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# Engineered bioremediation of deep hydrocarbon-contaminated sites


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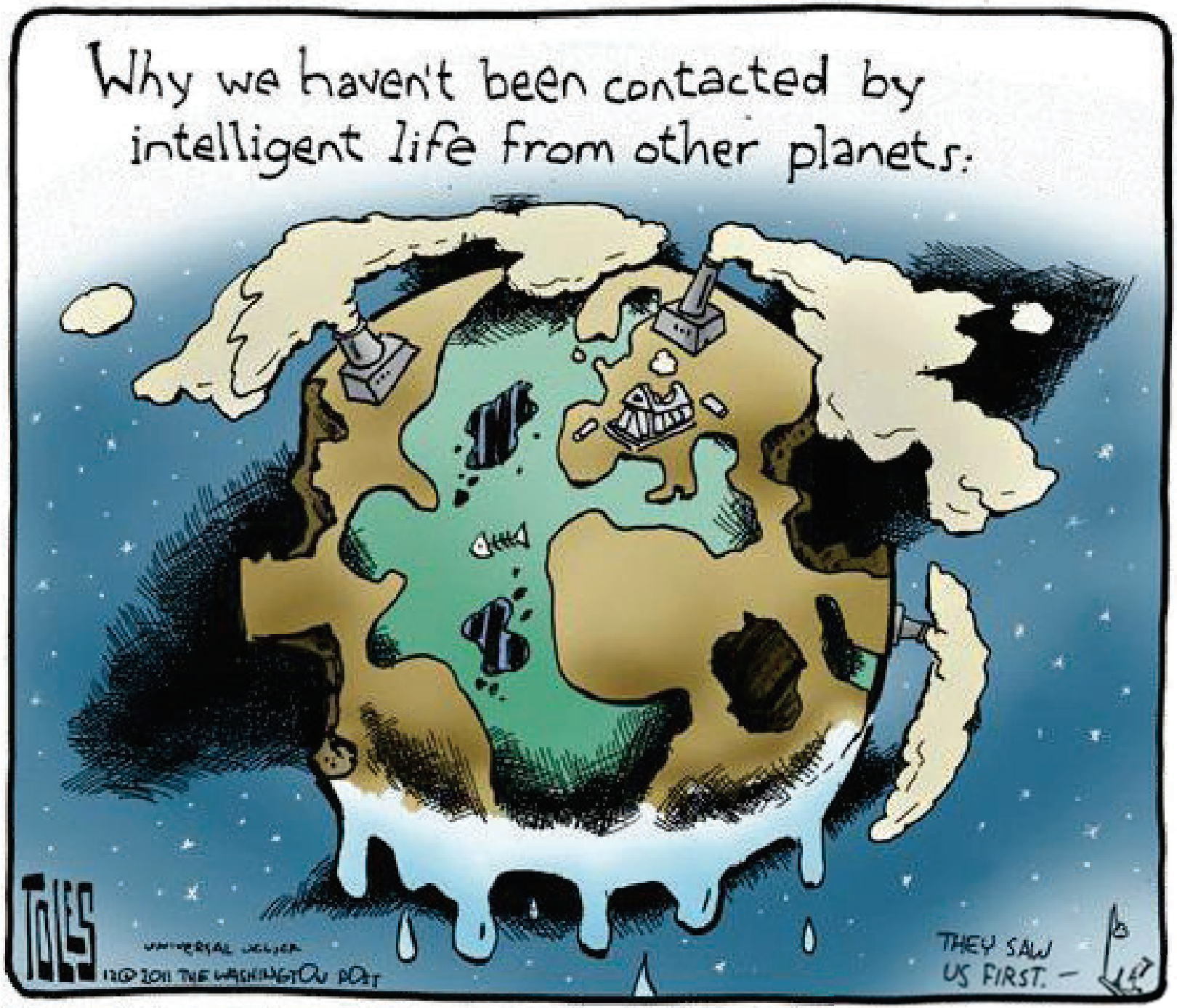


http://www.gocomics.com/tomtoles/2011/12/07

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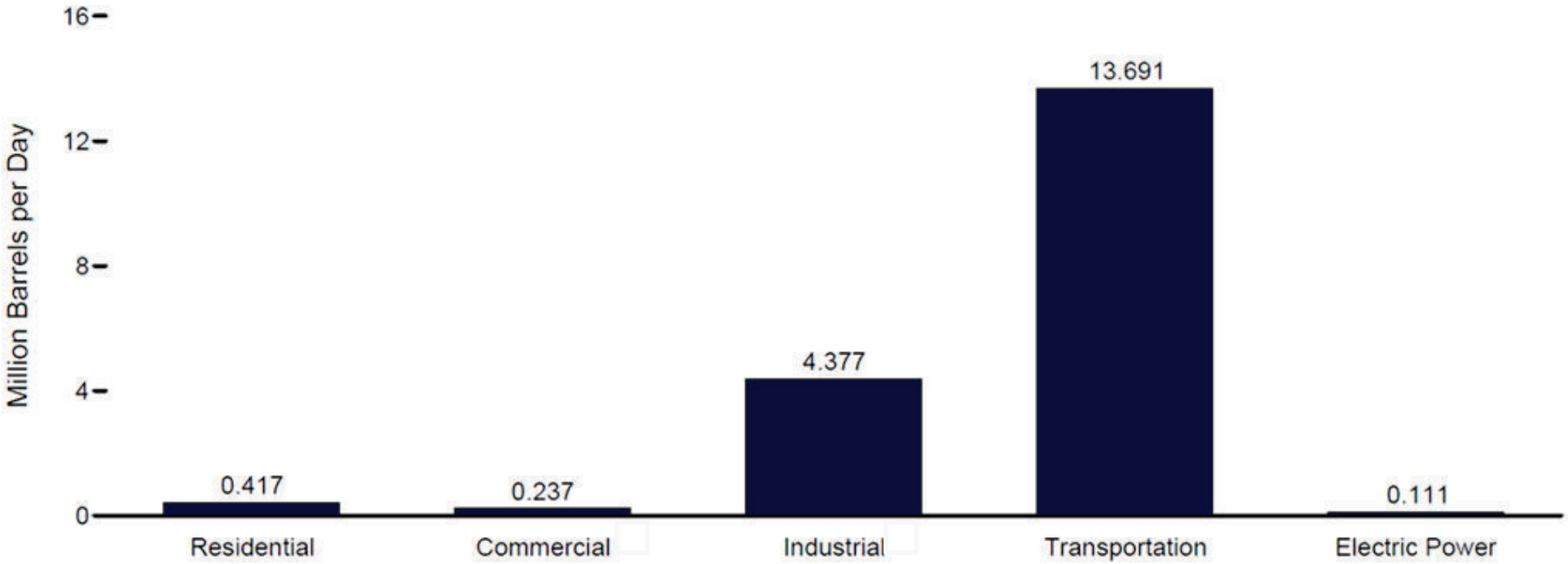
# Petroleum consumption by sector in the US

