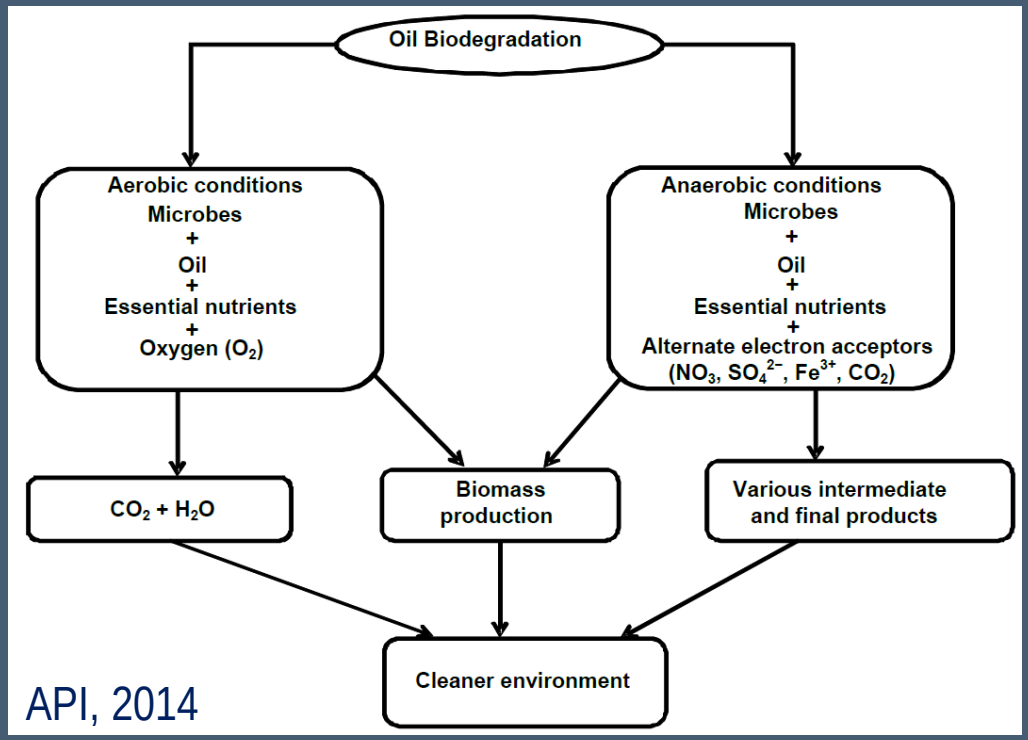
Capacity of certain organisms to assimilate oil as a source of energy

- Bacteria
- Fungi
- Yeast



The bioremediation of a contaminated environment involves influencing environmental conditions to optimise the natural biodegradation of the contaminant.

