Bioremediation of marine matrices contaminated by organic persistent pollutants

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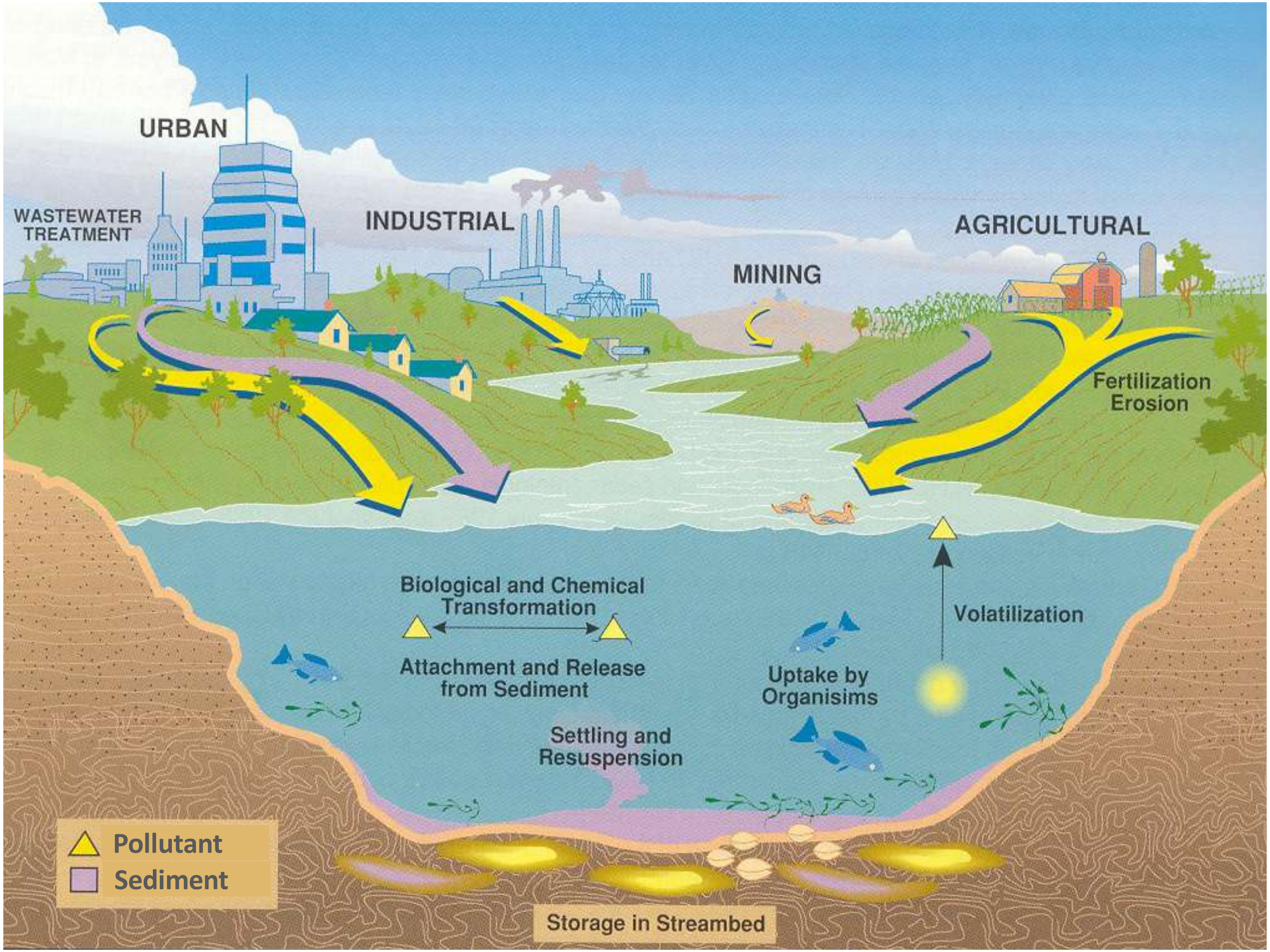
FishMed-PhD

Outline

1. Main sources, types and distribution of persistent organic pollutants in the marine environment
2. Recall on microbial metabolism of organic matter under aerobic and anaerobic conditions
3. Overview on the biodegradation mechanisms of the main persistent organic pollutants under aerobic and anaerobic conditions
4. Examples of technologies/approaches for the enhancement of biodegradation processes (bioremediation) in marine environments

1. Main sources, types and distribution of persistent organic pollutants in the marine environment

Main sources and environmental fate of pollutants (a)



Main sources of xenobiotic compounds (*a*)

The chemical industry produces more than 850 different synthetic chemical products. Some of them (more than 50) are produced extensively (2-10 million tons/year) for a variety of industrial and domestic purposes.

The main producers and/or users of synthetic chemicals are:

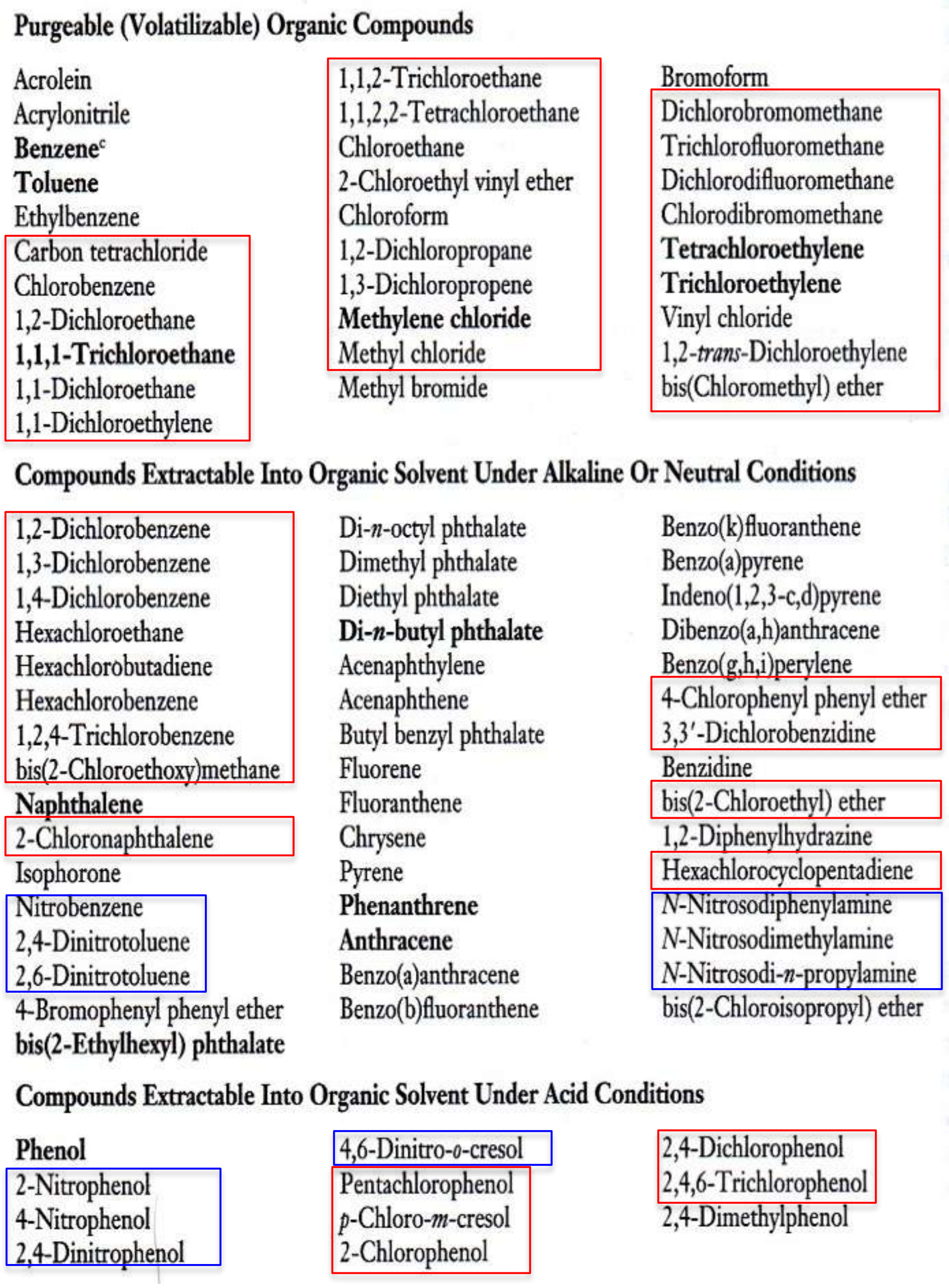
- the **petrochemical industry** which produces refined petroleum products (*mixtures of haliphatic and aromatic hydrocarbons*) as well as pure chemicals (*haliphatic and aromatic hydrocarbons, alcohols, ethers, phenols, aldehydes, carboxylic acids, frequently substituted with chlorine atoms or amino- or nitro-groups*).
- **the pulp and paper industry** (more than 200 *halogenated haliphatic and aromatic compounds* produced during the chlorine bleaching of pulp);
- the **plastic industry** (uses *styrene, vinyl chloride, aniline, terephtalic acids, methyl methacrylate, solvents, antioxidants, plasticizers, cross-linking agents*, etc. to produce *polymers*);

Main sources of xenobiotic compounds (*b*)

- the **pesticide industry** (produces and uses *benzene and heterocyclic derivatives*, as well as *organophosphorous compounds*, *carbamates, acetanilides and organometal compounds substituted with halogen-, hydroxy-, alkoxy-, aryl-, nitrile-, nitro-, and amino-groups*);
- the **cosmetic, medical and pharmaceutical industry** (uses and produces a *large array of complex synthetic organic compounds*);
- the **textile industry** (uses monomers and reagents to produce *synthetic fibres, halogenated haliphatic hydrocarbons* for cleaning, *surfactants, dyes, etc*);
- the **energy industry/combustion of fossil fuels** (uses *gasoline, i.e. haliphatic hydrocarbon (70%) + aromatic hydrocarbons (30%)* and *diesel fuel* and produces *polyaromatic hydrocarbons and nitrated-hydrocarbons*);

Finally, **transport through sea or roads**, as well as the **use of chemicals in agriculture** (pesticides, herbicides, inorganic nutrients, etc) and at the **domestic level**, (paints, cosmetics, personal care products, cleaning and disinfecting products, etc.), represent additional important sources of contamination.

Main priority pollutants released in the environment



Purgeable (Volatilizable) Organic Compounds

Acrolein
Acrylonitrile
Benzene[c]
Toluene
Ethylbenzene
Carbon tetrachloride
Chlorobenzene
1,2-Dichloroethane
1,1,1-Trichloroethane
1,1-Dichloroethane
1,1-Dichloroethylene
1,1,2-Trichloroethane
1,1,2,2-Tetrachloroethane
Chloroethane
2-Chloroethyl vinyl ether
Chloroform
1,2-Dichloropropane
1,3-Dichloropropene
Methylene chloride
Methyl chloride
Methyl bromide
Bromoform
Dichlorobromomethane
Trichlorofluoromethane
Dichlorodifluoromethane
Chlorodibromomethane
Tetrachloroethylene
Trichloroethylene
Vinyl chloride
1,2-*trans*-Dichloroethylene
bis(Chloromethyl) ether

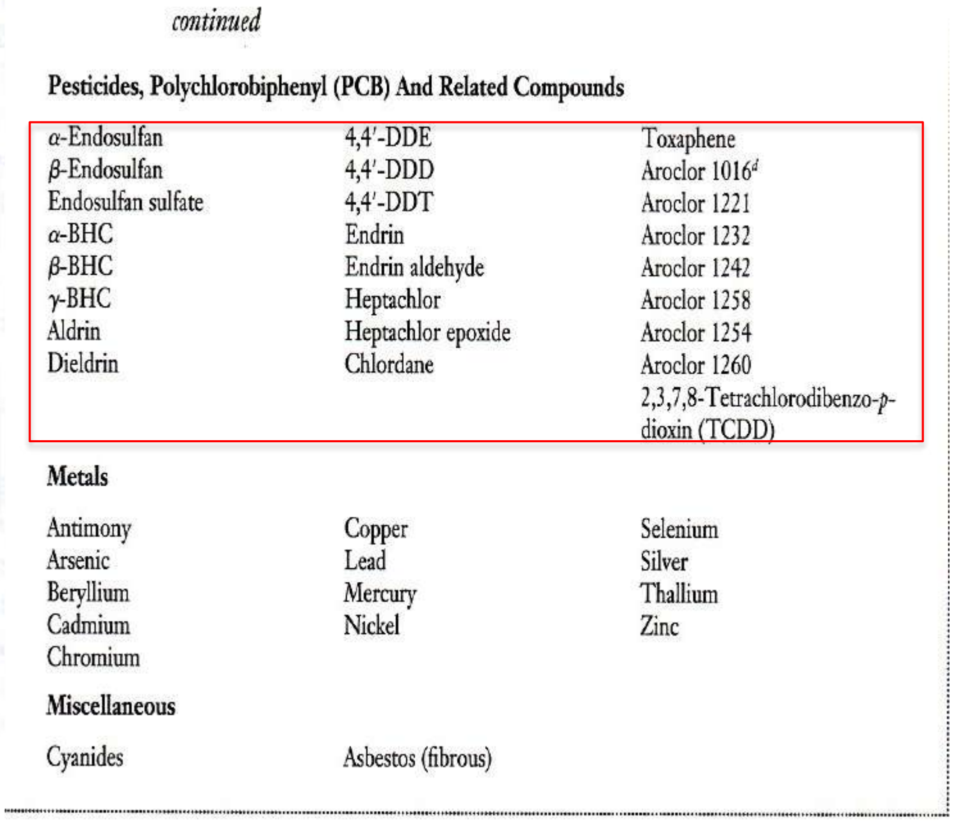
Compounds Extractable Into Organic Solvent Under Alkaline Or Neutral Conditions

1,2-Dichlorobenzene
1,3-Dichlorobenzene
1,4-Dichlorobenzene
Hexachloroethane
Hexachlorobutadiene
Hexachlorobenzene
1,2,4-Trichlorobenzene
bis(2-Chloroethoxy)methane
Naphthalene
2-Chloronaphthalene
Isophorone
Nitrobenzene
2,4-Dinitrotoluene
2,6-Dinitrotoluene
4-Bromophenyl phenyl ether
bis(2-Ethylhexyl) phthalate
Di-*n*-octyl phthalate
Dimethyl phthalate
Diethyl phthalate
Di-*n*-butyl phthalate
Acenaphthylene
Acenaphthene
Butyl benzyl phthalate
Fluorene
Fluoranthene
Chrysene
Pyrene
Phenanthrene
Anthracene
Benzo(a)anthracene
Benzo(b)fluoranthene
Benzo(k)fluoranthene
Benzo(a)pyrene
Indeno(1,2,3-c,d)pyrene
Dibenzo(a,h)anthracene
Benzo(g,h,i)perylene
4-Chlorophenyl phenyl ether
3,3′-Dichlorobenzidine
Benzidine
bis(2-Chloroethyl) ether
1,2-Diphenylhydrazine
Hexachlorocyclopentadiene
N-Nitrosodiphenylamine
N-Nitrosodimethylamine
N-Nitrosodi-*n*-propylamine
bis(2-Chloroisopropyl) ether

Compounds Extractable Into Organic Solvent Under Acid Conditions

Phenol
2-Nitrophenol
4-Nitrophenol
2,4-Dinitrophenol
4,6-Dinitro-*o*-cresol
Pentachlorophenol
p-Chloro-*m*-cresol
2-Chlorophenol
2,4-Dichlorophenol
2,4,6-Trichlorophenol
2,4-Dimethylphenol

Major priority pollutants (according to EPA) identified according to their documented **bioaccumulation, toxicity** towards humans and living organisms, and **persistence** in the environment.



continued

Pesticides, Polychlorobiphenyl (PCB) And Related Compounds

α-Endosulfan
β-Endosulfan
Endosulfan sulfate
α-BHC
β-BHC
γ-BHC
Aldrin
Dieldrin
4,4′-DDE
4,4′-DDD
4,4′-DDT
Endrin
Endrin aldehyde
Heptachlor
Heptachlor epoxide
Chlordane
Toxaphene
Aroclor 1016[d]
Aroclor 1221
Aroclor 1232
Aroclor 1242
Aroclor 1258
Aroclor 1254
Aroclor 1260
2,3,7,8-Tetrachlorodibenzo-*p*-dioxin (TCDD)

Metals

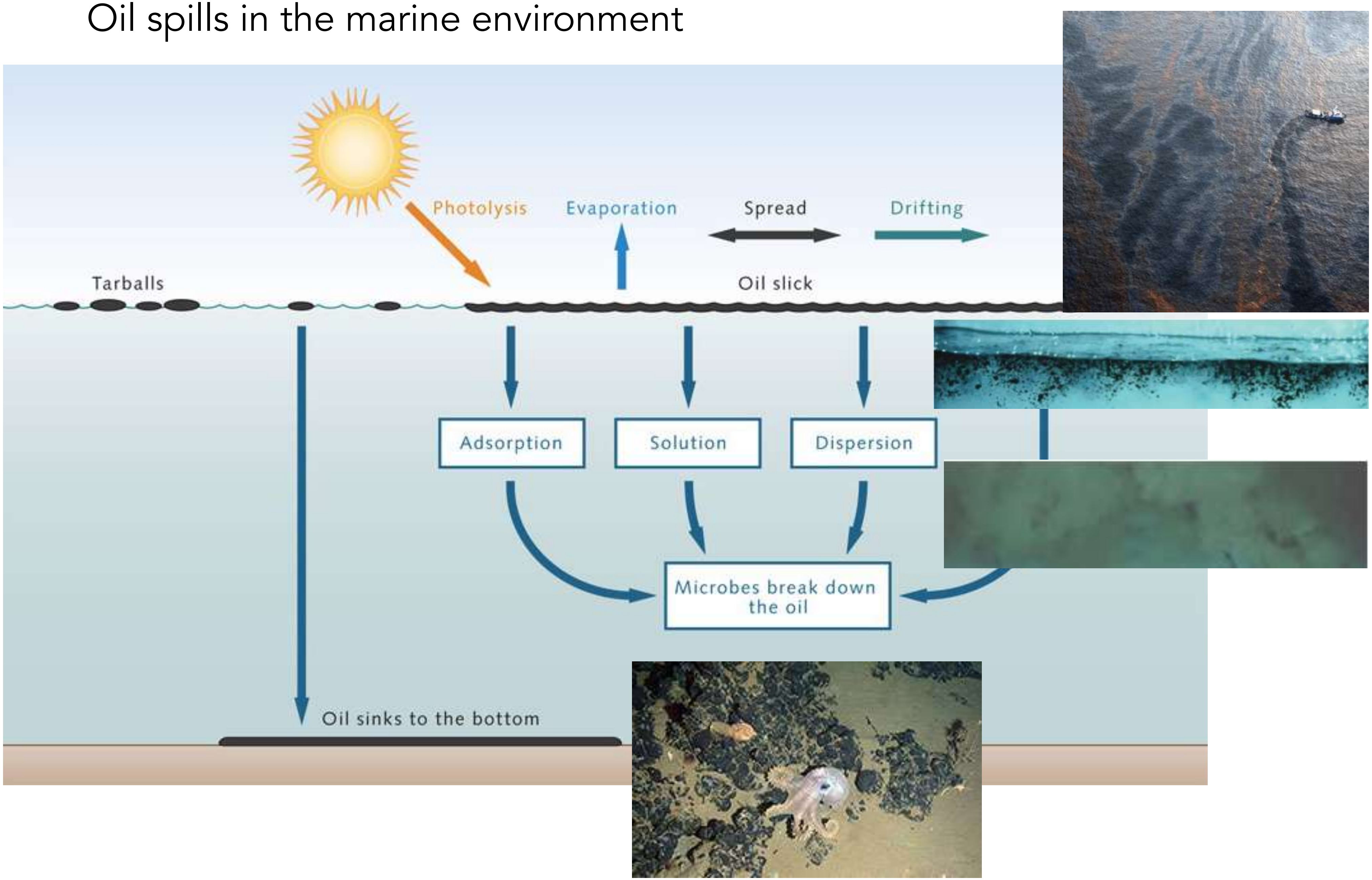
Antimony
Arsenic
Beryllium
Cadmium
Chromium
Copper
Lead
Mercury
Nickel
Selenium
Silver
Thallium
Zinc

Miscellaneous

Cyanides
Asbestos (fibrous)

Main sources and environmental fate of pollutants (b)

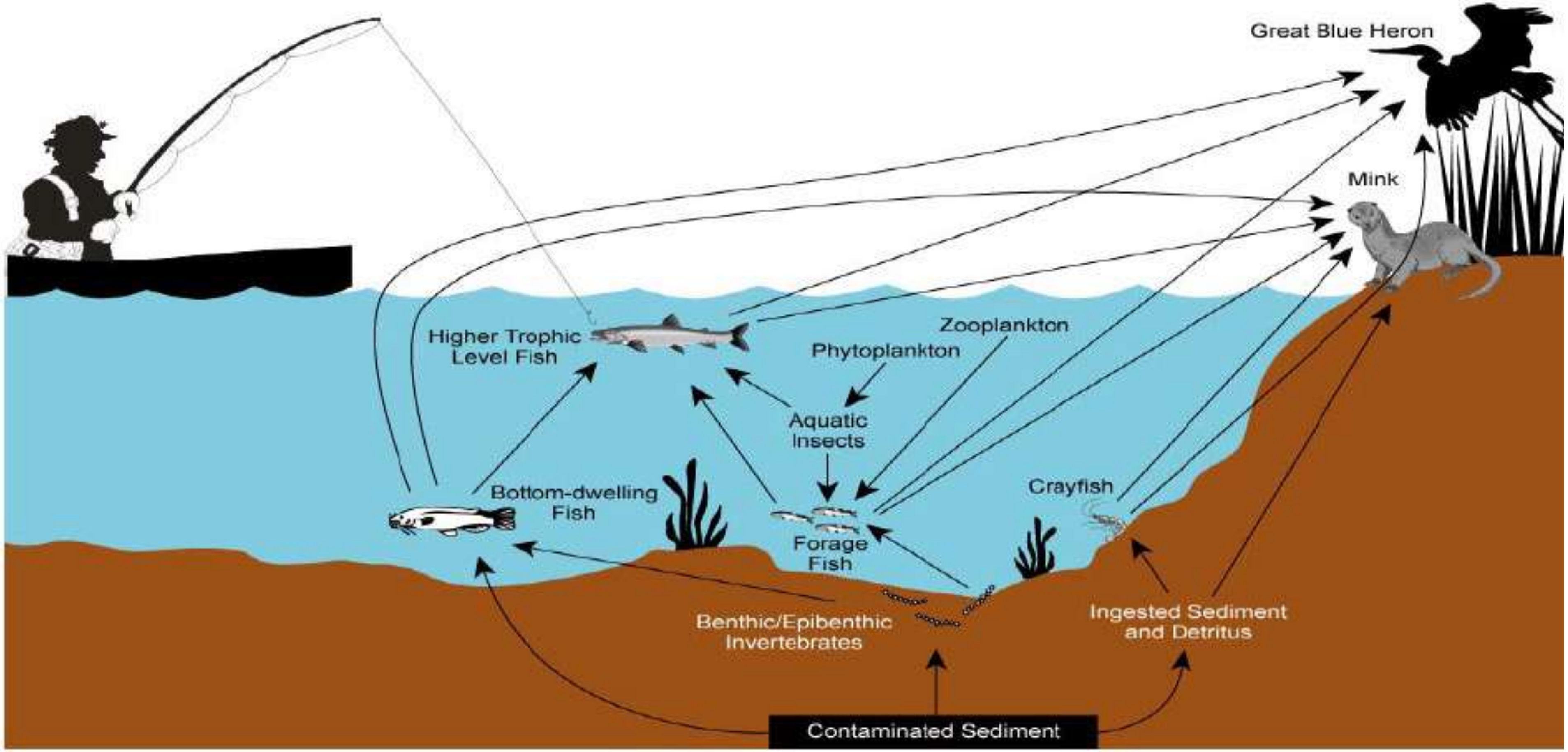
Oil spills in the marine environment



Photolysis
Evaporation
Spread
Drifting
Tarballs
Oil slick
Adsorption
Solution
Dispersion
Microbes break down the oil
Oil sinks to the bottom

Main sources and environmental fate of pollutants (c)

From seawater and sediment to biota up to the top of the food chain (humans)



2. Recall on microbial metabolism of organic matter under aerobic and anaerobic conditions

Main microorganisms with potential xenobiotic biodegradation capabilities

Bacteria are the main phylogenetic group of microorganisms that have evolved/acquired the capability of degrading several xenobiotic compounds in different habitats due to their:

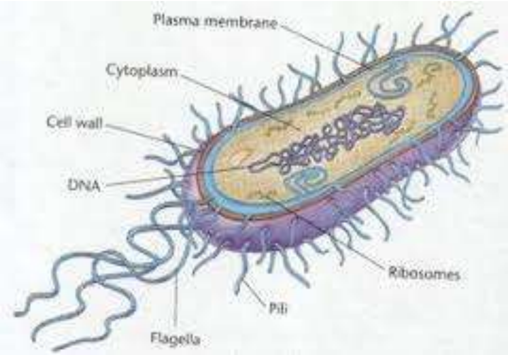
a) extensive distribution/ubiquity in the environment,

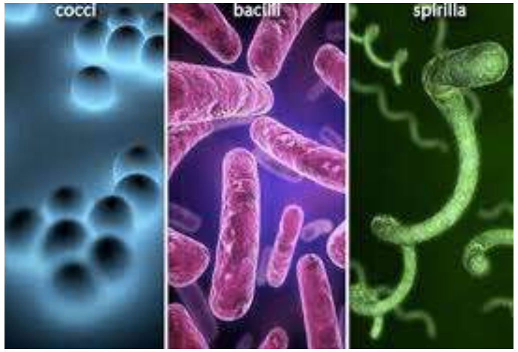
b) large native metabolic versatility (some bacteria are able to recognize over than 100 distinct organic substrates)

c) large diversity and high reproduction rates (higher biomass concentration, higher mutations rates) and

d) high susceptibility to undergo horizontal gene transfer.

They normally import the pollutants into the cell, where they are attacked by specific enzymes, that progressively degrade the pollutants towards simpler compounds, sometimes up to CO_2 and water.





Main requirements for growth of microorganisms

a) **Carbon (C).** Possible sources of C may be **organic molecules (heterotrophs)**, such as sugars, proteins, lipids, organic acids, hydrocarbons, xenobiotic compounds, etc., **or CO_2 (autotrophs)**;

b) **Nitrogen (N).** Sources of nitrogen may be organic molecules (proteins, amino acids, urea, amines), or inorganic (e.g., NH_4^+, NO_3^- and N_2);

c) **Phosphorous (P).** Sources may be organic or more commonly inorganic molecules (e.g., $HPO_4^=$, $H_2PO_4^-$, PO_4^{3-});

d) **Sulphur (S).** Sources may be organic (some amino acids) or inorganic molecules (e.g., $SO_4^=$);

e) **Hydrogen (H)**. Sources are water or organic compounds

f) **Oxygen (O).** Sources are water, molecular oxygen (O_2) or organic compounds.

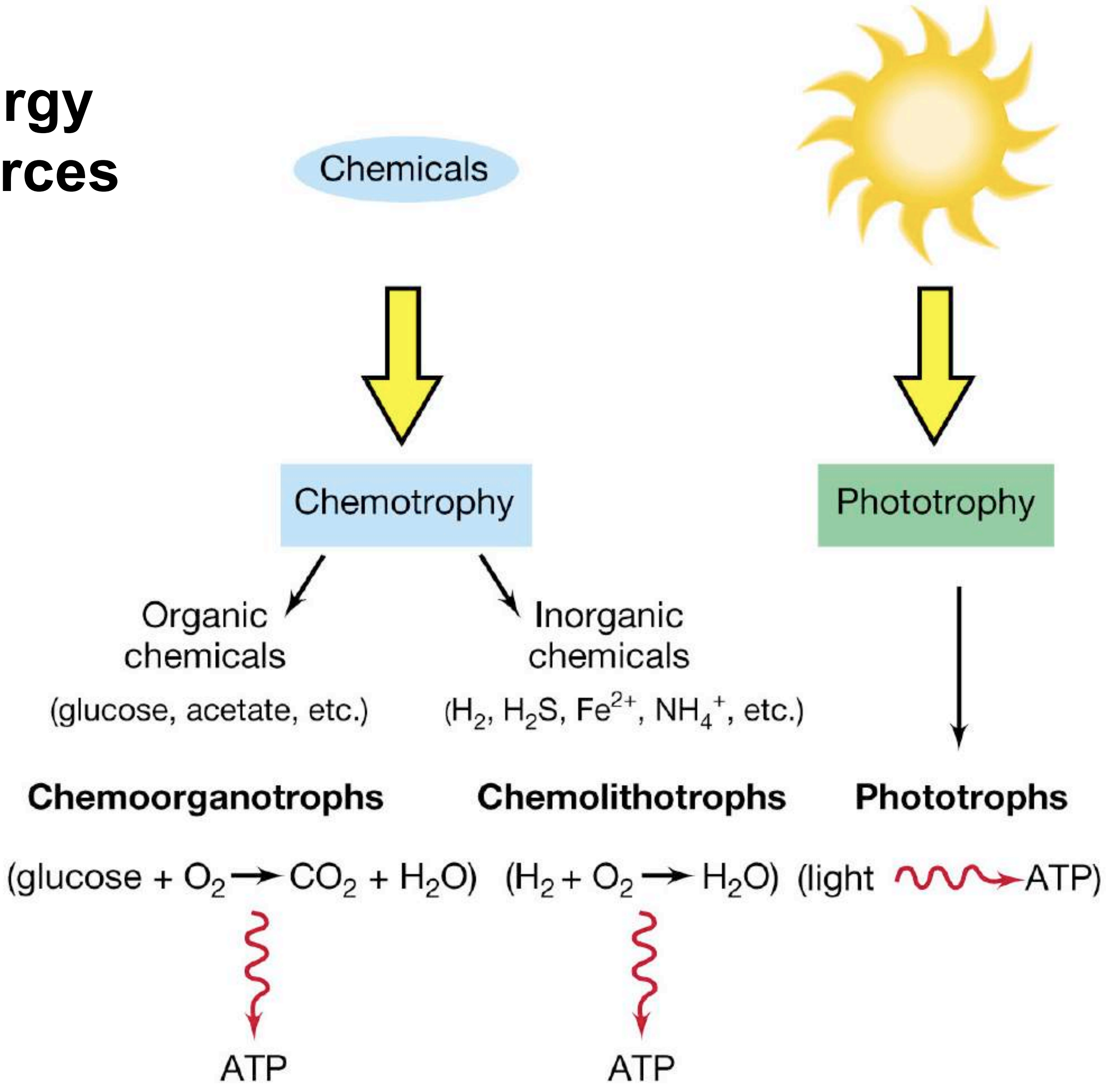
In addition, (micro)organisms require an **energy source**, that may be:

- an organic compound (chemoorganotrophs). In case of heterotrophs, the same organic compound is often used also as carbon source.
- an inorganic compound (chemolitotrophs)
- light (phototrophs)

(micro)organisms also require an **"electron acceptor"** (O_2 or other compounds) for the utilization of the energy source.

Metabolisms: sources of energy and carbon

Energy sources



Chemicals

Chemotrophy

Phototrophy

Organic chemicals

(glucose, acetate, etc.)

Inorganic chemicals

(H_2, H_2S, Fe^{2+}, NH_4^+, etc.)

Chemoorganotrophs

($glucose + O_2 \rightarrow CO_2 + H_2O$)

ATP

Chemolithotrophs

($H_2 + O_2 \rightarrow H_2O$)

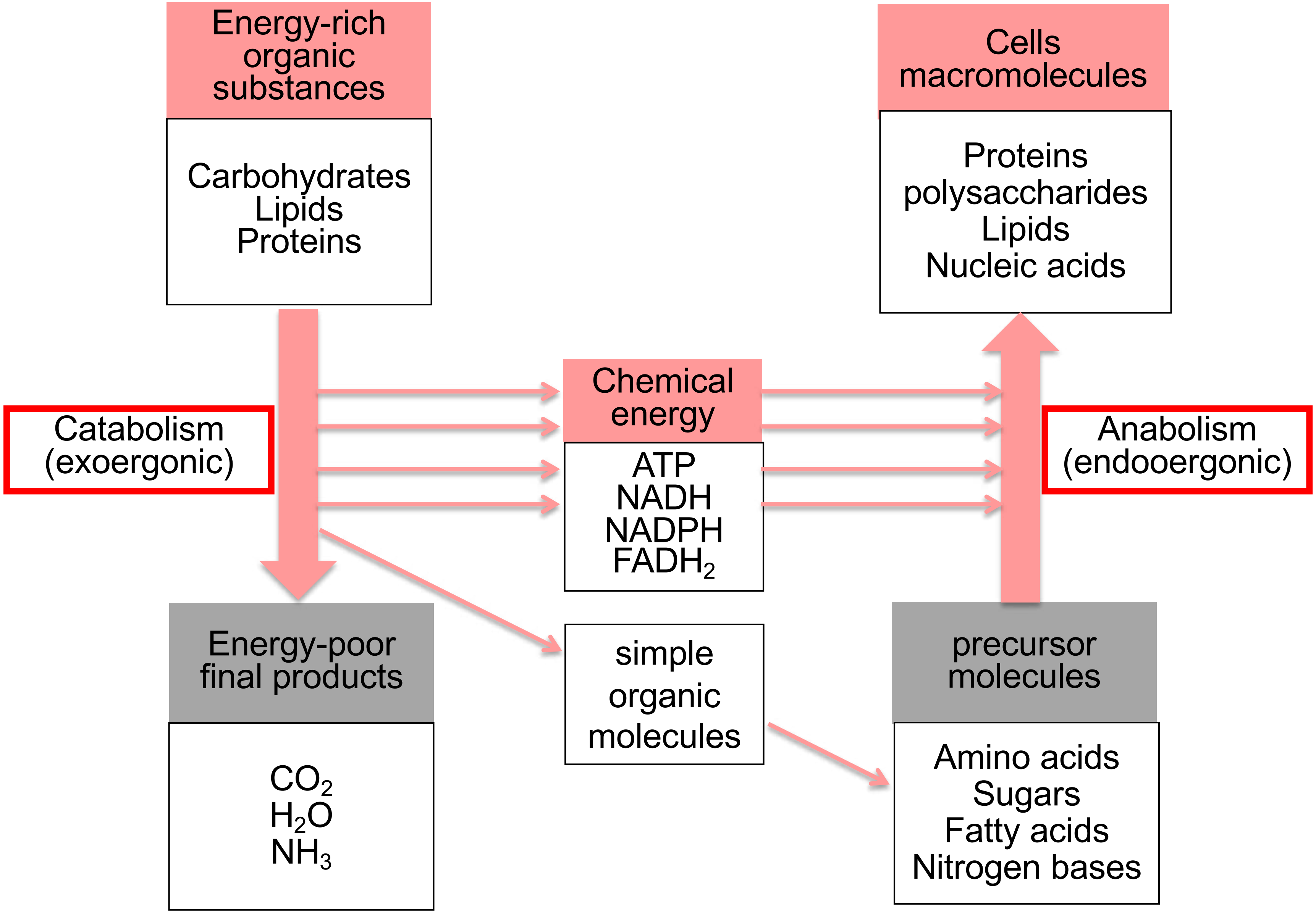
ATP

Phototrophs

(light → ATP)

C sources: **CO_2 ,autotrophy**, or **organic, heterotrophy**

Chemorganotrophic eterotrophic metabolism



Energy-rich organic substances

Carbohydrates
Lipids
Proteins

Catabolism (exoergonic)

Chemical energy

ATP
NADH
NADPH
$FADH_2$

Energy-poor final products

CO_2
H_2O
NH_3

simple organic molecules

Cells macromolecules

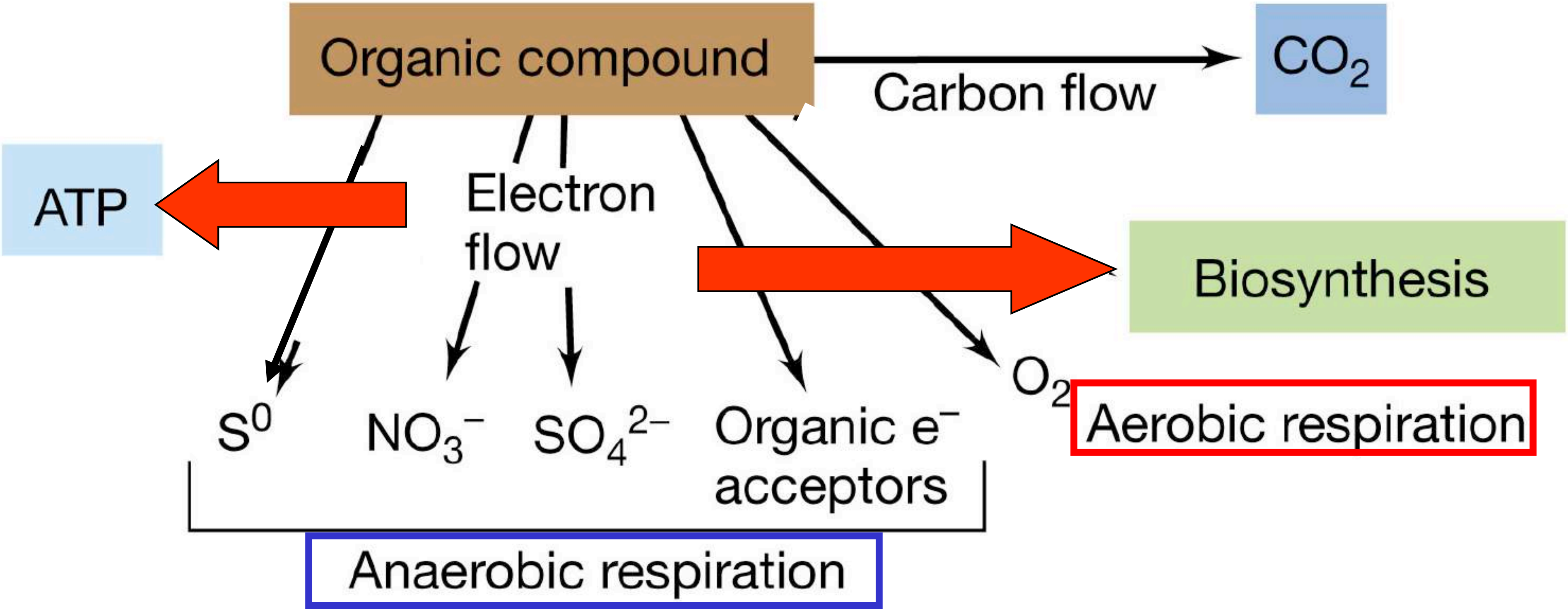
Proteins
polysaccharides
Lipids
Nucleic acids

Anabolism (endooergonic)

precursor molecules

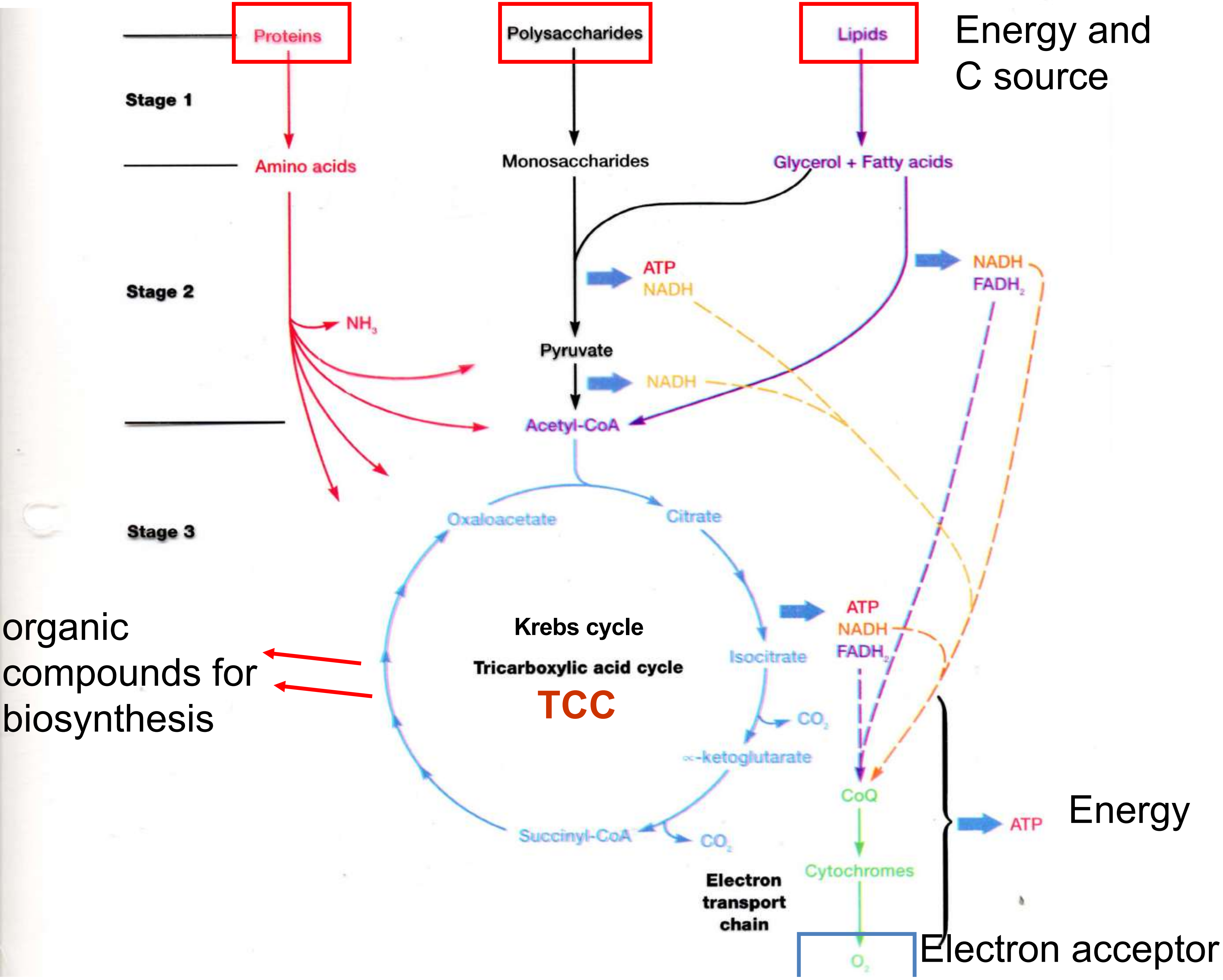
Amino acids
Sugars
Fatty acids
Nitrogen bases