## June 2014




U.S. Energy Information Administration, Monthly Energy Review September 2014
www.eia.gov/mer

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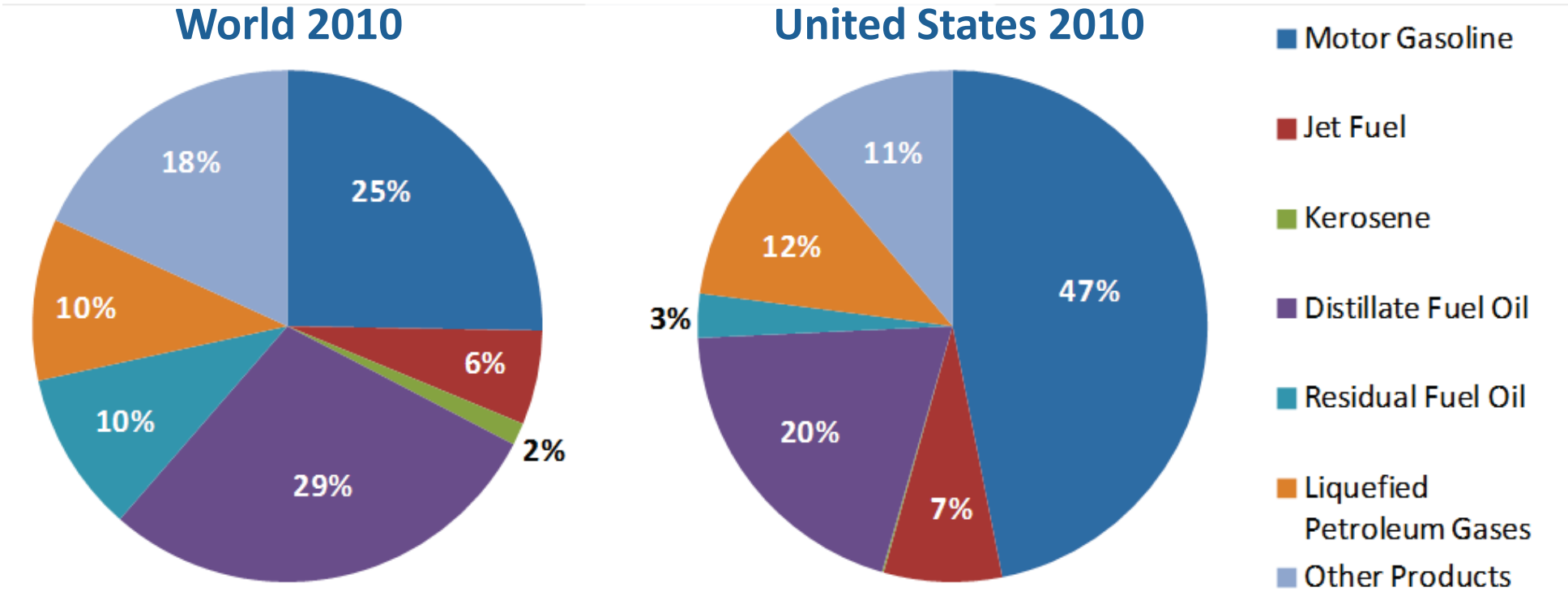
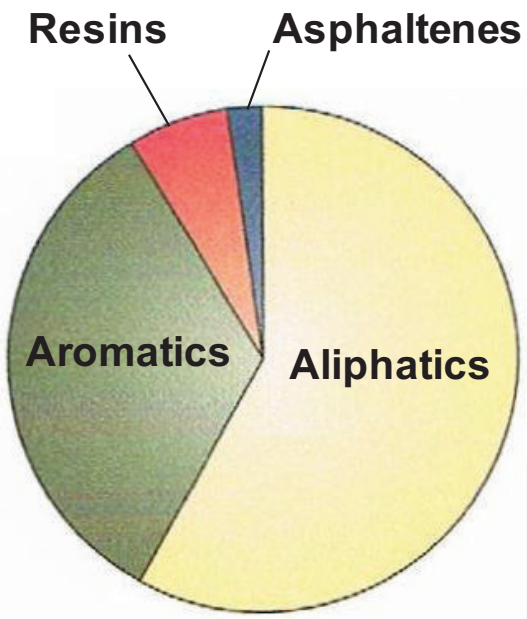
# Refined petroleum consumption by type




Prepared with data from U.S. Energy Information Administration
http://www.eia.gov/cfapps/ipdbproject/IEDIndex3.cfm?tid=5&pid=5&aid=2