2 forms of degradation:
- oxic
- anoxic

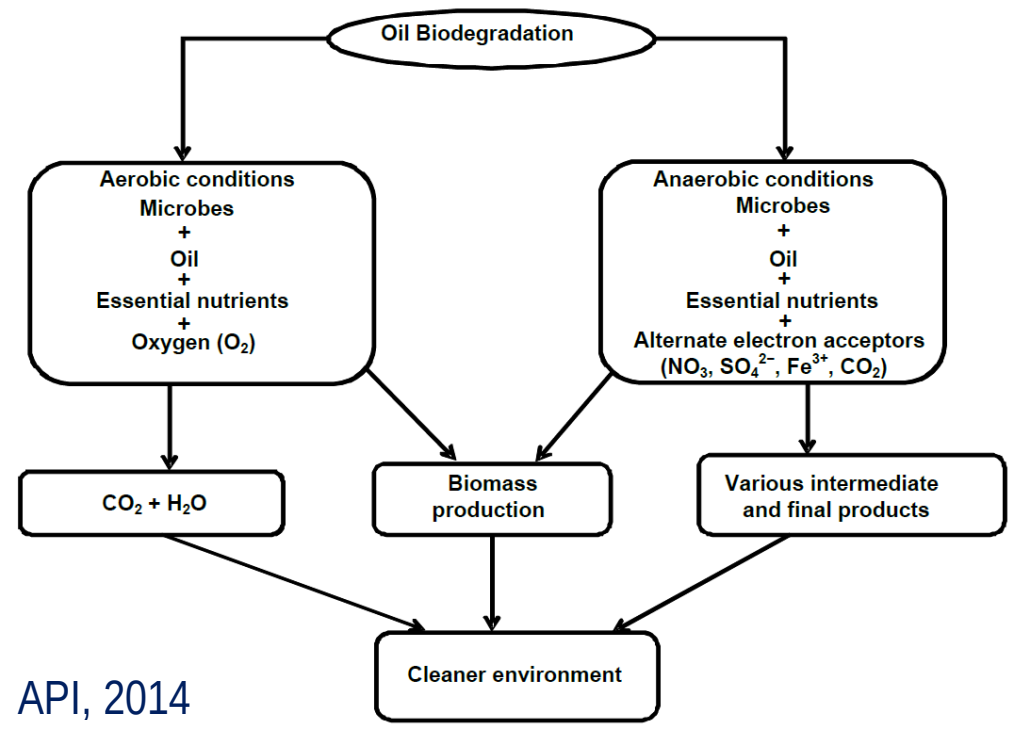


API, 2014

The bioremediation of a contaminated environment involves influencing environmental conditions to optimise the natural biodegradation of the contaminant.

2 forms of degradation:
- oxic
- anoxic

In the best case scenario
CO2 + H2O

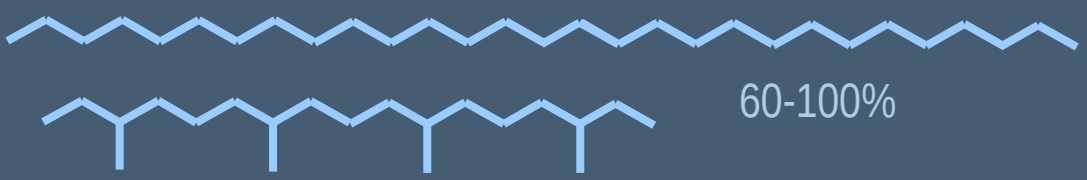


API, 2014

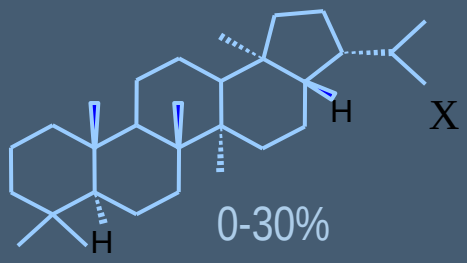
The bioremediation of a contaminated environment involves influencing environmental conditions to optimise the natural biodegradation of the contaminant.

Type of contamination

According to the chemical composition, the biodegradability of the oil will vary:

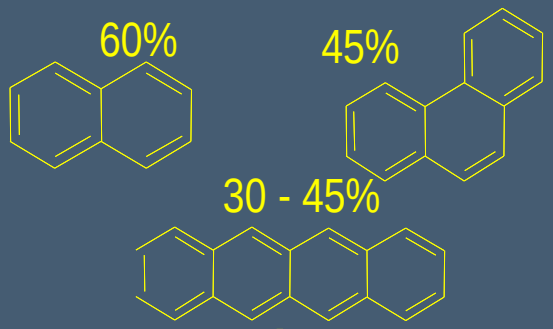


60-100%

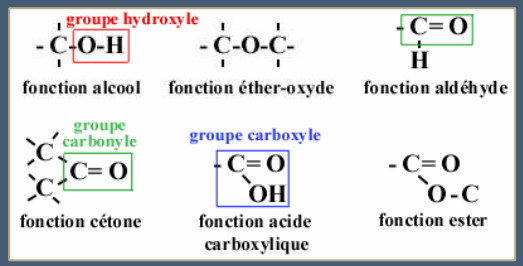


Alkanes

0-30%



Aromatics



Resins

10 - 30%

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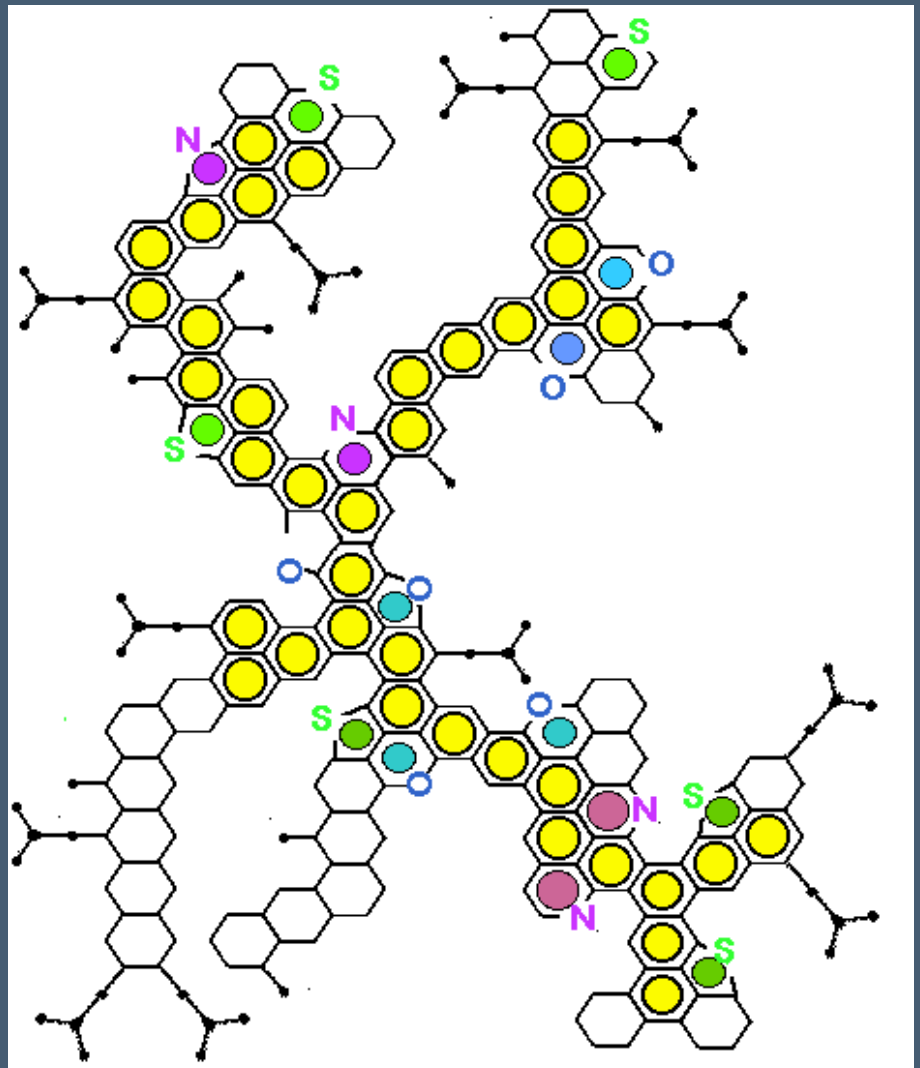

Asphaltenes

0 – 10%

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The bioremediation of a contaminated environment involves influencing environmental conditions to optimise the natural biodegradation of the contaminant.

Type of contamination

According to the chemical composition, the biodegradability of the oil will vary:

Type of oil	Biodegradability (%)
Petrol	> 90%
Kerosene	> 80%
Diesel	60 – 80%
Lubricants	< 50%
Crude oil (variable)	30 – 70%
Heavy fuel oil	10 - 20%
Bitumen	negligible

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The bioremediation of a contaminated environment involves influencing environmental conditions to optimise the natural biodegradation of the contaminant.

