Linking life and technology to shape the future

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Anaerobic bacteria with an appetite for oil

Ana Júlia Cavaleiro

CEB - Centre of Biological Engineering

University of Minho

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Industrial and Food Biotechnology

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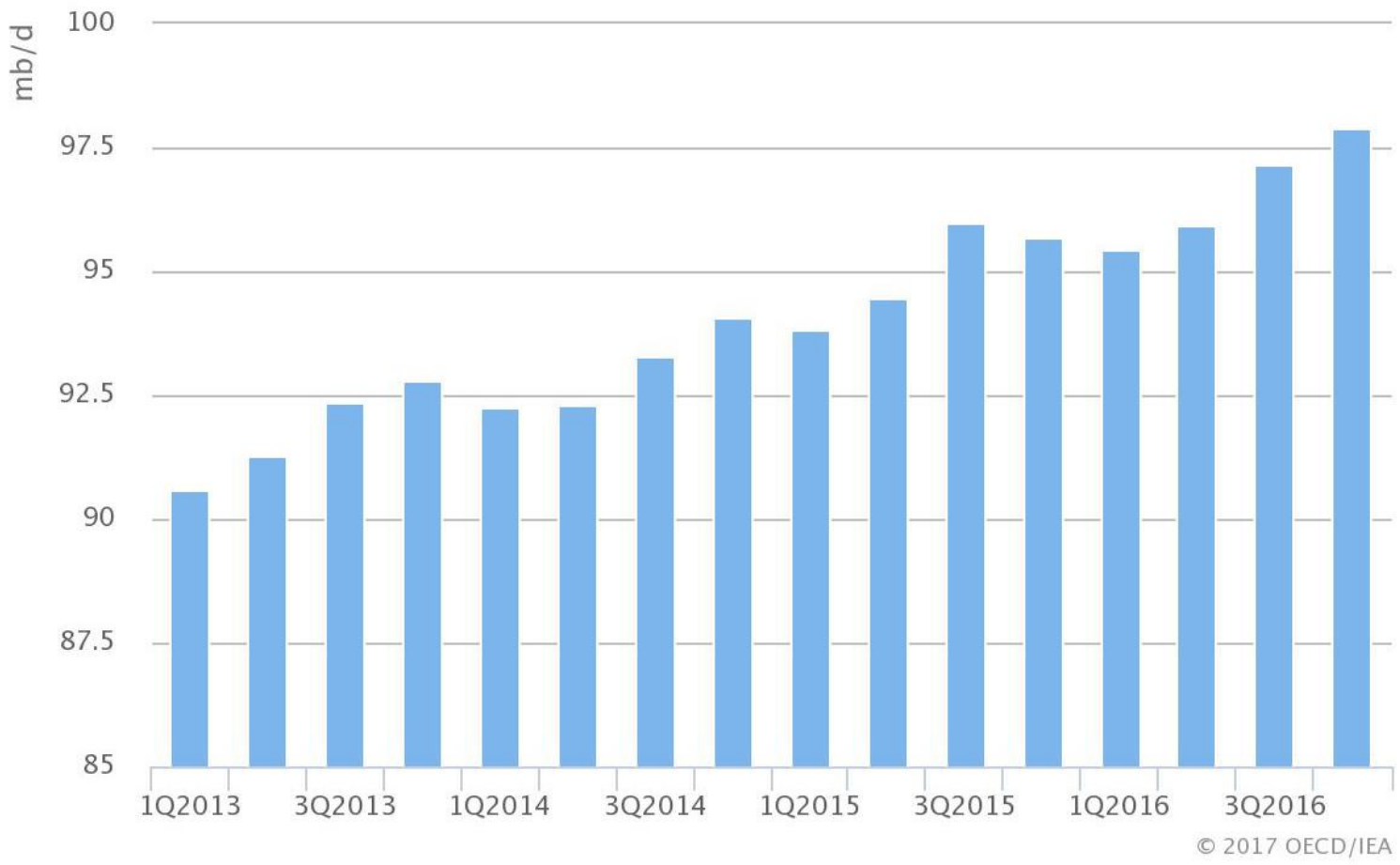
World Oil Demand



International Energy Agency, https://www.iea.org/oilmarketreport/omrpublic/

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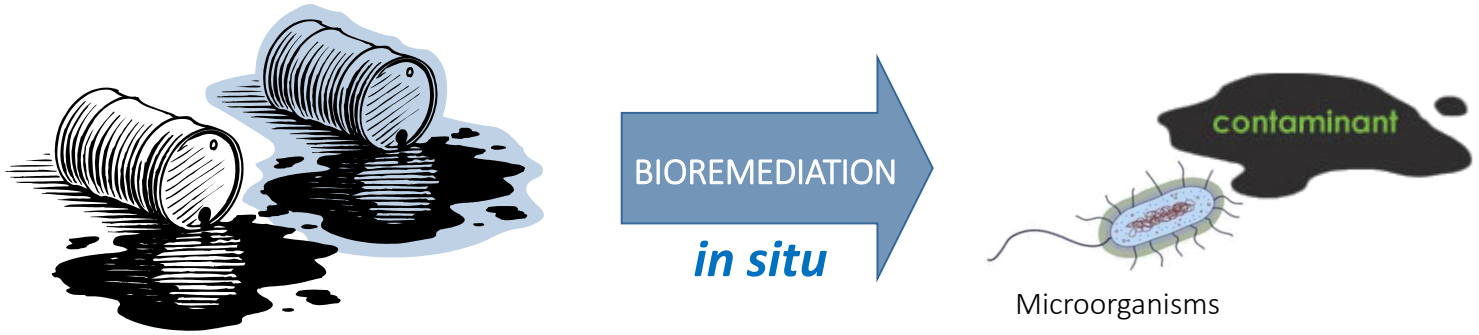
Gulf of Mexico, 2010