Chemorganotrophic, eterotrophic metabolism: electron donors and acceptors, sources of energy and carbon



Fermentation: anaerobic process in which electrons deriving from the oxidation of the organic matter are transferred to a biomolecule that is generated as intermediate within the cell. This molecule is thus reduced (electron acceptor) into a fermentation product, that is excreted into the environment

Aerobic biodegradation of natural organic compounds



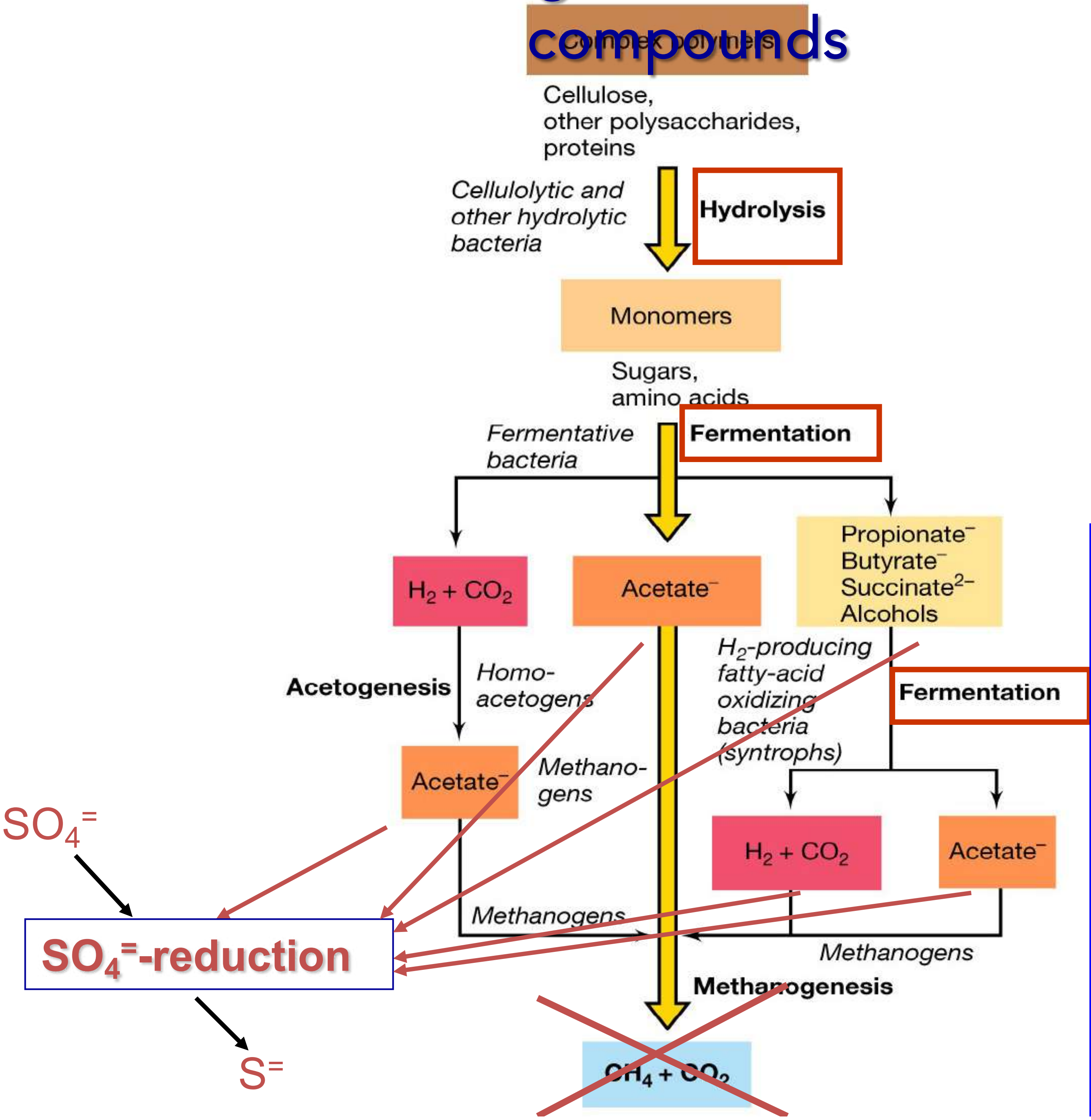
Energy and C source

organic compounds for biosynthesis

Energy

Electron acceptor

Anaerobic biodegradation of natural organic compounds



Complex polymers

Cellulose,
other polysaccharides,
proteins

Cellulolytic and other hydrolytic bacteria

Hydrolysis

Monomers

Sugars,
amino acids

Fermentative bacteria

Fermentation

$H_2 + CO_2$

Acetate$^-$

Propionate$^-$
Butyrate$^-$
Succinate^{2-}
Alcohols

Acetogenesis

Homo-acetogens

H_2-producing fatty-acid oxidizing bacteria (syntrophs)

Fermentation

Acetate$^-$

Methano-gens

$H_2 + CO_2$

Acetate$^-$

Methanogens

Methanogens

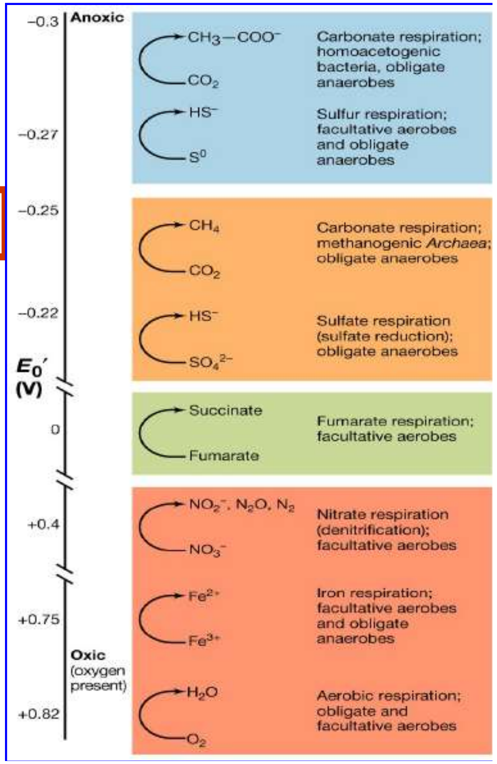
Methanogenesis

$CH_4 + CO_2$

$SO_4^=$

$SO_4^=$-reduction

$S^=$



E_0' (V)			
−0.3	Anoxic	$CO_2 \rightarrow CH_3-COO^-$	Carbonate respiration; homoacetogenic bacteria, obligate anaerobes
−0.27		$S^0 \rightarrow HS^-$	Sulfur respiration; facultative aerobes and obligate anaerobes
−0.25		$CO_2 \rightarrow CH_4$	Carbonate respiration; methanogenic *Archaea*; obligate anaerobes
−0.22		$SO_4^{2-} \rightarrow HS^-$	Sulfate respiration (sulfate reduction); obligate anaerobes
0		Fumarate → Succinate	Fumarate respiration; facultative aerobes
+0.4		$NO_3^- \rightarrow NO_2^-, N_2O, N_2$	Nitrate respiration (denitrification); facultative aerobes
+0.75		$Fe^{3+} \rightarrow Fe^{2+}$	Iron respiration; facultative aerobes and obligate anaerobes
+0.82	Oxic (oxygen present)	$O_2 \rightarrow H_2O$	Aerobic respiration; obligate and facultative aerobes

3. Overview on the biodegradation mechanisms of the main persistent organic pollutants under aerobic and anaerobic conditions

Catabolism of xenobiotic compounds

Fate of the xenobiotic compound:

a) MINERALIZATION (complete oxidation of carbon into CO_2)

Xenobiotic compounds → organic Intermediates → CO_2 + Energy

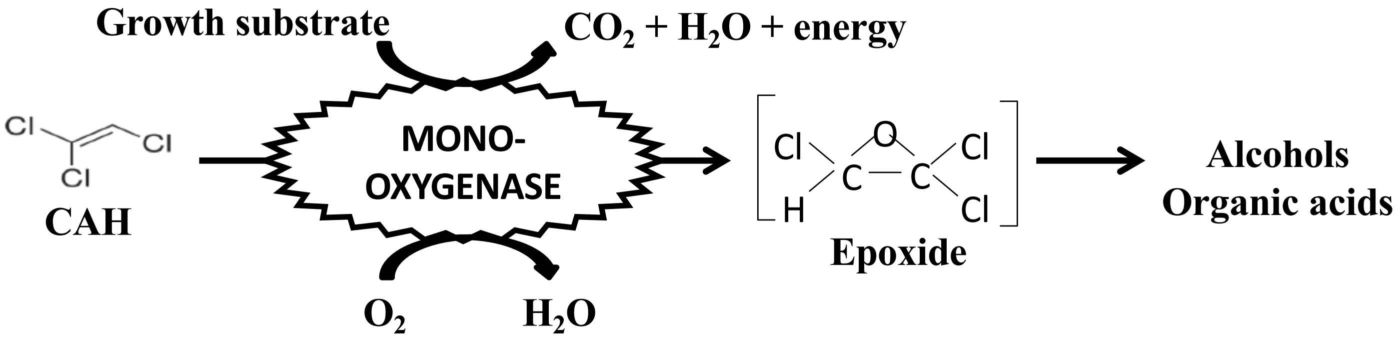
b) BIOCONVERSION (Partial oxidation of carbon)

Xenobiotic compounds → organic products (+ CO_2 + Energy)

Utilization of the xenobiotic compound by microorganisms:

a) GROWTH SUBSTRATE (direct metabolism, i.e., used as energy and/or carbon source → the degradation sustains growth)

b) CO-SUBSTRATE (co-metabolism, i.e., the degradation requires the utilization of another compound as energy/carbon source)

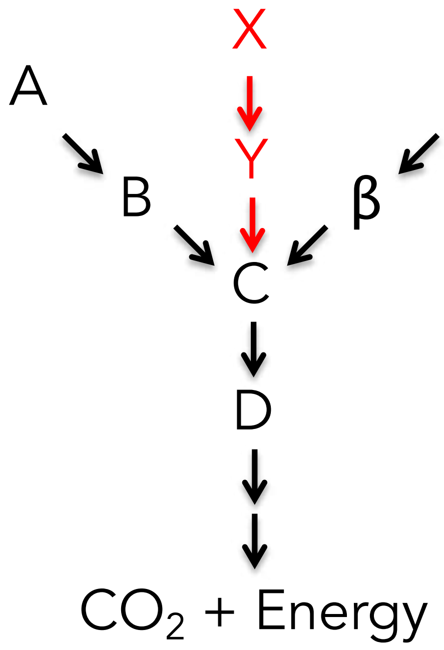


Evolution of microorganisms towards the capability to degrade xenobiotics:
Vertical expansion of metabolic pathways

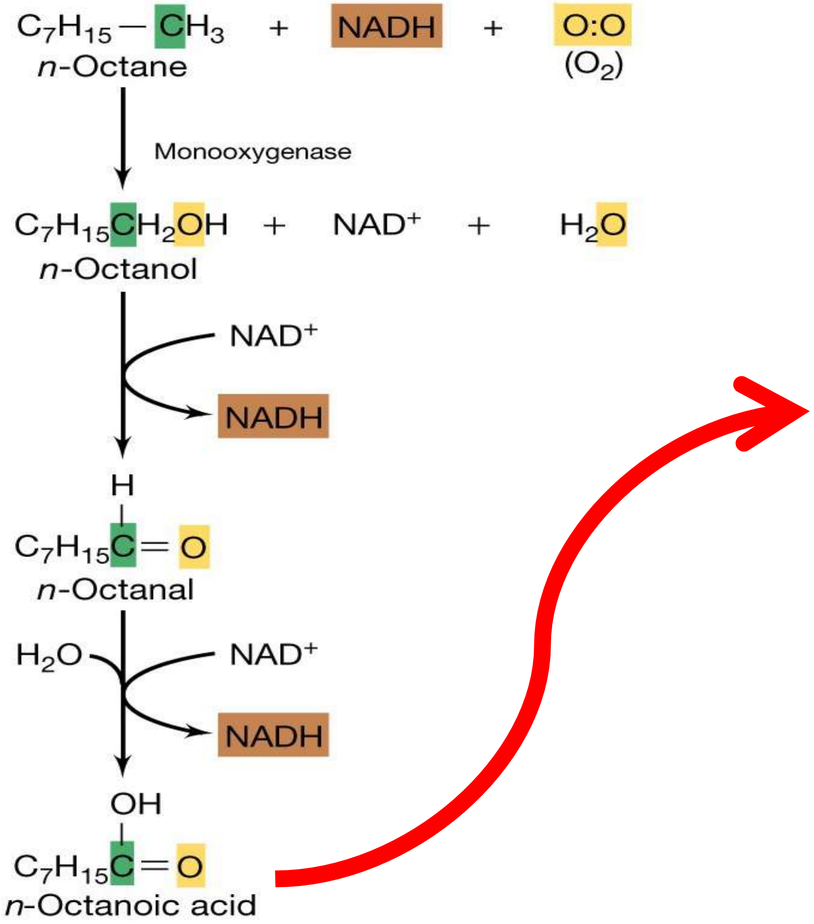
The vertical expansion of a metabolic pathway allows the microbe to transform an organic xenobiotic into a natural substrate or an intermediate of its metabolism. The latter can thus enter the central metabolic pathways (e.g., tricarboxylic acid cycle) through the existing catabolic routes.

The microbe becomes therefore able to catalyse a new sequence of reactions (catabolic pathway) upstream a metabolic pathway initially existing, which is thus expanded vertically.

The new molecule can be typically be biodegraded via direct metabolism (used as carbon and energy source) and often fully mineralized.

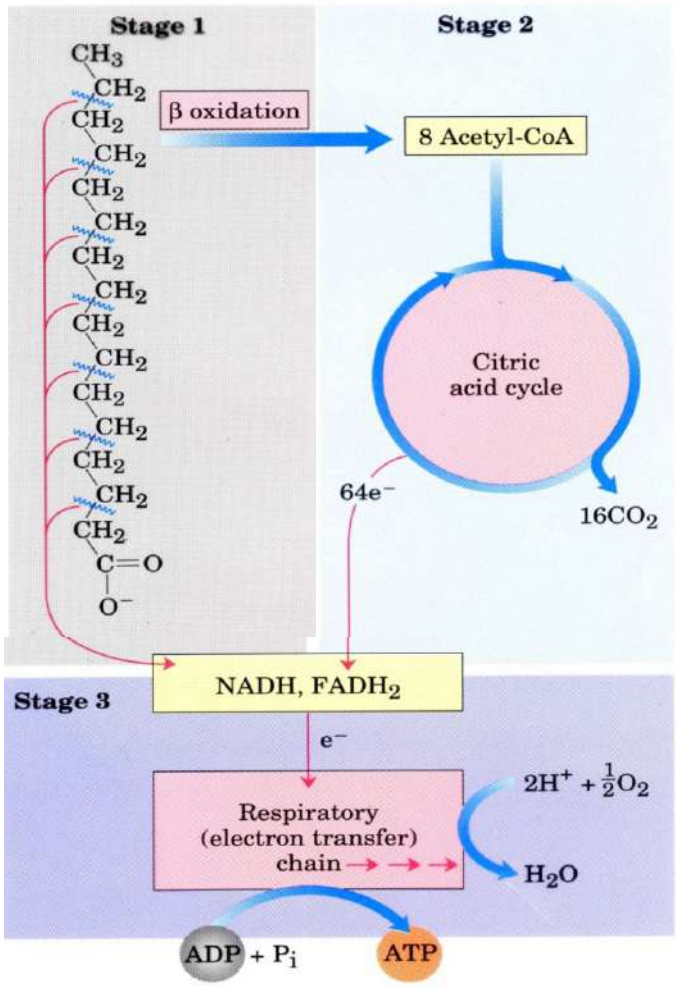


Example of vertical expansion of metabolic pathways: Aerobic metabolism of aliphatic hydrocarbons

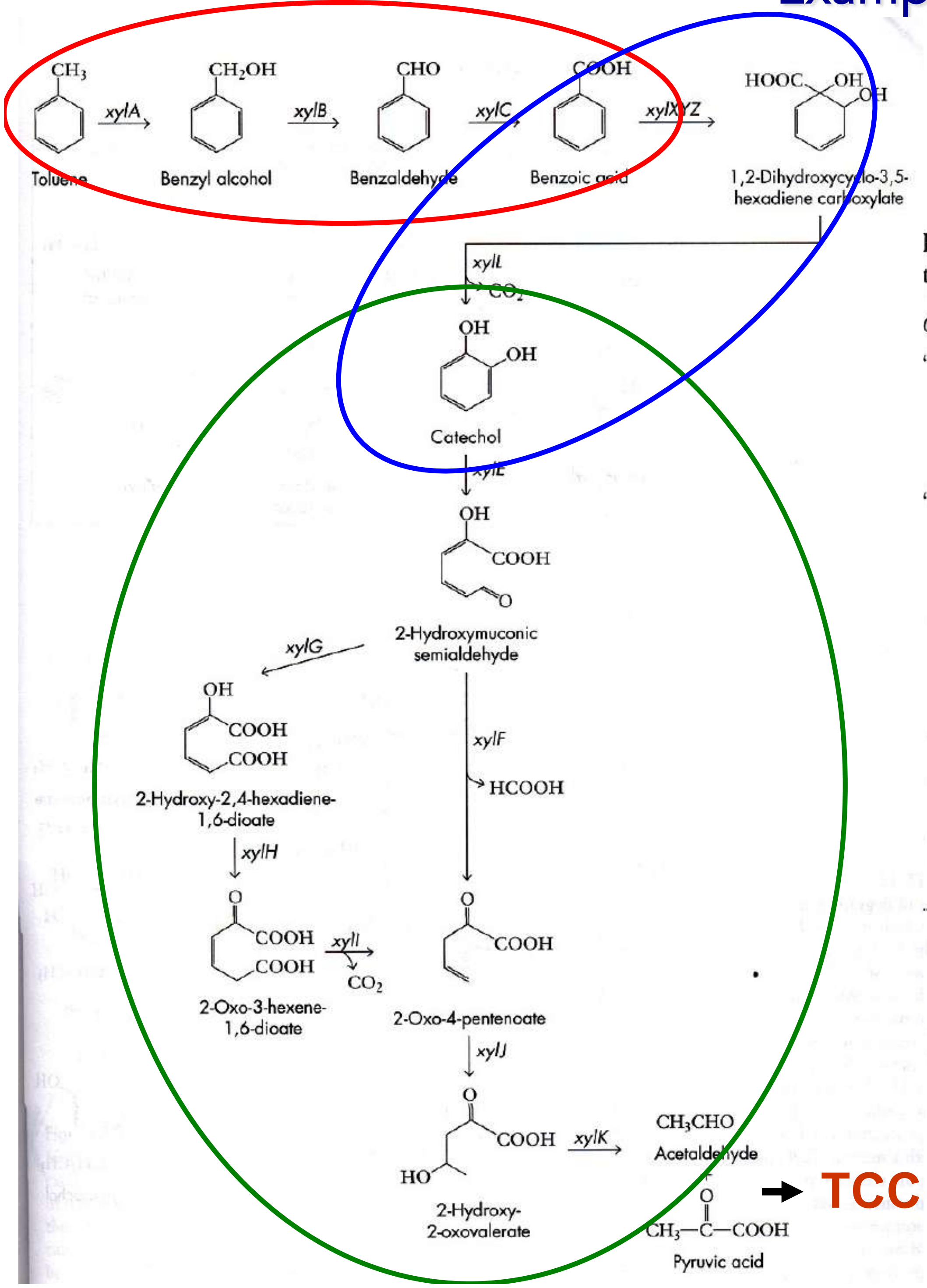


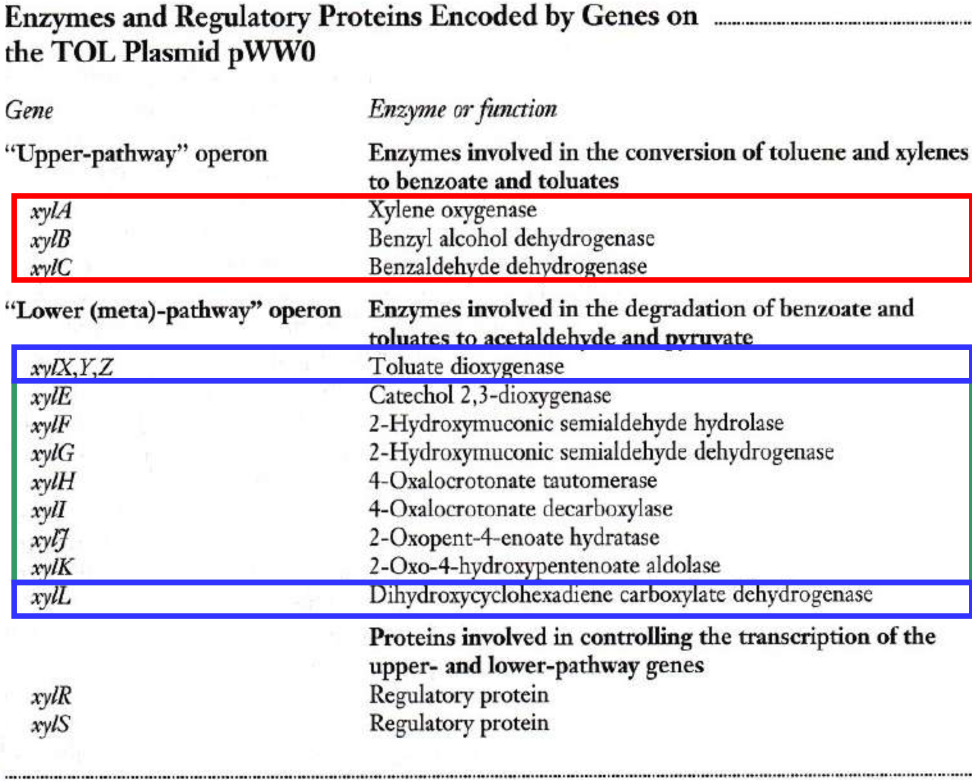
Biodegradation of aliphatic hydrocarbons:
vertical expansion of the **β**-oxidation pathway

Biodegradation of fatty acids (biomolecules)



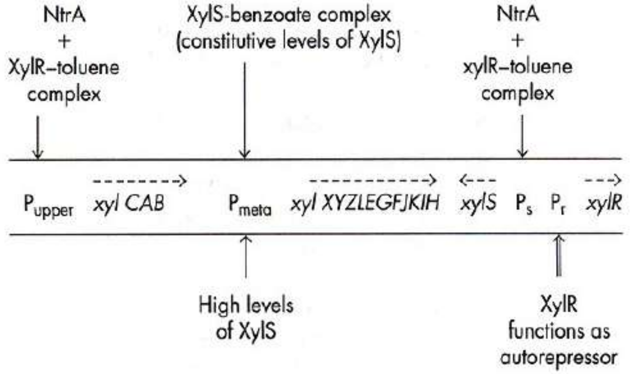
Example of vertical expansion of metabolic pathway: toluene biodegradation





Enzymes and Regulatory Proteins Encoded by Genes on the TOL Plasmid pWW0

Gene	*Enzyme or function*
"Upper-pathway" operon	**Enzymes involved in the conversion of toluene and xylenes to benzoate and toluates**
xylA	Xylene oxygenase
xylB	Benzyl alcohol dehydrogenase
xylC	Benzaldehyde dehydrogenase
"Lower (meta)-pathway" operon	**Enzymes involved in the degradation of benzoate and toluates to acetaldehyde and pyruvate**
xylX,Y,Z	Toluate dioxygenase
xylE	Catechol 2,3-dioxygenase
xylF	2-Hydroxymuconic semialdehyde hydrolase
xylG	2-Hydroxymuconic semialdehyde dehydrogenase
xylH	4-Oxalocrotonate tautomerase
xylI	4-Oxalocrotonate decarboxylase
xylJ	2-Oxopent-4-enoate hydratase
xylK	2-Oxo-4-hydroxypentenoate aldolase
xylL	Dihydroxycyclohexadiene carboxylate dehydrogenase
	Proteins involved in controlling the transcription of the upper- and lower-pathway genes
xylR	Regulatory protein
xylS	Regulatory protein

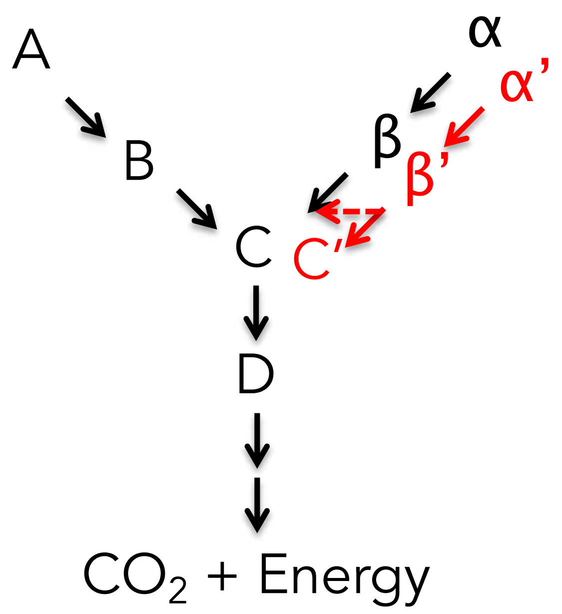


Evolution of microorganisms towards the capability to degrade xenobiotics:
Horizontal expansion of metabolic pathways

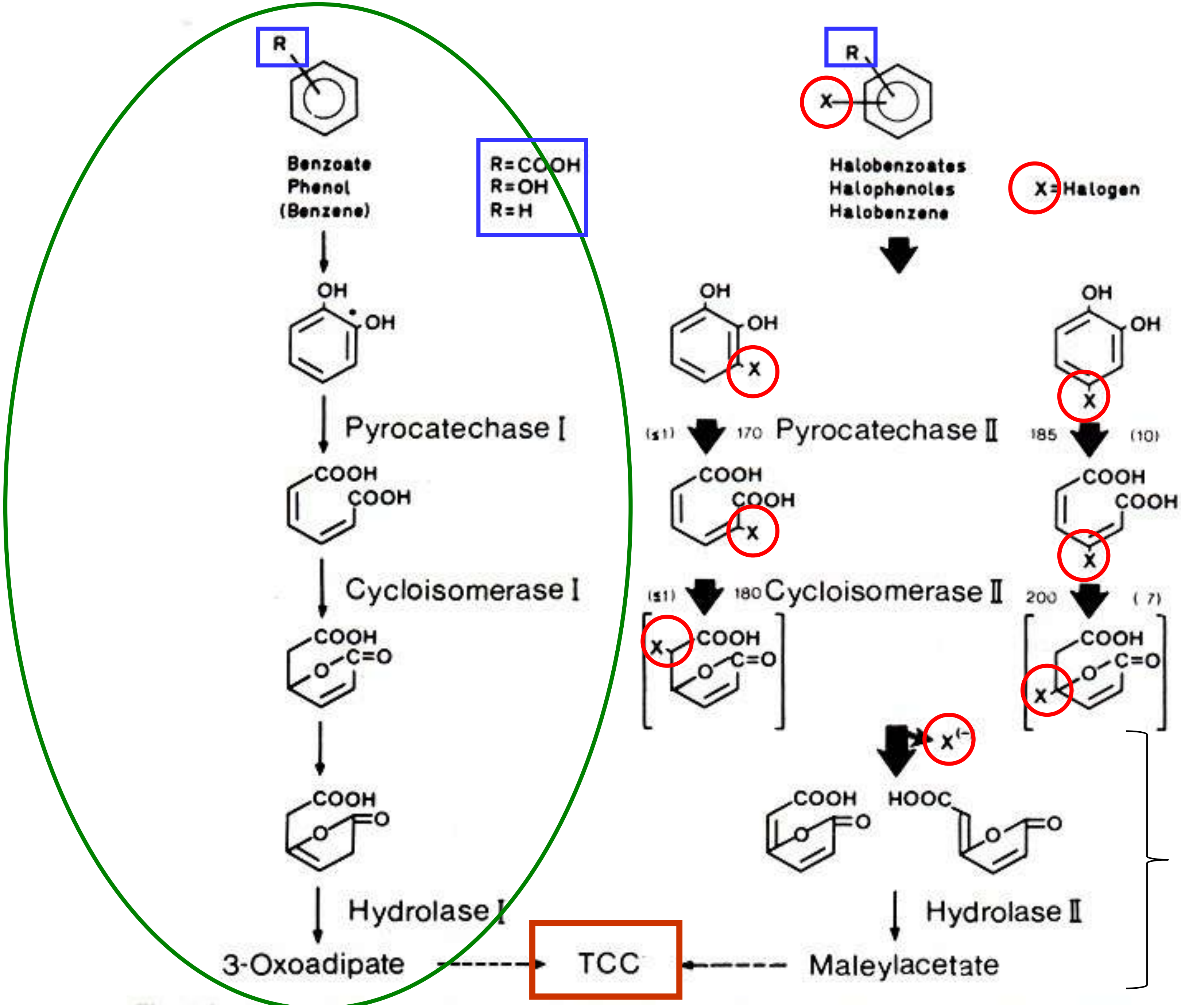
A horizontal expansion of a metabolic pathway allows the microbe to transform a new organic molecule (xenobiotic), which is structurally similar to one of its natural substrates, thanks to random mutations of existing enzymes involved in the substrate utilization.

The cell becomes therefore able to carry out the same sequence of reactions that was initially using on the natural substrate, also using the xenobiotic as substrate. The metabolic pathway initially present is thus expanded horizontally/duplicated, increasing the number of substrates that can be processed.

Very often, the new molecule is NOT transformed into an intermediate of the central metabolism, since the structural difference with the natural substrate is not eliminated. Thus it is typically processed through co-metabolism (the natural growth substrate must be present since required as energy and carbon source).



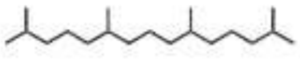
Example of horizontal expansion of metabolic pathways



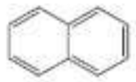
NOT always this step occurs

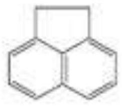
Aerobic and anaerobic biodegradation of aliphatic and aromatic hydrocarbons by bacteria.

$CH_3-CH_2-CH_2-(CH_2)_n-CH_2-CH_2-CH_3$

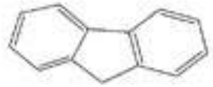

Pristane

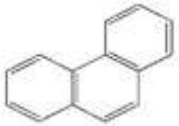

Phytane

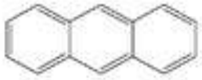

1. Naphthalene

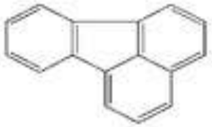

2. Acenaphthene


3. Acenaphthylene


4. Fluorene


5. Phenanthrene


6. Anthracene


7. Fluoranthene

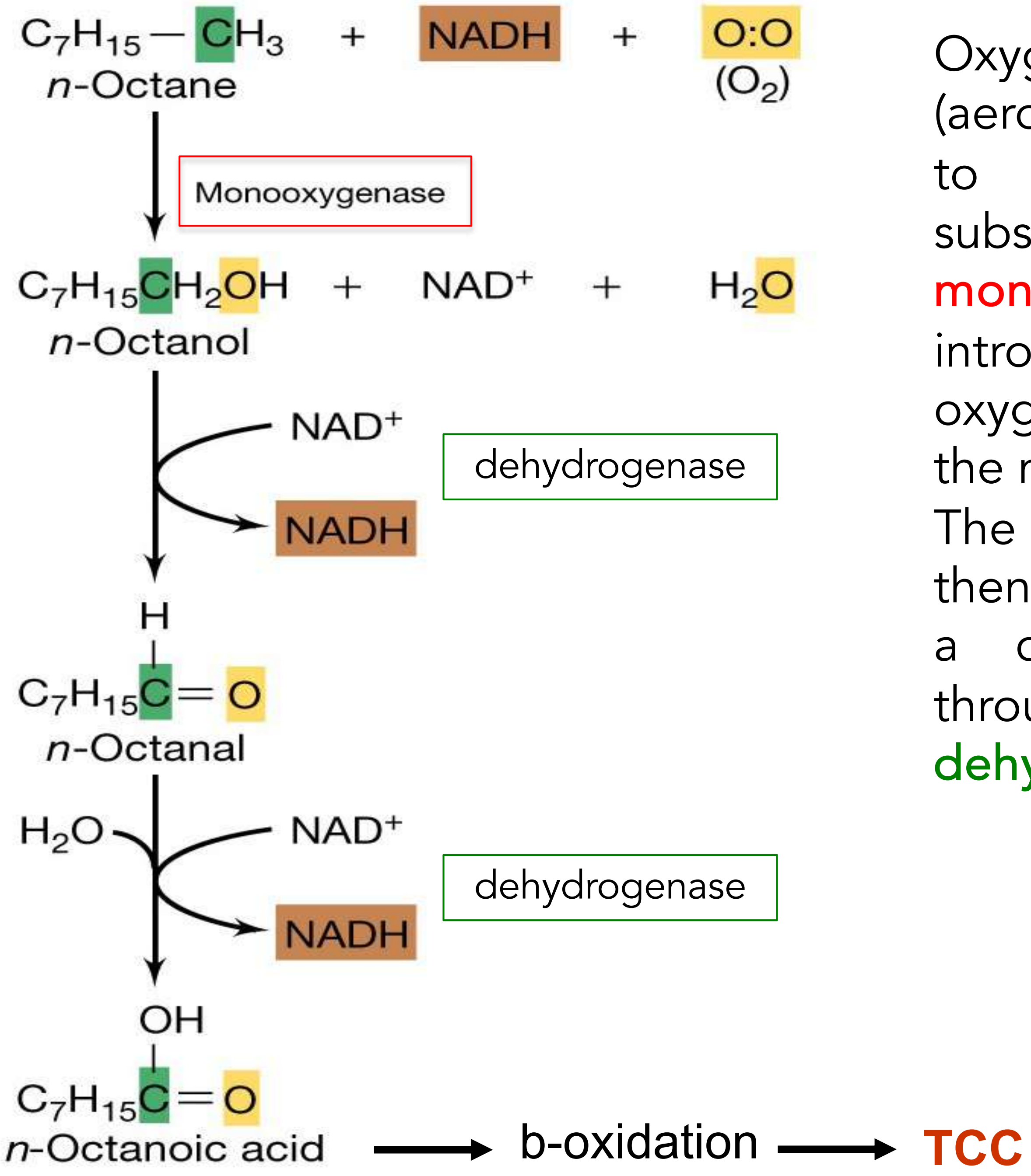

8. Pyrene

Several aliphatic and aromatic hydrocarbons can be biodegraded by aerobic bacteria through vertical expansions of metabolic pathways.

They are often biodegraded via direct metabolism (used as carbon and energy source) and fully mineralized to CO_2 and water.

→ biodegradation in contaminated environments occurs only if all other elements required by the degraders (O_2, sources of N and P, etc.) are present in sufficient amounts.

Aerobic biodegradation of aliphatic hydrocarbons (a)



$C_7H_{15}—CH_3$ + NADH + O:O (O_2)

n-Octane

Monooxygenase

$C_7H_{15}CH_2OH$ + NAD^+ + H_2O

n-Octanol

NAD^+ → NADH **dehydrogenase**

$C_7H_{15}C(H)=O$

n-Octanal

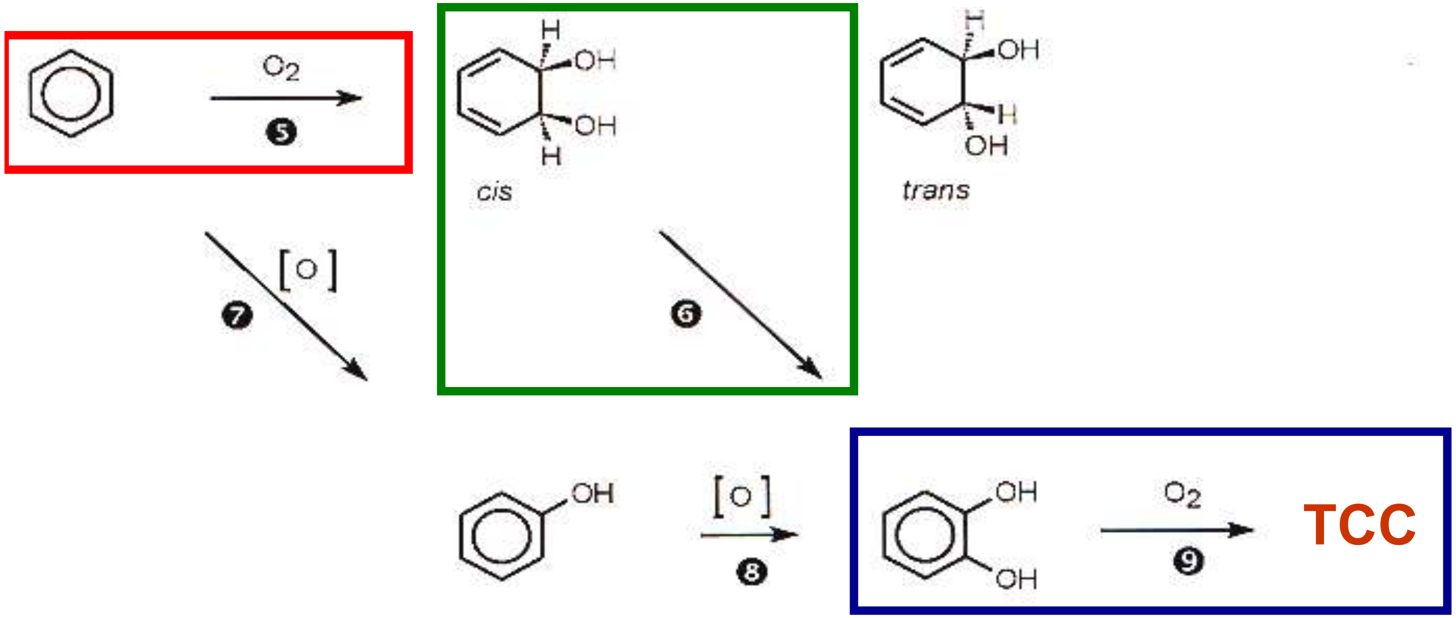
H_2O, NAD^+ → NADH **dehydrogenase**

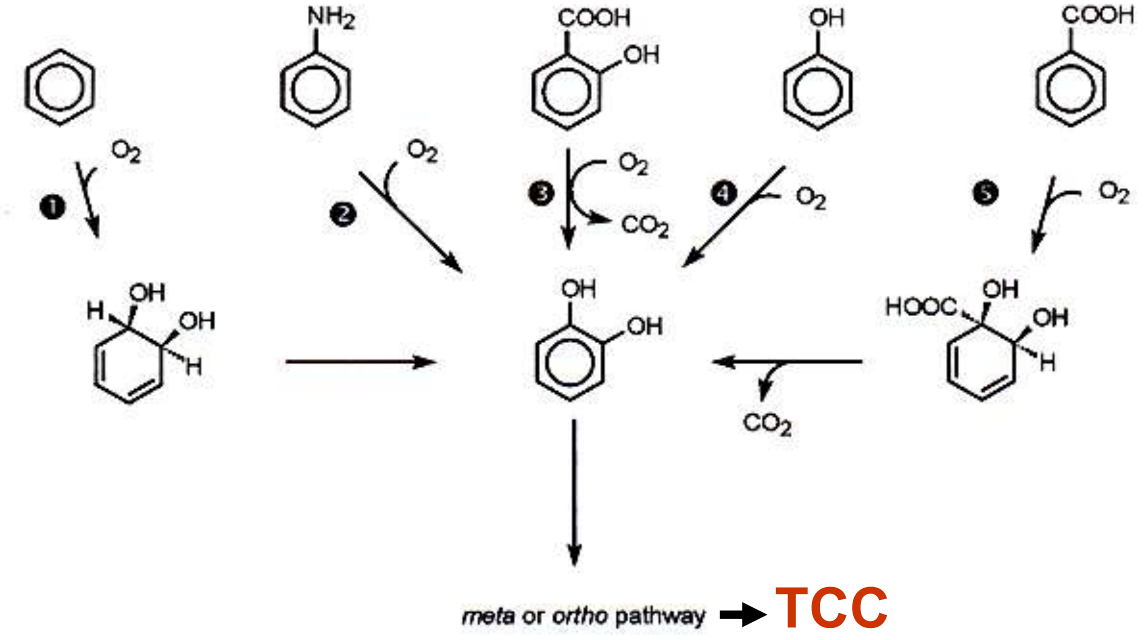
$C_7H_{15}C(OH)=O$

n-Octanoic acid → b-oxidation → **TCC**

Oxygen is used (aerobic conditions) to activate the substrate through a **monooxygenase**: introduction of 1 oxygen atom into the molecule.
The intermediate is then converted into a carboxylic adic through **dehydrogenases**.