Type of contamination

Physical state of oil influences its bioavailibility

- dispersed or concentrated
- emulsified
- film, slick, …

Oil concentration

Bioremediation should be implemented following initial clean-up of the site (polishing)

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The bioremediation of a contaminated environment involves influencing environmental conditions to optimise the natural biodegradation of the contaminant.

- Temperature, salinity, pH
- Water, oxygen concentration
- Bacterial flora autochotnous
- Abundance of nutrients (N, P, K)

Bioremediation ….

- Is considered as a "green" techniques compared to others

- Can be limited due to oil nature / concentration and environmental parameters (Temperature, Oxygen, Nutrients) => these parameters need to be assessed systematically before bioremediation deployment

- Implies that commercial products are used to increase oil biodegradation

Different bioremediation methods

- Biostimulation involves boosting the activity of indigenous microflora in a given environment by compensating for a lack of a fundamental element in the oil biodegradation process. This is implemented by providing nutrients and/or terminal electron acceptors (oxygen, nitrate, sulphate).

3 types of solutions:

- Water-soluble mineral fertilisers for agricultural or horticultural use composed of nitrogen and phosphorous

- Slow-release fertilisers in solid form: N and P combined with a solid carbonated element

- Liquid oleophilic formulations developed to provide nutrients close to bacterial activity (i.e. at the water-oil interface).

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Different bioremediation methods

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- Bioaugmentation involves adding exogeneous micro-organisms in an environment characterised by the absence or low abundance of hydrocarbonoclastic bacteria. The micro-organisms are generally applied by spraying a rehydrated freeze-dried culture.

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Cedre's activities in this field

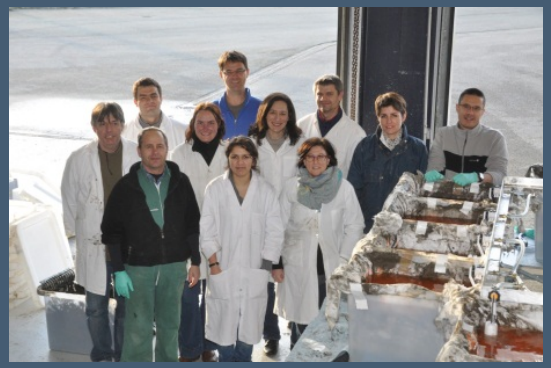
- 1990 – 2000: many laboratory and in situ experimental studies to test and improve techniques.

- 2006 – 2015: 2 ANR projects on natural biodegradation in mudflats (DHYVA / DECAPAGE) and adaptation of communities to oil pollution

Oil degradation in coastal muddy areas and anoxic ecosystems

*In **DE**pth characterization of HC-degradation **CAPA**cities of marine sediment microbial communities: adaptation, metabolic processes and influence of oxy**GE**nation regimes*