Oil Contamination

Revista "Visão", 10 Novembro 2016

BIOREMEDIATION

Hydrocarbon contamination of soil and water



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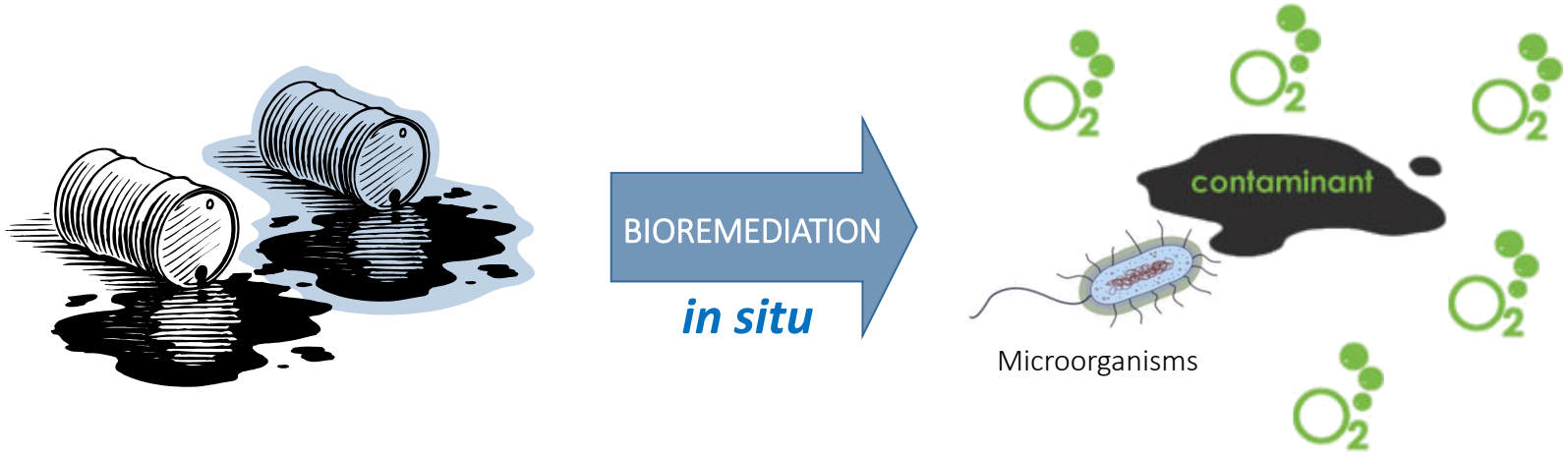
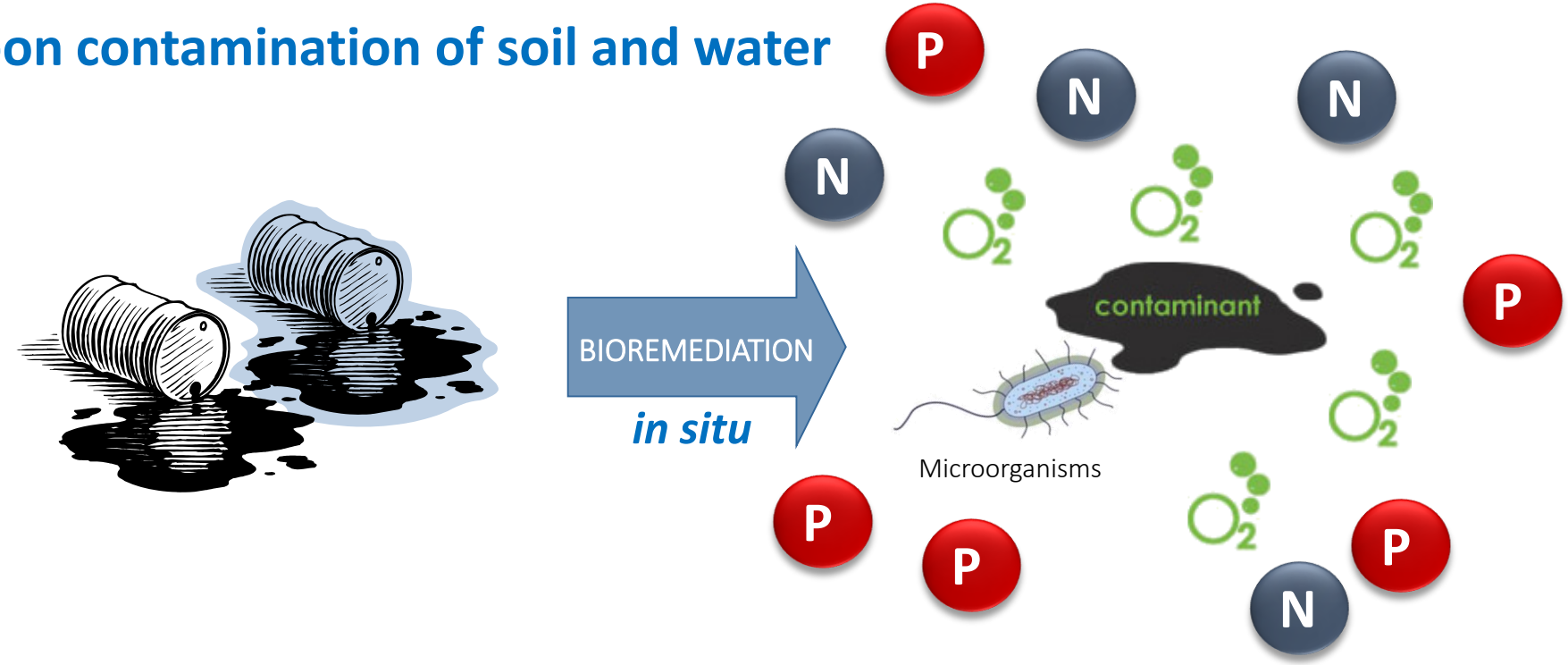
Hydrocarbon contamination of soil and water