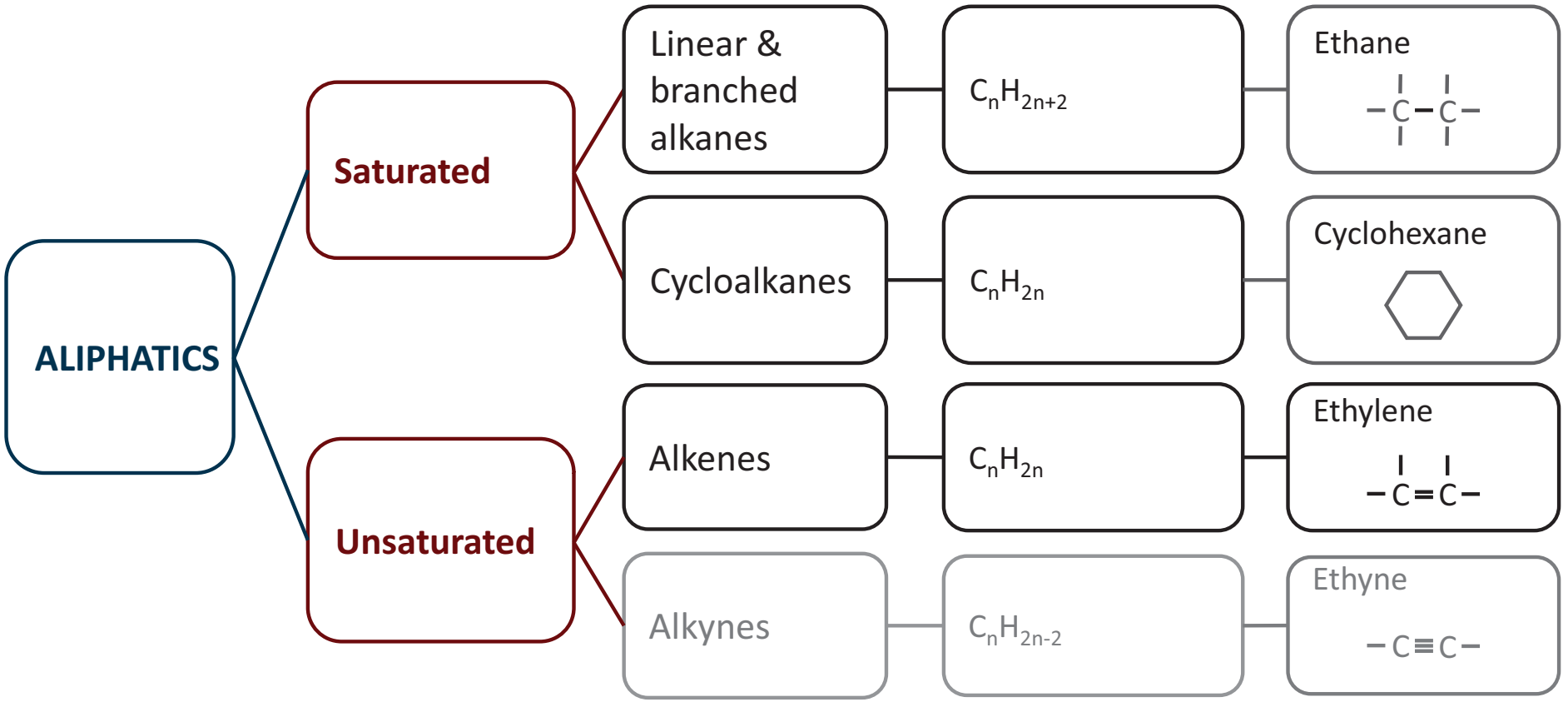
# Composition of crude oil and petroleum products



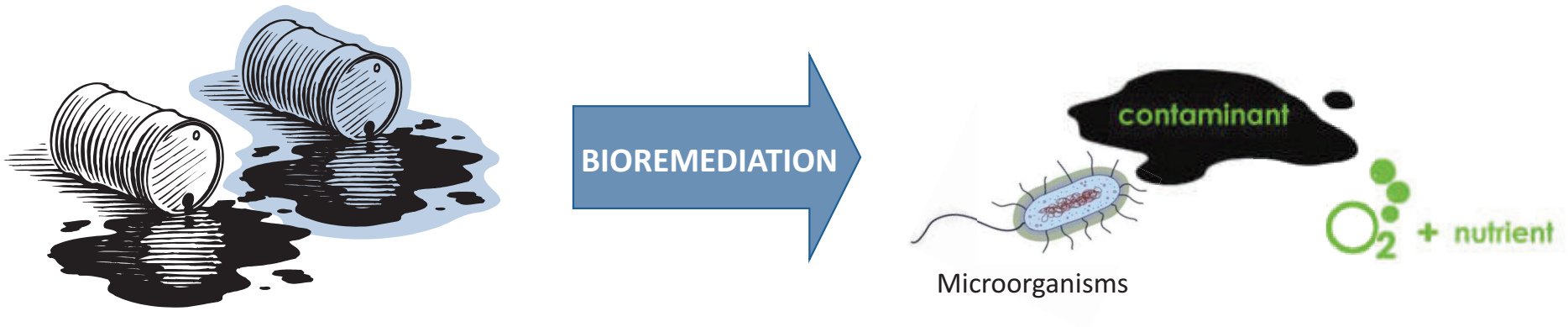

(Head et al., 2006. Nature Reviews 4, 173-182)

| Gasoline | JP-4 | Lubricants, motor oil |
|---|---|---|
| 40-70% aliphatic | 79% aliphatic | 70-90% aliphatic |
| 20-50% aromatic | < 25% aromatic | 10-30% aromatic |