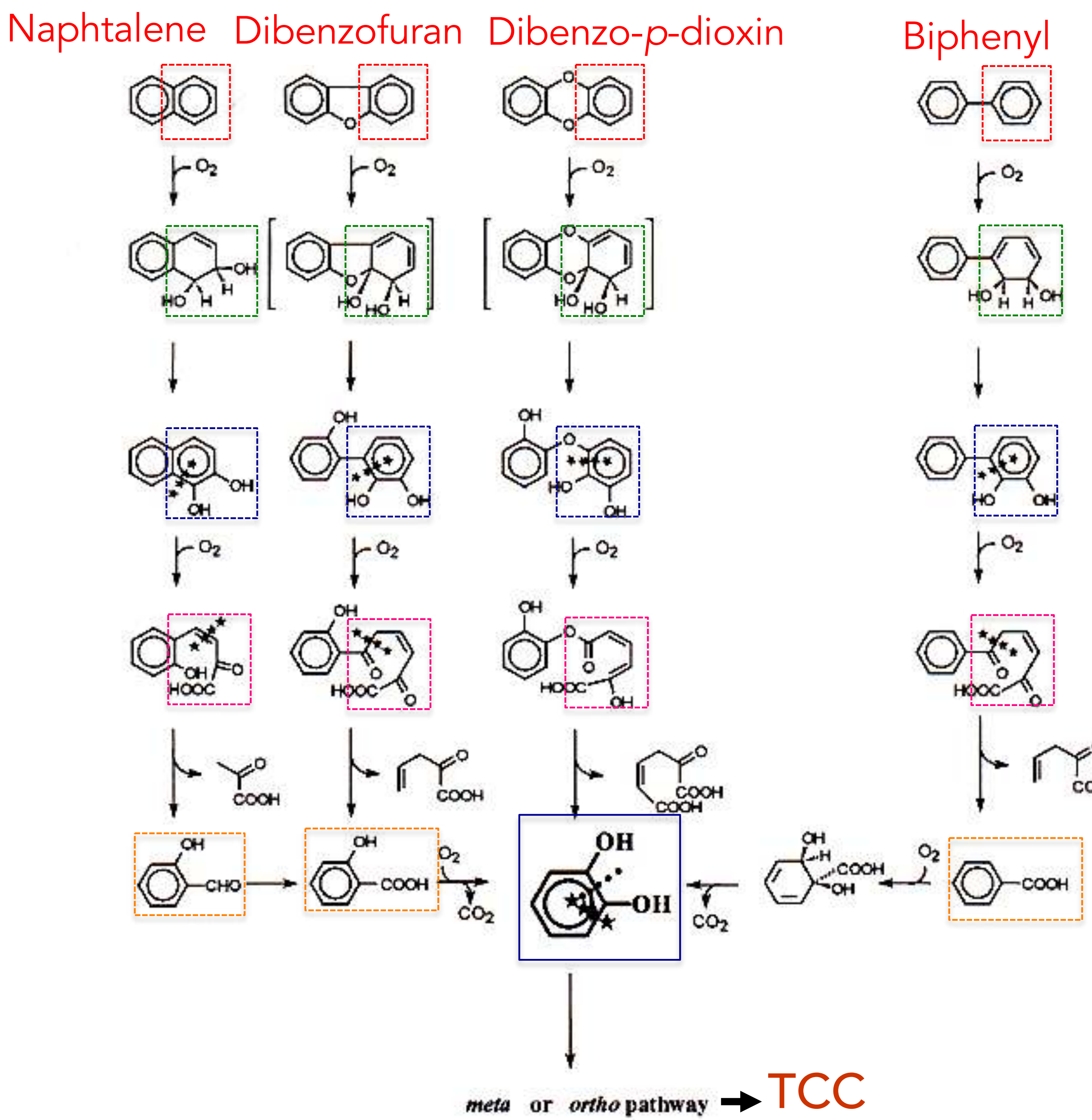
Aerobic biodegradation of aromatic hydrocarbons (a)





Oxygen is used (aerobic conditions) to activate the aromatic ring through a dioxygenase: introduction of 2 oxygen atoms into the molecule. A dehydrogenase converts then the intermediate into cathecol, which undergoes ring opening and degradation via the cathecol patway and TCC.

Aerobic biodegradation of aromatic hydrocarbons (b)



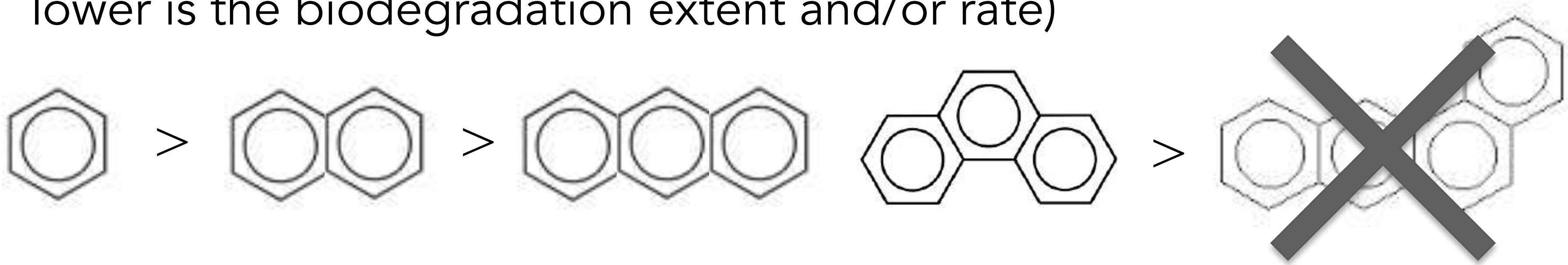
Reactions occurring sequentially on each aromatic ring

dioxygenase	activation
dehydrogenase or spontaneous	conversion to cathecol-like intermediate
meta cleaving dioxygenase	ring opening of the cathecol-like intermediate
hydrolase	degradation of the cathecol-like intermediate

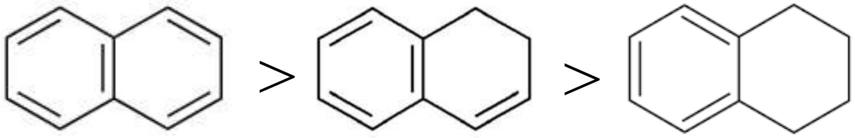
Mono-aromatic intermediate that undergoes conversion to cathecol

Main features affecting the aerobic biodegradation of hydrocarbons

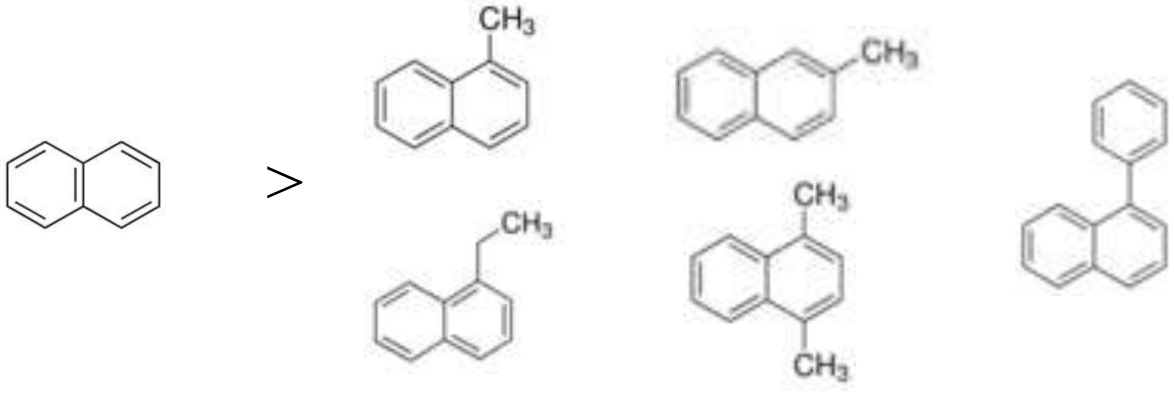
1) **Molecular weight** (MW)/number of fused rings (the higher it is, the lower is the biodegradation extent and/or rate)



2) **Degree of saturation** (the higher it is, the lower is the biodegradation extent and/or rate)

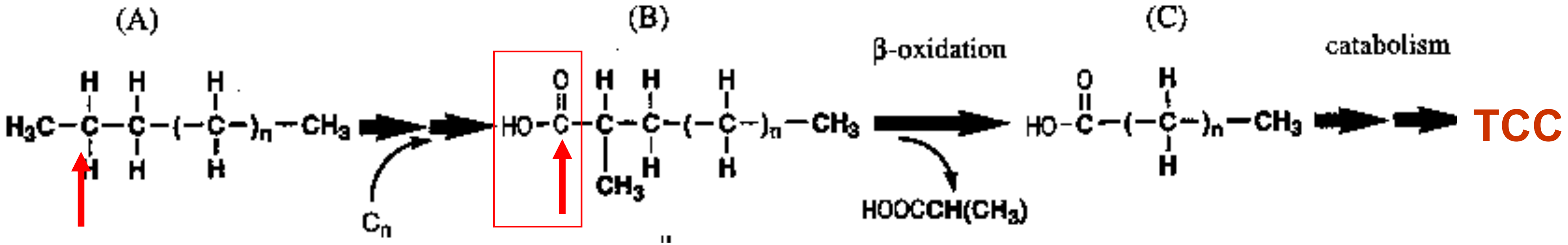


3) **Presence, number, type (alkyl, phenyl) and position of substituents**

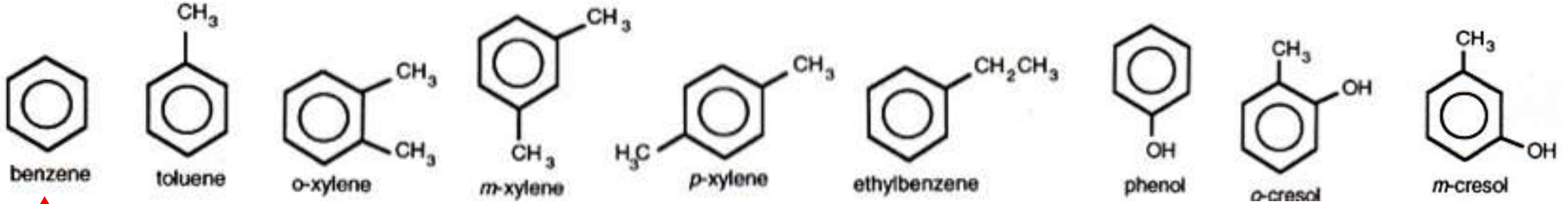


Anaerobic biodegradation of aliphatic and aromatic hydrocarbons (a)

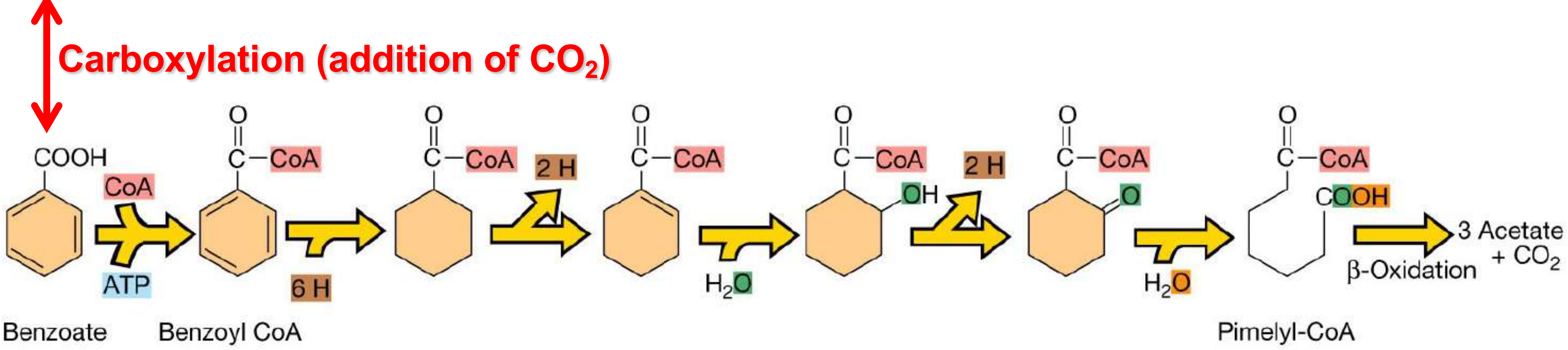
1) Activation via carboxylation (addition of CO_2)



Carboxylation (addition of CO_2)

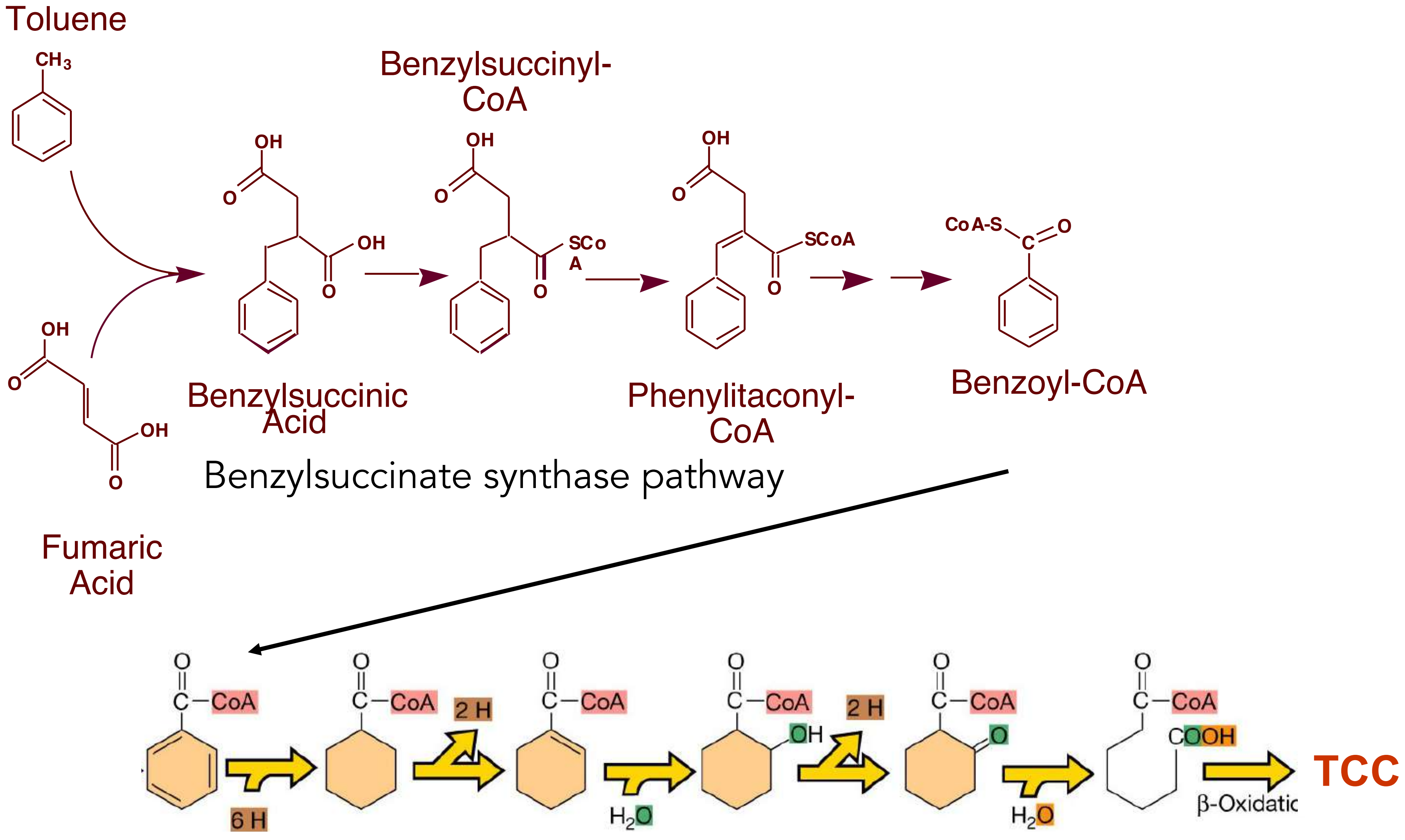


Carboxylation (addition of CO_2)



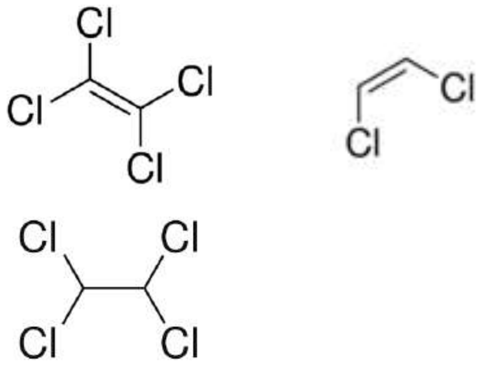
Anaerobic biodegradation of aliphatic and aromatic hydrocarbons (b)

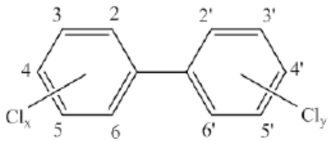
2) Activation via addition of fumaric acid



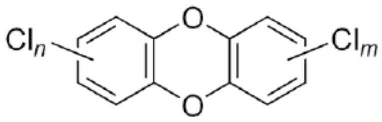
Benzylsuccinate synthase pathway

Aerobic biodegradation of chlorinated aliphatic and aromatic hydrocarbons by bacteria.





Polychlorinated biphenyls (PCBs)



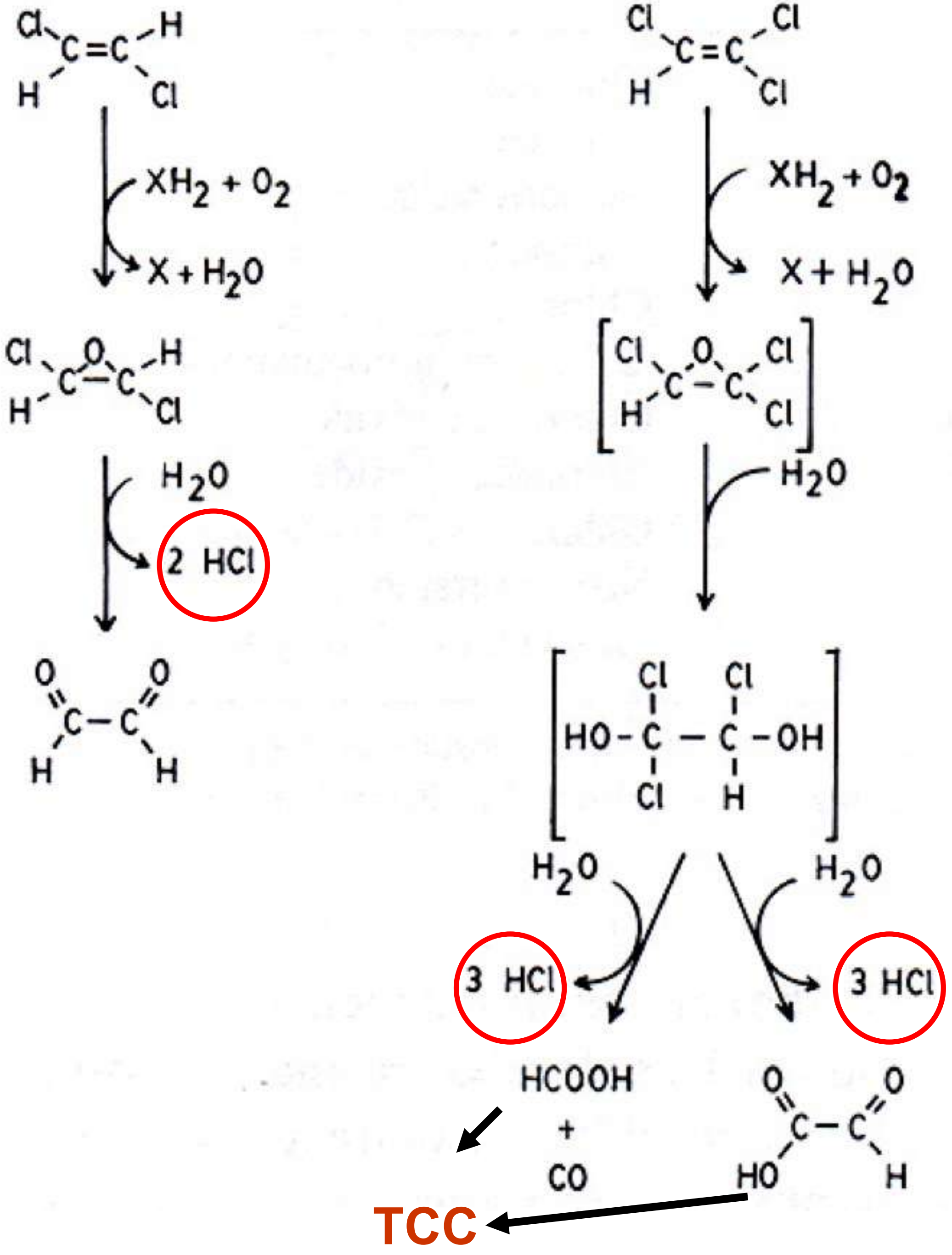
Polychlorinated dioxins (PCDDs)

Chlorinated aliphatic and aromatic hydrocarbons can be often biodegraded by aerobic bacteria through horizontal expansions of vertically expanded metabolic pathways.

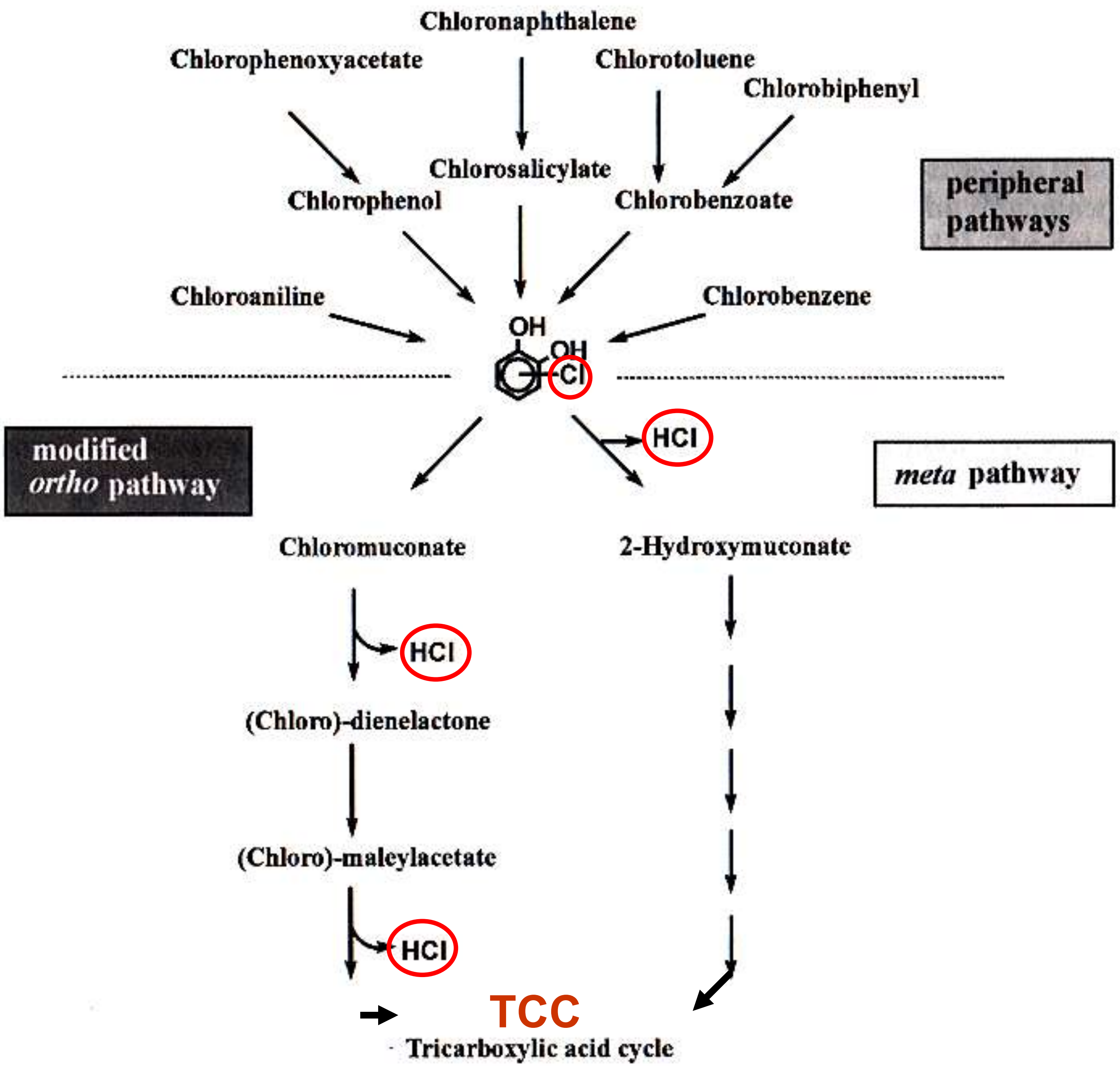
They are often biodegraded via co-metabolism (need of a growth substrate, along with sources of N, P, in sufficient amount); only some monochlorinated ones can be used as carbon and energy sources.

Since the removal of the chlorine substituents is generally fortuitous, low-chlorinated hydrocarbons can be typically biodegraded under aerobic conditions, while fully or highly chlorinated ones are not.

Aerobic biodegradation chloro-aliphatic hydrocarbons



Aerobic biodegradation chloro-aromatic hydrocarbons



Chloronaphthalene
Chlorophenoxyacetate
Chlorotoluene
Chlorobiphenyl
Chlorosalicylate
Chlorophenol
Chlorobenzoate

peripheral pathways

Chloroaniline
Chlorobenzene

OH
OH
Cl

HCl

modified *ortho* pathway

***meta* pathway**

Chloromuconate
2-Hydroxymuconate

HCl

(Chloro)-dienelactone

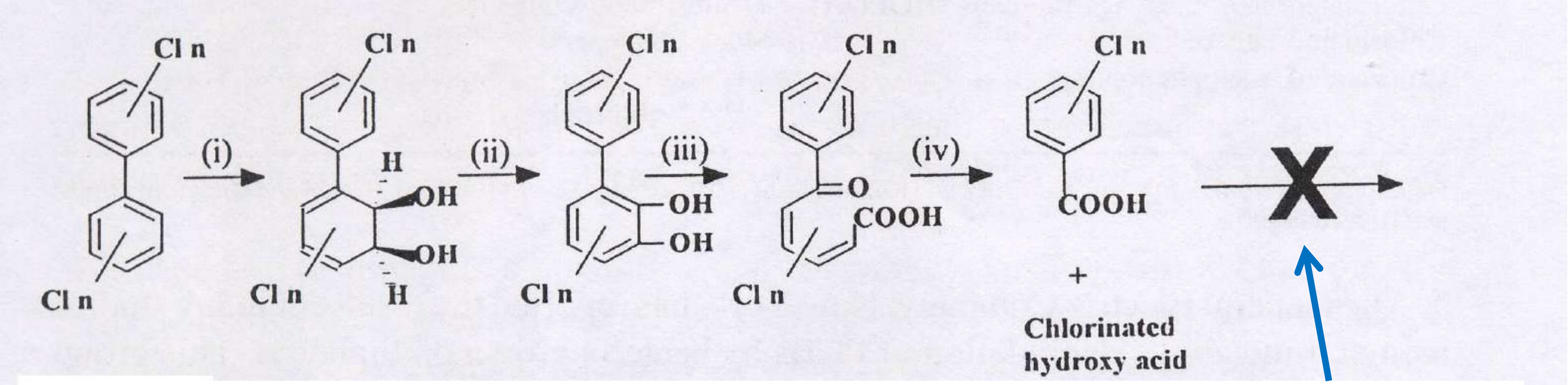
(Chloro)-maleylacetate

HCl

TCC
Tricarboxylic acid cycle

Aerobic biodegradation of polychlorinated biphenyls

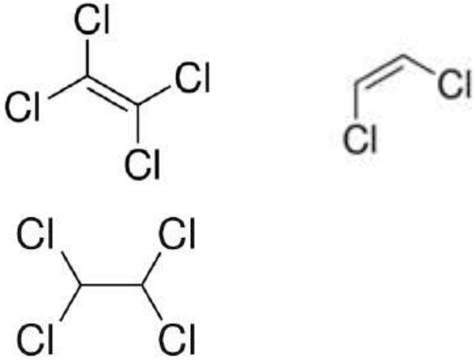
Low chlorinated PCBs (up to approx. 3-4 Cl per biphenyl molecule)

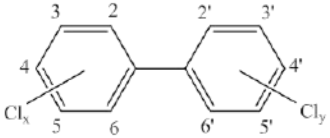


PCB degradation stops here, since horizontal expansion for chlorinated benzoic and chlorinated hydroxy acids has not happened in the same bacteria:

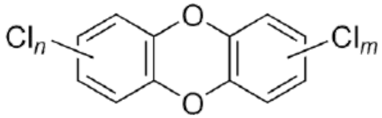
- CO-METABOLISM with biphenyl
- full biodegradation possible only by mixed microbial cultures having complementary biodegradation activities.

Anaerobic biodegradation of chlorinated aliphatic and aromatic hydrocarbons by bacteria



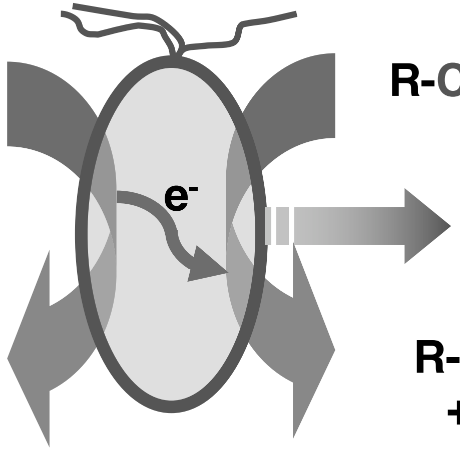


Polychlorinated biphenyls (PCBs)



Polychlorinated dioxins (PCDDs)

Under anaerobic conditions, highly halogenated aliphatic and aromatic hydrocarbons can undergo **dehalorespiration**: they are used as **final electron acceptors** of the anaerobic respiration and, while reduced, they are dehalogenated.



Electron Donor Reduced

R-Cl

Electron Acceptor Oxidized

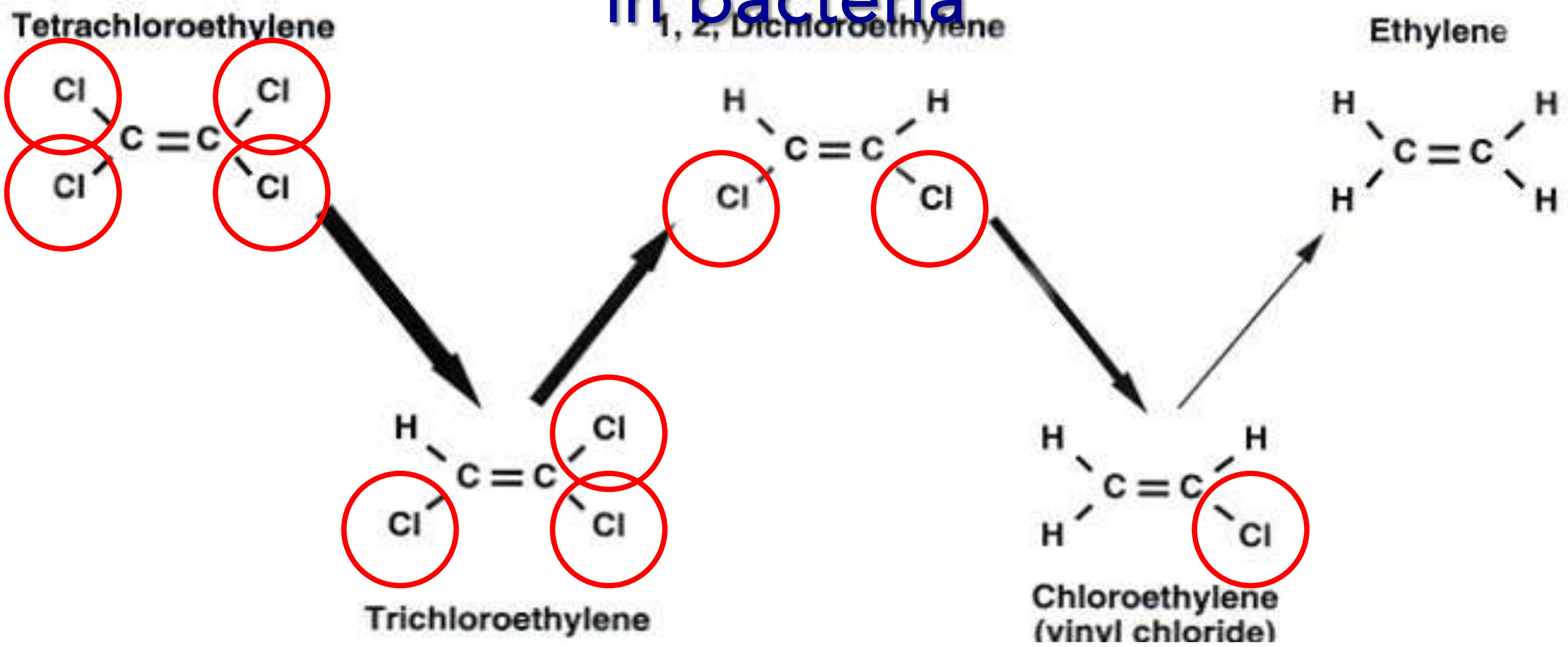
n ATP

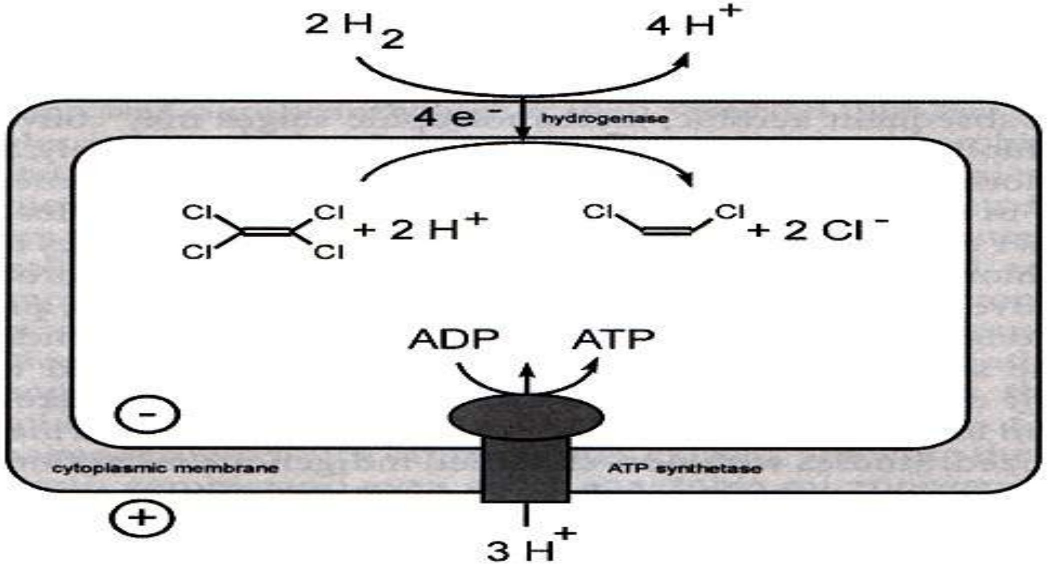
Electron Donor Oxidized

R-H + H^+ + Cl^-

Electron Acceptor Reduced

Anaerobic biodegradation pathways for chlorinated aliphatic and aromatic hydrocarbons in bacteria

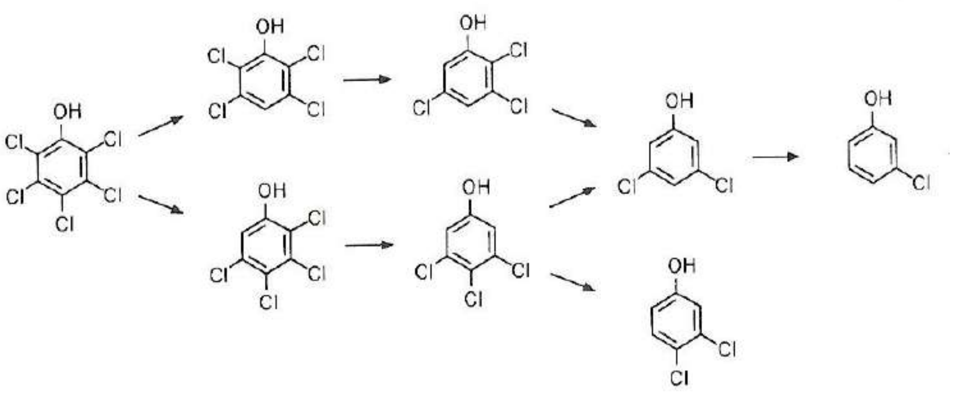


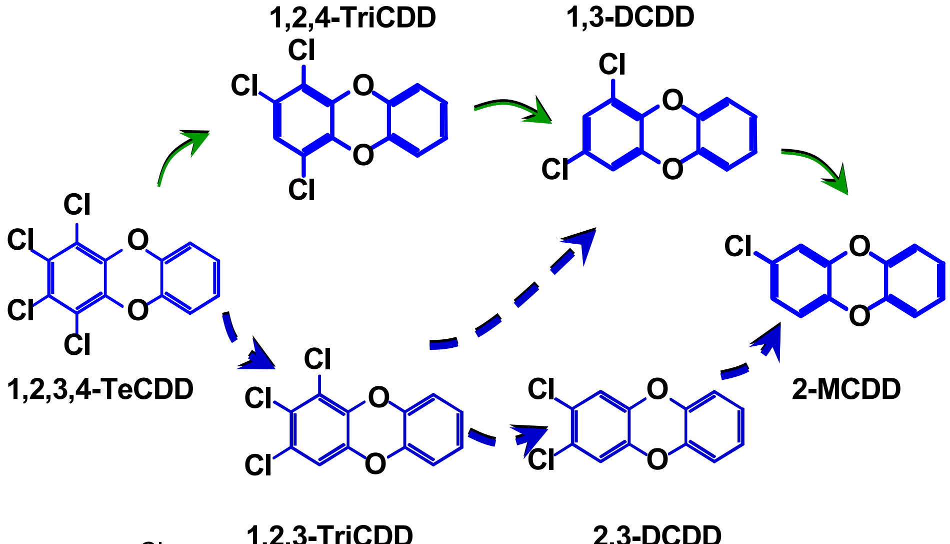


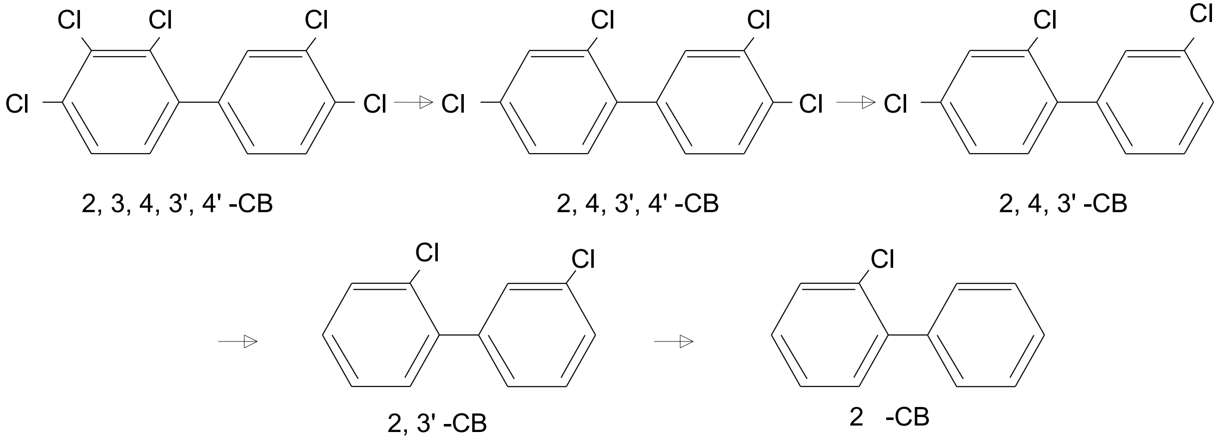
Dehalorespiration carried out by *Dehalococcoides* spp. *(mccartyi)*

Anaerobic biodegradation pathways for chlorinated aliphatic and aromatic hydrocarbons in bacteria

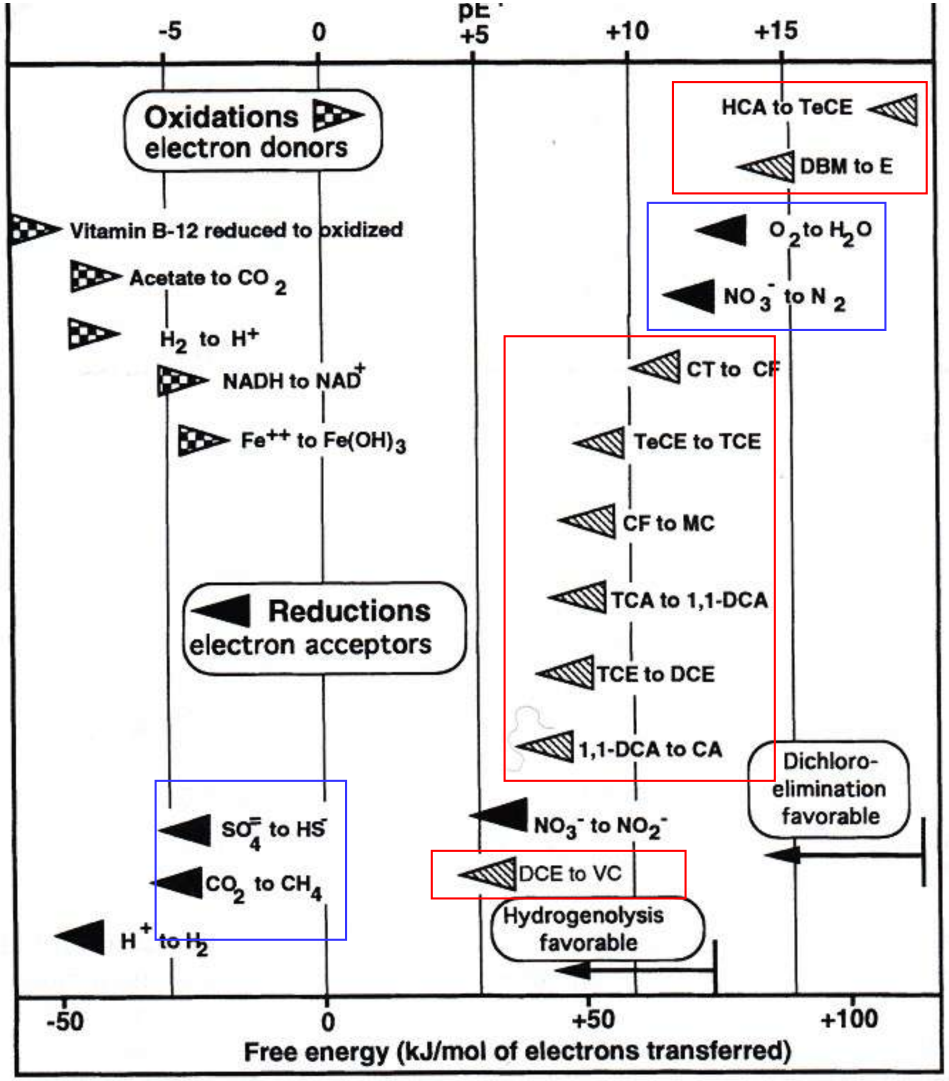
With few exceptions, dehalorespiration is active only towards medium-highly chlorinated compounds, which are therefore only partially dechlorinated and thus converted into low-chlorinated products.