Biostimulation

BIOREMEDIATION

Hydrocarbon contamination of soil and water



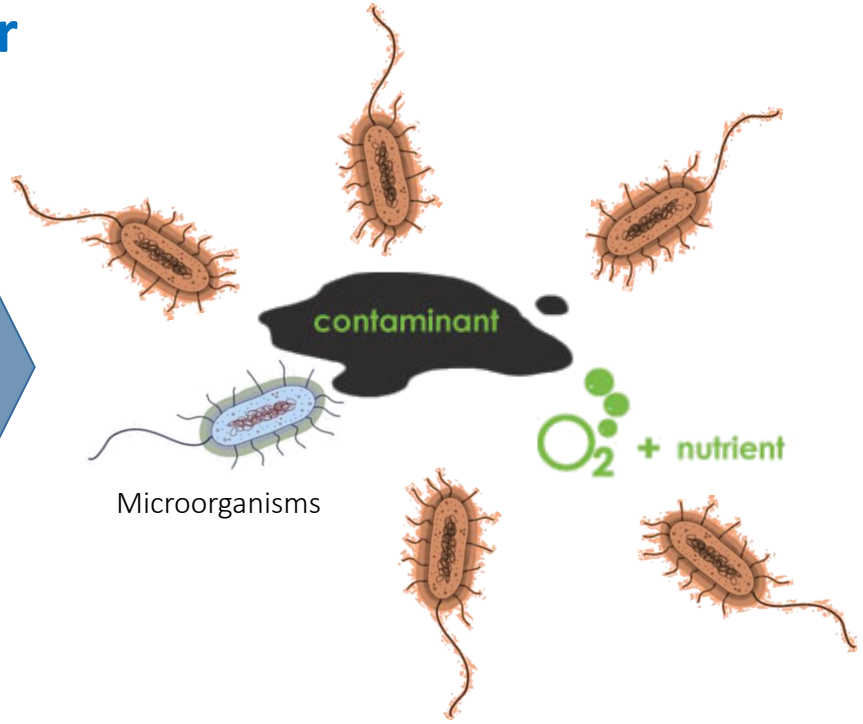
Biostimulation

BIOREMEDIATION

Hydrocarbon contamination of soil and water

BIOREMEDIATION

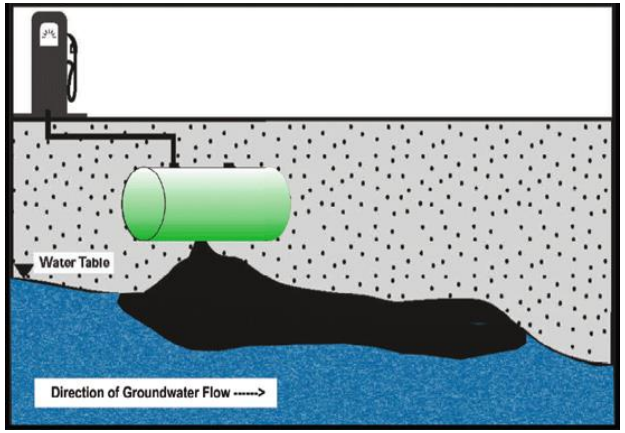
in situ



Bioaugmentation

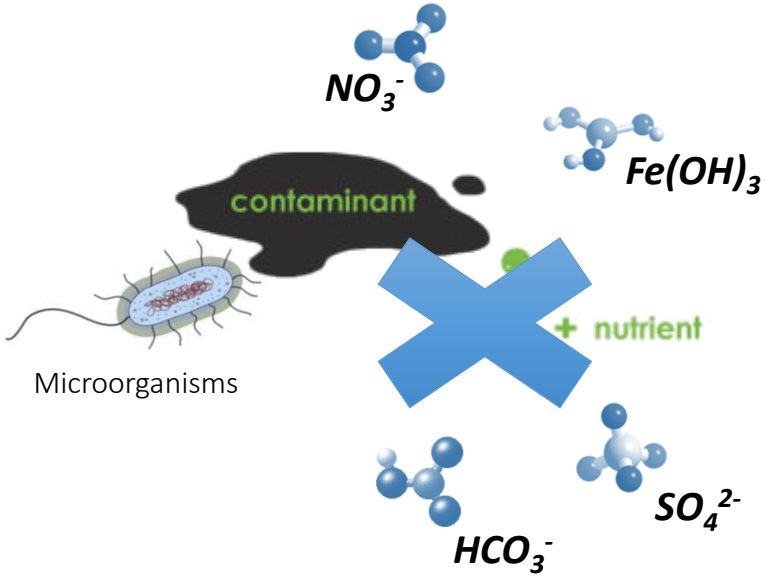
BIOREMEDIATION

Hydrocarbon contamination of deep soil and groundwater



BIOREMEDIATION

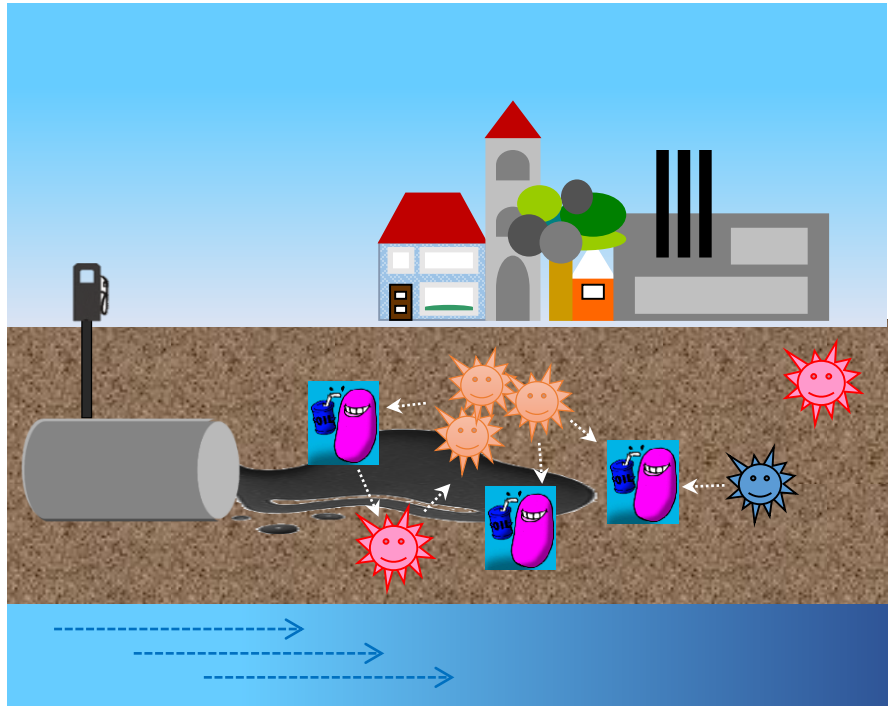
in situ



[Removal of a leaking storage tank from a Superfund site. Image via Massachusetts Department of Environmental Protection photostream]