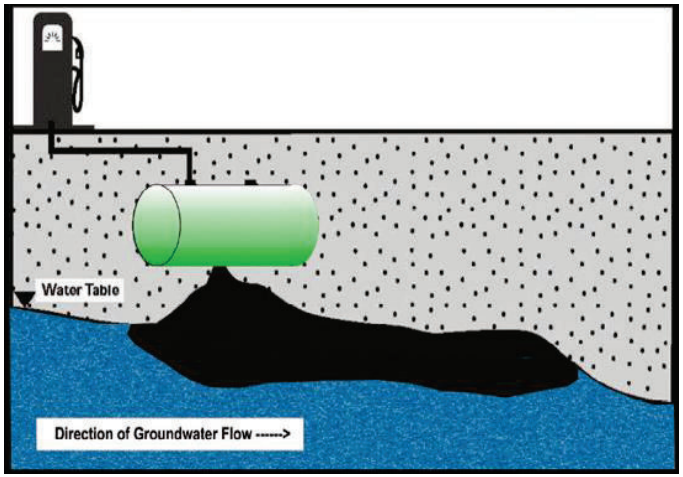
# Aliphatic hydrocarbons (AHC)



# Hydrocarbon contamination of soil and water

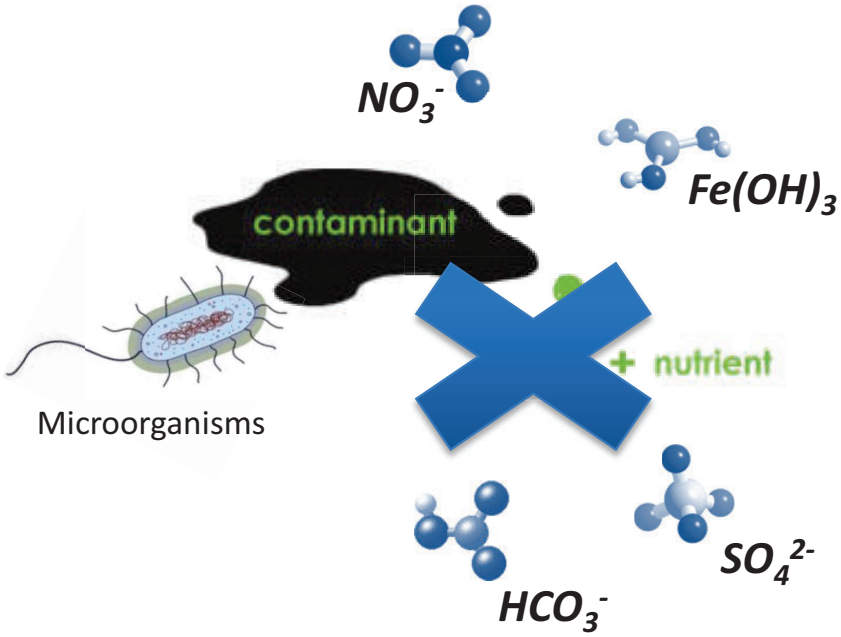


# Hydrocarbon contamination of deep soil and groundwater




http://www.all-electronic-devices.com

BIOREMEDIATION

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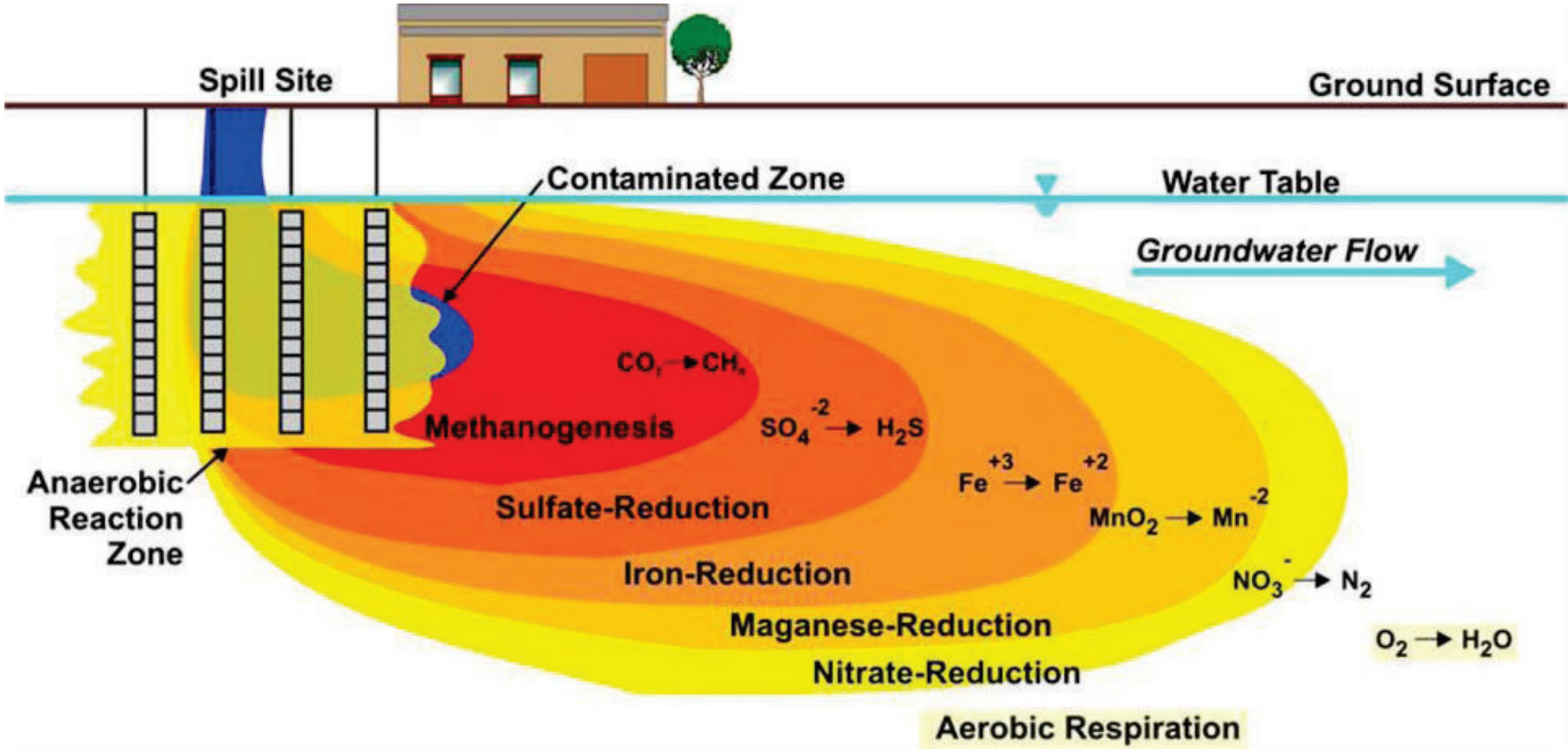