Nov. 2011 - Nov. 2015
1500 k€

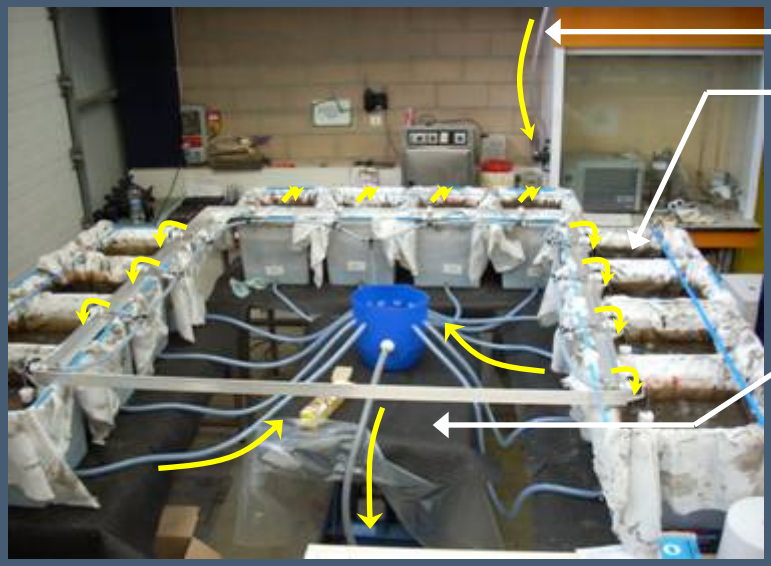


- University of Toulouse
- University of Pau (2 laboratories)
- University of Marseille
- *Cedre*

Main Objectives of the project:

- Understanding how microbial communities respond, adapt and degrade petroleum compounds in anoxic area
- How bacterial metabolisms interact in the degradation of oil ?
- Estimating the bioturbation effects on these bacterial activities.
- Assessing the influence of dispersant on the oil distribution in sediment, on microbial and macrofauna communities

Mesoscale Experimentation (Jan. - Nov. 2012)
Microcosms of a mud type ecosystem



Seawater supply

16 microcosms (30L of mud each) equipped of :

- *geotextile membrane*
- *ball cock*
- *evacuation pipes of tides water*

Lifting table with collector of tides water

Conditions :

- *negative control (only sediments)*
- *sediments with oil pollution*
- *sediments with Hediste diversicolor (bioturbation)*
- *sediments with oil pollution + bioturbation*
- *sediment with dispersed oil with or without Hediste.*

10 sampling rounds of sediment cores (10 x 3 cm) were dispatched to the different laboratories during the 10 months of experiment.

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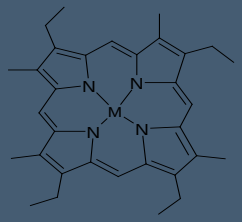
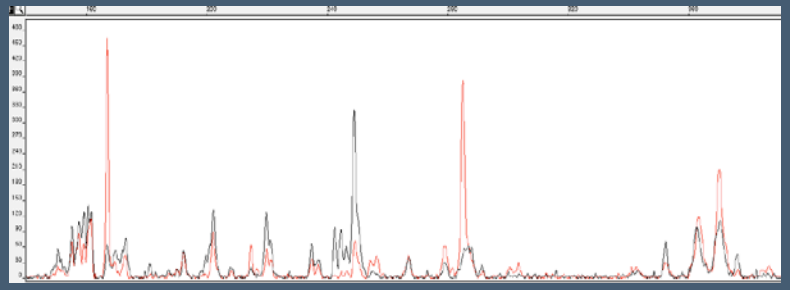
ICP-MS analysis of metals in petroleum



Metal - complex type in petroleum:

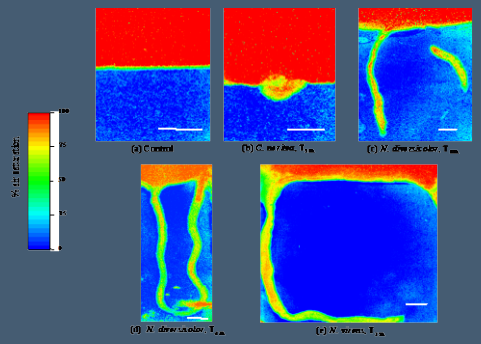
ETIO porphyrin

Bacterial diversity (DNA and RNA)



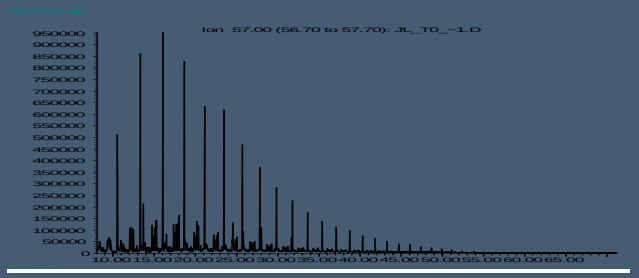
A T-RFLP profile of extracted DNA from mud polluted (black) or not (red)