Research Questions

Bioremediation of petroleum-contaminated environments in the absence of molecular oxygen



Who is there?

Who is doing what?

With whom?

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Research Questions

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Bioremediation of petroleum-contaminated environments in the absence of molecular oxygen

Project **MORE**

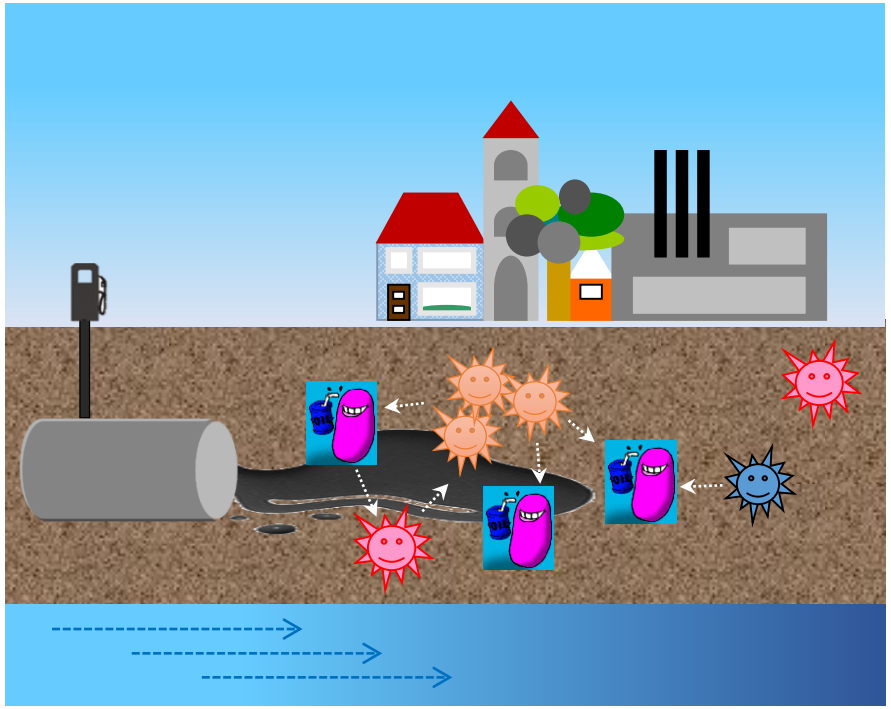
(PTDC/AAG-TEC/3500/2014)

Project **OIL-Less**

(PTDC/AAG-TEC/3428/2012)

Project **Novel Anaerobes**

(ERC Grant Agreement no. 323009)



Who is there?