http://www.clu-in.org

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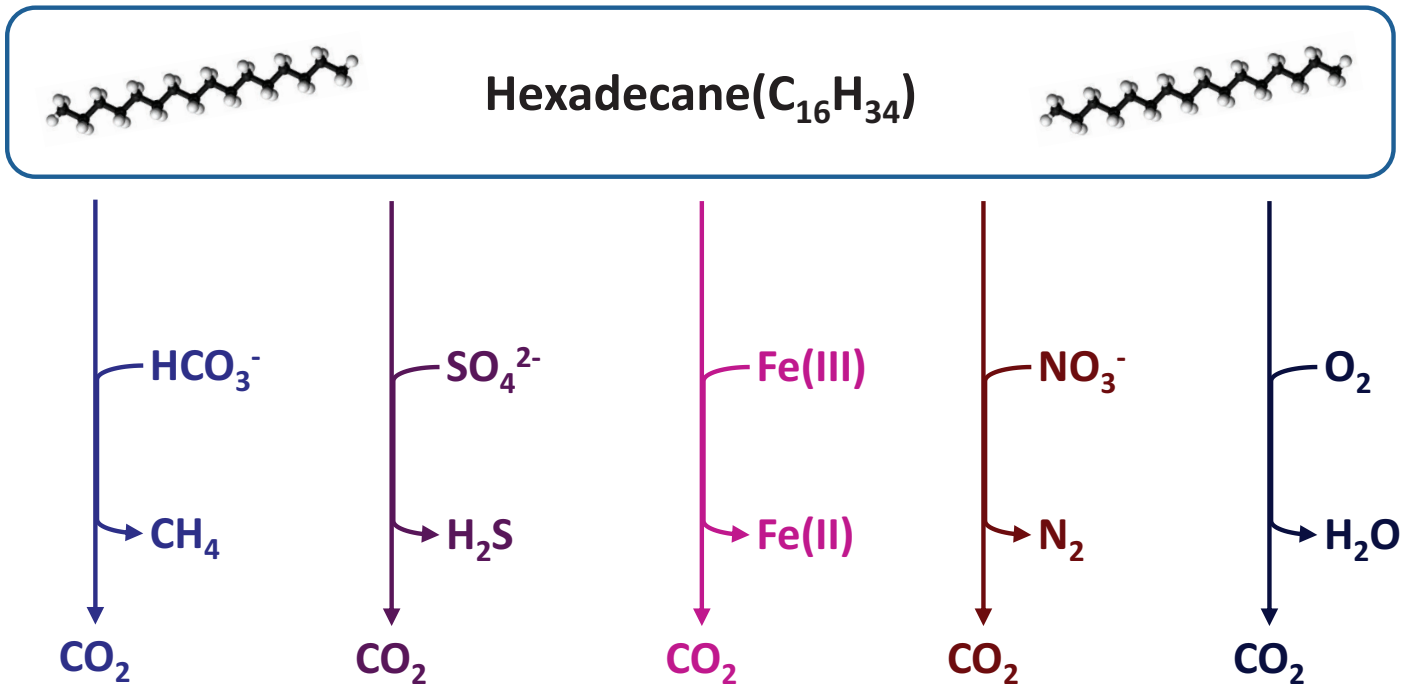


| $\Delta G^{0'}$ (kJ reaction$^{-1}$): | -354 | -558 | -9892 | -9676 | -10316 |
|---|---|---|---|---|---|

(values from Mbadinga et al. (2011) *International Biodeterioration and Biodegradation*, 65:1-13)

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**AHC-degrading bacteria**

**Proteobacteria**

- **6 nitrate-reducing isolates**
- **1 chlorate-reducing isolates**
- **9 sulfate-reducing isolates**
- ✗ **no iron-reducing isolates**

# Microbial processes under engineered conditions for in situ bioremediation of oil-contaminated sites

# OIL-LESS Project

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# Microbial processes under engineered conditions for in situ bioremediation of oil-contaminated sites

# OIL-LESS Project

**Objectives:** **Who is there?**

Identification of key microorganisms in anaerobic aliphatic hydrocarbon degradation

# Microbial processes under engineered conditions for in situ bioremediation of oil-contaminated sites

# OIL-LESS Project

**Objectives:** **Who is there?** **Who is doing what ?**

Identification of key microorganisms in anaerobic aliphatic hydrocarbon degradation

Functional characterization of these microorganisms

# Microbial processes under engineered conditions for in situ bioremediation of oil-contaminated sites

# OIL-LESS Project

**Objectives:** **Who is there?** | **Who is doing what ?** | **With whom?**

Identification of key microorganisms in anaerobic aliphatic hydrocarbon degradation

Functional characterization of these microorganisms

Study possible relations between different microbial groups

# Microbial processes under engineered conditions for in situ bioremediation of oil-contaminated sites

# OIL-LESS Project

**Objectives:** **Who is there?** | **Who is doing what ?** | **With whom?**

Identification of key microorganisms in anaerobic aliphatic hydrocarbon degradation

Functional characterization of these microorganisms

Study possible relations between different microbial groups

**How can we use what we have learned?**

Isolation of novel aliphatic hydrocarbon degrading microorganisms

Develop innovative engineered bioremediation strategies

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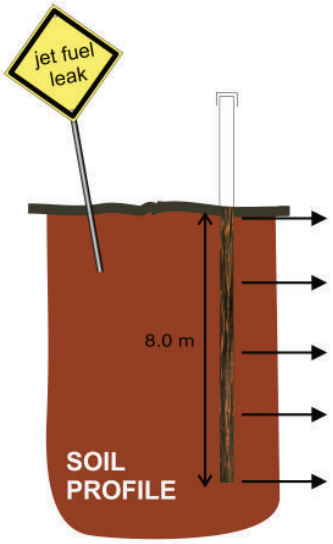



AHC-contaminated soil samples

Who is there? Who is doing what?