Reduction potential decreases with chlorination degree

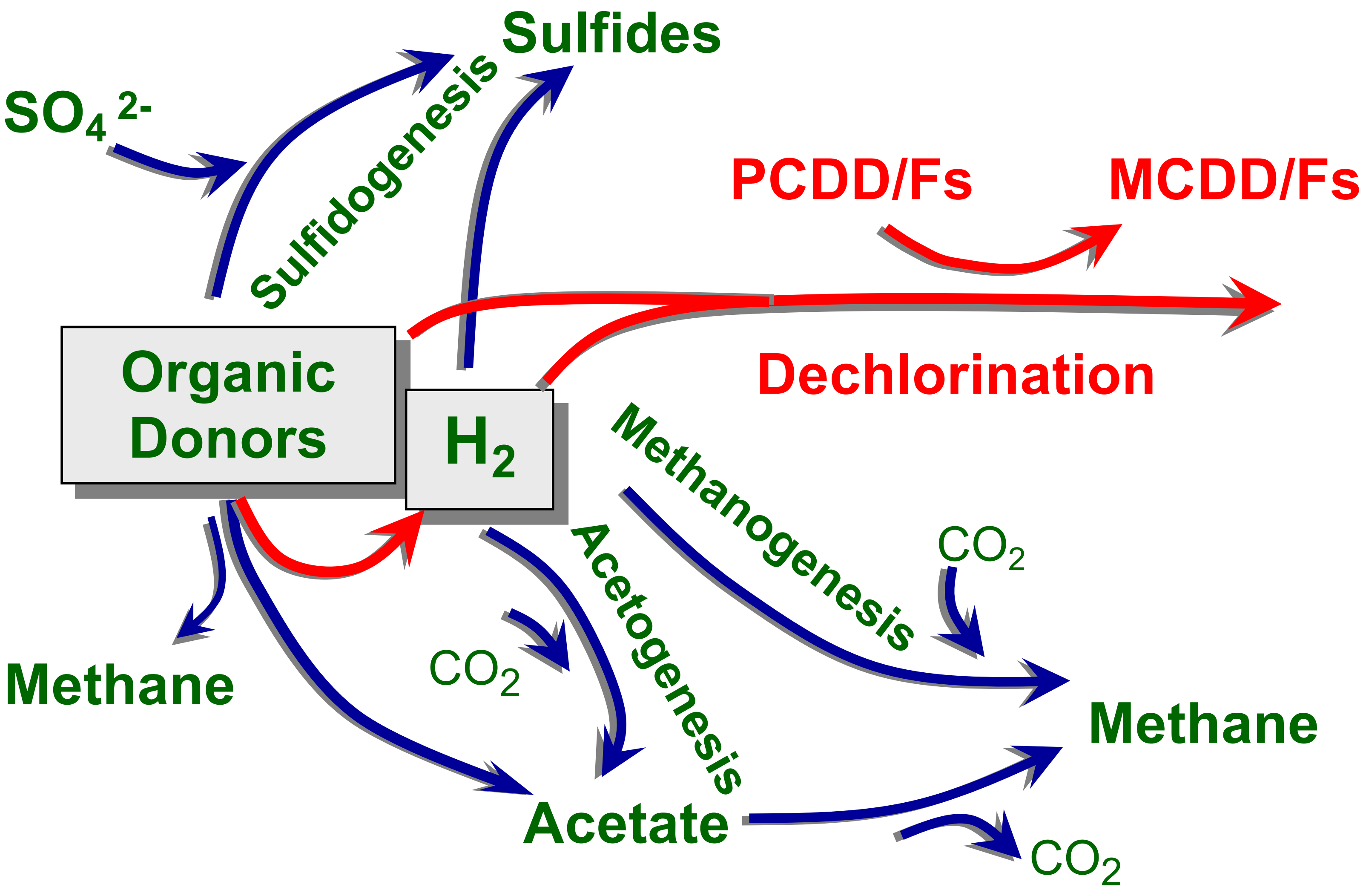


Bases of arrows align with the potentials of the half-reaction shownin volts.

COMPOUND	ABBREVIATION
METHANE	CH_4
CHLOROMETHANE	CM
DIBROMOMETHANE	DBM
DICHLOROMETHANE (METHYLENE CHLORIDE)	MC
TRICHLOROMETHANE (CHLOROFORM)	CF
TETRACHLOROMETHANE (CARBON TETRACHLORIDE)	CT
DICHLOROETHYLENE	DCE
TRICHLOROETHYLENE	TCE
TETRACHLOROETHENE (PERCHLOROETHYLENE)	TeCE
ETHANE	E
CHLOROETHANE	CA
1,2-DIBROMOETHANE	EDB
PENTACHLOROETHANE	PCA
HEXACHLOROETHANE	HCA
DICHLOROETHANE	DCA

Abbreviations used for chemical species

Dehalorespires compete for electron donors (H_2) with other anaerobic respirers



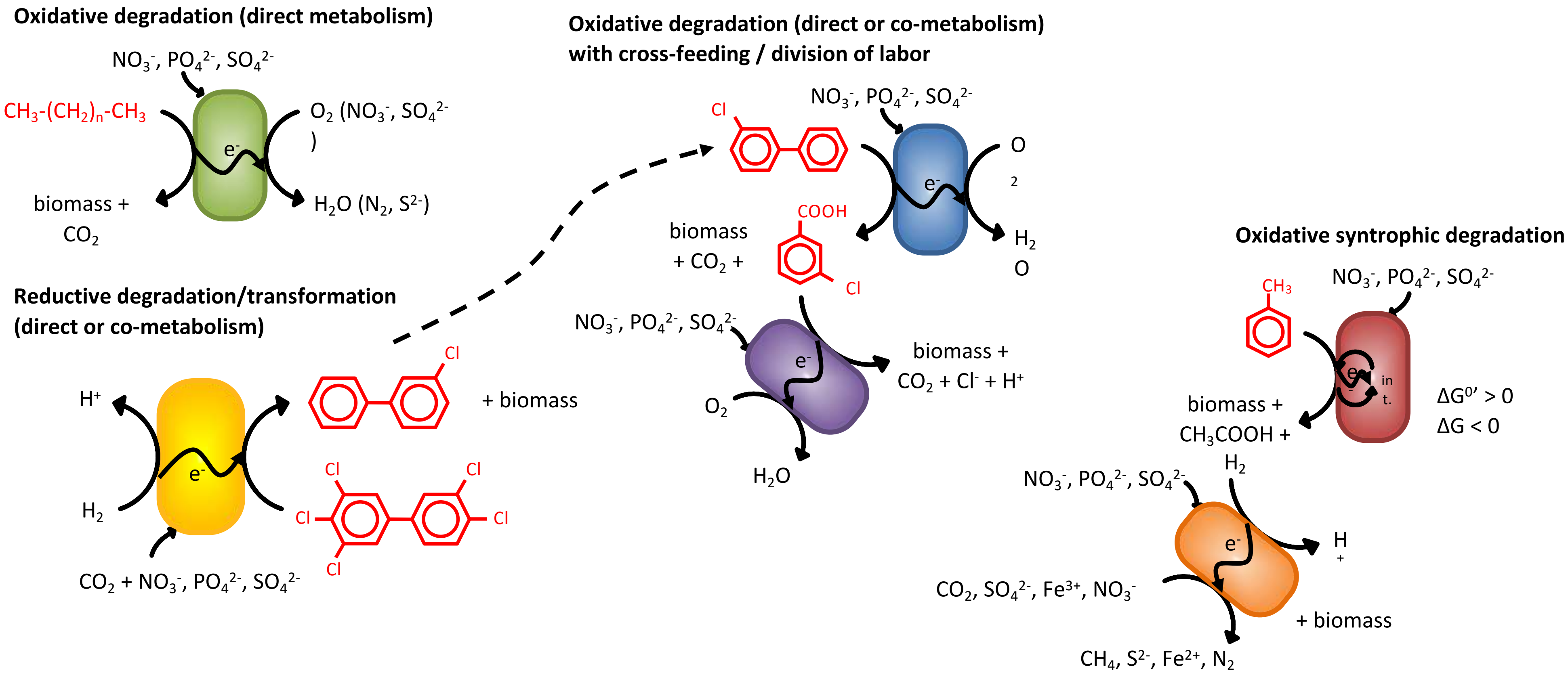
Competing electron flow pathways in anaerobic sediments

Affinity for H_2 determines predominant electron accepting processes

Electron Acceptor Process	Hydrogen Concentration (nM)
Aerobic (O_2) respiration	<0.1
Denitrification	<0.1
Iron(III) reduction	0.2 - 0.6
Dehalorespiration	< 0.31
Sulfate reduction	1 - 4
Methanogenesis	>5
Acetogenesis	>336

4. Examples of technologies/approaches for the enhancement of biodegradation processes (bioremediation) in marine environments

Microbial degradation of pollutants: single and multiple players involved



Oxidative degradation (direct metabolism)

NO_3^-, PO_4^{2-}, SO_4^{2-}

CH_3-$(CH_2)_n$-CH_3

e^-

O_2 (NO_3^-, SO_4^{2-})

biomass + CO_2

H_2O (N_2, S^{2-})

Reductive degradation/transformation (direct or co-metabolism)

H^+

e^-

H_2

CO_2 + NO_3^-, PO_4^{2-}, SO_4^{2-}

Cl

+ biomass

Cl Cl Cl Cl Cl

Oxidative degradation (direct or co-metabolism) with cross-feeding / division of labor

Cl

NO_3^-, PO_4^{2-}, SO_4^{2-}

e^-

O_2

biomass + CO_2 +

COOH

Cl

H_2O

NO_3^-, PO_4^{2-}, SO_4^{2-}

e^-

O_2

biomass + CO_2 + Cl^- + H^+

H_2O

Oxidative syntrophic degradation

CH_3

NO_3^-, PO_4^{2-}, SO_4^{2-}

e^-

int.

$\Delta G^{0\prime} > 0$

$\Delta G < 0$

biomass + CH_3COOH + H_2

NO_3^-, PO_4^{2-}, SO_4^{2-}

e^-

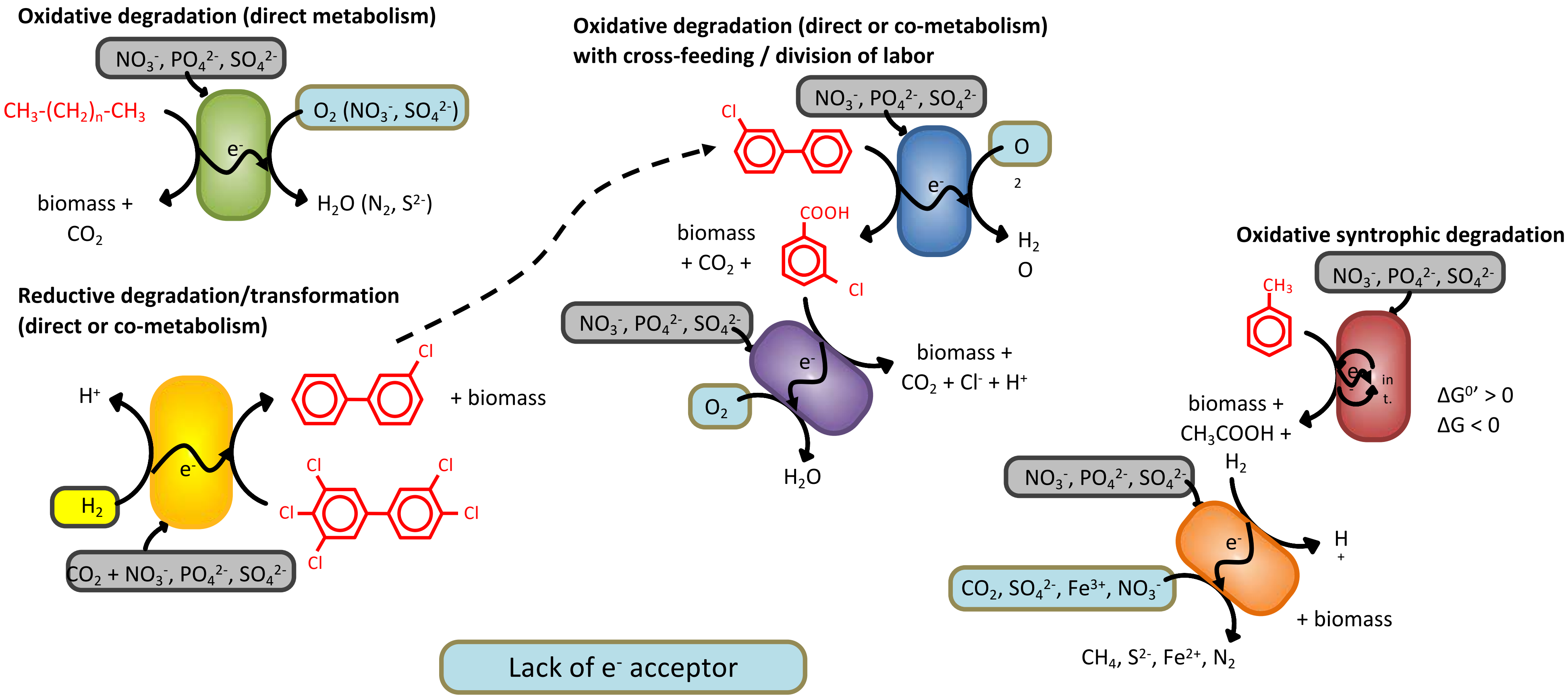
H^+

CO_2, SO_4^{2-}, Fe^{3+}, NO_3^-

+ biomass

CH_4, S^{2-}, Fe^{2+}, N_2

Main microbiological factors limiting biodegradation in contaminated environments addressed by bioremediation approaches



Lack of e- acceptor

Lack of nutrients

Lack of e- donor

Lack of suitable redox, pH, environmental conditions

Marine oil spills: a serious problem

The need for action

- Marine shorelines are important public and ecological resources
- Oil spills have posed great threats and caused extensive damage to the marine coastal environments
- Also to marine mammals, a significant reduction in population of many intertidal and subtidal organisms, and many long term adverse environmental effects.

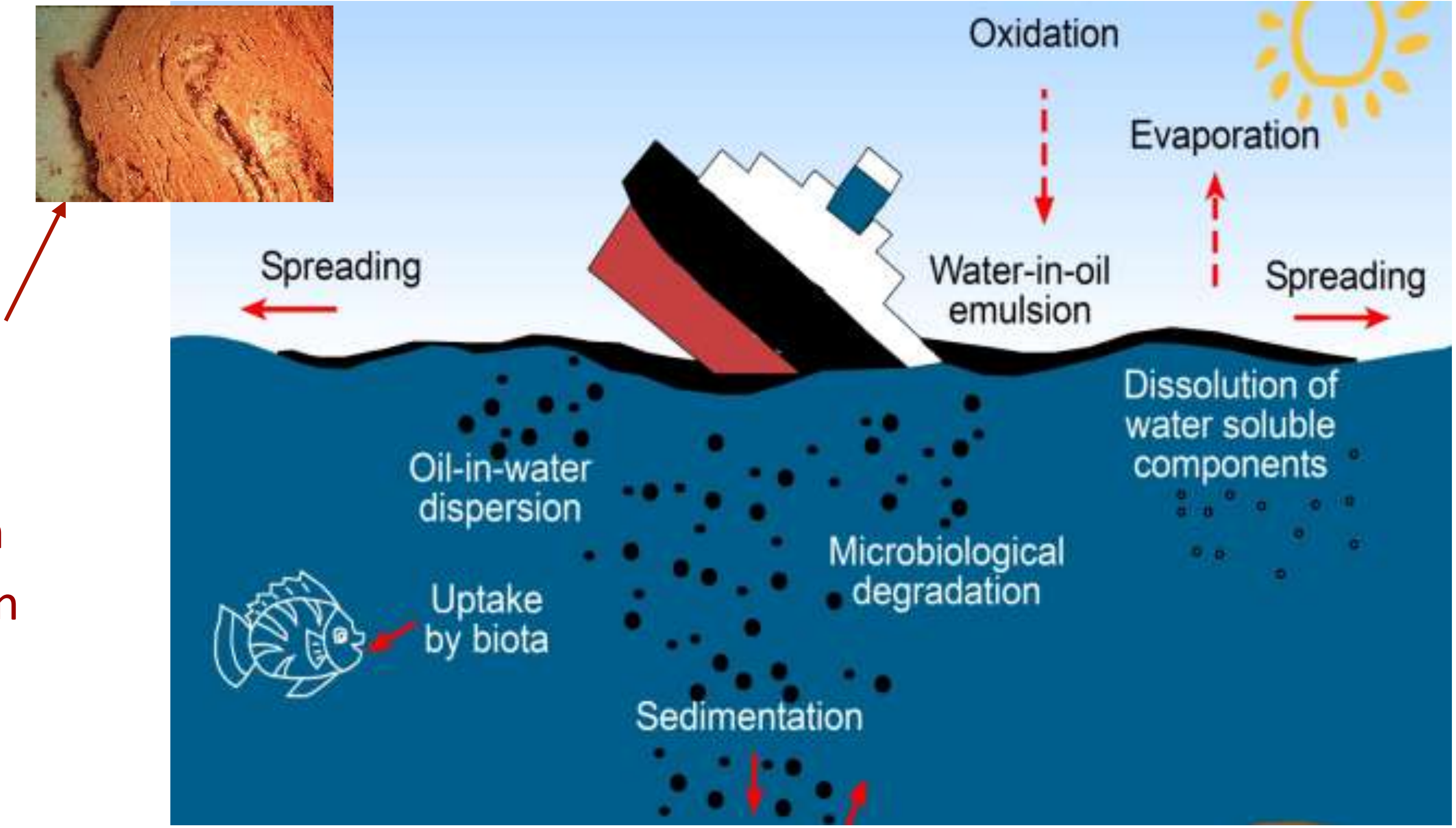
Marine oil spills: the issue

- **Oil spills remain** one of the most **serious risks** for the **oil and shipping industries** as the environment and livelihoods can be significantly affected in the event of a major incident.
- **Accidents still happen**. Although large spills from tankers and oil industry operations **have become less frequent** in the last few decades.
- Initial focus must be on **prevention**; however, the oil industry & governments also give **high priority to developing capability to respond to spills**.
- When oil spills do happen, **prompt action** minimizes the impact.

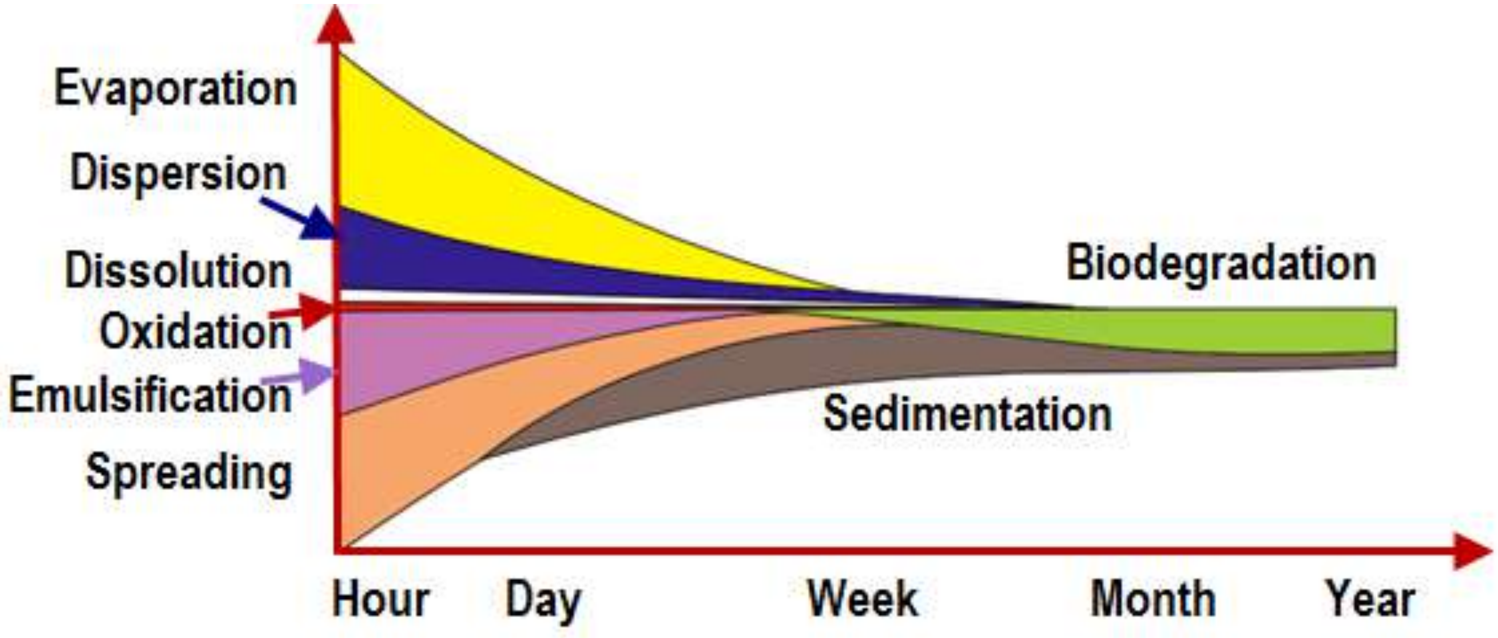
Fate of marine oil spills

Oil, when spilled at sea, will normally break up and be dissipated or scattered into the marine environment over time. This dissipation is a result of a number of chemical, physical and biological processes that change the compounds that make up oil when it is spilled. The processes are collectively known as **weathering**.

1. Spreading
2. Evaporation
3. Dispersion
4. Emulsification
5. Dissolution
6. Oxidation
7. Sedimentation
8. Biodegradation



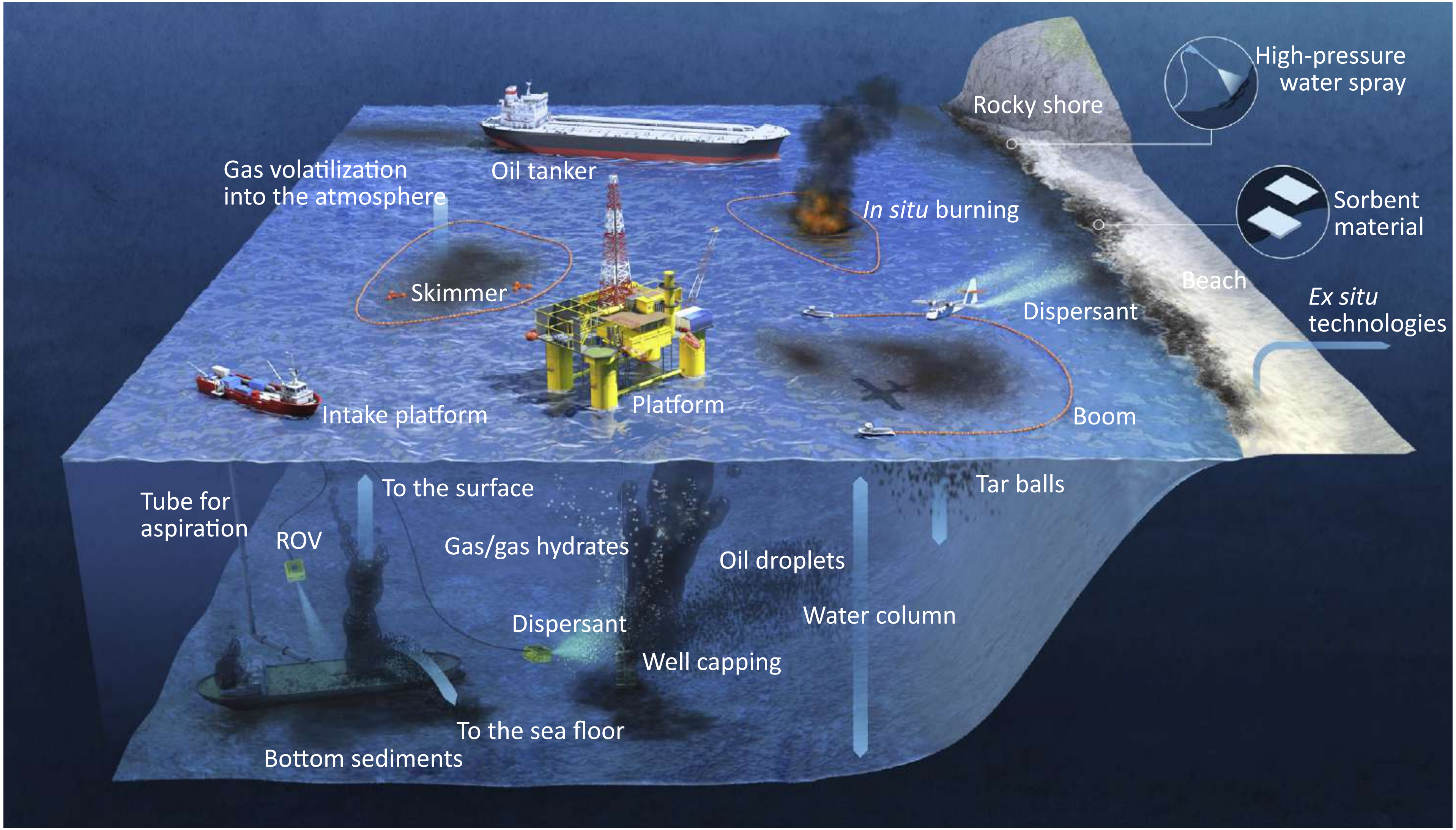
Weathering processes and periods of activation



- Bioremediation, the only process that can actually restores the ecosystem to its prior state, starts at a later time (it takes at least 1 week for the hydrocarbon degraders to increase their concentration)

PROMPT ACTIONS MINIMIZE THE IMPACT!

Oil spill responses and remediation options



Emergency response options

Current techniques are classified as:

- ☐ **Physical** (booms, skimmers and adsorbent materials)
- ☐ **Chemical** (dispersants and solidifiers)
- ☐ **Thermal** (in situ burning)
- ☐ **Biological** (bioremediation: intrinsic vs. enhanced)
- ☐ **Natural attenuation** (open seas with high energy waves – monitoring only)

Wave action on spilled oil

Physical options in emergency response to oils spills

- Containment and removal with **booms** and **skimmers**

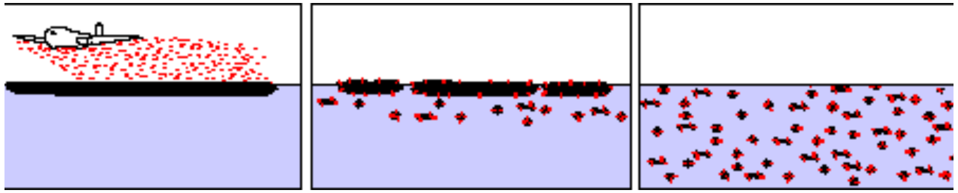
Physical options in emergency response to oils spills

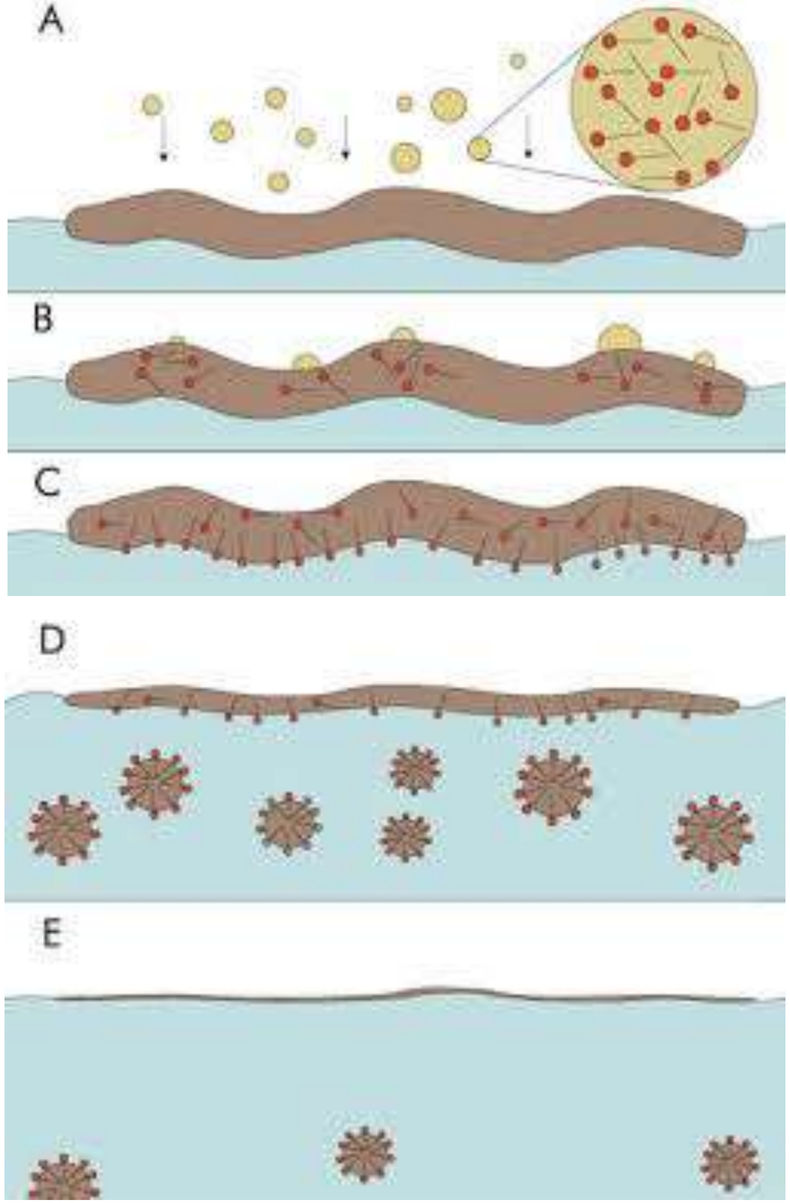
- Containment and removal with **booms**, **skimmers** and **pads**


KOMARA 20

Only about 10% (max 15%) of spilled oil is recovered in a successful operation (only 3% was recovered in the DWH incident)

Chemical options in emergency response to oils spills:
chemical dispersants (surfactants and solvents)





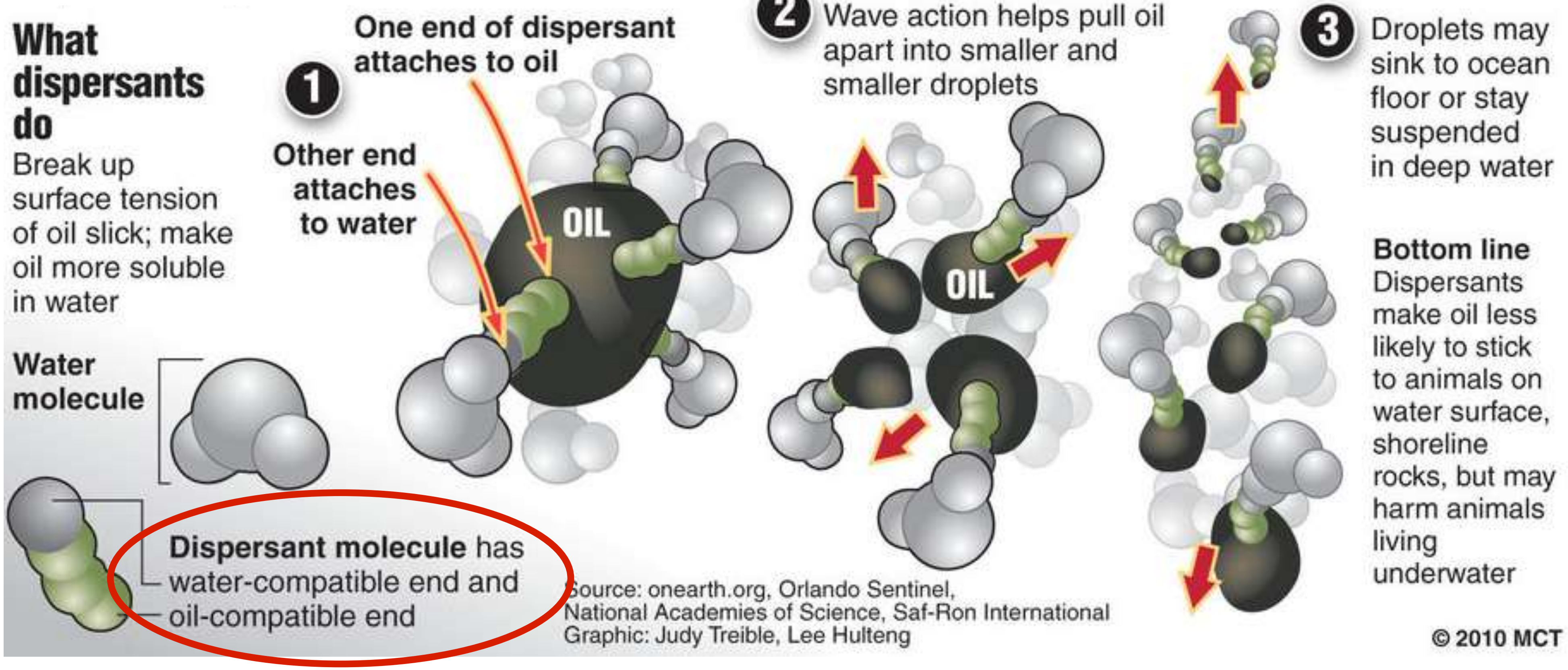
Goal: to remove the oil from the surface (transfer in the water column)

…however, the water column and the sediment may become more and more toxic (not only because of oil hydrocarbons but also due to dispersant.

Oil dispersion might enhance biodegradation (higher surface), but dispersant should be non toxic and biodegradable

Dispersants: mode of action

Dispersants are amphiphilic compounds acting on an oil droplet:



What dispersants do

Break up surface tension of oil slick; make oil more soluble in water

Water molecule

Dispersant molecule has water-compatible end and oil-compatible end

1 **One end of dispersant attaches to oil**

Other end attaches to water

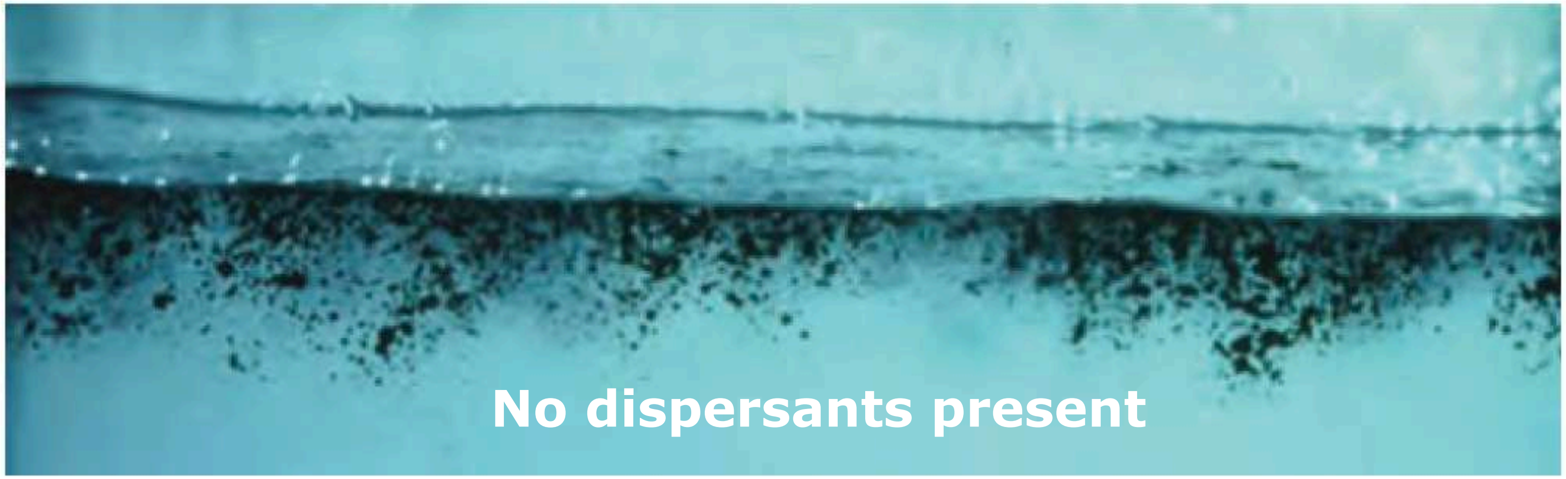
2 Wave action helps pull oil apart into smaller and smaller droplets

3 Droplets may sink to ocean floor or stay suspended in deep water

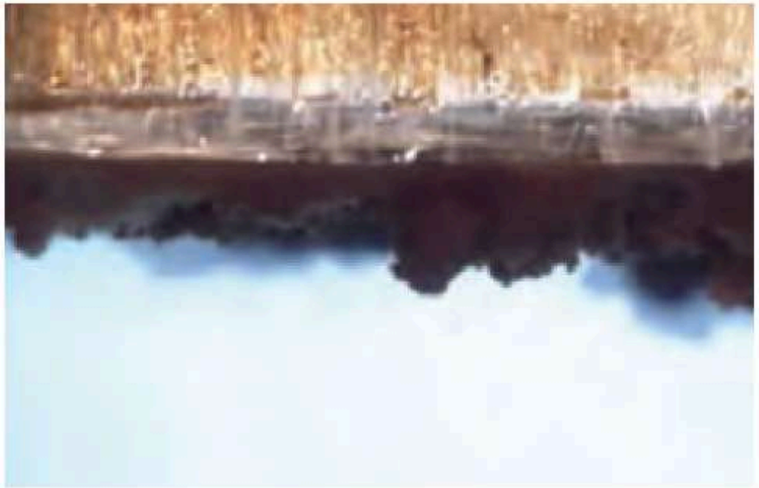
Bottom line
Dispersants make oil less likely to stick to animals on water surface, shoreline rocks, but may harm animals living underwater

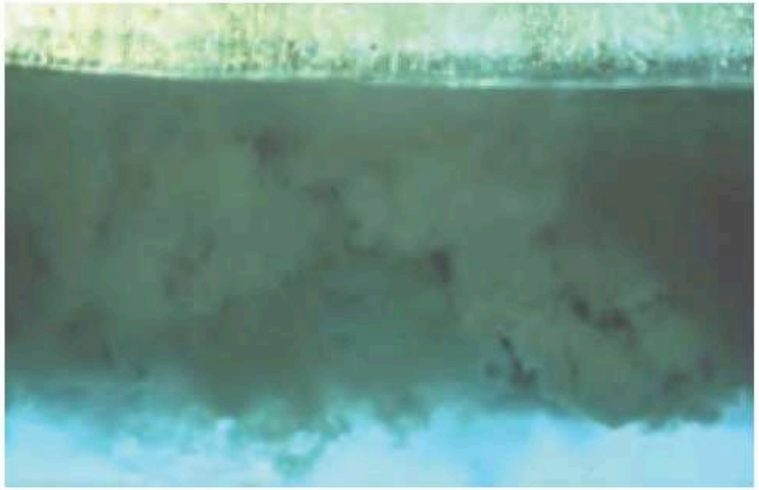
Source: onearth.org, Orlando Sentinel, National Academies of Science, Saf-Ron International
Graphic: Judy Treible, Lee Hulteng

Dispersants: mode of action – visual observation (lab scale)



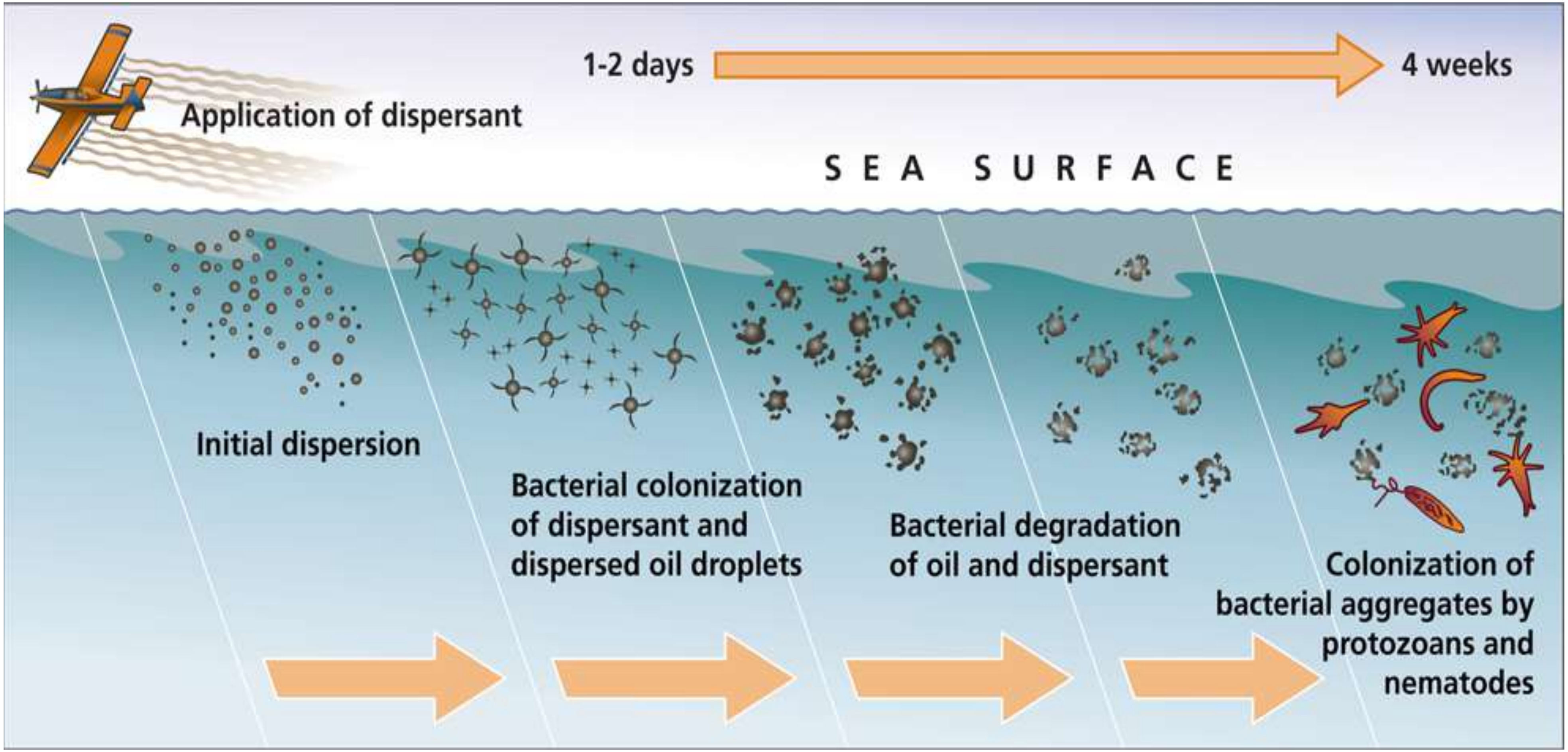
No dispersants present





Oil + dispersants ($t=0^+$) **Oil + dispersants ($t=30$ s)**

Dispersants: fate *vs* time



Dispersants: window of opportunity

As time progresses the light components of the spilled oil evaporate or dissolve in seawater:

→the viscosity of the remaining spilled oil increases

→"quickly" (**few hours to one-two days**) the spill can become not dispersable.