Who is doing what?

With whom?

Experimental Approach

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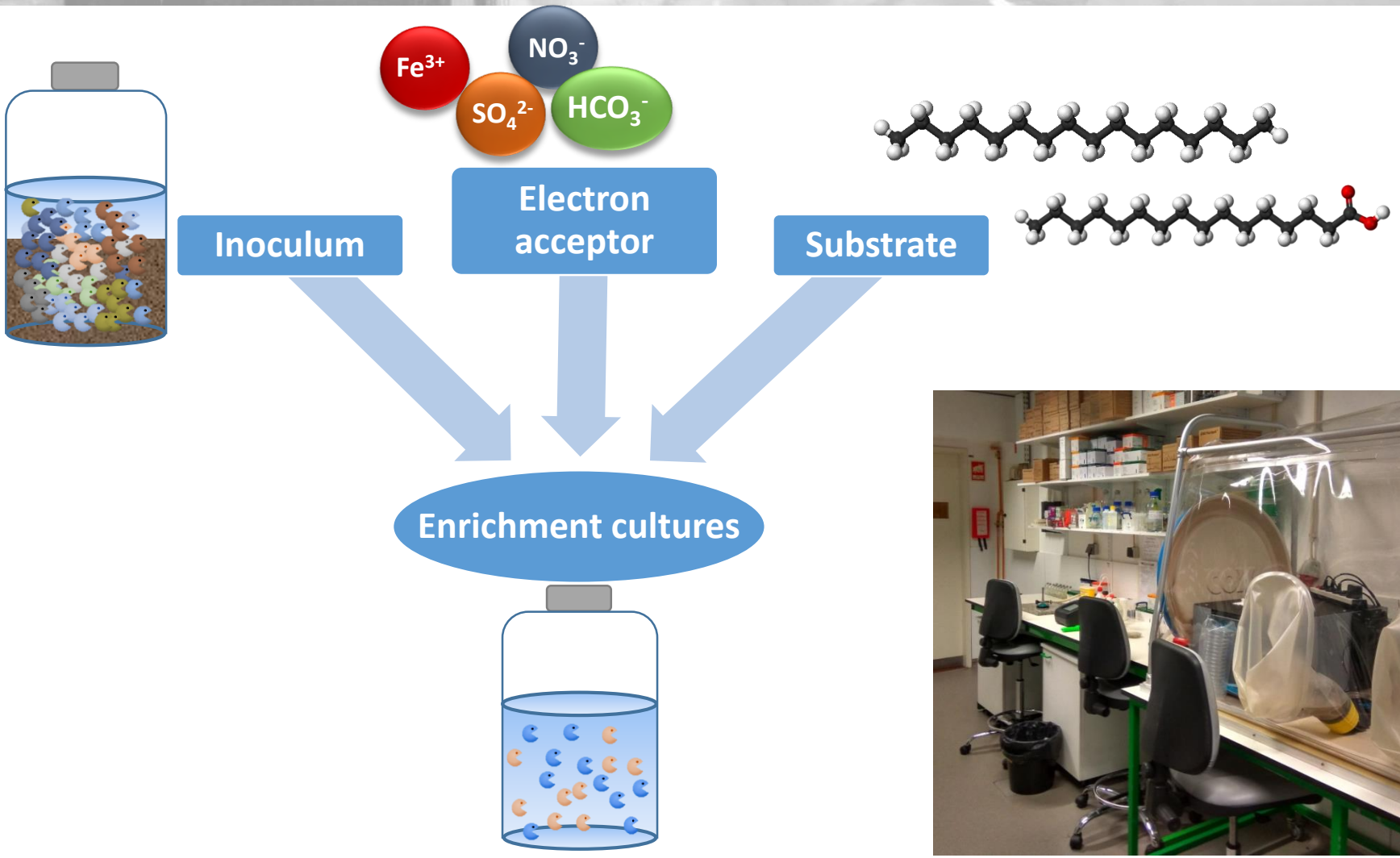
1 - Enrichment cultures

Contaminated soil
Anaerobic granular sludge
Aerobic sludge
Marine sediment
Freshwater sediments



Experimental Approach

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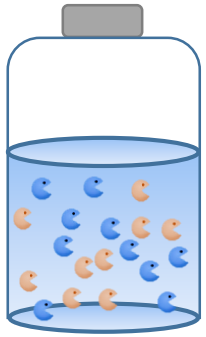
2 - Microbial diversity and composition