**Task 1**

Microbial enrichments:
$NO_3^-$-reducing
$Fe^{3+}$-reducing
$SO_4^{2-}$-reducing
methanogenic

stable AHC-degrading enrichment cultures

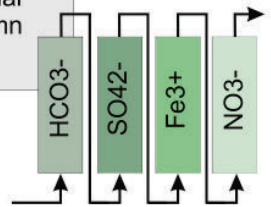
**Task 2**

Microbial composition and function of alkane-degrading communities

enriched cultures degrading AHC

**Task 3**

Bioremediation trials in laboratorial soil column




What are the prospects for the bioremediation of n-alkanes under anoxic conditions?

*Nitrate, $KNO_3$ 10 mM*
*Iron(III), $Fe(OH)_3$ 100 mM*
*Sulfate, $Na_2SO_4$ 20 mM*
*Bicarbonate, $NaHCO_3$ 50 mM*

*Contaminated soil*

*Anaerobic granular sludge from a brewery wastewater treatment plant*

*Aerobic sludge from the wastewater treatment plant of an industry cleaning machine engines*

*Marine sediment*

*Freshwater sediments*

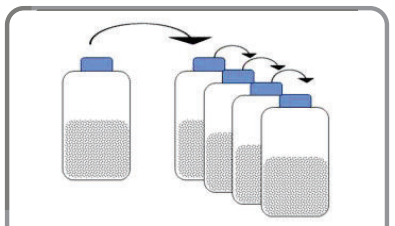