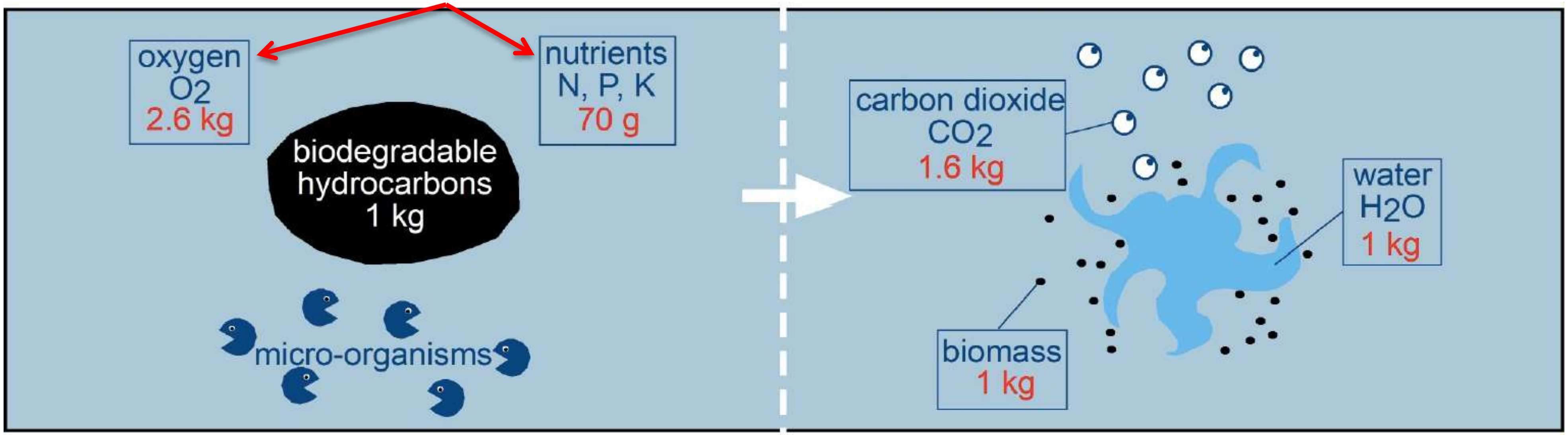
In situ burning

Toxic by-products (gases & liquids) are generated. Air pollution can reach places >100 km away… **BUT, impact is much less compared to the oil spill reaching the shoreline**…it is the industry-preferred option.

Bioremediation of oil spills

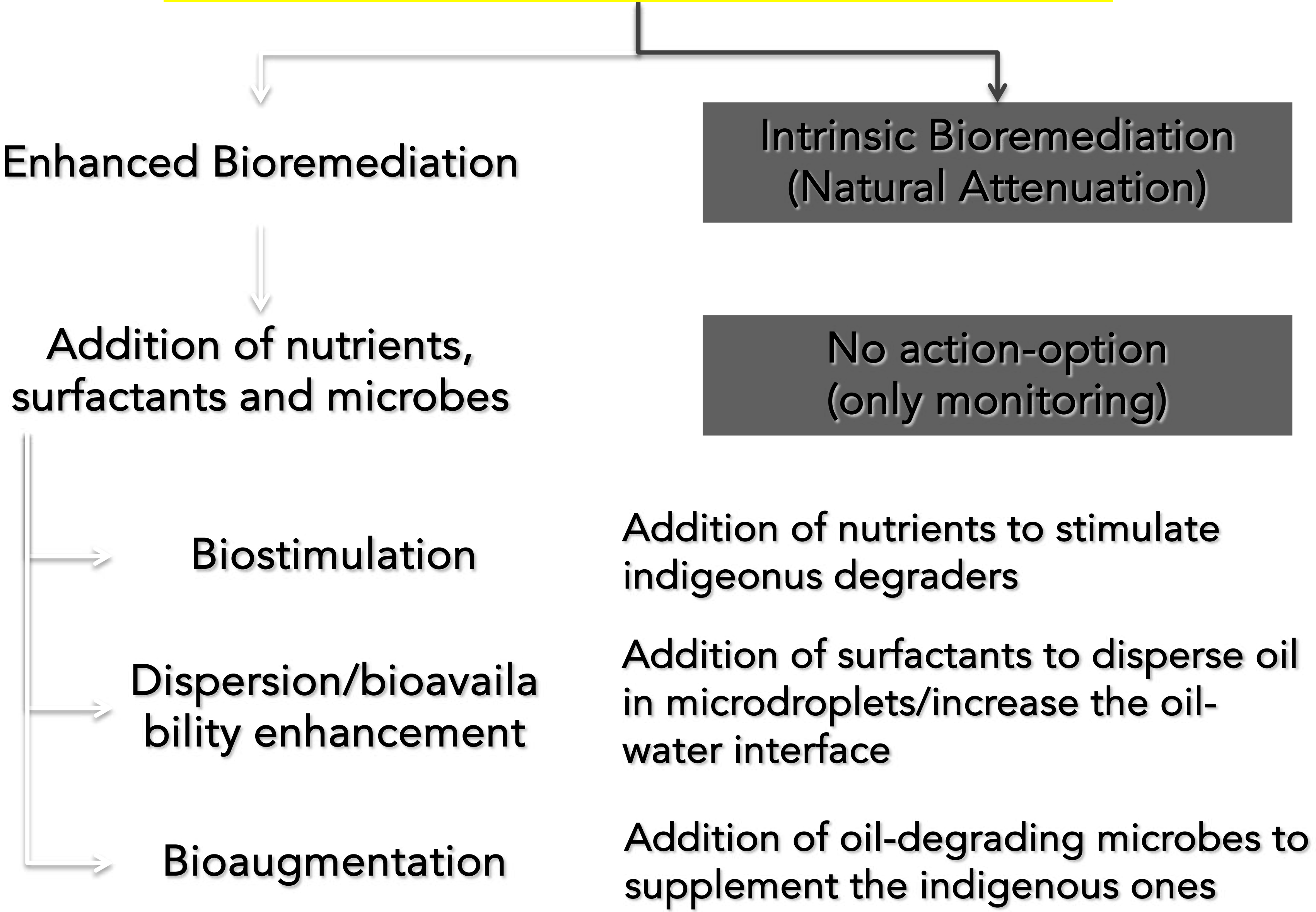
During the last 20 years many marine bacteria with hydrocarbon degrading capabilities have been found:
Alkanivorax, Cycloclasticus, Marinobacter, Fundibacter, Phycroserpens

WHERE DO THEY COME FROM?



- **BIOSTIMULATION**: addition on nutrients (mainly N and P) in order to establish/maintain the C:N:P (100:5:1) optimal for a quick and complete biodegradation of oil hydrocarbons by the indigenous microbes
- **BIOAUGMENTATION**: addition of specialized marine bacteria able to biodegrade hydrocarbons (increase of biocatalyst concentration)

Bioremediation of oil spills (after first response)



Enhanced Bioremediation

Addition of nutrients, surfactants and microbes

Intrinsic Bioremediation (Natural Attenuation)

No action-option (only monitoring)

Biostimulation	Addition of nutrients to stimulate indigeonus degraders
Dispersion/bioavailability enhancement	Addition of surfactants to disperse oil in microdroplets/increase the oil-water interface
Bioaugmentation	Addition of oil-degrading microbes to supplement the indigenous ones

Bioremediation of oil spills: biostimulation *vs* bioaugmentation

- Research studies have failed to prove conclusively that seeding is effective (with bioengineered organisms or organisms enriched from different environments and grown in the laboratory to high numbers).

Bioaugmentation is usually found less effective than Biostimulation in the long run.

Bioaugmentation offers only short term gains.

Challenges and needs in Biostimulation

Commercial inorganic fertilizers and mineral nutrient salts are **washed out by the wave action**!

- ❑ design nutrient delivery systems that overcome the wash out problems: formulations that are able to adhere to oil and provide (possibly in a controlled way) nutrients at the oil-water interface, where oil biodegradation mainly occurs, without the need to increase the nutrient concentrations in the bulk water.
  - ❖ use of slow release fertilizers, e.g., inorganic nutrients coated with hydrophobic materials like paraffins or vegetable oils
  - ❖ use oleophilic organic nutrients, like uric acid (source of nitrogen) or lecithins (source of phosphorous), as oil biodegradation mainly occurs at the oil-water interface
  - ❖ development of smart nutrient-releasing formulations able to interact with oil and release nutrients upon contact with oil

Development of an oleophilic nutrient formulation

- **Source of nitrogen: Uric acid**

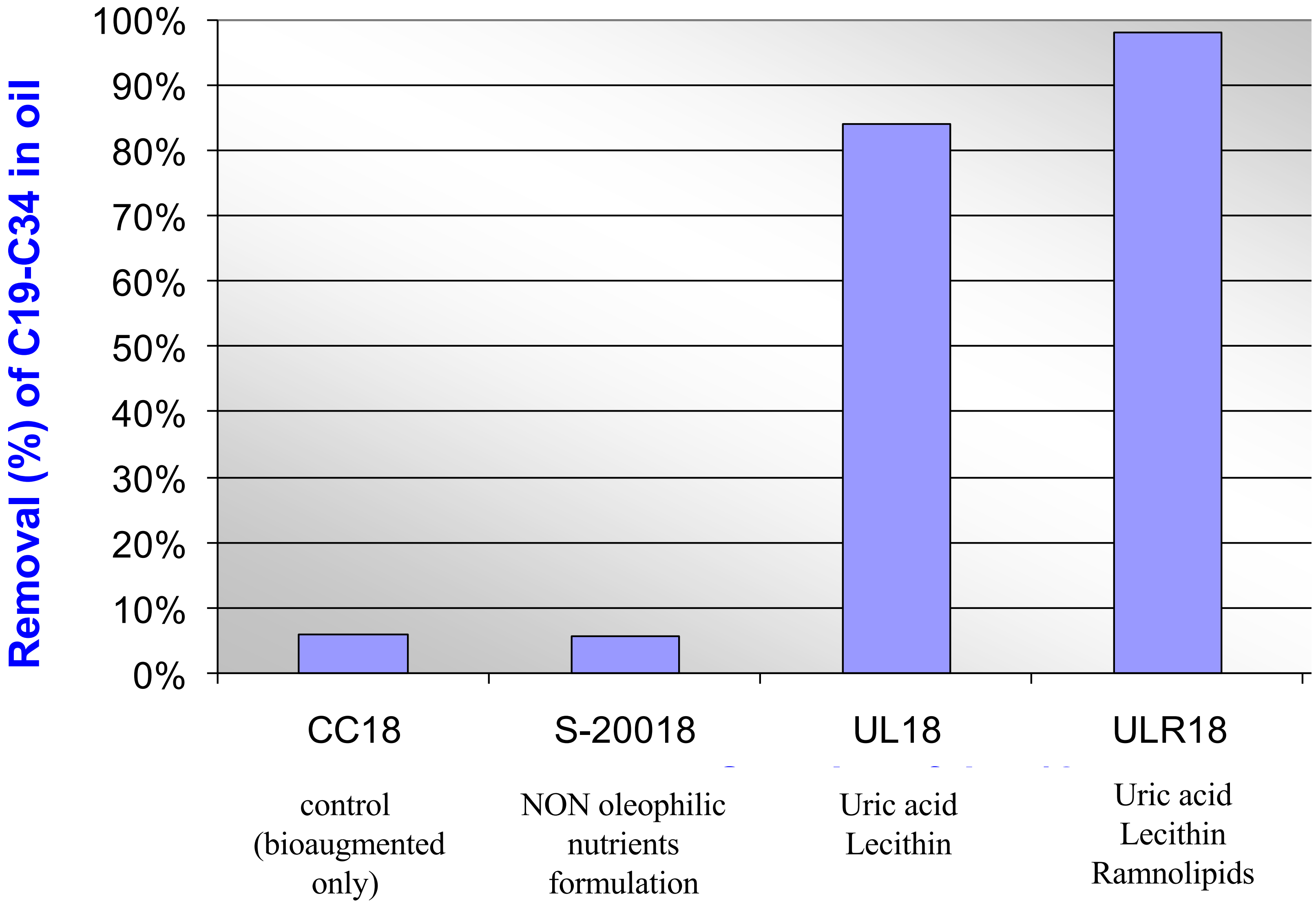
  It is a cost effective natural origin waste product of birds etc., it has low solubility in water (it is not readily washed out) binds to crude oil and therefore it is available for bacteria growing at the hydrocarbon-water interface.

- **Source of phosphorous: Lecithin**

  It is a natural phospholipid, oil soluble, easy to get at low cost as by-product of the Vegetable Oil Industry and has good dispersant properties (can also serve as a biosurfactant).

☐ An additional biosurfactant (e.g., rhamnolipids) may be added to further disperse hydrocarbons and increase their bioavailability

Effect of an oleophilic nutrient formulation (+ bioaugmentation with autochtonous HC degraders)



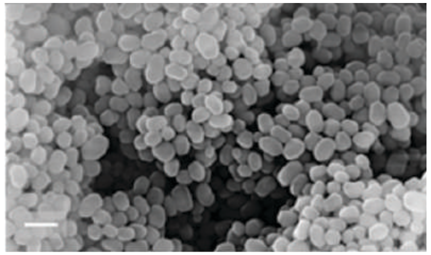
Nikolopoulou, M., Kalogerakis, N. 2009 Journal of Chemical Technology and Biotechnology84(6), pp. 802-807

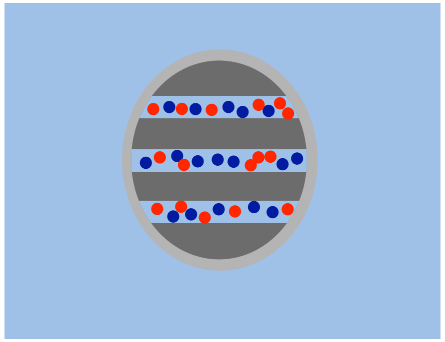
SmartGate particles for the controlled release of nutrients

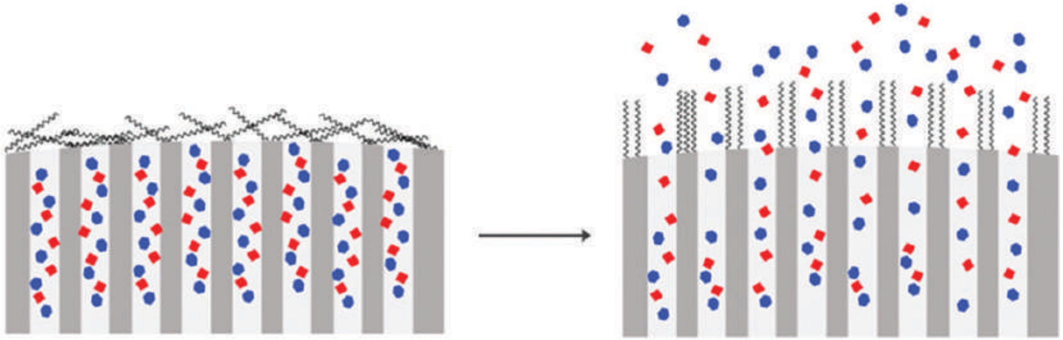
Corvini N et al., 2019, Chemical Communications55(52), pp. 7478-7481

SmartGate Concept

- mesoporous silica nanoparticles loaded with active ingredients (nutrients, N and P) to intensify oil hydrocarbons biodegradation (biostimulation)
- After suited surface modification, the particles sorb to oil slick and remain associated to the hydrophobic phase
- Active ingredients (here: N and P) encapsulated in the mesoporous material are only released at oil/water interface

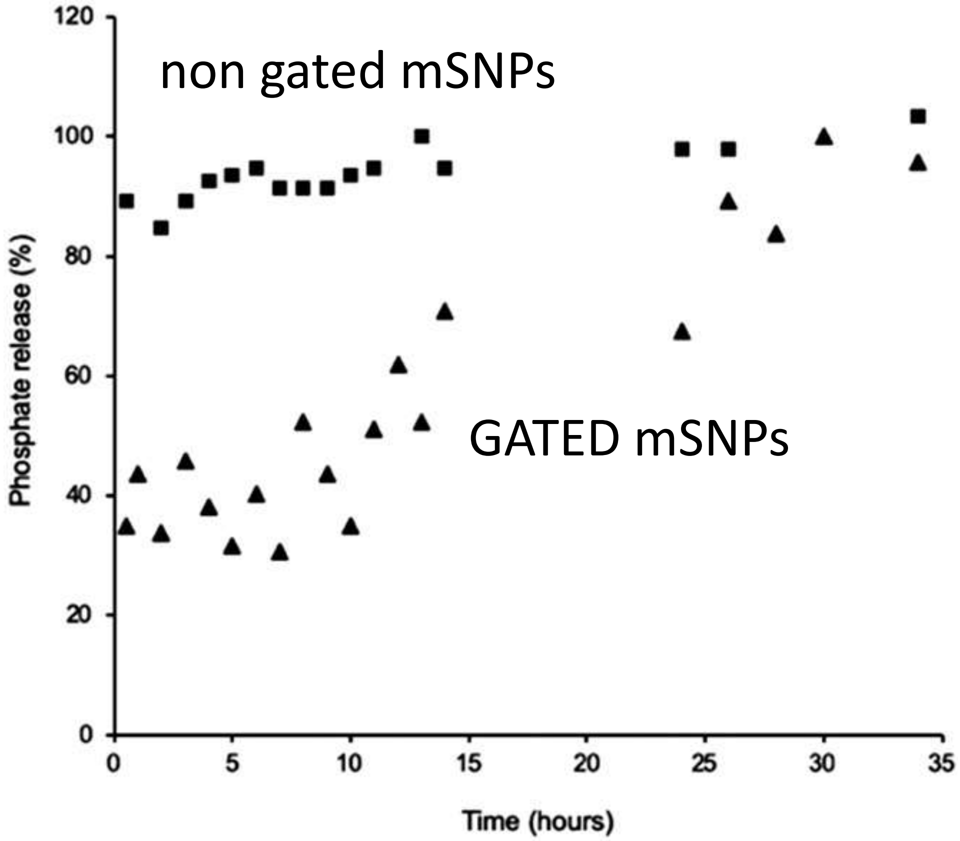






SmartGate particles for the controlled release of nutrients

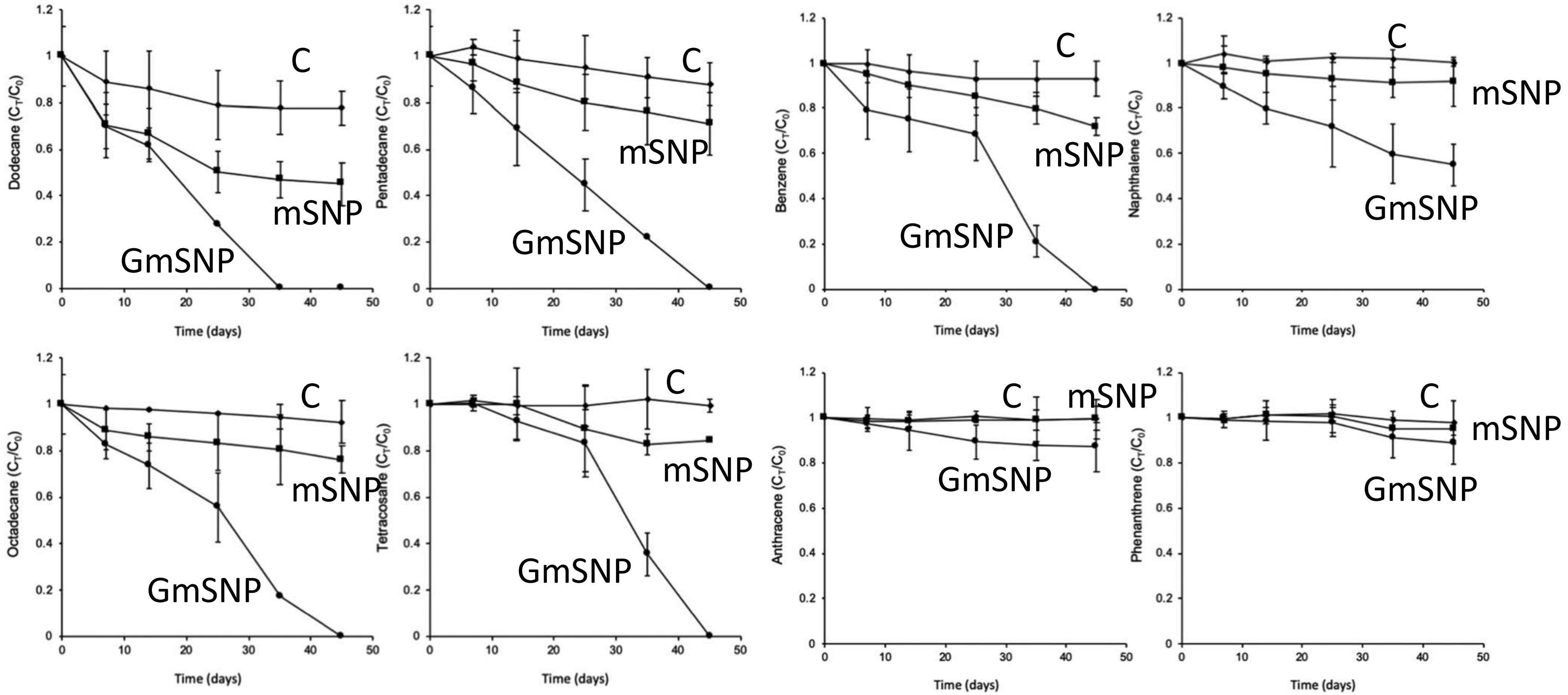
Release of the encapsulated nutrients by mSNPs and gated mSNPs in a solution mimicking an oil-contaminated water environment (water/heptane mixture)



Corvini N et al., 2019, Chemical Communications55(52), pp. 7478-7481

SmartGate particles for the controlled release of nutrients

Biodegradation of oil hydrocarbons by a hydrocarbonoclastic marine bacterium with mSNP and gated mSNPs (GmSNP)

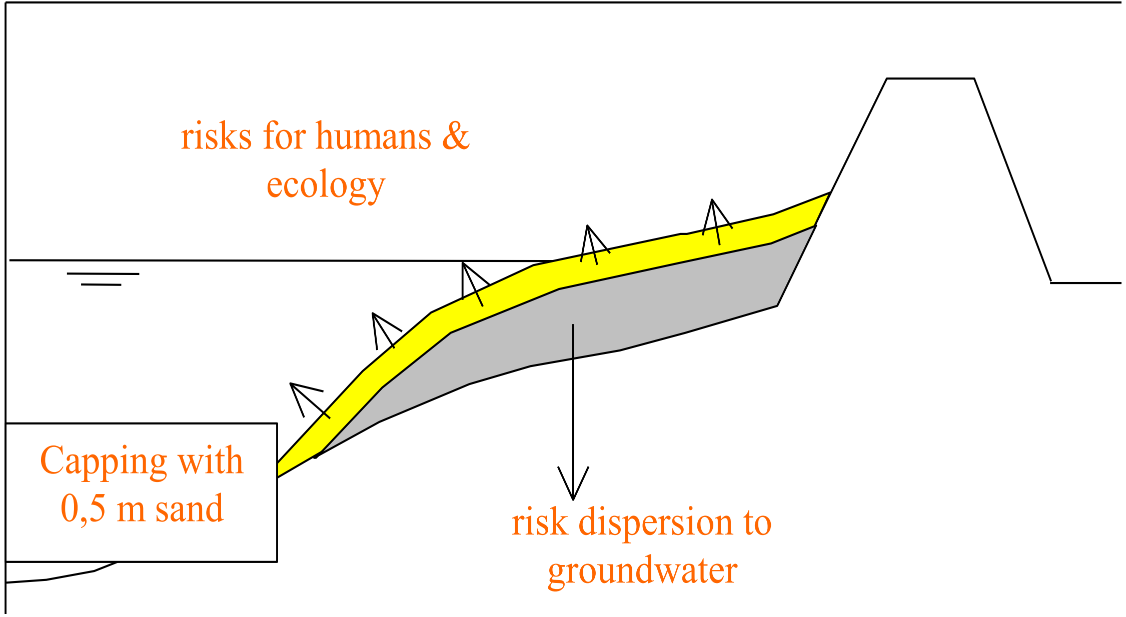


Corvini N et al., 2019, Chemical Communications55(52), pp. 7478-7481

In situ management of contaminated sediments: Capping

Action mechanism of sand:

- Armors sediment for containment
- Separates contaminants from benthic organisms
- Reduces diffusive/advective flux
- Provides opportunities for habitat development.



In situ management of contaminated sediments: Capping

Objectives:

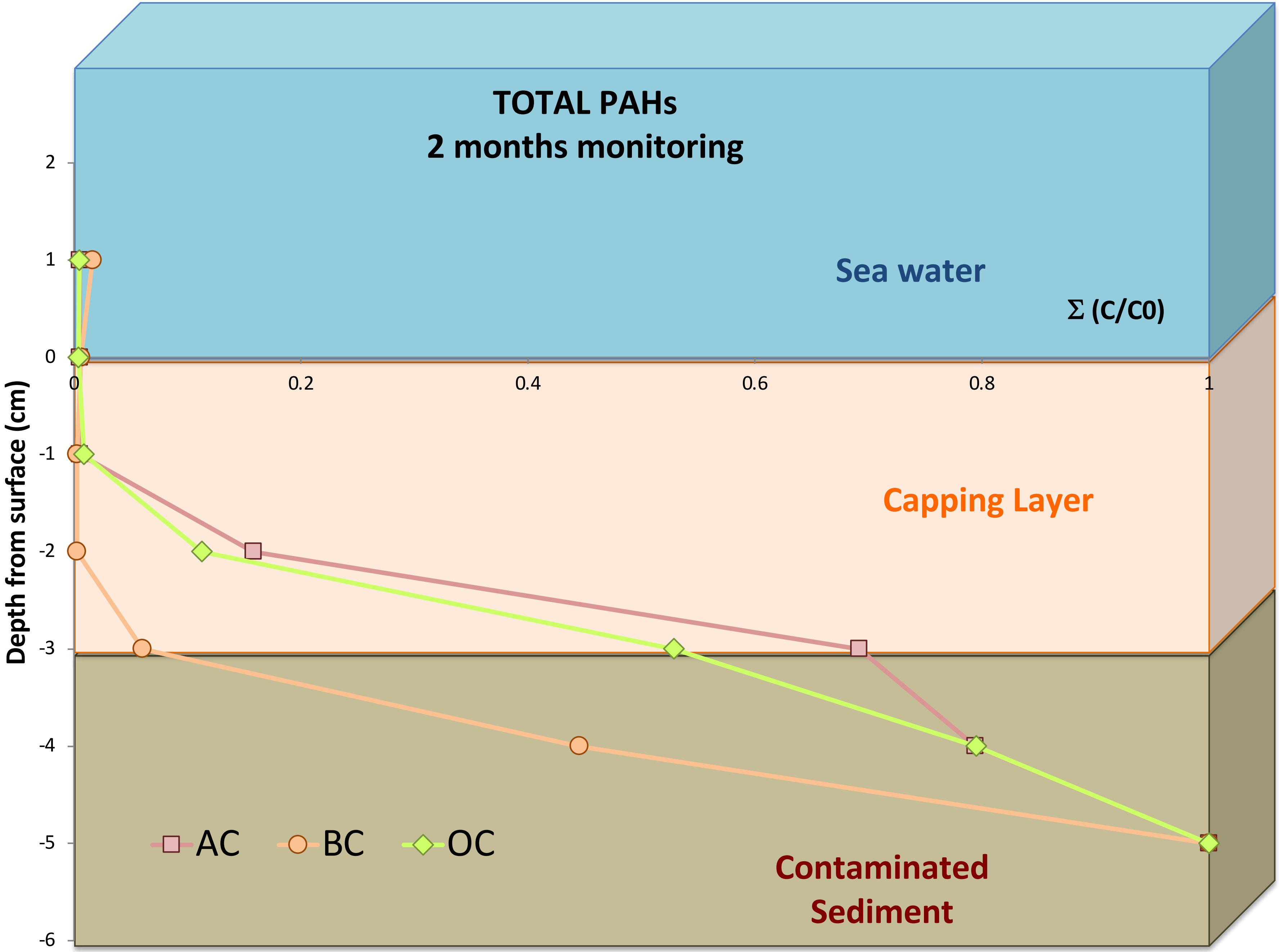
- Physical isolation of sediment contaminants
- Reduce contaminant flux to benthos

Active capping can enhance chemical isolation

Reactive layer

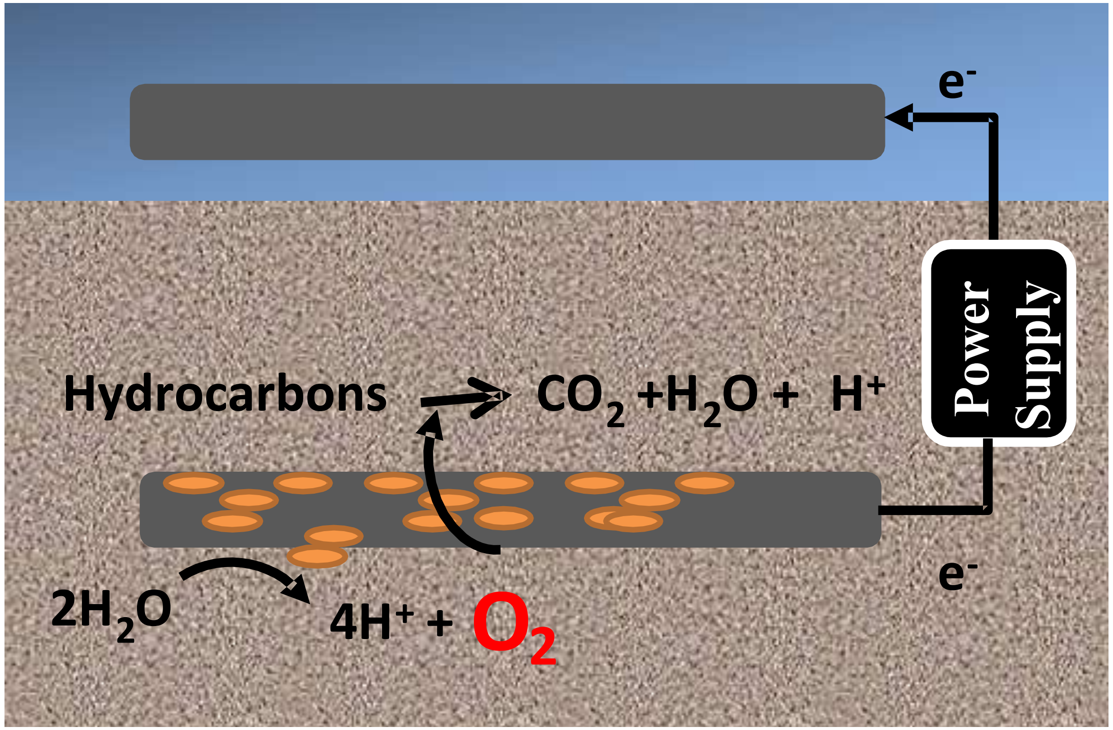
- Organoclay (low soluble organics and NAPL)
- Activated carbon (soluble organics and some metals)
- Apatite (heavy metals)
- Zero-valent Iron
- Combinations of the above

Capping: Activated carbon (AC), organoclay (OC), biochar (BC)



Silvani et al., 2017, Chemical Communications55(52), pp. 7478-7481

Bioelectrochemical stimulation of aerobic hydrocarbons biodegradation in sediments

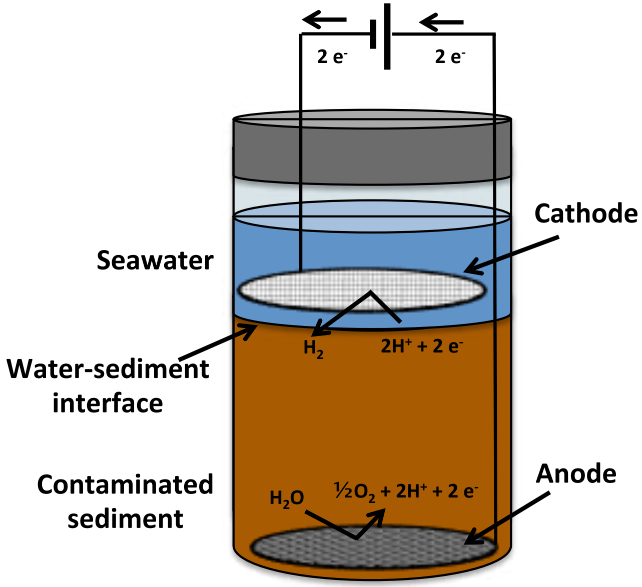


- ✓ Bioremediation of sediments is challenged by the lack of oxygen which is needed to “activate” hydrocarbons and sustain their fast biodegradation
- ✓ dimensionally stable anodes (i.e., Ti mesh electrodes coated with mixed metal oxides) are deployed within the contaminated sediment and exploited to generate oxygen from seawater electrolysis

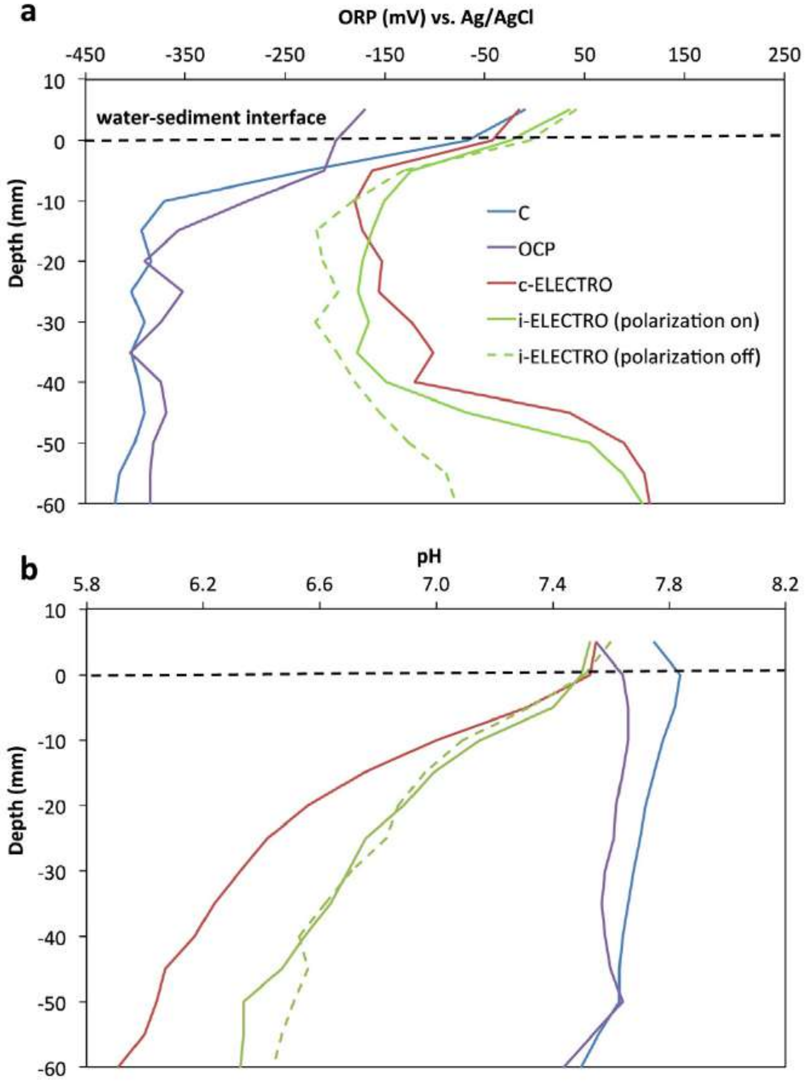
➔ Rates of O_2 production can be controlled finely and easily

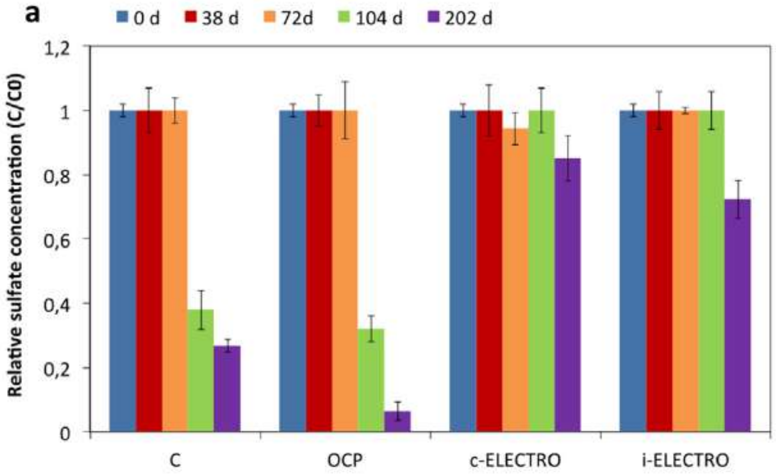
Bellagamba M. *et al.* **New Biotechnology** 2016. doi: 10.1016/j.nbt.2016.03.003

Bioelectrochemical stimulation of aerobic hydrocarbons biodegradation in sediments



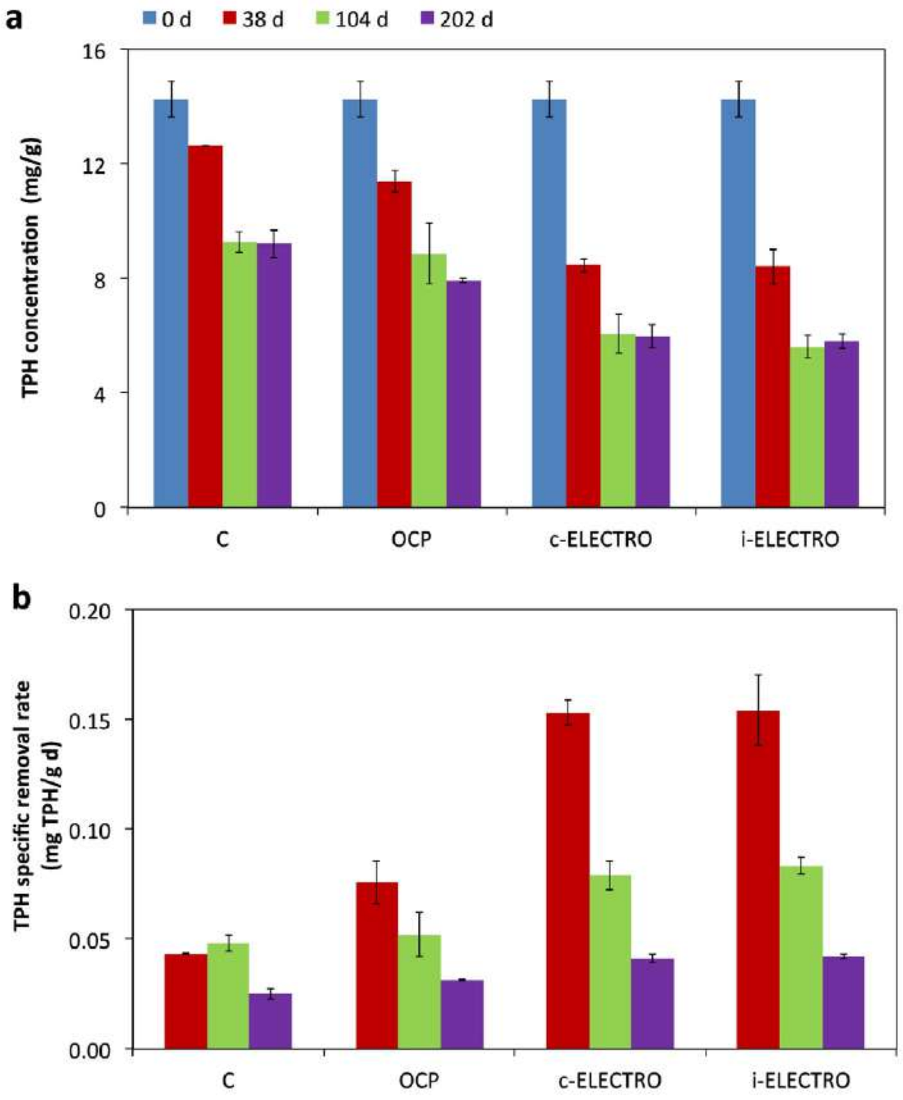
Electrolysis (2 V) results in ar increased redox potential of the marine sediment





Bellagamba M. *et al.* **New Biotechnology** 2016. doi: 10.1016/j.nbt.2016.03.003

Bioelectrochemical stimulation of aerobic hydrocarbons biodegradation in sediments

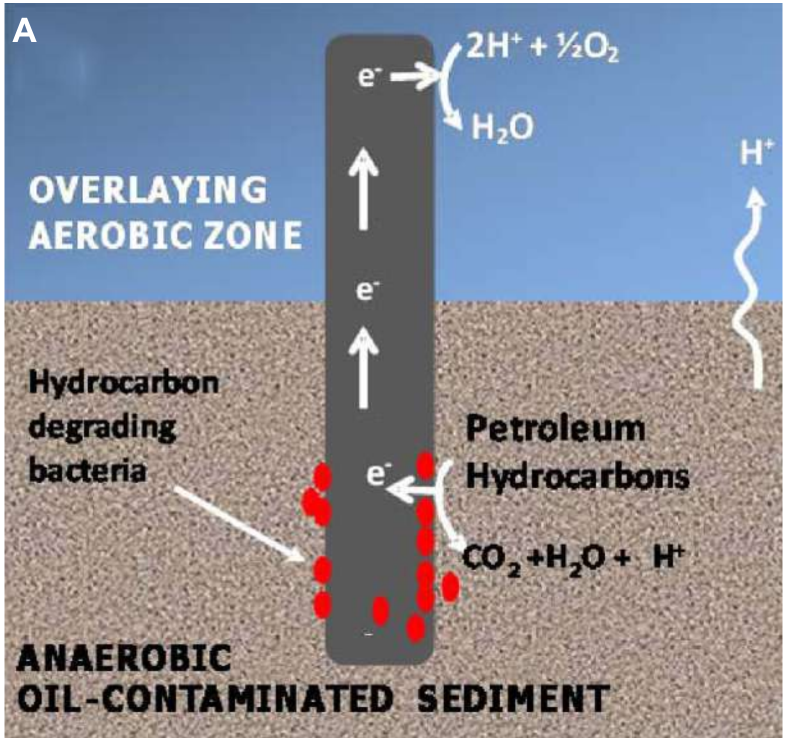


- Up to 3-fold enhancement of total hydrocarbons biodegradation compared to control sediments
- Energy consumption amounts to 0.11 KWh energy consumed per kg TPH degraded
- Cost of anode material (≈ 1k€/ m^2 geometric surface area) represents a possible bottleneck

➔ in view of field-scale application, the determination of the radius-of-influence of the technology becomes critically important!