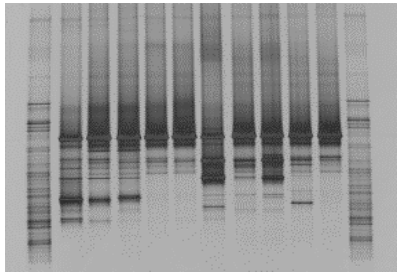


DNA

Microbial diversity

16S rRNA PCR

DGGE

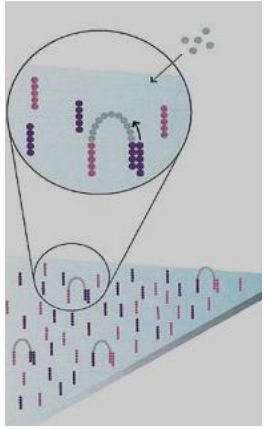


Microbial identification

16S rRNA extraction

Sequencing

Illumina MiSeq



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MAIN FINDINGS

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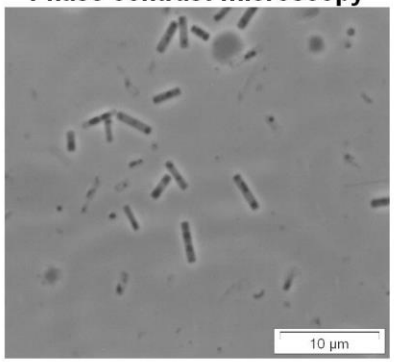
Hexadecane-degrading enrichment

Hexadecane degradation:

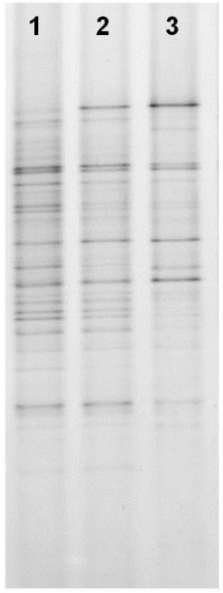
$C_{16}H_{34} + 12.25\ SO_4^{2-} + 8.5\ H^+ \rightarrow 16\ HCO_3^- + 12.25\ H_2S + H_2O$

An enriched culture capable of hexadecane degradation was obtained

Phase contrast microscopy



BACTERIAL COMMUNITIES



PCR-DGGE of 16S rRNA genes

1 = 1st incubation, 2 = 2nd incubation, 3 = 3rd incubation

Palmitate-degrading culture

Palmitate degradation:

$C_{16}H_{32}O_2 + 11.5\ SO_4^{2-} + 7\ H^+ \rightarrow 16\ HCO_3^- + 11.5\ H_2S$

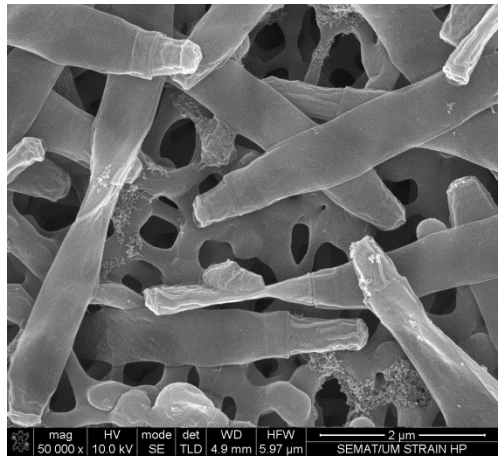
Incubation with palmitate and sulfate:

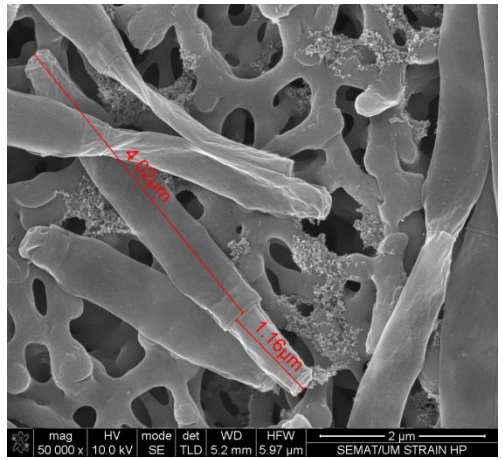
Serial dilutions

Novel *Desulfomonile* strain

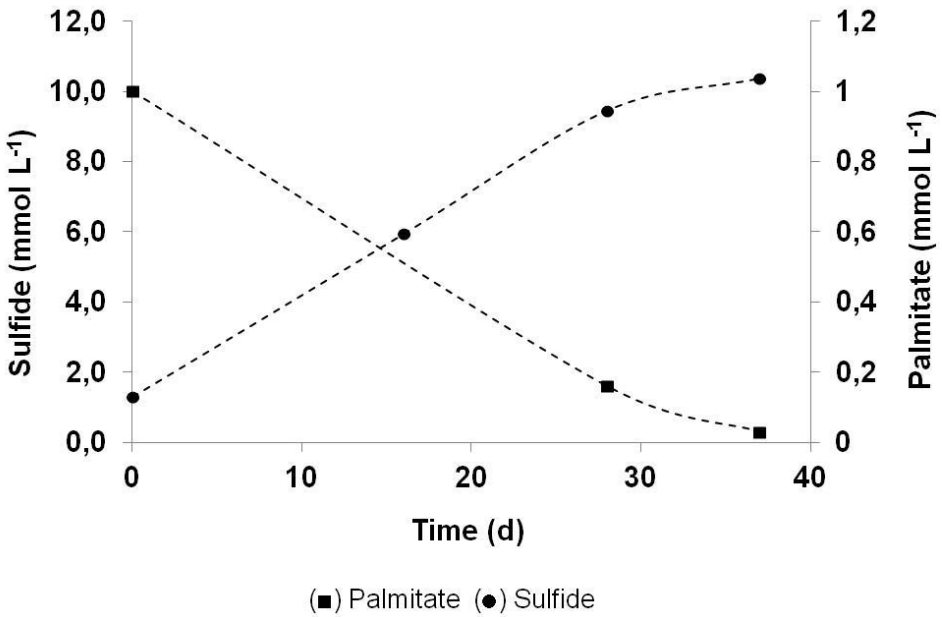
Closest relative	% Identity
Desulfomonile limimaris	94
Desulforhabdus amnigena	99