**Electron acceptor**

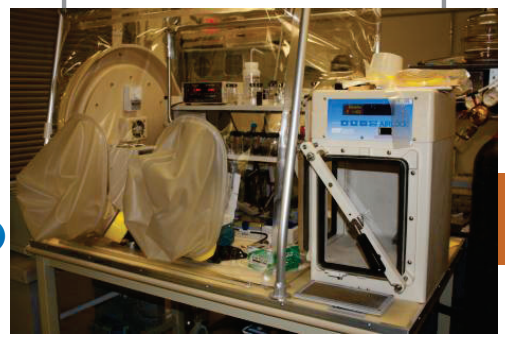
**Inoculum**

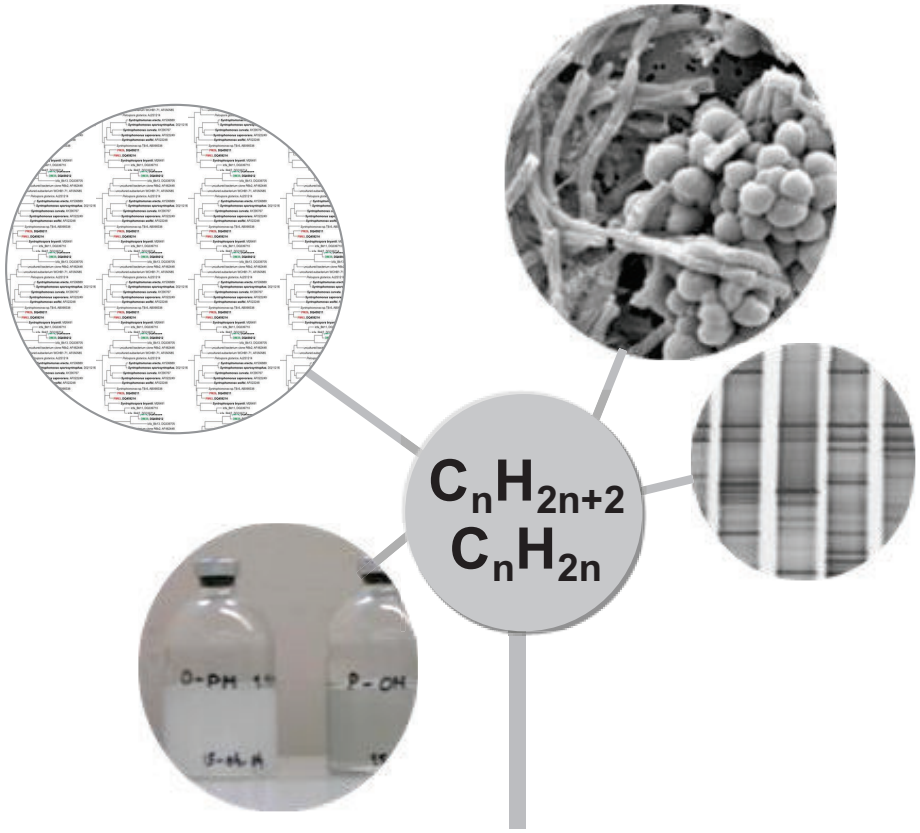
**Substrate (1 mM)**

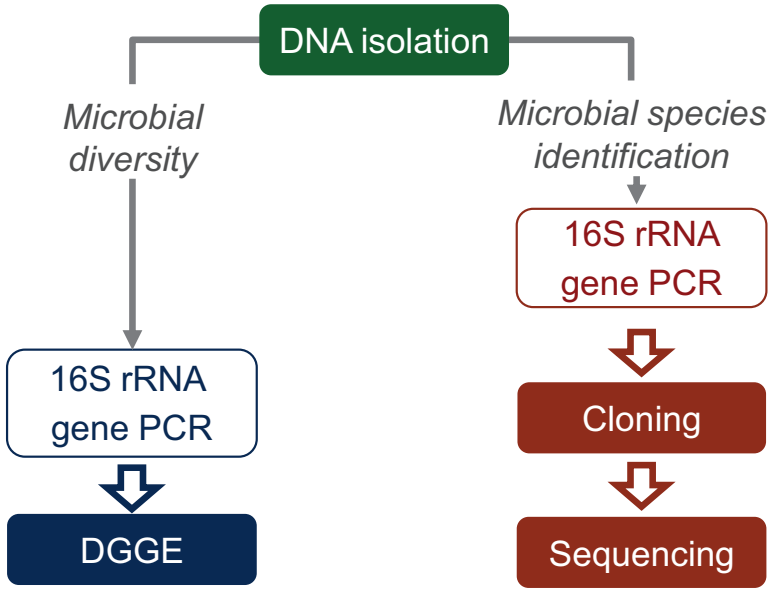
*Hexadecane*
*Hexadecene*

**Enriched culture**





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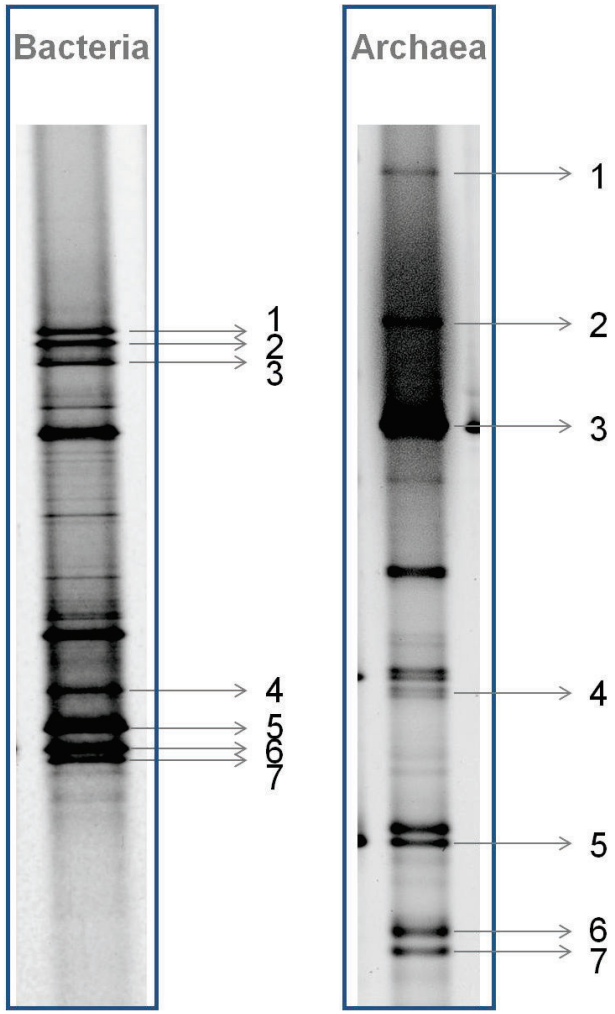
## Complex microbial culture enriched under methanogenic conditions




*(Numbers show the bands chosen for cloning and sequencing)*

- A *Syntrophus*-like microorganism was identified and is possibly involved in the degradation of the AHC

- Known methanogens utilizing acetate and $H_2/CO_2$ were identified, and were likely the syntrophic partners in AHC degradation

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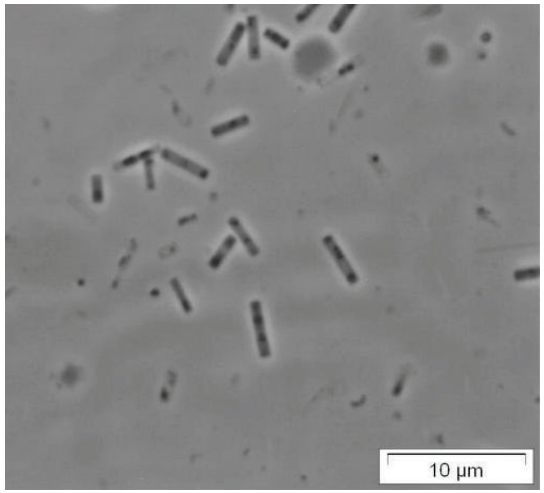
## Sulfate-reducing microbial culture