Bellagamba M. *et al.* **New Biotechnology** 2016. doi: 10.1016/j.nbt.2016.03.003

Bioelectrochemical stimulation of anaerobic hydrocarbons biodegradation in sediments



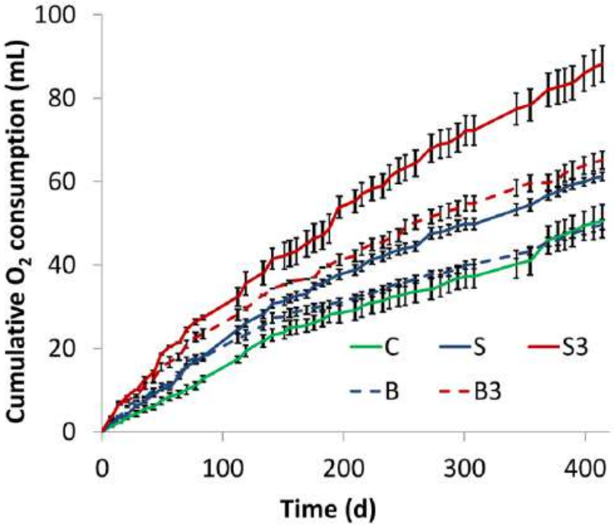
Viggi et al., 2015, Frontiers in Microbiology6(SEP),881

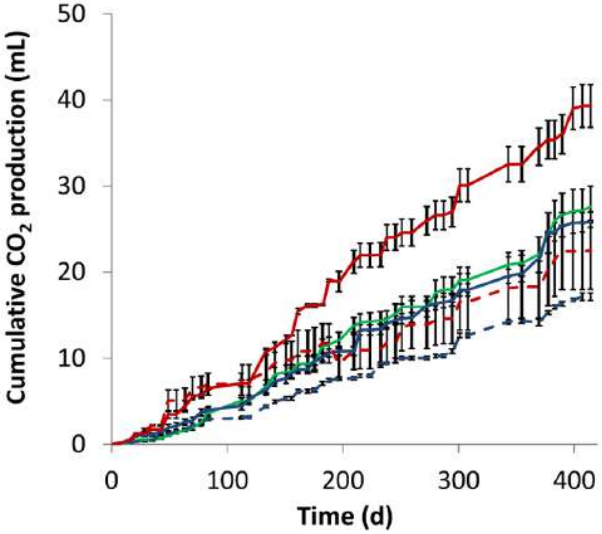
A single conductive material (the snorkel) positioned suitably to create an electrochemical connection between the anoxic zone (the contaminated sediment) and the oxic zone (the overlying O_2-containing water). The segment of the electrode buried within the sediment plays a role of anode, accepting electrons deriving from the oxidation of contaminants. Electrons flow through the snorkel up to the part exposed to the aerobic environment (the cathode), where they reduce oxygen to form water.

Bioelectrochemical stimulation of anaerobic hydrocarbons biodegradation in sediments

The snorkel promote the oxidation of organic matter in the sediment (higher O_2 consumption and CO_2 accumulation in the water phase)

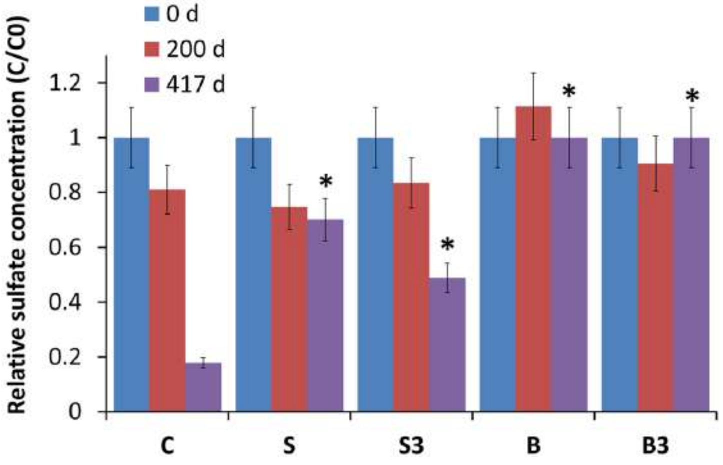
Viggi et al., 2015, Frontiers in Microbiology6(SEP),881



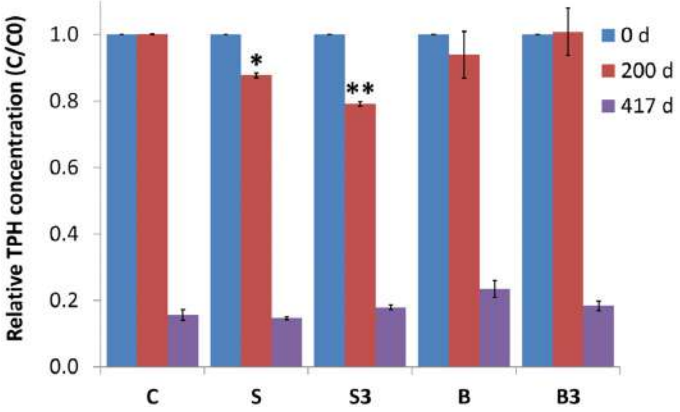


C: biotic control
S1–S3: 1-3 snorkels
B1-B3: sterile controls with 1-3 snorkels

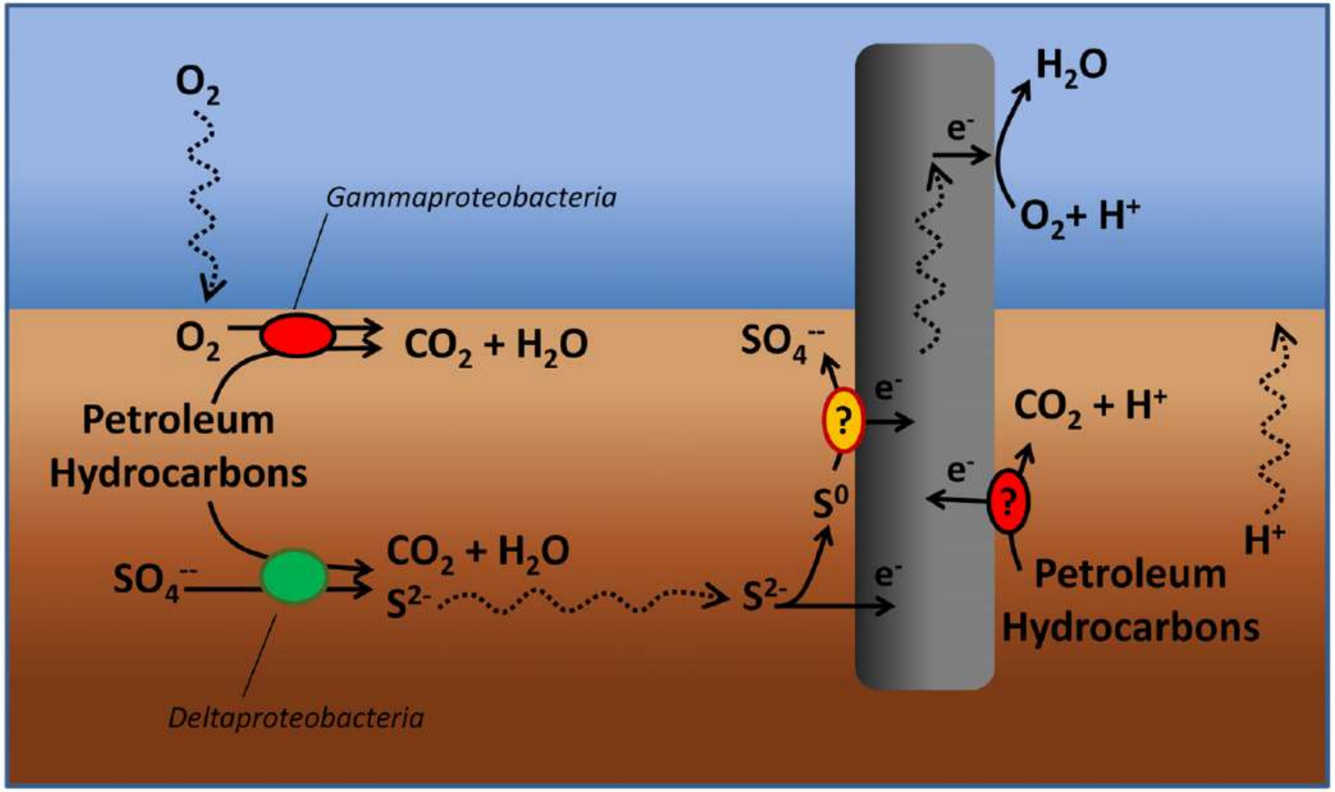
The snorkel slows down sulfate-reduction



The snorkel increases the initial rate of hydrocarbons biodegradation



Bioelectrochemical stimulation of anaerobic hydrocarbons biodegradation in sediments



Viggi et al., 2015, Frontiers in Microbiology6(SEP),881

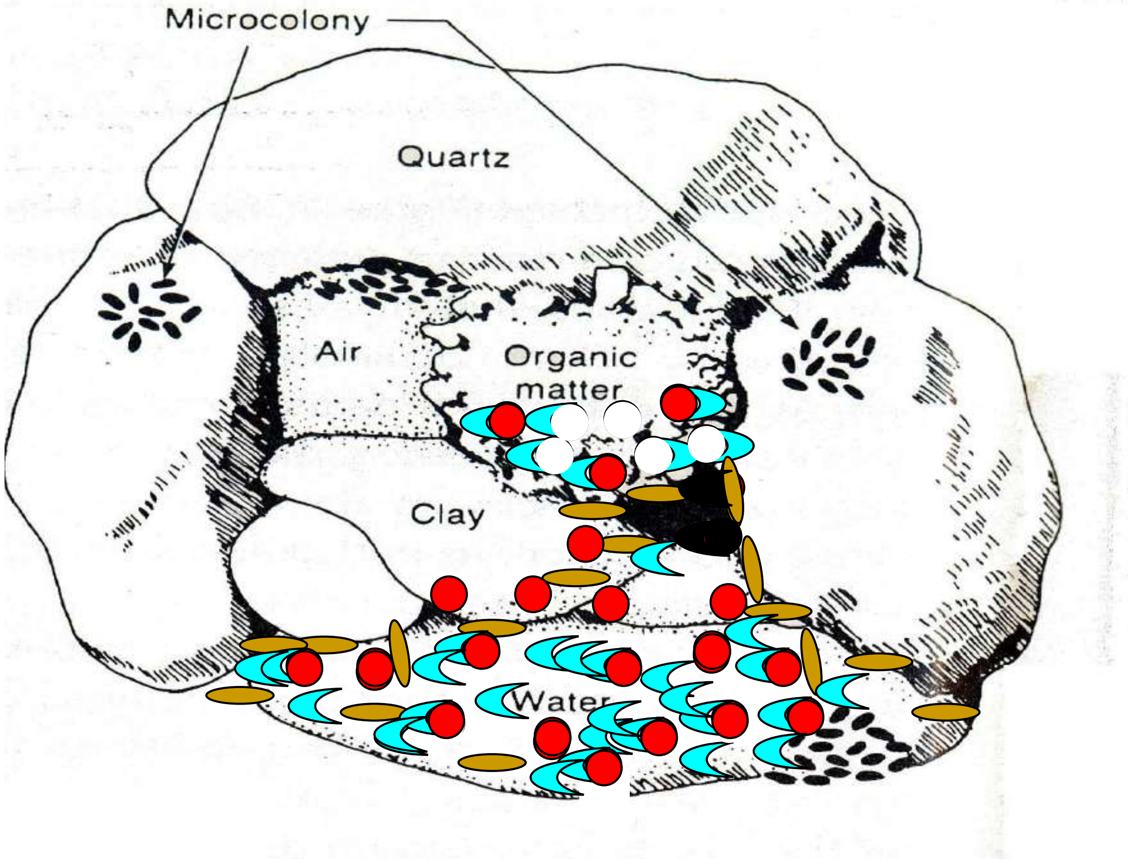
Still unclear whether the effect of the snorkel on hydrocarbons biodegradation is direct (e.g., the graphite electrode served as a direct electron acceptor for hydrocarbons oxidation), indirect (e.g., the electrode somehow stimulated the activity of hydrocarbon-oxidizing sulfate-reducing bacteria) or both.

The issue of pollutant bioavailability in contaminated sediments

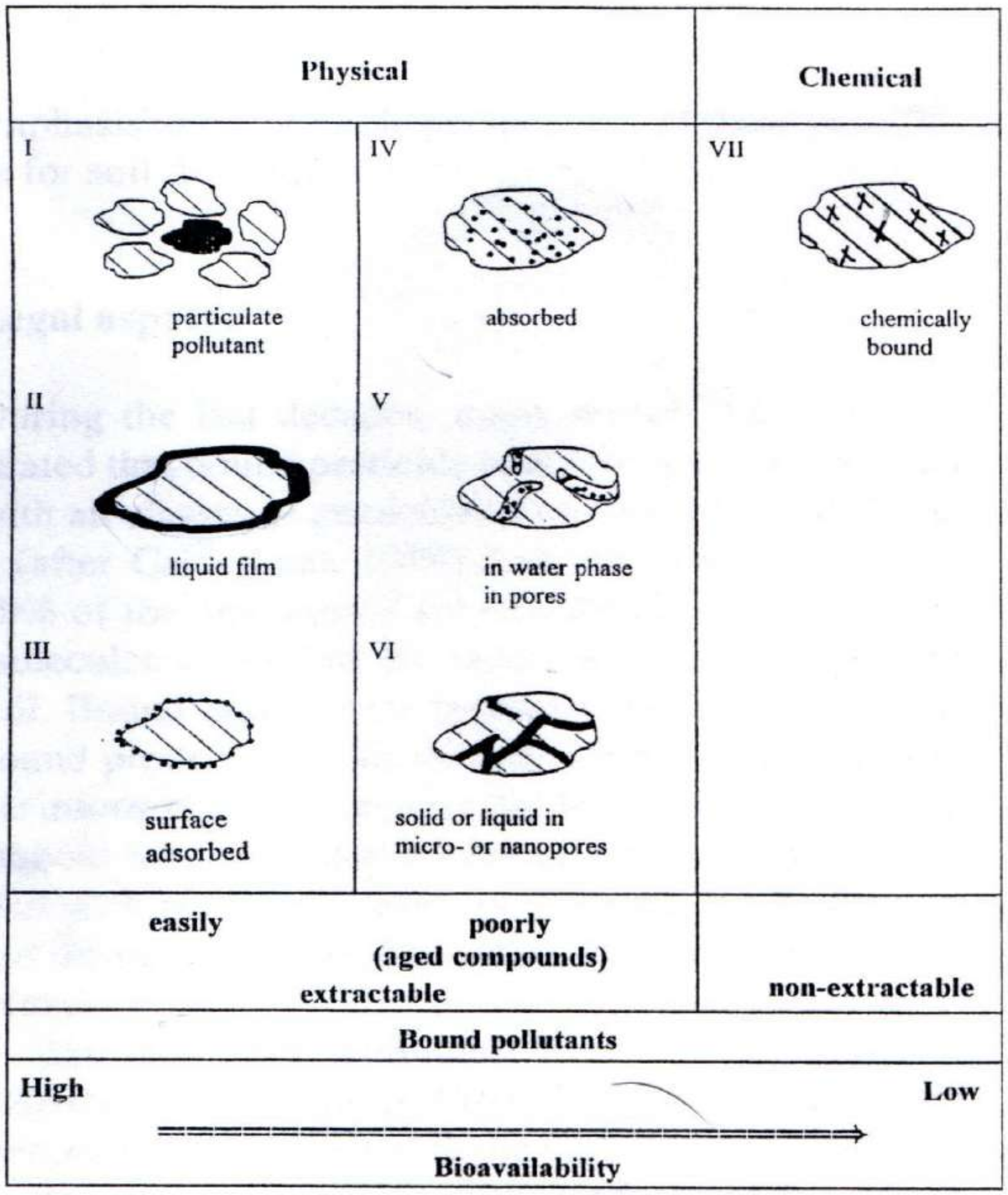
● **Hydrophobic pollutant**

☾ **Solubilizing agent**

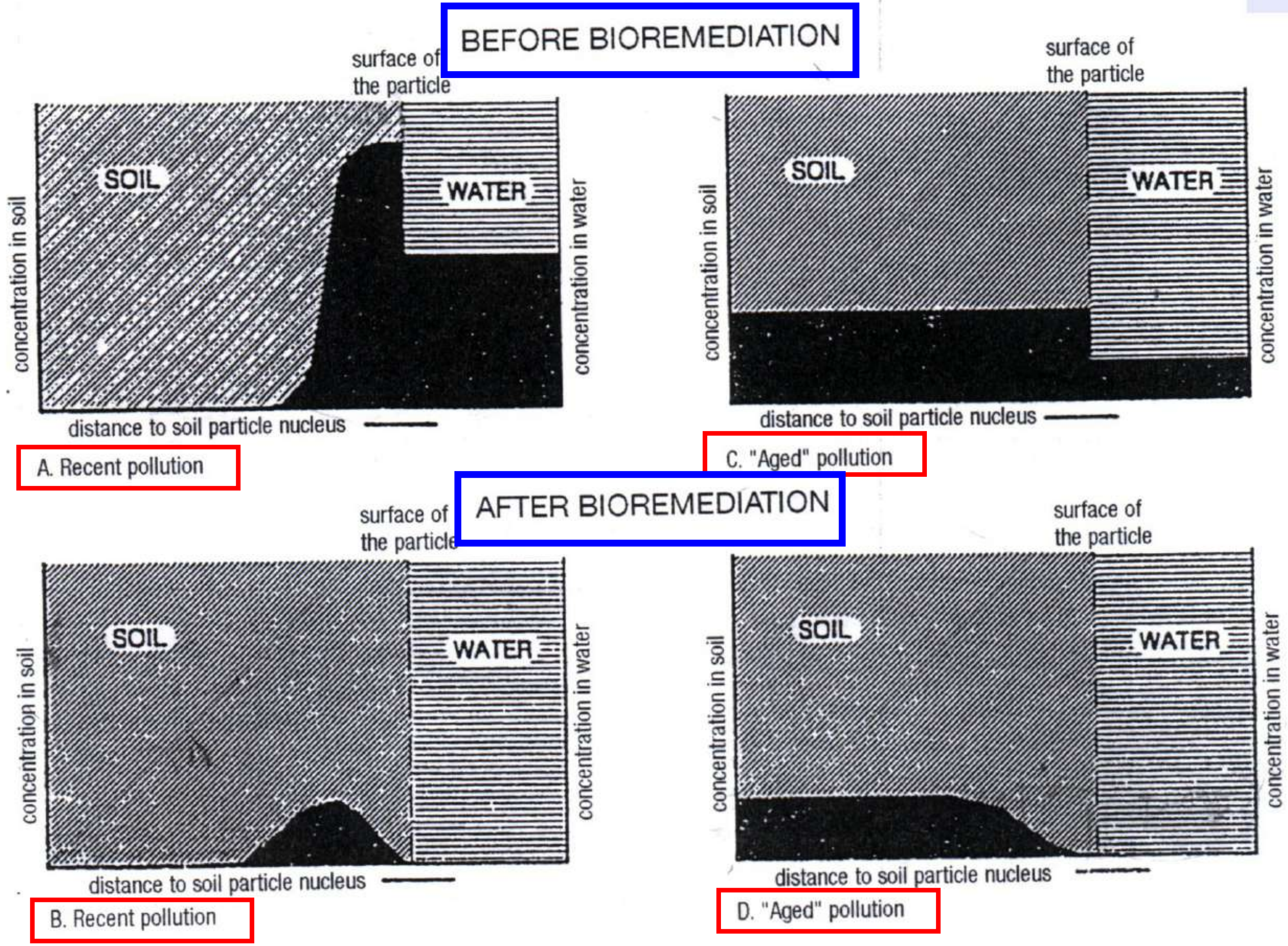
⬬ **Microbial cells**



The issue of pollutant bioavailability in contaminated sediments



The issue of pollutant bioavailability in contaminated sediments



Schematic visualisation of the distribution of a pollutant in a soil particle and surrounding liquid. The vertical scales are different in a way that the soil concentration >> water concentration (after Bosma 1994).

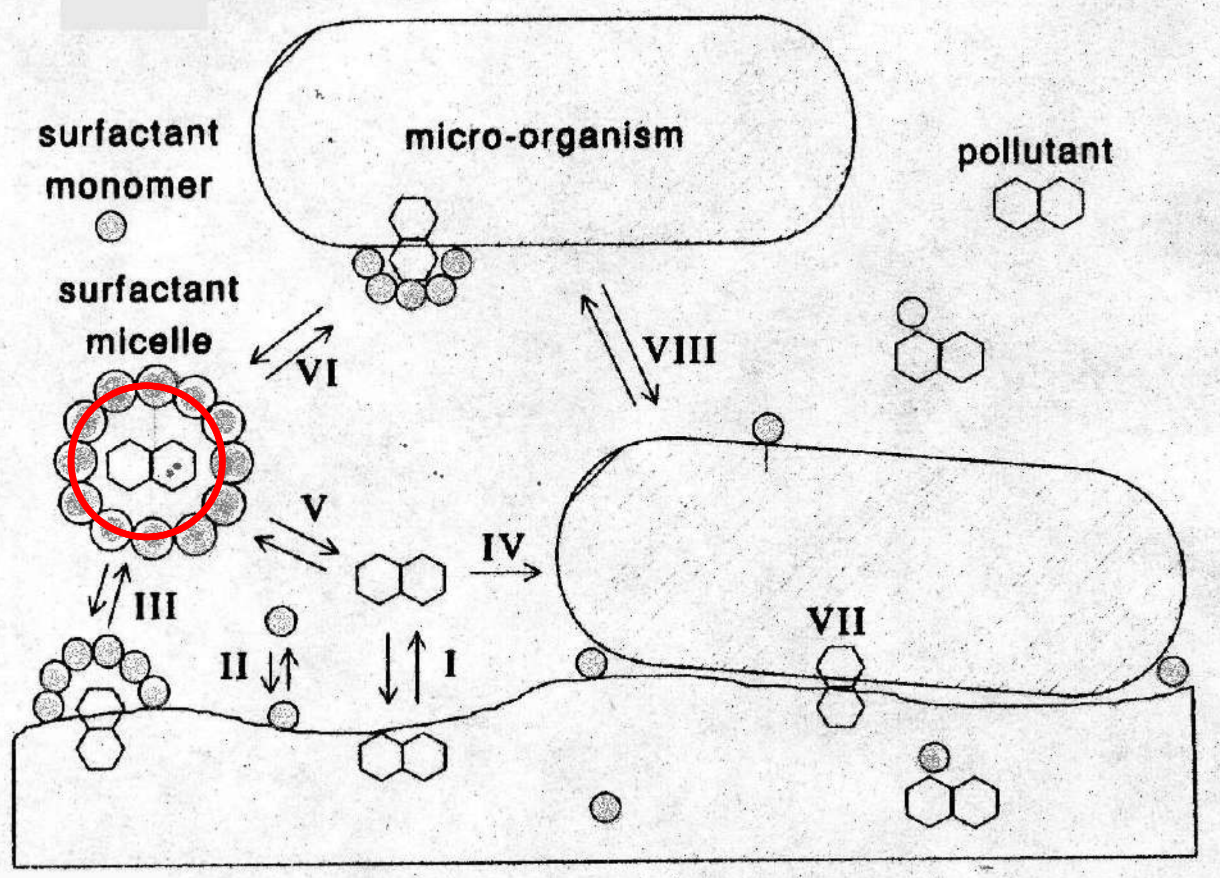
The issue of pollutant bioavailability in contaminated sediments

Surfactants can enhance the bioavailability of PCBs through[12]:

a)micellar-solubilization (at concs.≥ CMC);

b) reducing the interfacial tension between water and the solid-adsorbing phase;

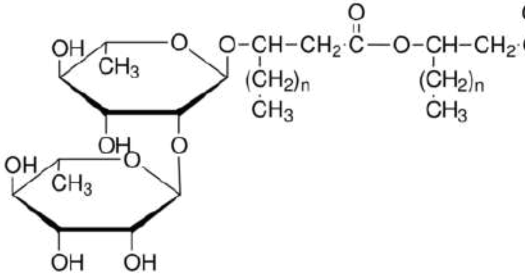
c) swelling of the organic matter.

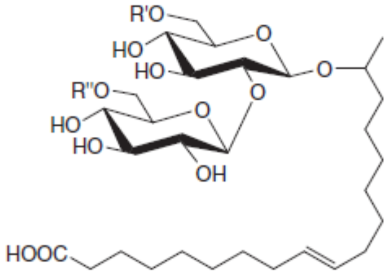


Schematic overview of the interactions between microorganisms, soil, pollutant, and surfactants. I sorption of pollutant, II sorption of surfactant molecules onto soil, III solubilisation of pollutant, IV uptake of pollutant from the water phase by micro-organisms, V partitioning of pollutant between the water phase and the micelles, VI sorption of micelles to micro-organisms, VII direct uptake of pollutant from the solid phase by micro-organisms, VIII sorption of micro-organisms onto soil.

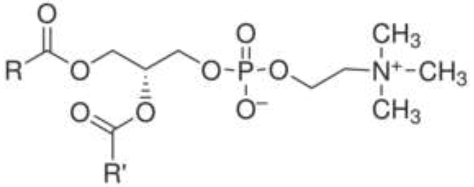
Potential biogenic surfactants/pollutant mobilising agents for increasing bioavailabiliity and biodegradation

- Rhamnolipids
- Sophorolipids



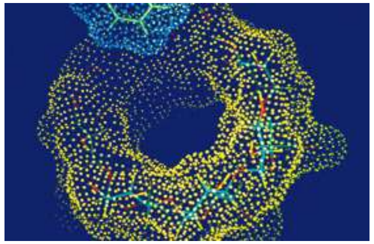


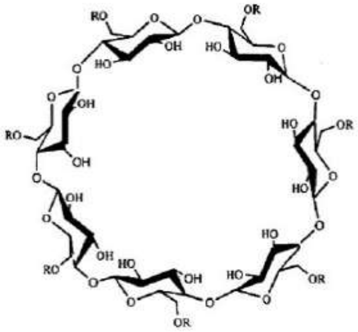
- Soy lecithin (TEXTROL F-10 ; HLB=4)
- Deoiled soy lecithin (SOLEC C ; HLB=7)



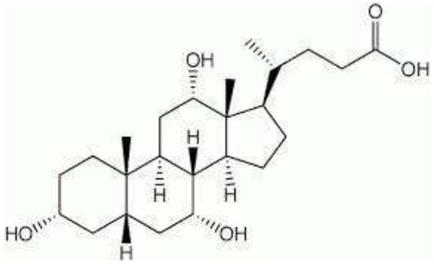
R, R' = fatty acid residues

- Hydroxy propyl-β-cyclodextrins (HPB-CD)
- Randomly methylated β-cyclodextrins (RAMEB-CD)





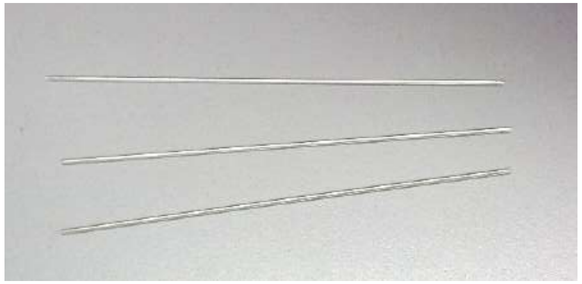
- Bile acids (cholic acid, taurocholic acid, glycocholic acid, deoxycholic acid, etc.)

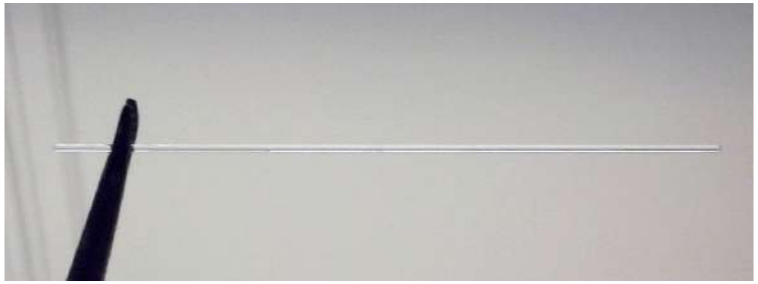


Measurement of oil hydrocarbons bioavailability (pore-water concentration in a contaminated sediment

passive sampling with **polydimethylsiloxane (PDMS) fibers**.

558.8 μm outer diameter, 486 μm inner diameter (i.e., annulus of PDMS 35.4 μm; fiber volume 0.597 μL/cm)





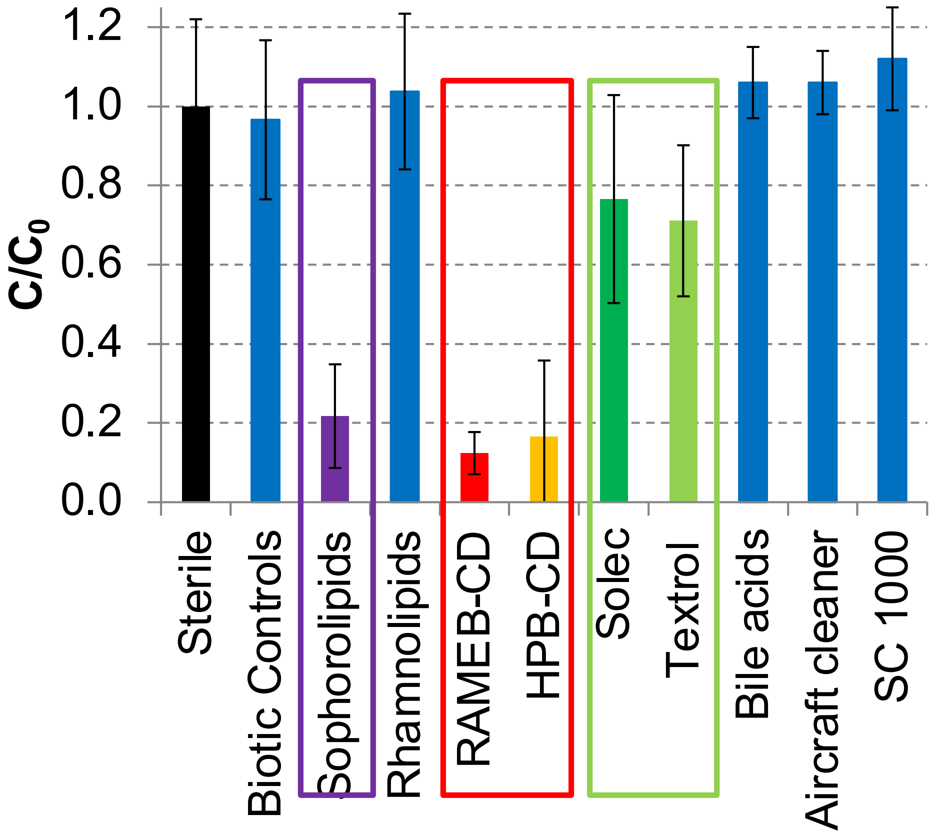
Fibers (5 cm) are incubated in the sediment and replaced every 20 days.

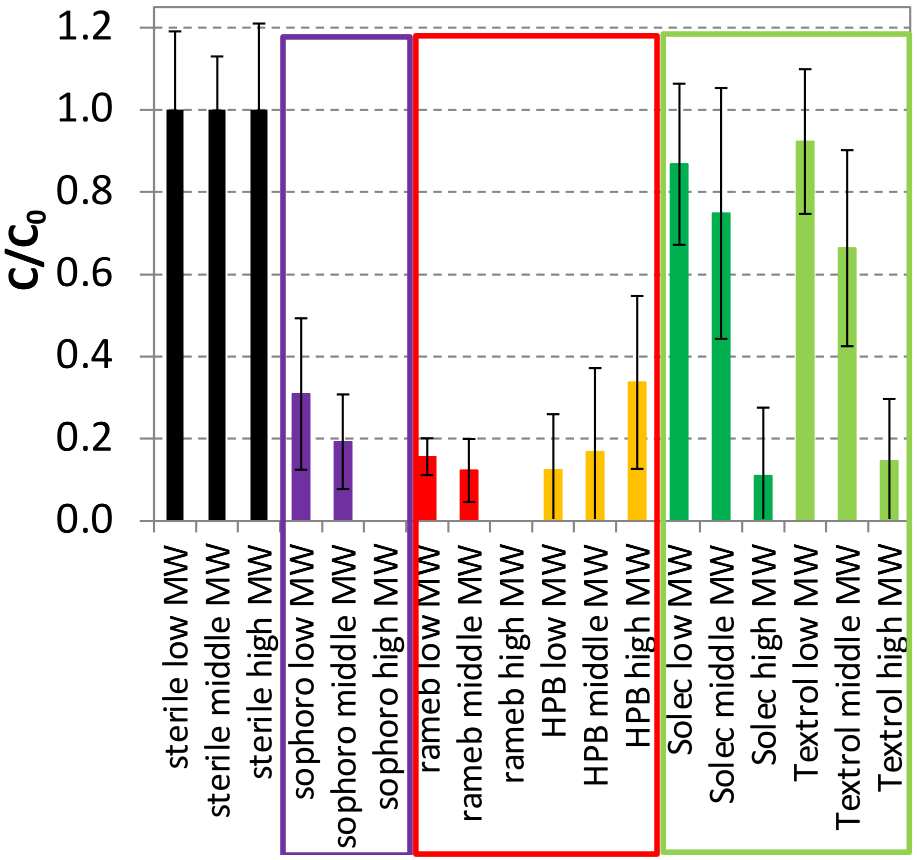
After elution into organic solvent (hexane), *n*-alkanes concentration is analyzed via GC-FID and the pore-water concentration is calculated using fiber/water partition coefficients for oil hydrocarbons.

Effects of biogenic surfactants/pollutant mobilising agents oil hydrocarbons biodegradation in a contaminated marine sediment

Biodegradation of *n*-alkanes (*n*C10-*n*C33): **40 weeks of incubation**

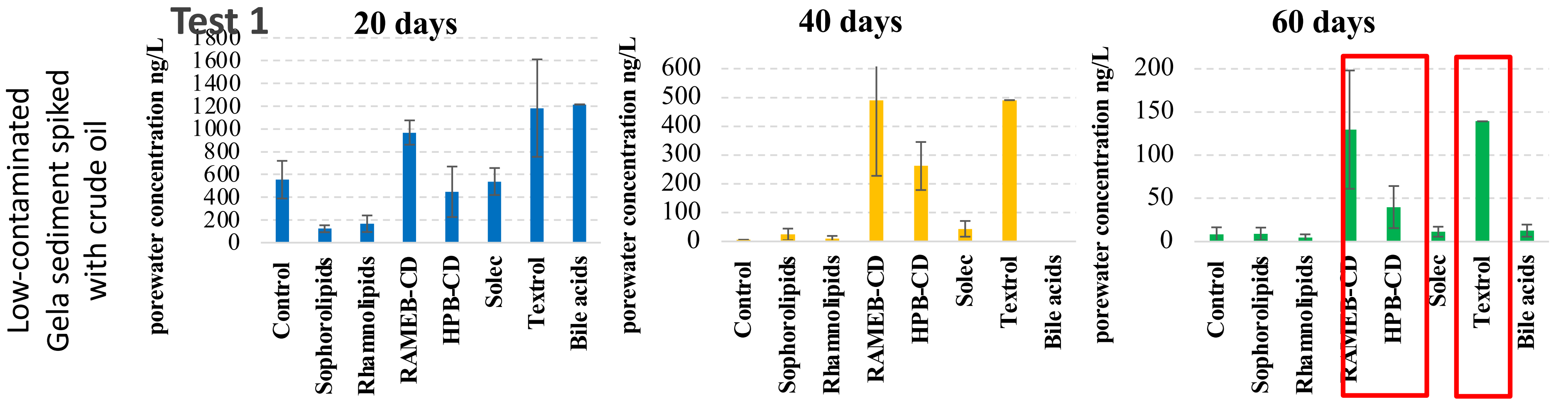
Extensive biodegradation (80 to 88%) occurred in the presence of **cyclodextrins** and **sophorolipids**, and to less extent, of **soy lecithins**.

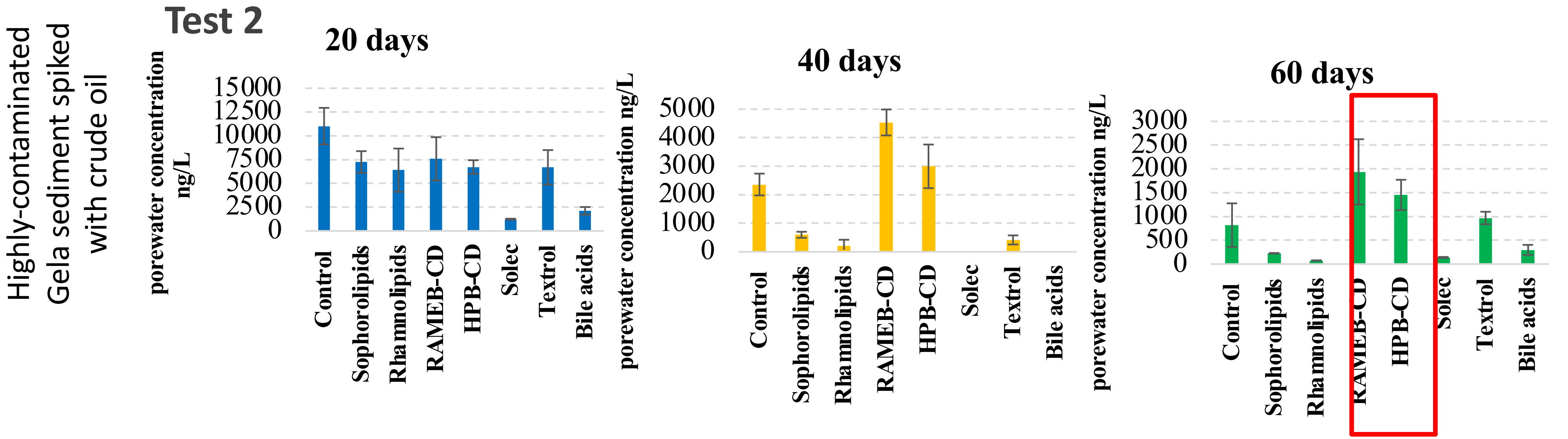




Higher MW hydrocarbons were preferentially degraded in the presence of **sophorolipids** and **soy lecithins**.