Main Findings





1. LCFA biodegradation



2. Hydrocarbons biodegradation

Hexadecane

Hexadecene

3. Dehalogenation ability

3-Clorobenzoate

2-Clorobenzoate

3-Bromobenzoate

MAIN FINDINGS

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1-Hexadecene

Model compound for oleofin-based products

- surfactants
- lubricating fluids
- others

Production of **olefins** in petrochemical plants generates **olefin-contaminated wastewater**

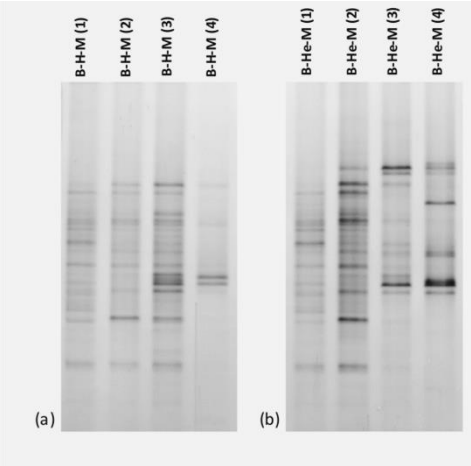
can be treated by **anaerobic digestion** coupling **wastewater treatment** to **methane production**

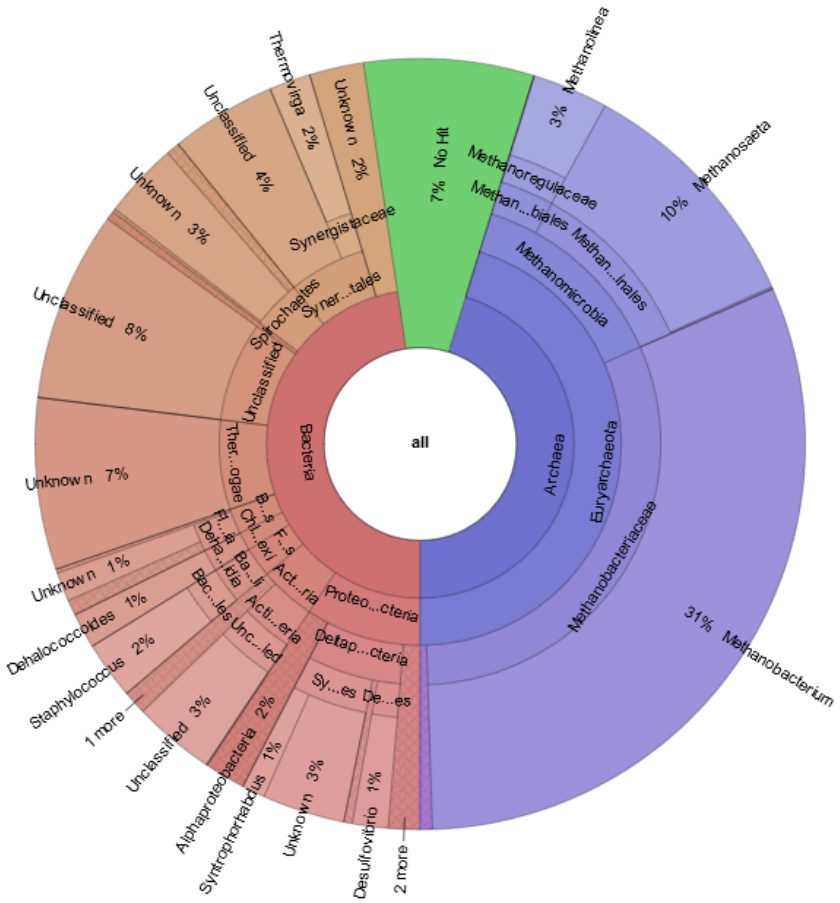
Anaerobic Digestion

Wastewater

Main Findings

Methanogenic culture degrading 1-Hexadecene





Main Findings

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Methanogenic culture degrading 1-Hexadecene