Bioturbation analysis



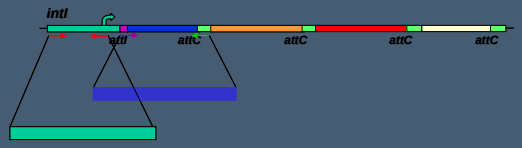
Examples of oxygen distribution in sediments.

Hydrocarbons titration



Ion chromatogram of *n*-alcanes (m/z=57) of the initial oil

Mechanisms of adaptation and genes involved



Schematic representation of an integron and location of the fragment containing the “first gene cassette” targeted

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Cedre's activities in this field

- 1990 – 2000: many laboratory and in situ experimental studies to test and improve techniques.

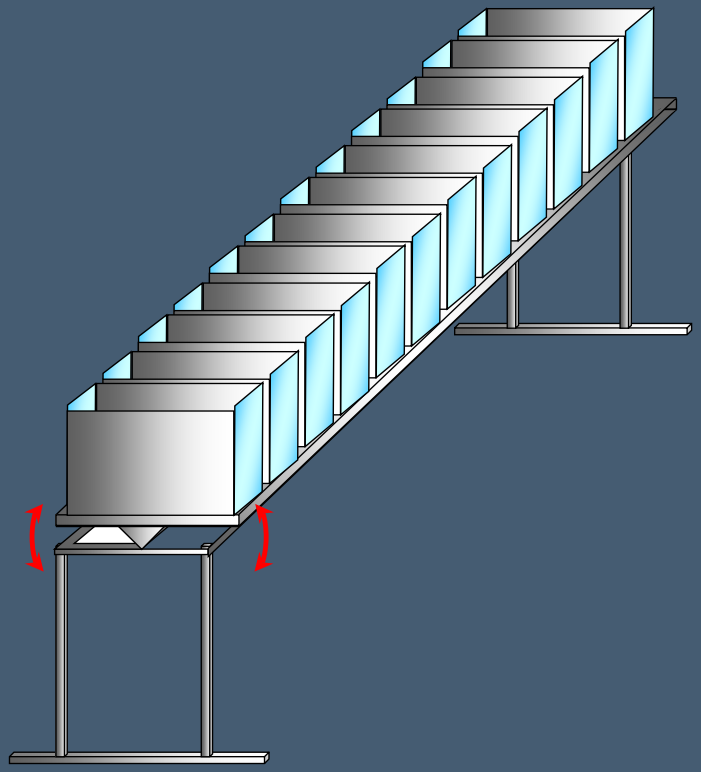
- 2004 – 2015: 2 ANR projects on natural biodegradation in mudflats (DHYVA / DECAPAGE) and adaptation of communities to oil pollution

- 2012: review of bioremediation techniques

- 2014 – 2015: development of an efficiency test for bioremediation agents

Development of an efficiency test for bioremediation agents

Objective: to develop an experimental system with tidal cycle simulation (dilution phenomenon) in a contaminated environment treated with a bioremediation agent.



12 tanks
(L = 40cm; l = 20 cm; h = 30 cm)

Oscillating table
(L = 4,80m ; l = 20 cm)

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Development of an efficiency test for bioremediation agents

Objective: to develop an experimental system with tidal cycle simulation (dilution phenomenon) in a contaminated environment treated with a bioremediation agent.

- shaker table with 12 tanks
- seawater tank
- programmable lifting table whose upward and downward movements control the emptying (low tide) or filling (high tide) of the tanks

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Development of an efficiency test for bioremediation agents

Action schedule:

- 2014: 1st series of trials on 2 biostimulants (inconclusive due to lack of bacteria)

- 2015: 2nd series (April)

Thank you for your attention