Porewater concentration of *n*-alkanes in presence of different biosurfactants



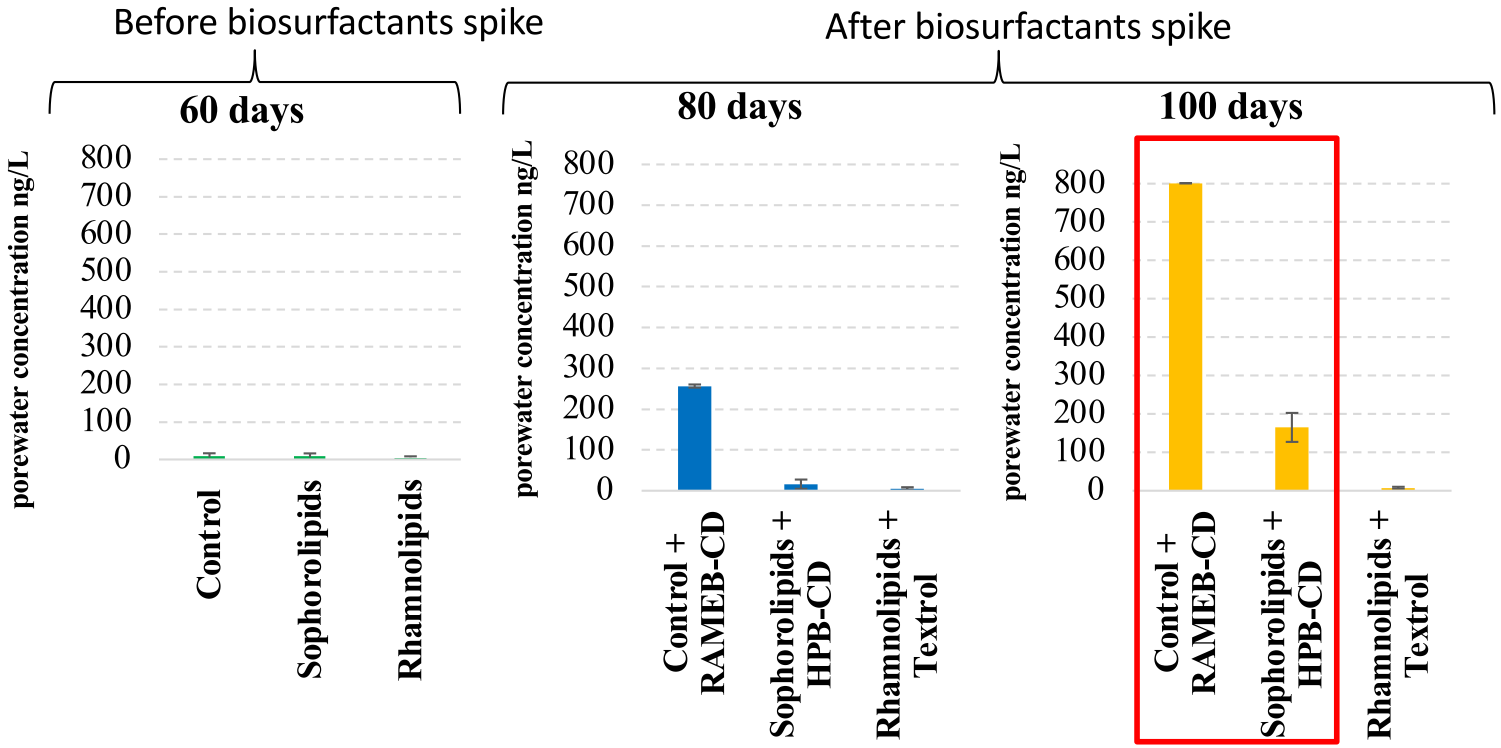


RAMEB-CD, HPB-CD and Textrol F reduce the adsorption rate of *n*-alkanes in the Gela sediment after 60 days of incubation.

Porewater concentration of adsorbed *n*-alkanes after biosurfactant re-spike

To test their effect on adsorbed *n*-alkanes, **RAMEB-CD**, **HPB-CD** and **Textrol F** were added to the un-amended controls, Sophorolipids and Rhamnolipids microcosms of the **Test 1**, respectively, after adsorption of spiked *n*-alkanes was completed:

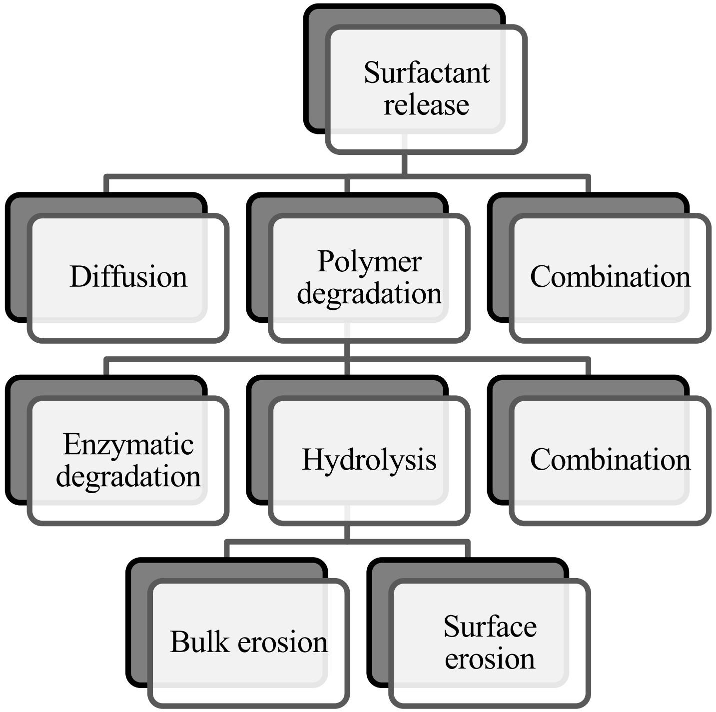
Un-amended controls	→	RAMEB-CD (5g/kg sed.)
Sophorolipids	→	HPB-CD (5g/kg sed.)
Rhamnolipids	→	Textrol F (5g/kg sed.)



RAMEB-CD and, to less extent **HPB-CD**, increase the bioavailability of adsorbed *n*-alkanes.

Encapsulation and release of surfactants

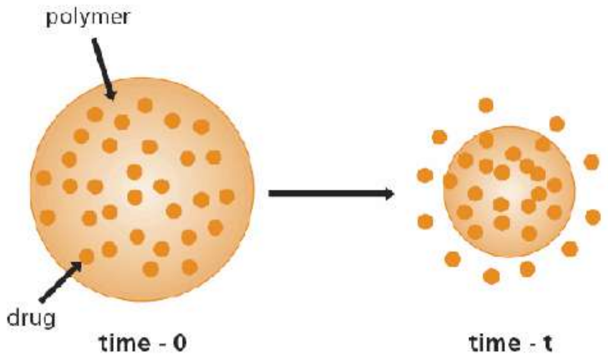
- **Organic Systems: natural or synthetic polymers.**

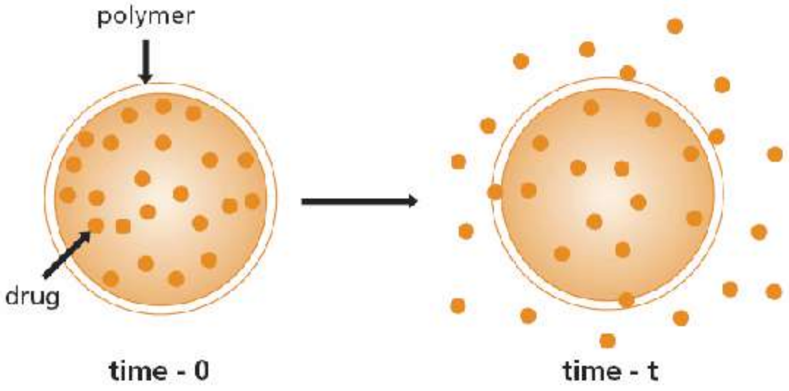


- Surfactant release
  - Diffusion
  - Polymer degradation
    - Enzymatic degradation
    - Hydrolysis
      - Bulk erosion
      - Surface erosion
    - Combination
  - Combination

Polymer degradation

Proteins, polysaccarides, PLA, PGA, PHB ….



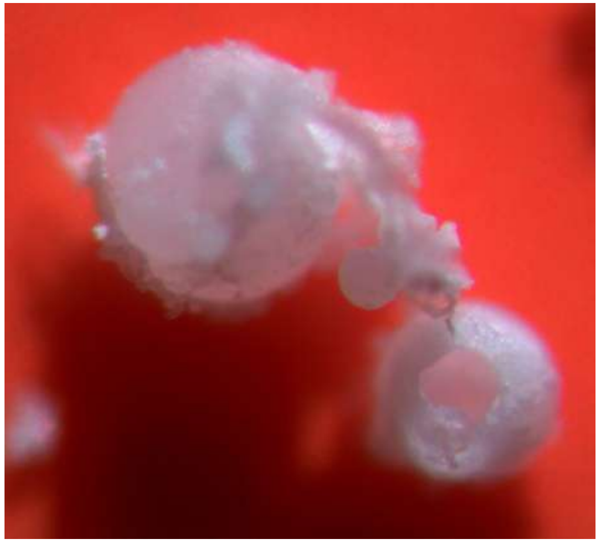


Diffusion and/or degradation (hydrogel or microcapsule)

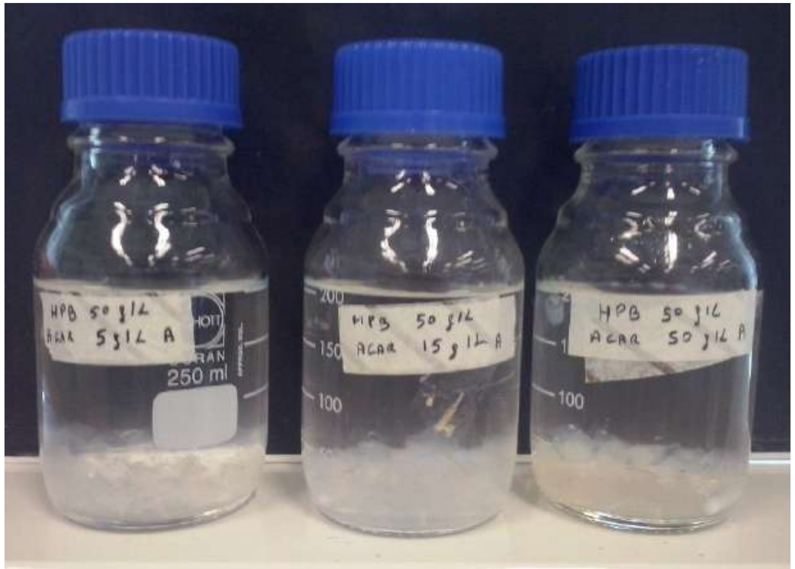
pHEMA, PVA, Chitosan, Gelatin, Agar, guar gum, etc…

Encapsulated surfactants

PBS-encaspulated sophorolipids



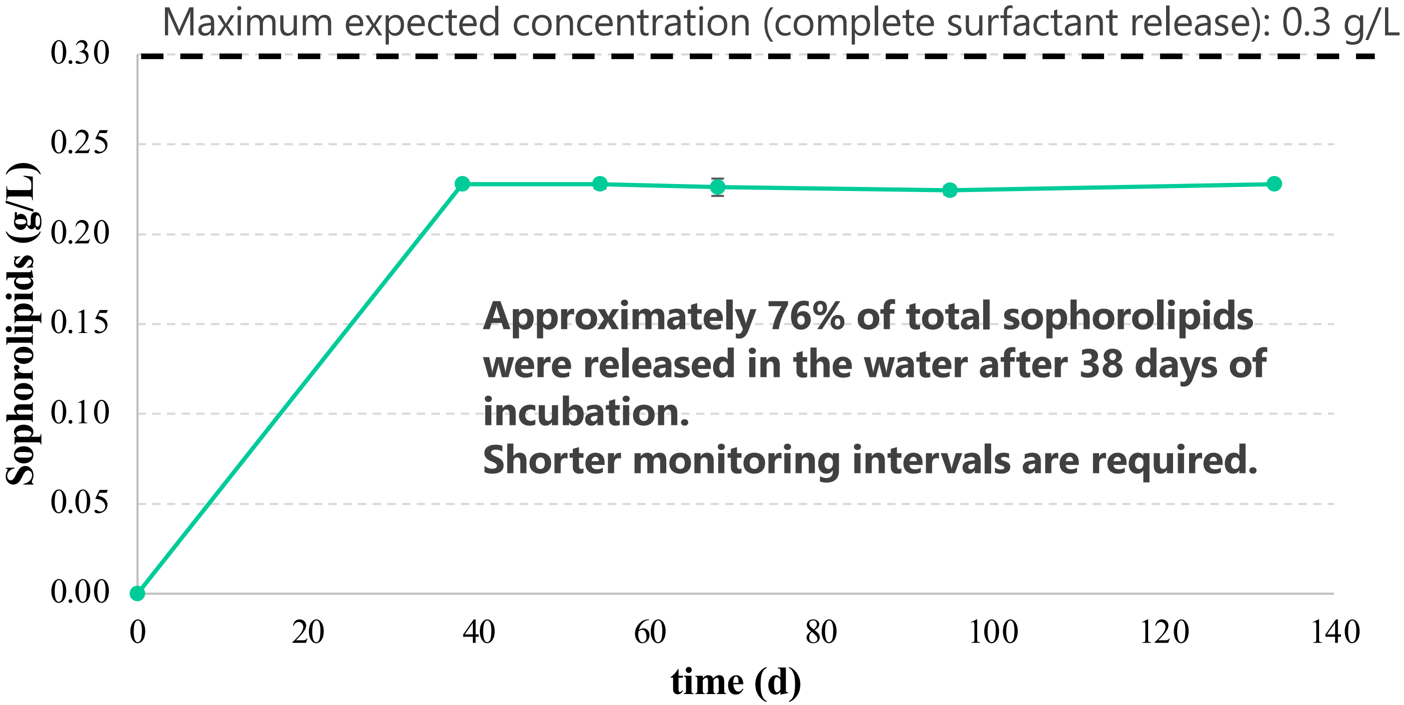
HPB-CD in hydrogel



Sophorolipids microspheres: Release of surfactant in sterile water

1 g of PBS microspheres was incubated statically at 20 °C in 100 mL of sterile water.

The tests were conducted in duplicate.



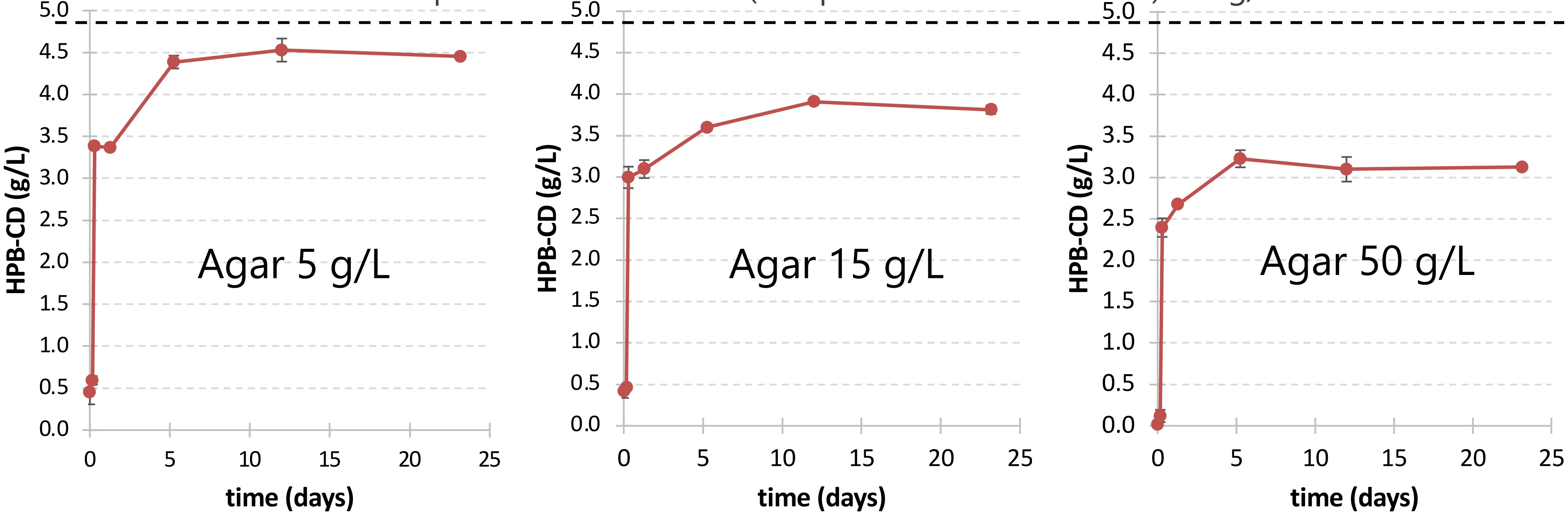
Maximum expected concentration (complete surfactant release): 0.3 g/L

Approximately 76% of total sophorolipids were released in the water after 38 days of incubation.

Shorter monitoring intervals are required.

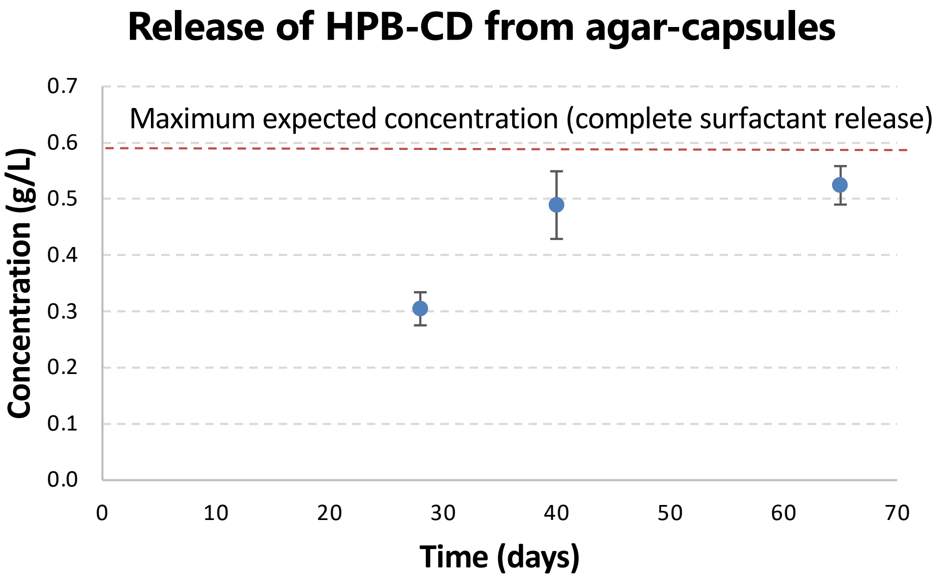
Release of HPB-CD from hydrogel capsules

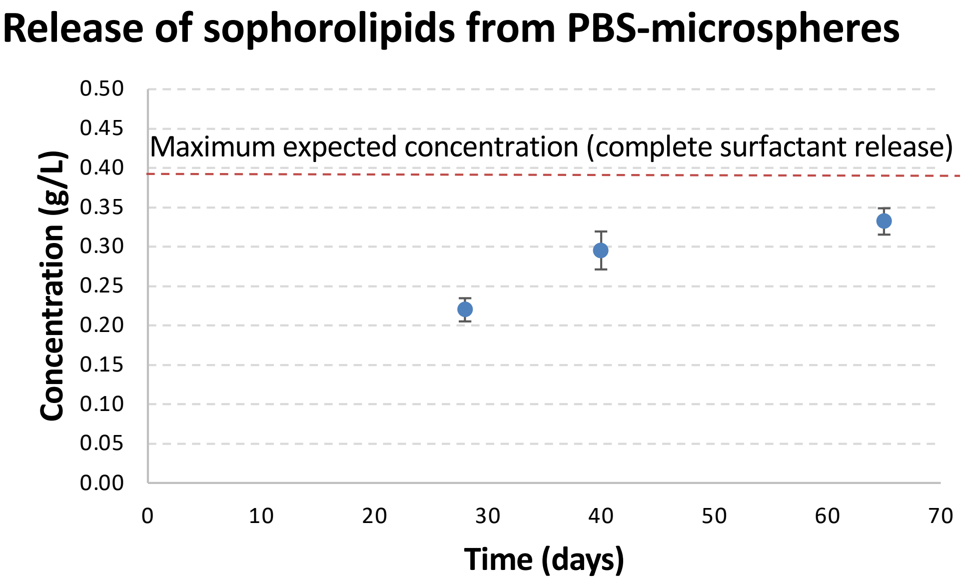
96 capsules with the **same concentration of HPB-CD** (50 g/L) and **different concentration of agar** (5, 15 and 50 g/L) were incubated statically at 20 °C in 200 mL of sterile marine water.


Maximum expected concentration (complete surfactant release): 4.8 g/L

1. Increasing agar concentration reduces the release of HPB-CD.
2. 50-70% (depending on agar concentration) of HPB-CD is released within the first 8 hours.

Application of encapsulated surfactants in sand slurries spiked with Dansk Blend crude oil – surfactants release



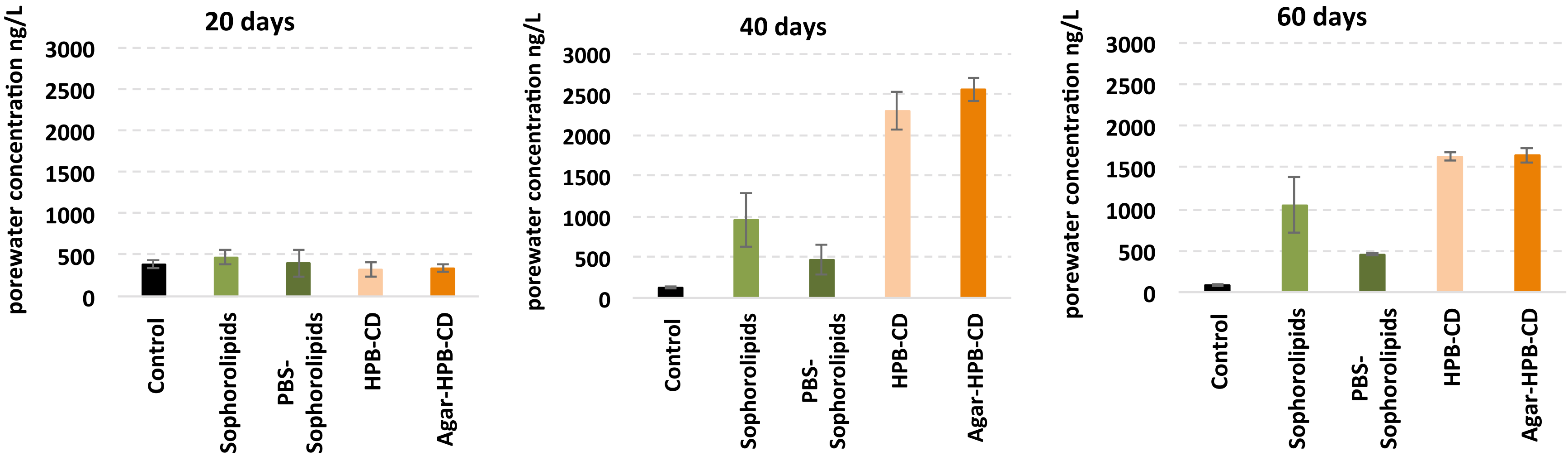


Approx. 87% of total HPB-CD released after 65 days of incubation.
Slower release when capsules are surrounded by sediment: possible limitations to diffusion

Approx. 81% of total sophorolipids were released in sand slurries after 65 days of incubation.

Application of encapsulated surfactants in sand slurries spiked with Dansk Blend crude oil – HC bioavailability

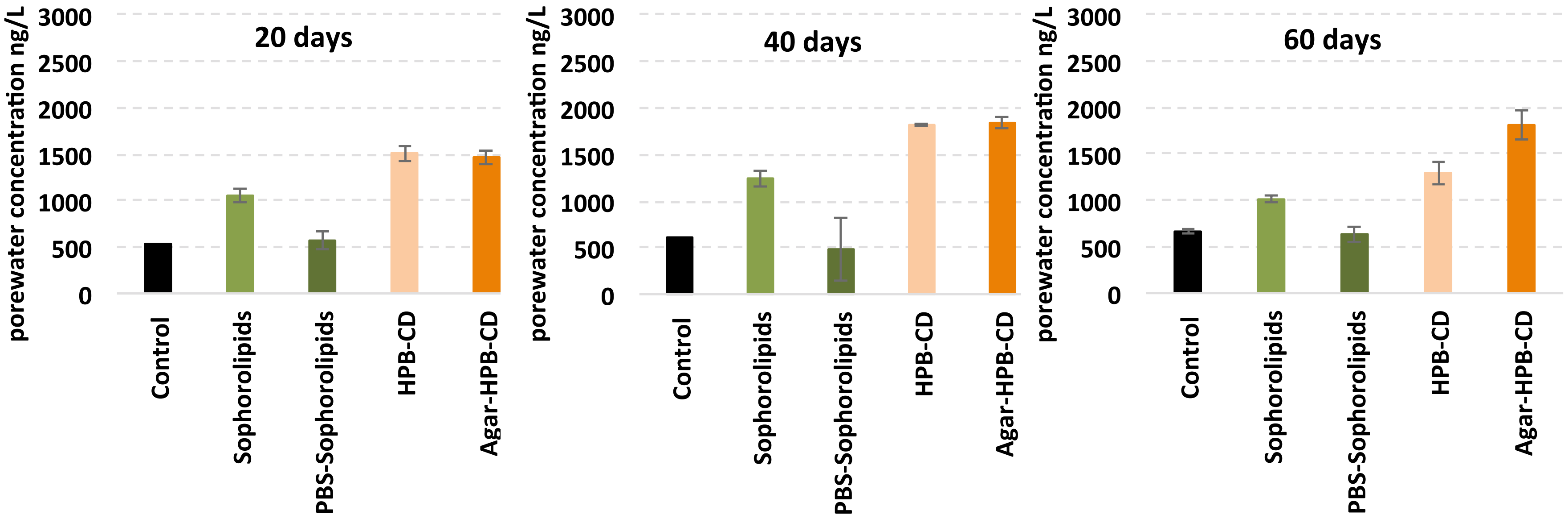
Porewater concentration of C_{10}-C_{40} *n*-alkanes in freshly spiked, sterile sand slurries



- ➢ While *n*-alkanes porewater concentration decreases over time in the un-amended controls (adsorption taking place), it increases when surfactants are added (increase of bioavailability).
- ➢ The effect of encapsulated surfactants on *n*-alkanes porewater concentrations is similar (**hydrogel-encapsulated HPB**) or approximately 50% (**PBS-encapsulated sophorolipids**) than that of free surfactants.

Application of encapsulated surfactants in sand slurries spiked with Dansk Blend crude oil – HC bioavailability (2)

Porewater concentration of C_{10}-C_{40} *n*-alkanes in weathered spiked, sterile sand slurries (encapsulated surfactants added 40 days after contamination)



- ➢ In weathered sand slurries, only **agar-encapsulated HPB** are able to increase *n*-alkanes pore similarly to the free agent.

Enhancement of PCB reductive dehalogenation in contaminated sediments

Several approaches have been successfully applied to stimulate PCB reductive dechlorination in sediment-free cultures or freshwater/estuarine sediment cultures developed with defined media:

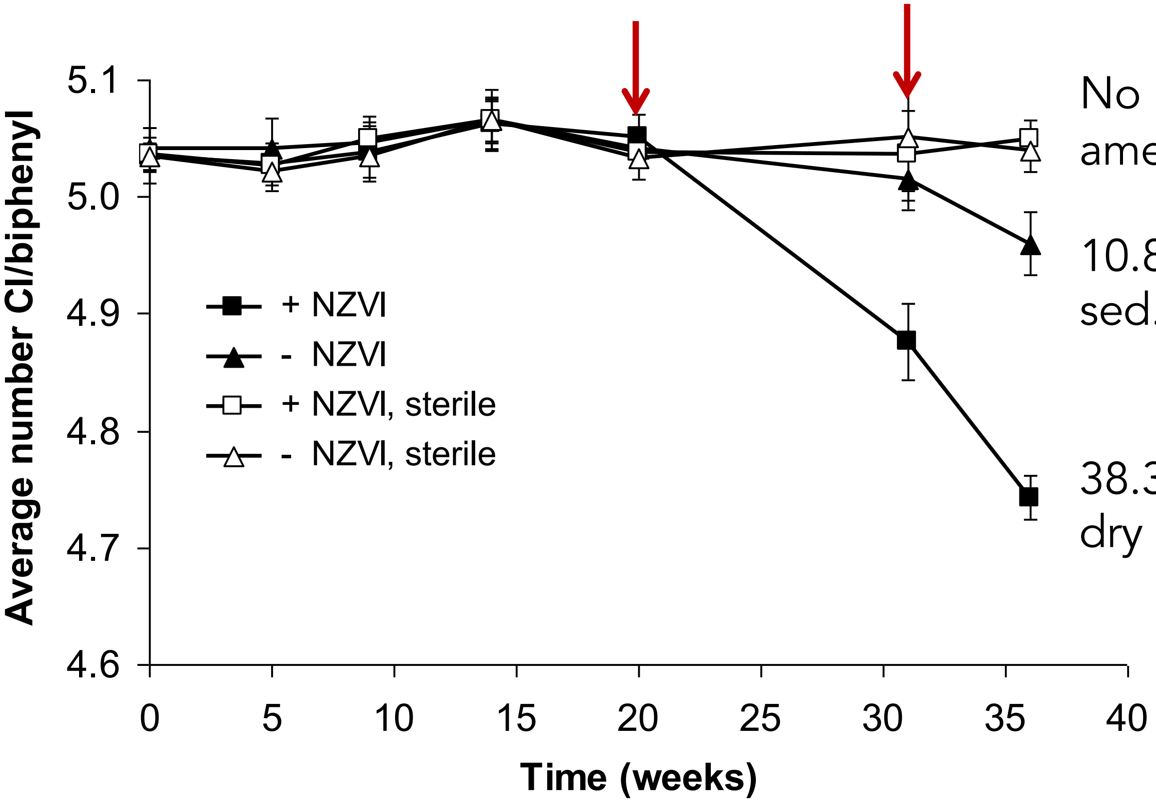
- Supplementation of electron donors:
- H_2 (not practical for in situ applications)
- Zerovalent iron ($Fe^0 + 2H_2O \rightarrow Fe^{2+} + 2OH^- + H_2$). Its positive effects on PCB dechlorination seem to be sediment-related
- Organic substrates (e.g., formate, acetate, pyruvate, lactate). Stimulation of competitors of dehalorespiring microbes, such as sulfate reducers and methanogens, was also reported

- Bioaugmentation with dehalorespiring exogenous bacteria

Enhancement of PCB reductive dehalogenation in contaminated sediments

Anaerobic slurry cultures of a VL sediment where a slow PCB dechlorination was previously detected after 5 months of incubation, suspended in the site water (20% v/v), spiked with Aroclor 1254 1000 mg/kg dry sed.

NZVI supplemented at the final concentration of 6.7 g Fe^0/kg dry sed.



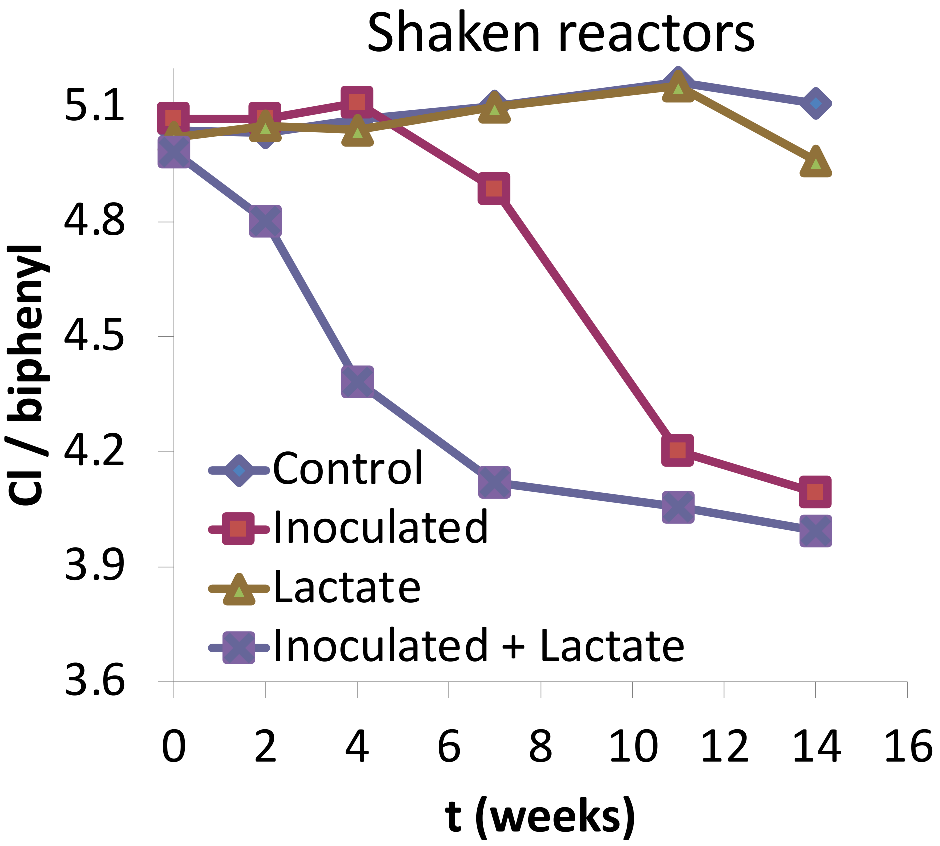
No PCB dechlorination in the NZVI amended sterile controls

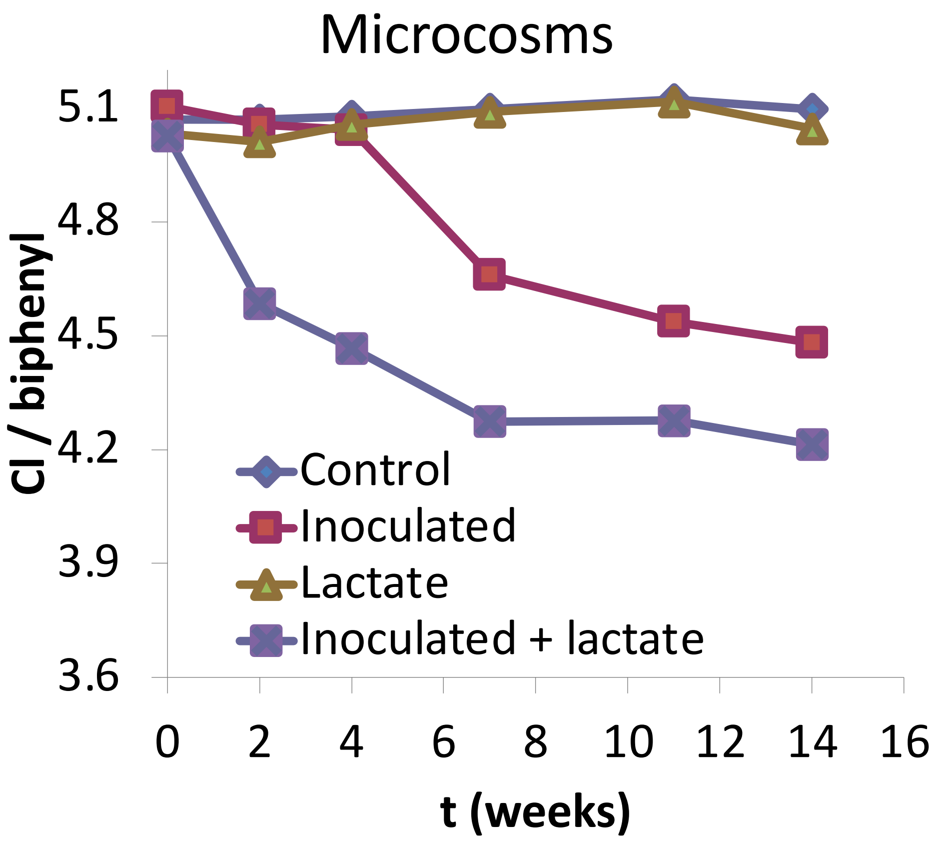
10.8±1.8 μmoles Cl removed kg dry sed.$^{-1}$ week^{-1}

38.3±5.7 μmoles Cl removed kg dry sed.$^{-1}$ week^{-1} (4-fold increase)

Zanaroli et al., 2012, J Chem Technol Biotechnol **87**:1246–1253

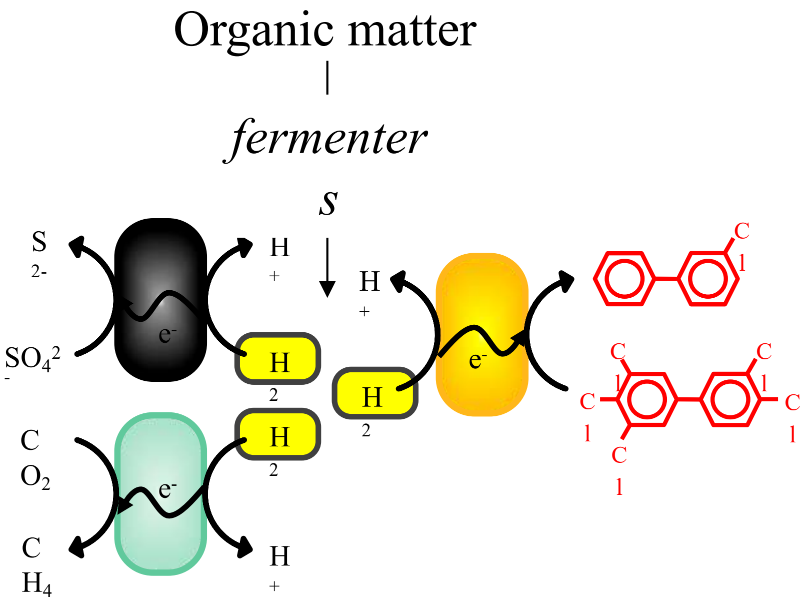
Enhancement of PCB reductive dehalogenation in contaminated sediments


Shaken reactors


Microcosms

- biostimulation alone with an electron donor (lactate) is not sufficient due to the low concentration of indigenous PCB halorespiring bacteria
- Autochnonous bioaugmentation (inoculated) remarkably stimulates the process, and its effect if further enhanced if combined with biostimulation (Inoculated + lactate)

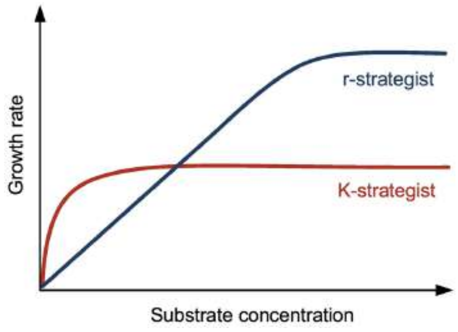
Main microbiological factors limiting biodegradation in contaminated environments addressed by bioremediation approaches



Competition for resources

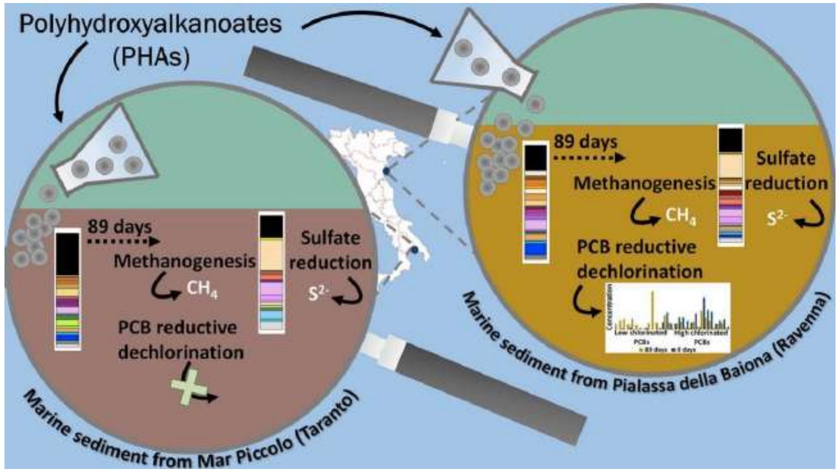
TEAP	H_2 Ks (mM)
Organohalide respiration	< 0.3
Sulfate reduction	1 - 3
Methanogenesis	> 5

k-strategist
r-strategist



→ need to release / generate in situ H_2 at controlled rate for long times to stimulate selectively organohalide respiration

Biostimulation of PCB reductive dechlorination in marine sediments through controlled H_2 release – use of biopolymers

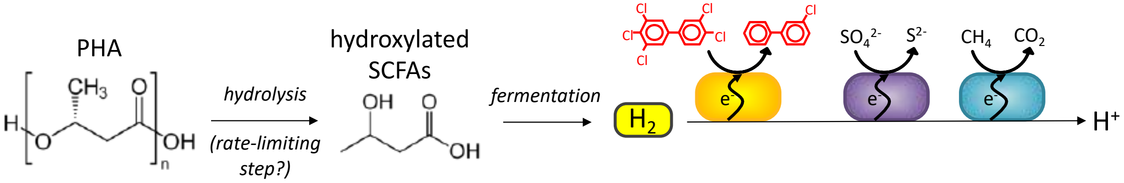


Mar Piccolo (MP)	**Piallassa Baiona (PB)**
Clay/silty	Sandy
Higher TOC	Lower TOC
Higher As, Cu, Pb, Zn	Higher Cr, Mn, PAH

Botti et al., Marine Pollution Bulletin 186 (2023) 114458
Botti et al., Science of the Total Environment 898 (2023) 165485

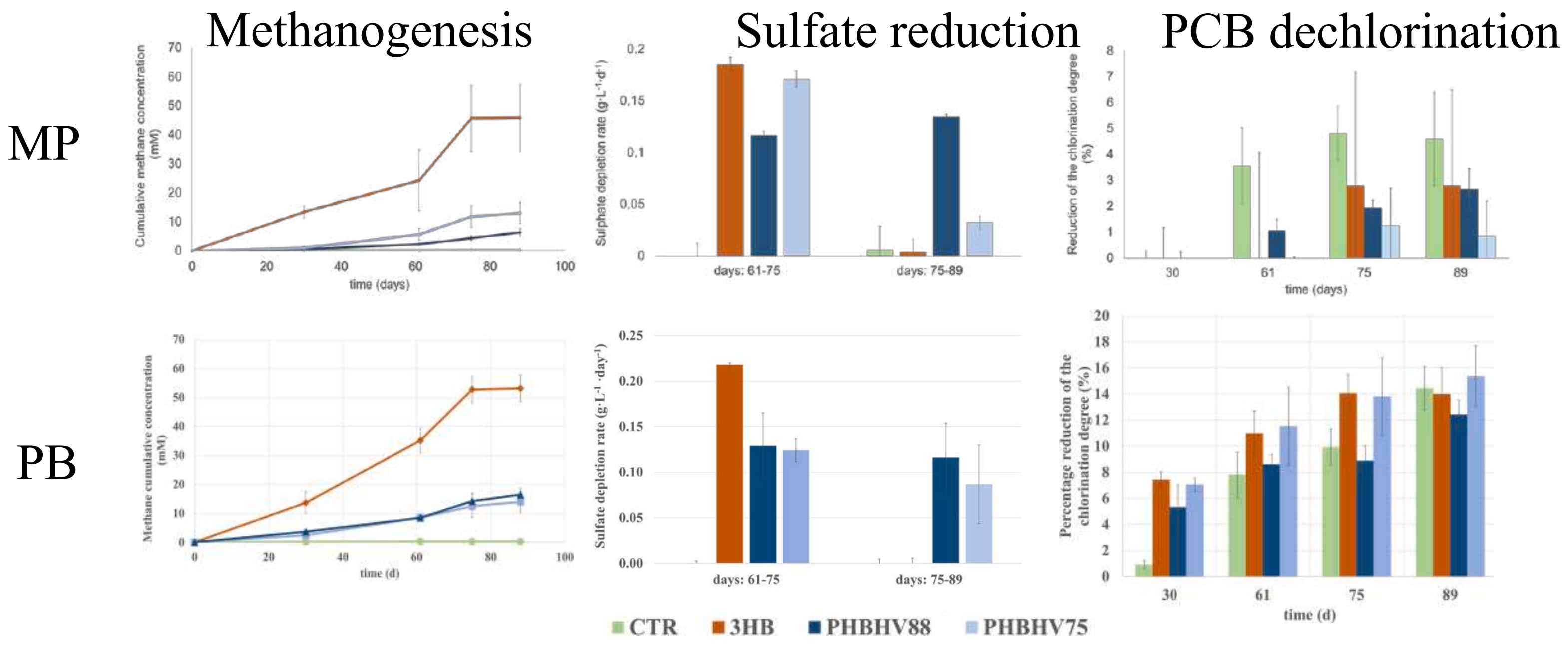
Sediments inoculated with a marine PCB dechlorinating culture and supplemented with PHA or 3HB to:

- evaluate if biopolymers (PHA) may be a selective, long-term, slow releasing electron donor for OHRB in marine environments



- assess their effect on the sediment microbiome

Biostimulation of PCB reductive dechlorination in marine sediments through controlled H_2 release – use of biopolymers

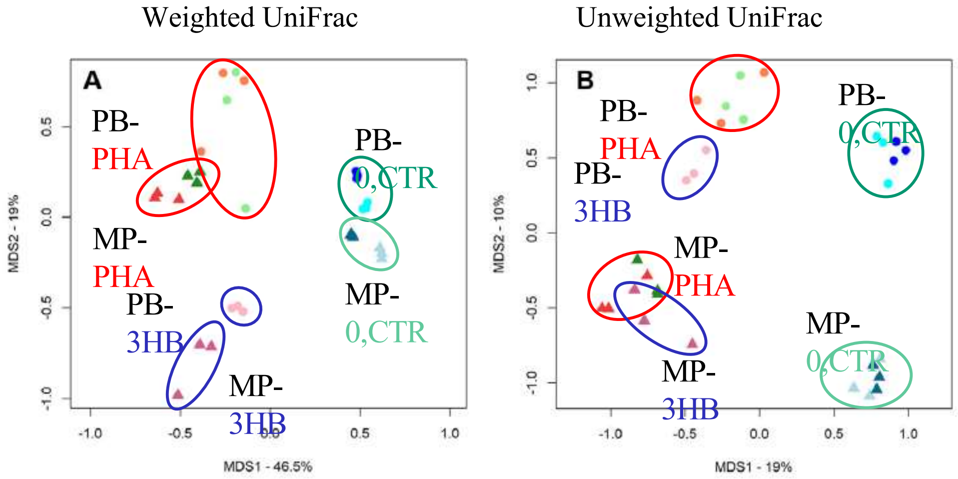


- The monomer (3HB) is fermented rapidly and strongly stimulates both sulfate reduction and methanogenesis;
- PHAs are fermented more slowly and stimulate sulfate reduction and methanogenesis at much lower extent;
- PCB reductive dechlorination is much lower in MP (where organic electron donors further inhibit it) than in PB, where PHAs do not significantly promote it.