| | Sulfate reduction |
|---|---|
| Hexadecane | 19 % |
| Hexadecene | 72 % |

Phase contrast microscopy



## Sulfate-reducing microbial culture

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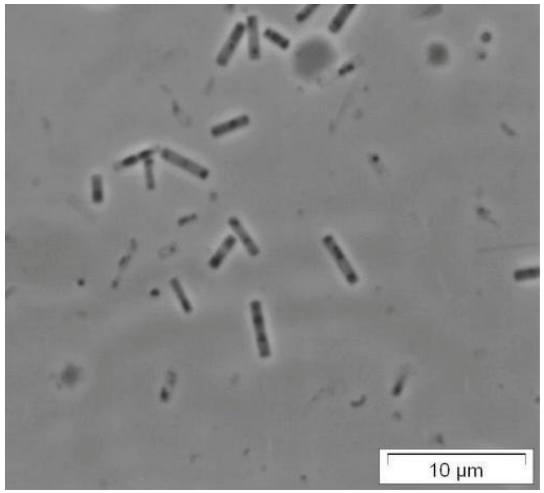
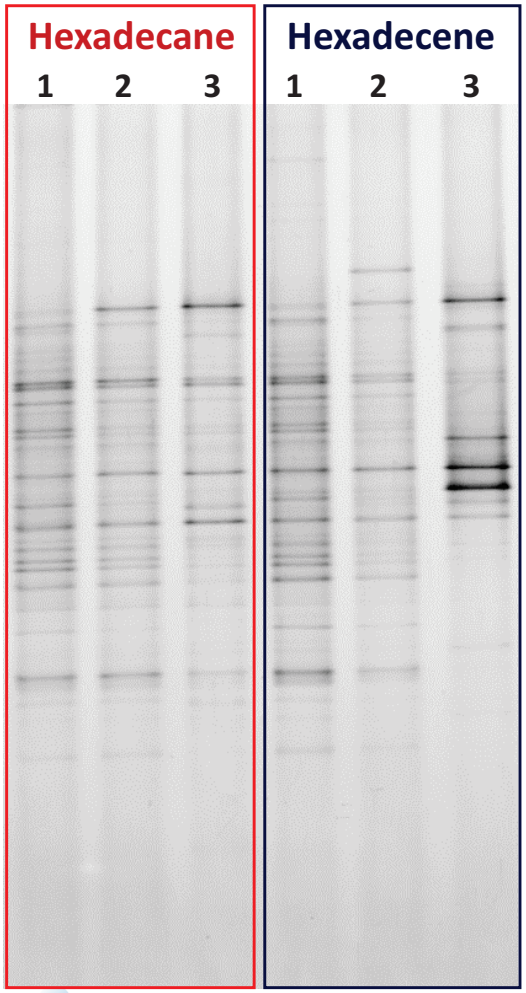
**Phase contrast microscopy**




**Microbial diversity analysis: PCR-DGGE of 16S rRNA genes**

BACTERIAL COMMUNITIES

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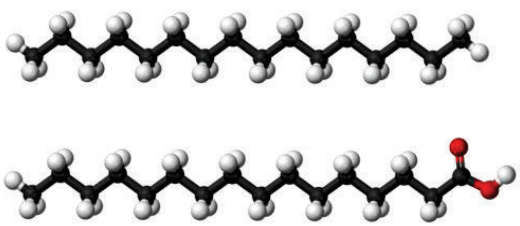
## Sulfate-reducing microbial culture

- Microbial diversity decreased with time
- Different microbial communities developed in the presence of Hexadecane or Hexadecene
- When incubated with palmitate, a *Desulfomonile*-like bacteria became predominant



**Hexadecane**



**Palmitic acid (C16:0)**


**Apparently is a new species!!!**

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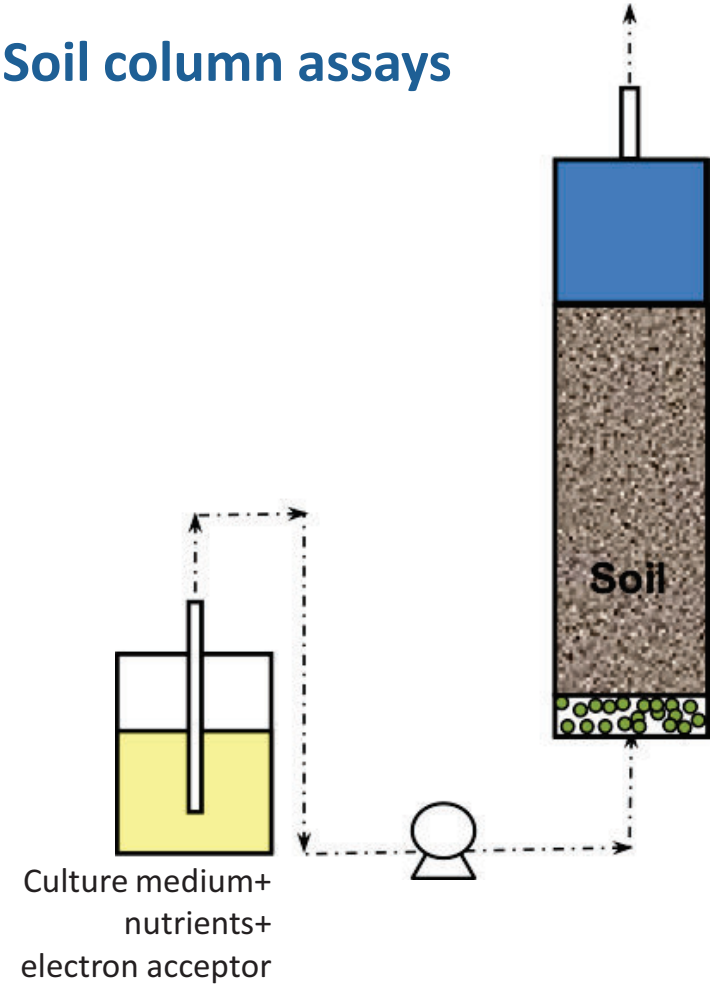
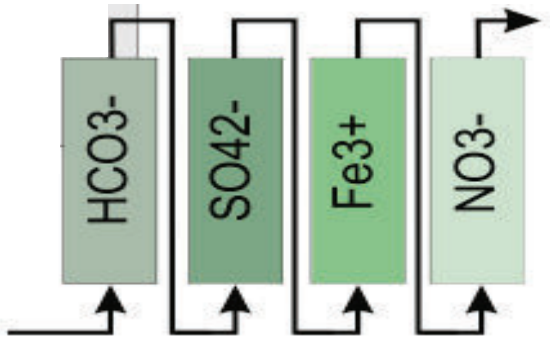
## Sulfate-reducing microbial culture

## Soil column assays




## Mesocosms assays

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# Thank you for your attention!

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Microbe Zoo

**Acknowledgements:**

**Alves, Madalena**
**Alves, Joana**
**Langenhof, Alette**
**Salvador, Andreia**
**Sousa, Diana**
**Stams, Fons**

FUNDAÇÃO
LUSO-AMERICANA

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This work was financially supported by FEDER funds, through COMPETE program, and by Portuguese funds, through the Portuguese Foundation for Science and Technology (FCT), in the frame of project FCOMP-01-0124-FEDER-027917.

COMPETE
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