Methanobacterium formicicum

Bioaugmentation with methanogens

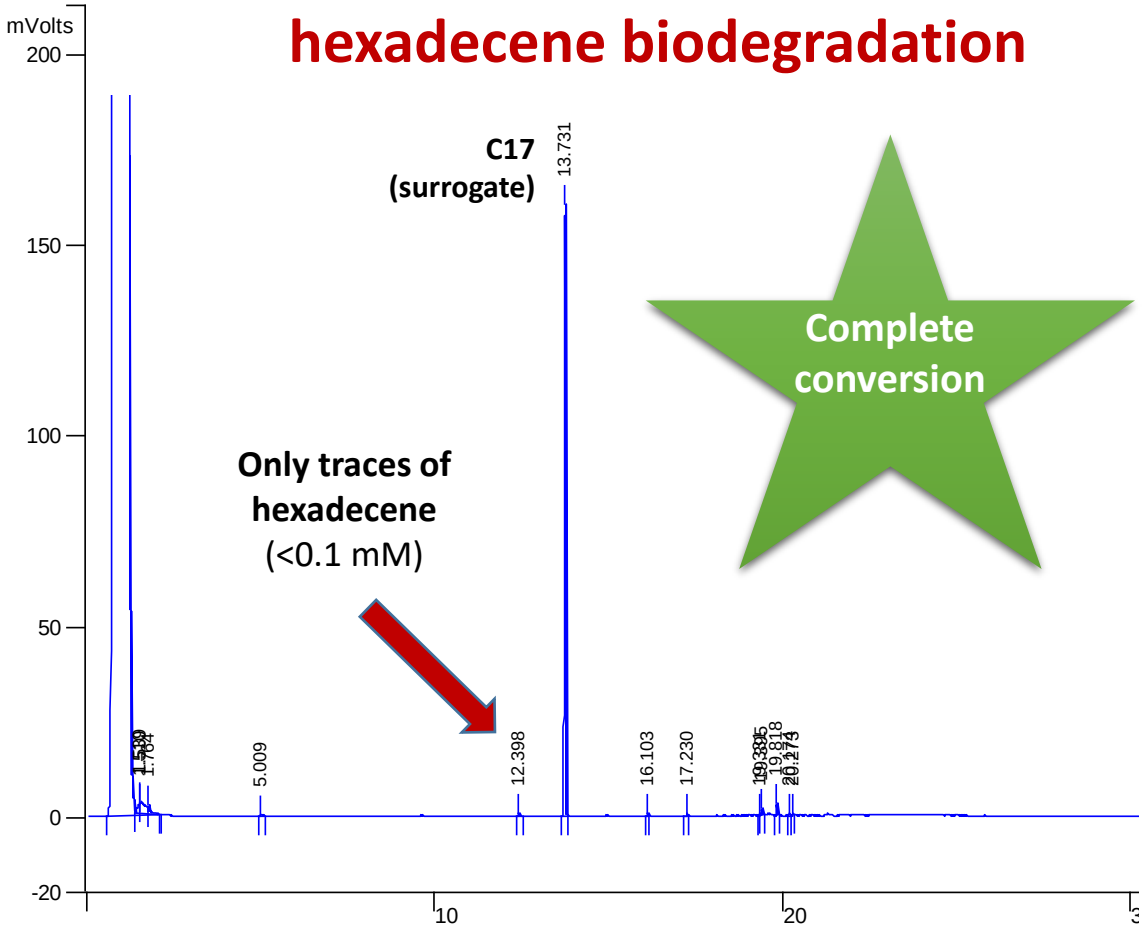
Yeast extract

Crotonate

Lactate

Co-substrate addition

improved 5x hexadecene biodegradation



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12th International Workshop on Environment and Alternative Energy (Cape Canaveral, USA, 2014)

https://www.ceb.uminho.pt/bridge/News/Details/82

Work in Progress

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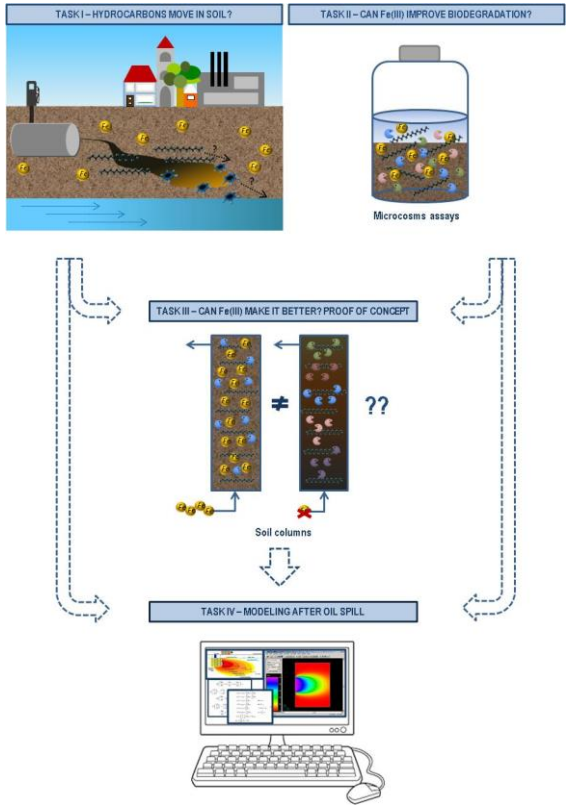
Can we improve anaerobic hydrocarbons bioremediation...

... with ferric minerals?



... with carbon nanomaterials?

Work in Progress

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Treatment and valorisation of petroleum-contaminated biosorbents

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Thank you for your attention!

Acknowledgments:

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NORTE-01-0145-FEDER-000004
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