→ PAHs are fermented too rapidly and cause the hyperproliferation of competing microbes

Botti et al., Marine Pollution Bulletin 186 (2023) 114458
Botti et al., Science of the Total Environment 898 (2023) 165485

Biostimulation of PCB reductive dechlorination in marine sediments through controlled H_2 release – use of biopolymers



PHAs separate from 3HB PB separates from MP

- Subdominant bacterial groups discriminated MP from PB
- Dominant bacterial groups discriminated 3HB from PHA

- Relevant proportion of the community (25% on average) putative SRB (up to 42% with 3HB)
- No enrichment of *Dehalococcoidia* and decrease of Chloroflexi with all amendments

→ **3HB enriched mainly for putative fermenters and syntrophs**, which represented the dominant bacterial groups (***Dethiosulfatibacteracea, Dethiosulfatibacter*** 21.5 %; *Synergistaceae, Thermovirga* 3.2-2.3 %; *Marinilabiliaceae* 9.2-4.3 %)

→ **PHA enriched mainly for putative hydrolytic/primary degraders** (dominant bacterial groups *Spirochaetaceae, Petrotogaceae* 6-1.6 %; *Williamwhitmaniaceae* 11.5 %)

Need to better understand interactions between the composition of resident microbial community and the chemical-physical parameters /inputs to design efficient bioremediation strategies

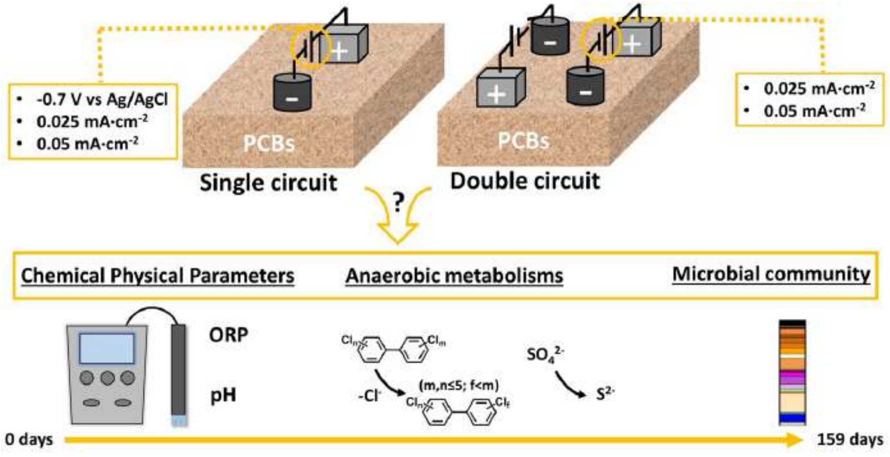
Botti et al., Marine Pollution Bulletin 186 (2023) 114458
Botti et al., Science of the Total Environment 898 (2023) 165485

Biostimulation of PCB reductive dechlorination in marine sediments through controlled H_2 release – electrostimulation

Sediment inoculated with a marine PCB dechlorinating culture and electrified to generate H_2 in situ (cathodic water electrolysis).

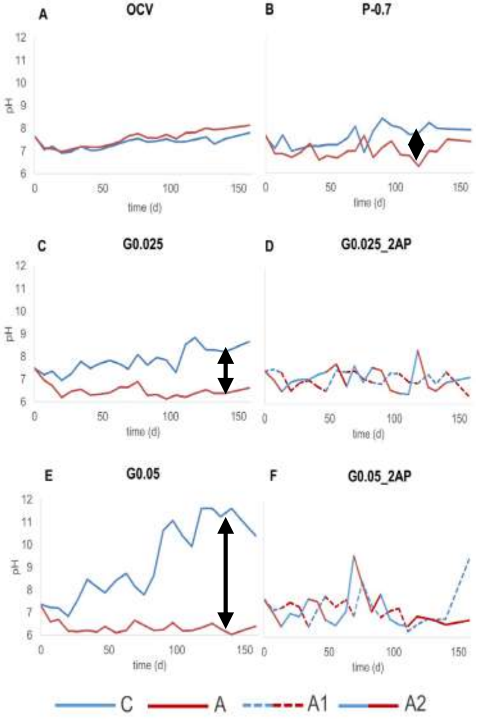
Effects on:

- sediment physical-chemical parameters (pH, ORP);
- PCB dechlorination and main competing TEAP (sulfate-reduction)
- sediment microbiome

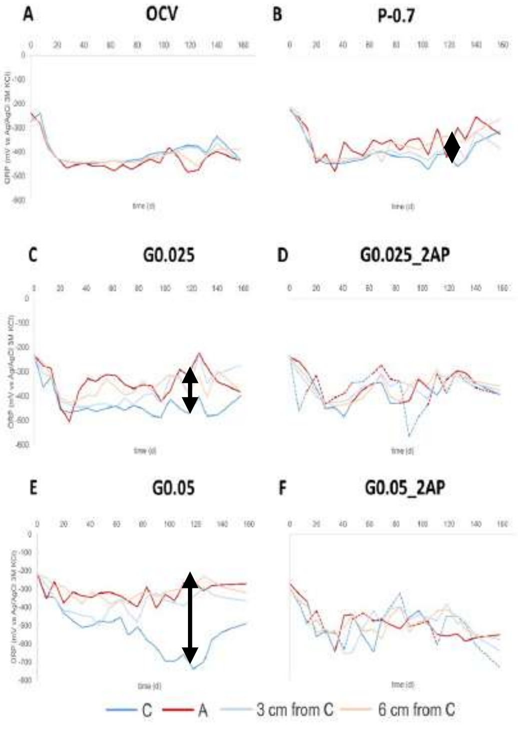


Botti et al. 2024, Journal of Hazardous Materials 469: 133878

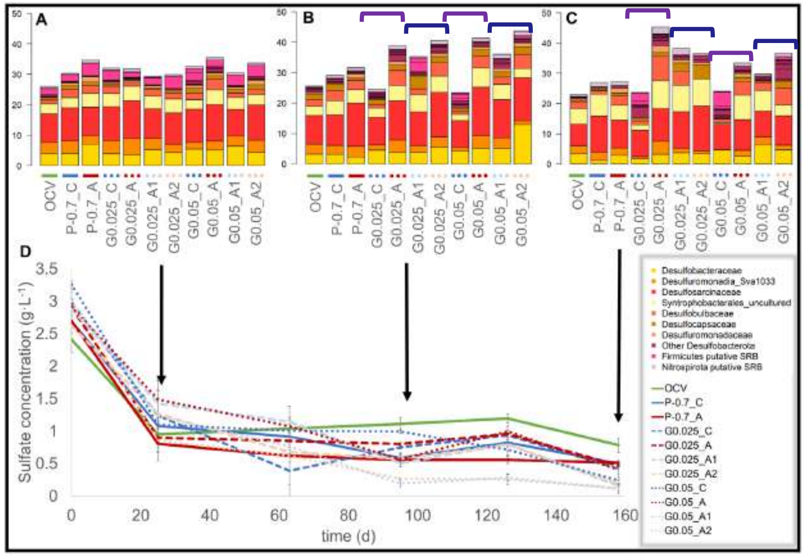
Sediment pH



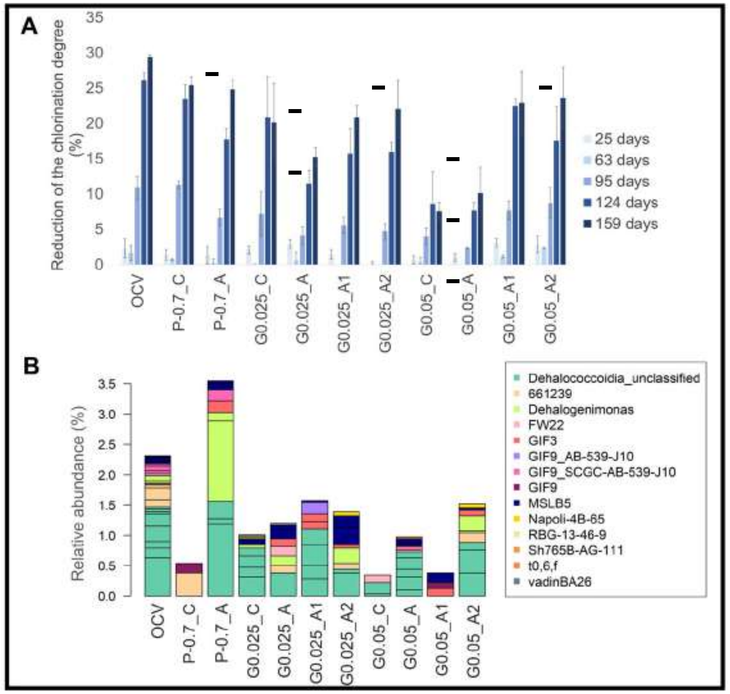
Sediment ORP



Biostimulation of PCB reductive dechlorination in marine sediments through controlled H_2 release – electrostimulation



- Sulfate reduction stimulated in electrified sediment (vs OCV) after day 21
- Selective enrichment in different putative SRB between anodic and cathodic areas, mitigated by polarity inversion

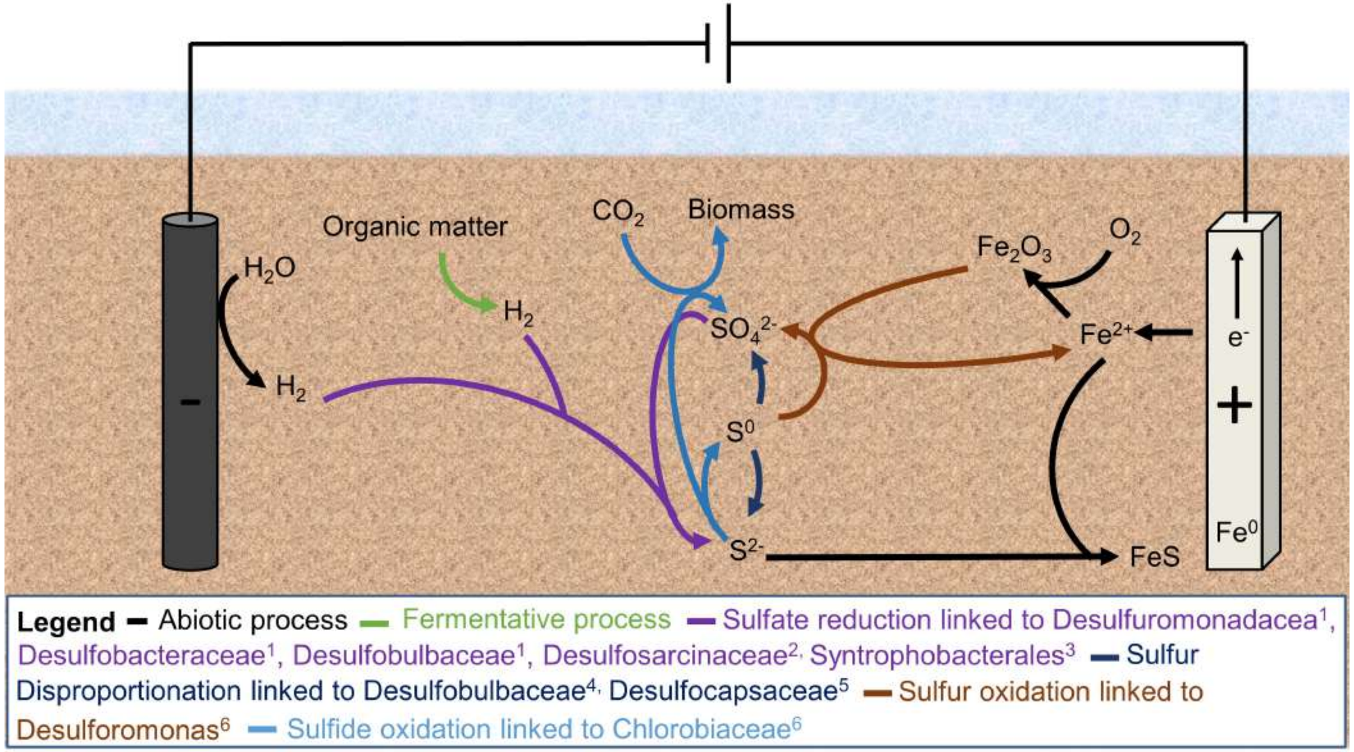


- Inhibition of PCB reductive dechlorination, proportional to electric input and mitigated by polarity inversion
- Decrease of putative OHRB

Botti et al. 2024, Journal of Hazardous Materials 469: 133878

Biostimulation of PCB reductive dechlorination in marine sediments through controlled H_2 release – electrostimulation

- Electron balance on sulfate reduction indicates more electrons are consumed by SRB as the current input increases;
- Coulombic efficiency >100% indicates electrons provided exceed those theoretically consumed by sulftate-reduction;
- Microbial community composition suggests S cycling may regenerate sulfate further supporting electrons consumtion by sulfate reduction



Botti et al. 2024, Journal of Hazardous Materials 469: 133878

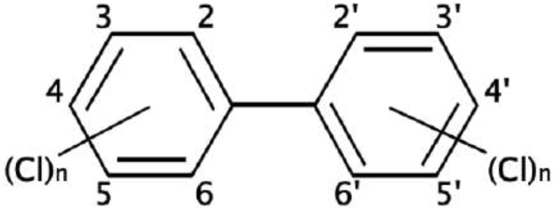
MPs-associated POPs

Polychlorinated Biphenyls (**PCBs**)
Polycyclic aromatic hydrocarbons (**PAHs**)
Dichlorodiphenyltrichloroethanes (**DDTs**)
Polybrominated diphenyl ethers (**PBDEs**)
Bisphenol A (**BPA**)

Organic contaminants in marine plastics debris

concentrations of 0.001–10 mg/kg *(3)*

Polychlorinated Biphenyls (PCBs)



- 209 congeners; complex commercial mixtures
- High persistence and hydrophobicity:
  - common **contaminants in marine sediments**
  - reported on **marine plastic pellets up to 7.5 mg/kg** *(4)* .
- May undergo **anaerobic reductive dechlorination processes** mediated by **organohalide respiring microorganisms occurring in marine sediments** *(5)*.

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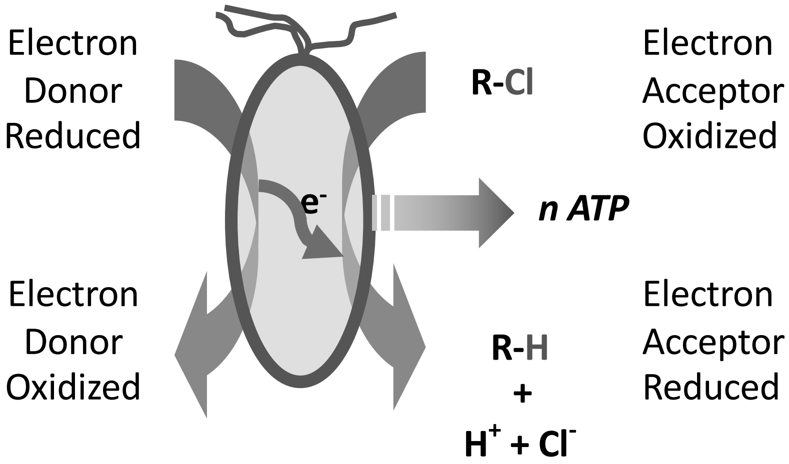
[3] Hirai, H. et al. *Marine Pollution Bulletin*, 62:1683-1692 (2011).

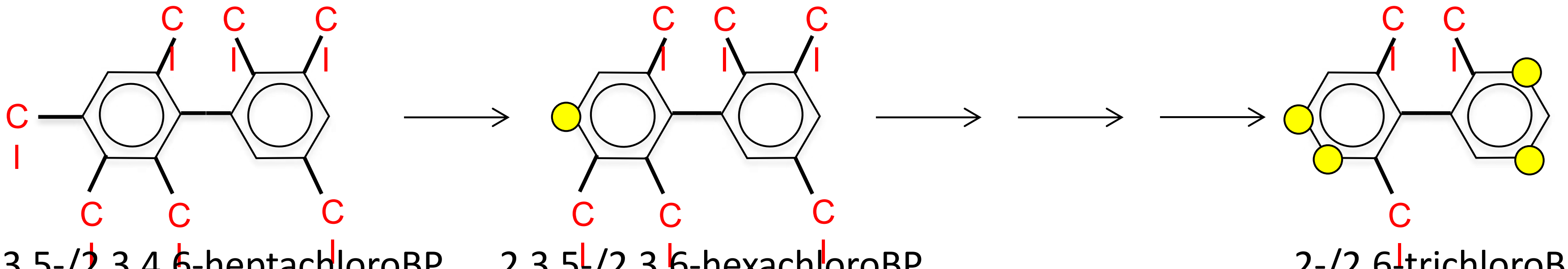
[4] Taniguchi, S., et al. *Marine Pollution Bulletin*, 106.1-2: 87-94 (2016).

[5] Zanaroli, G. et al. *Curr Opin Biotechnol* 33:287 (2015)

Anaerobic microbial
PCB reductive dehalogenation



- PCBs used as terminal electron acceptors by organohalide respiring microbes *(5-7)*
- Highly chlorinated congeners are bioconverted into less chlorinated products, which are often less toxic, less prone to bioaccumulation (less hydrophobic) and more amenable to aerobic degradation *(8)*



2,3,5-/2,3,4,6-heptachloroBP

2,3,5-/2,3,6-hexachloroBP

2-/2,6-trichloroBP

→ If taking place on MP-sorbed PCBs, this microbial process might change the composition, and thus the toxicity and bioavailabilty, of the sorbed PCB mixture.

[6] Bedard ,2008. *Annu Rev Microbiol* 62:253; [7] Field & Sierra-Alvarez, 2008. *Rev Environ Sci Biotechnol* 7:211; [8] McFarland & Clarke, 1989 *Environmental Health Perspectives* 81: 225-239.

Aim of the study

To investigate

- ✓the colonisation dynamics &
- ✓the potential biotransformation of sorbed PCBs

on different types of MPs (PE, PET, PS, PP, PVC; pristine *vs.* PCB-contaminated) by anaerobic marine sediment communities.

Experimental approach

- Low density Polyethyelene - **PE**
- Poly(ethylene terephthalate) - **PET**
- Polystyrene - **PS**
- Polypropylene - **PP**
- Poly(vinyl chloride) - **PVC**

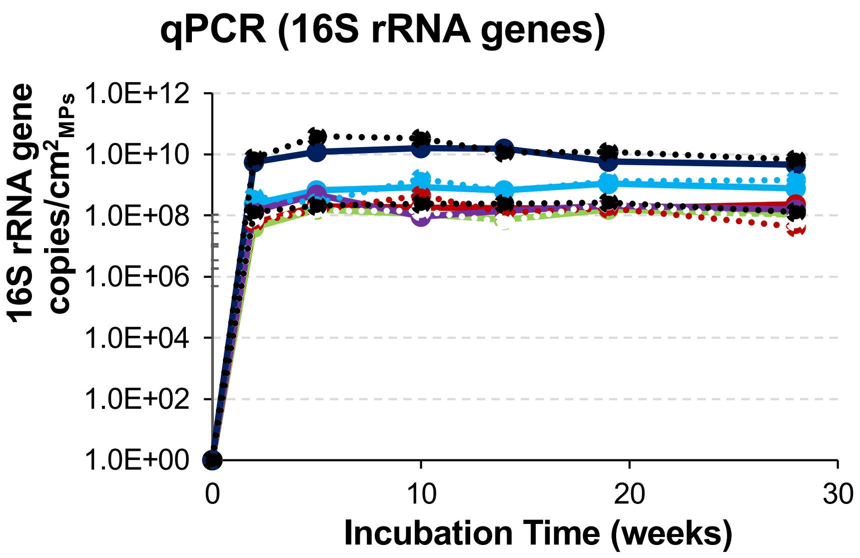
industrial pellets contaminated with a commercial mixture of **PCBs** (Aroclor 1254, **30 mg_{PCBs}/kg_{MPs}**).

- ➢ Incubation in sterilized slurry microcosms of marine sediment suspended in seawater (20 w/v) under **anaerobic conditions** and **inoculated with a marine, PCB-dechlorinating culture** enriched from a Venice lagoon sediment(*).

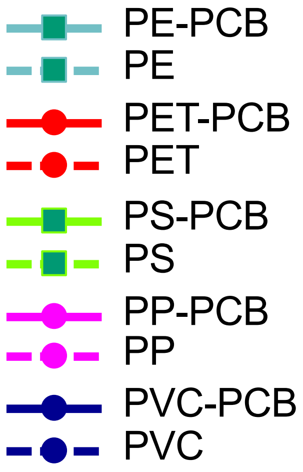
Treatments	MPs
MPs-**PCBs***	LDPE, PS, PET, PP, PVC
pristine MPs*	LDPE, PS, PET, PP, PVC
MPs-**PCBs** (sterile control)	LDPE, PS, PET, PP, PVC
Sediment-**PCBs** control*	NO MPs

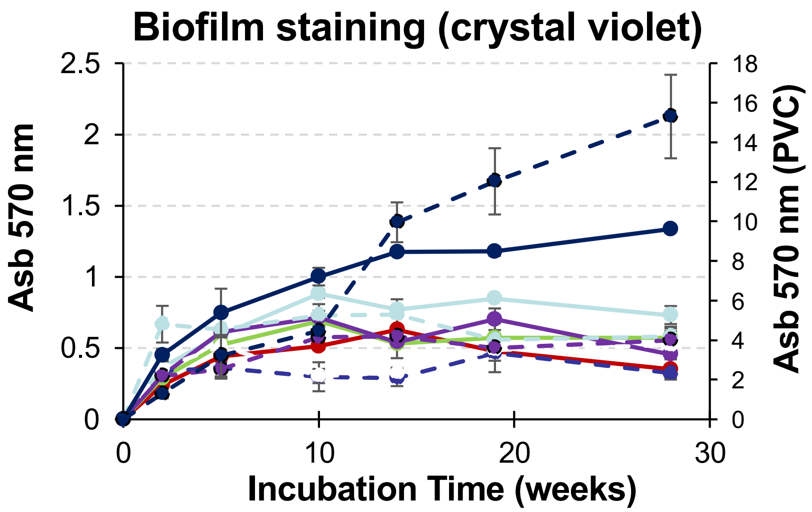
- ➢ Incubation time: 6 months; 6 samplings (0.5, 1, 2, 3, 4, 6 months)

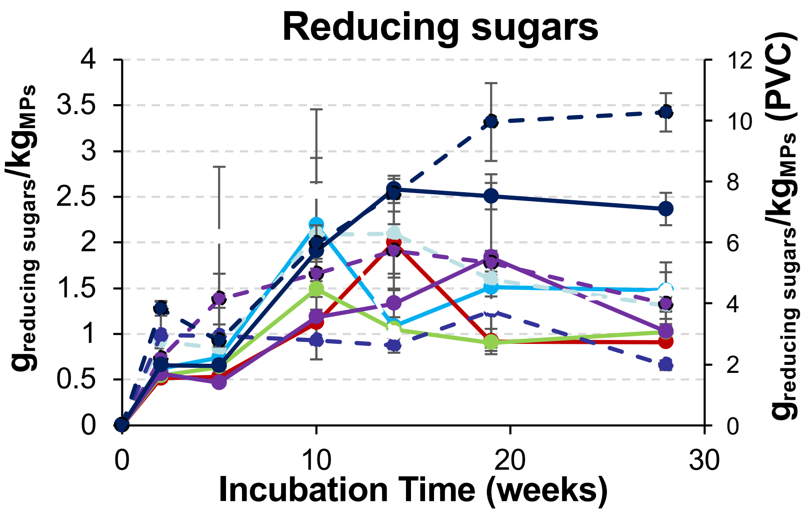
1. Quantification of biofilm


qPCR (16S rRNA genes)

- ✓ MPs were rapidly colonized by the microbial community (within 2 weeks).
- ✓ The most abundant cells concentration was observed on PVC pellets.
- ✓ Biofilm maturation occurred in the following weeks (further production of extracellular polymeric substance without remarkable increase of cell concentration).

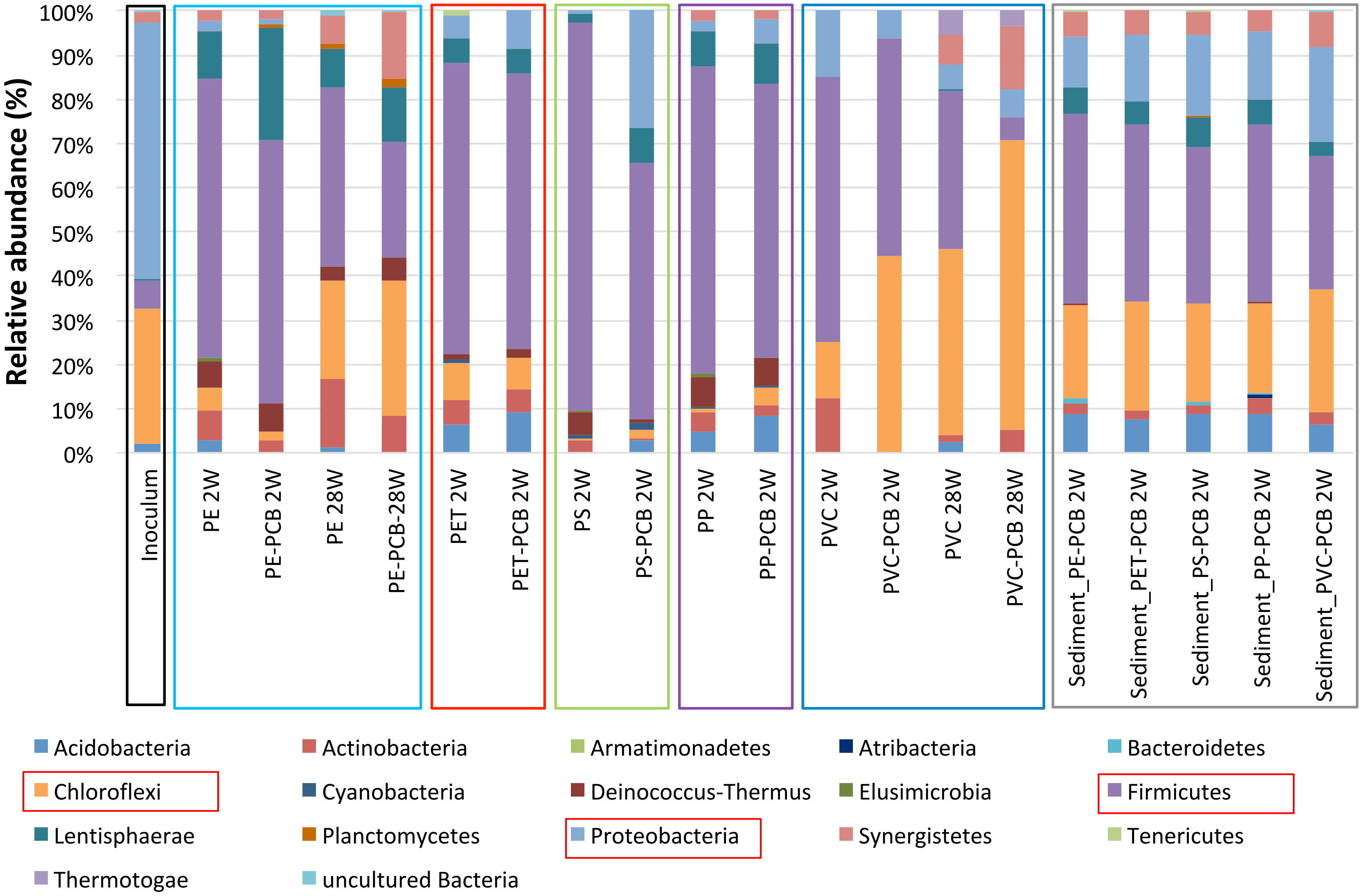



Biofilm staining (crystal violet)


Reducing sugars

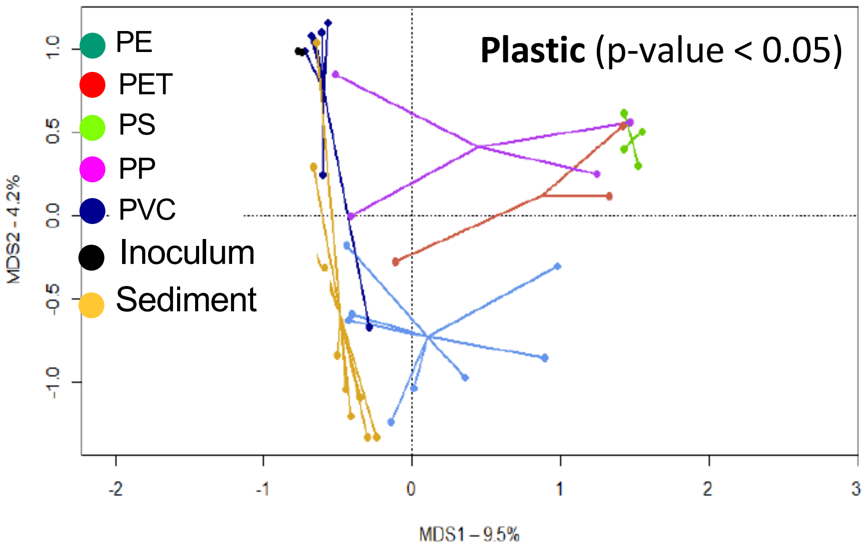
2. Characterization of biofilm

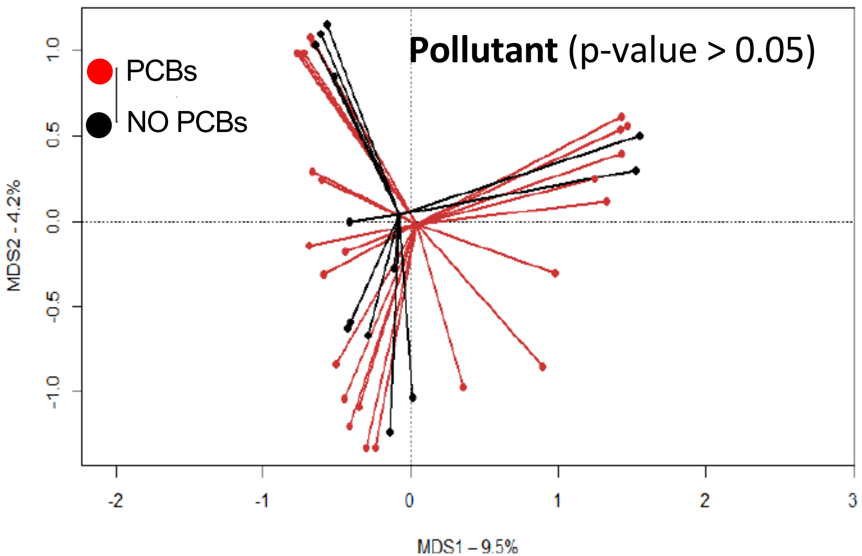
2.2 Illumina sequencing (16S rRNA genes)

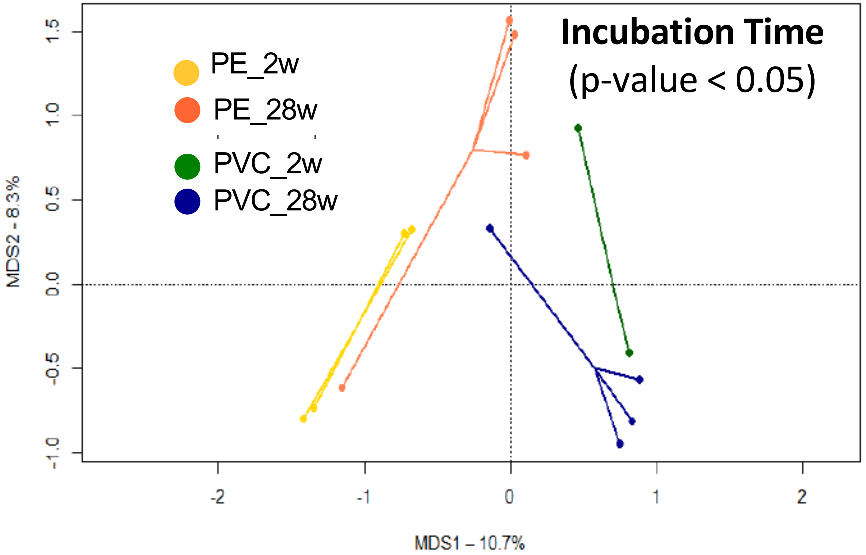


2. Characterization of biofilm

2.3 PCA – Beta diversity







The bacterial biofilm communities on MPs:

- ✓ significantly differ from the surrounding sediment and the inoculum communities;
- ✓ are affected by the type of MP;
- ✓ are not significantly correlated with the presence of sorbed PCBs.
- ✓ significantly change over time on PE and PVC.

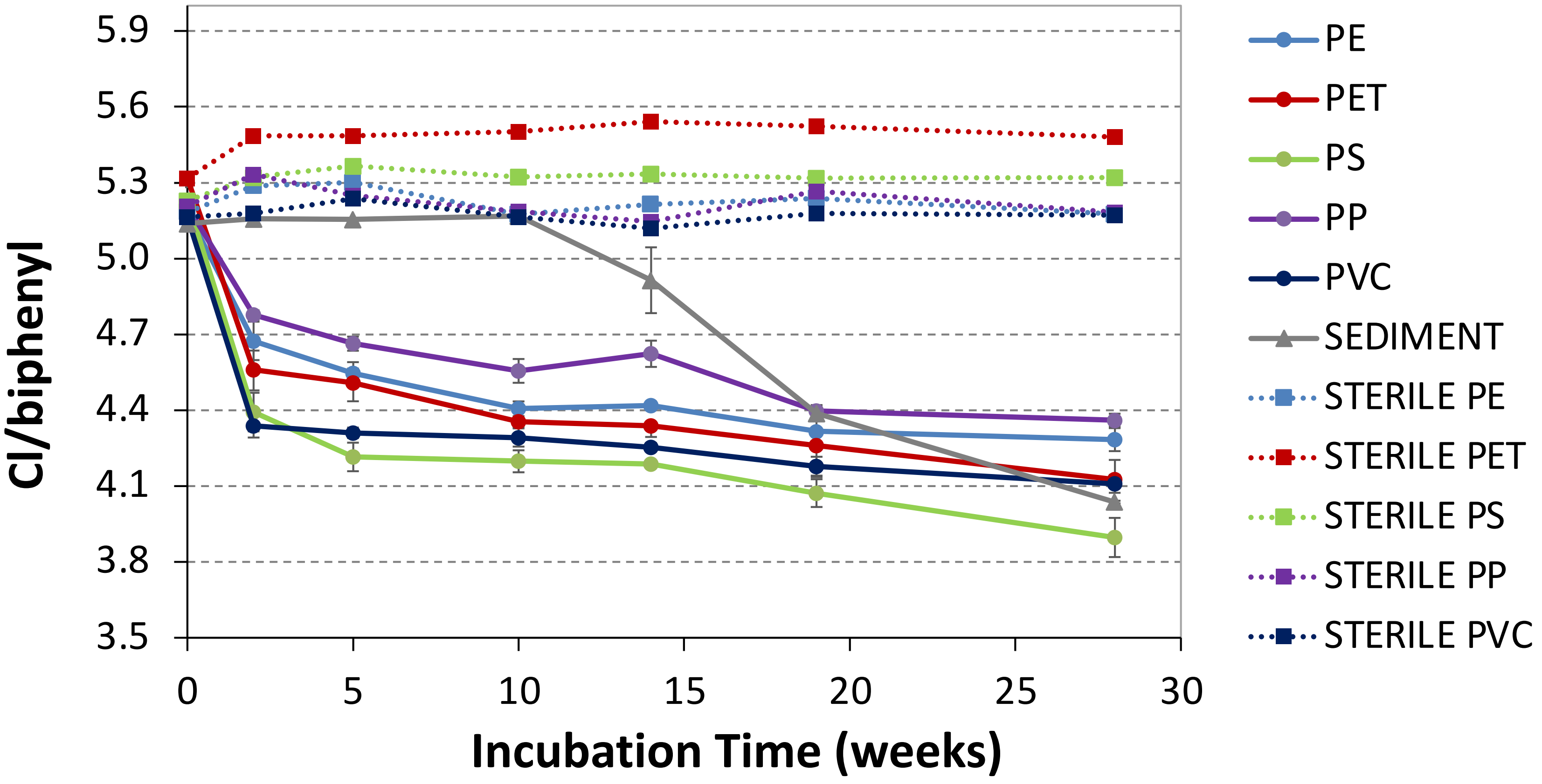
2. Characterization of biofilm

2.4 Organohalide respiring members

The presence of PCBs enriched the community in **Dehalococcoidia**, the organohalide respiring Chloroflexi members.

MPs	SAMPLE	% of total community	% of Phylum Chloroflexi
PE	No PCB	0.6	11
	PCB	1.4	75
	sediment	0.0	0
PET	No PCB	0.9	11
	PCB	2.7	39
	sediment	2.6	10
PS	No PCB	0.0	0
	PCB	1.7	91
	sediment	1.7	8
PP	No PCB	0.9	95
	PCB	3.2	80
	sediment	1.7	8
PVC	No PCB	10.0	80
	PCB	39.4	88
	sediment	1.2	4

3. PCB biotransformation

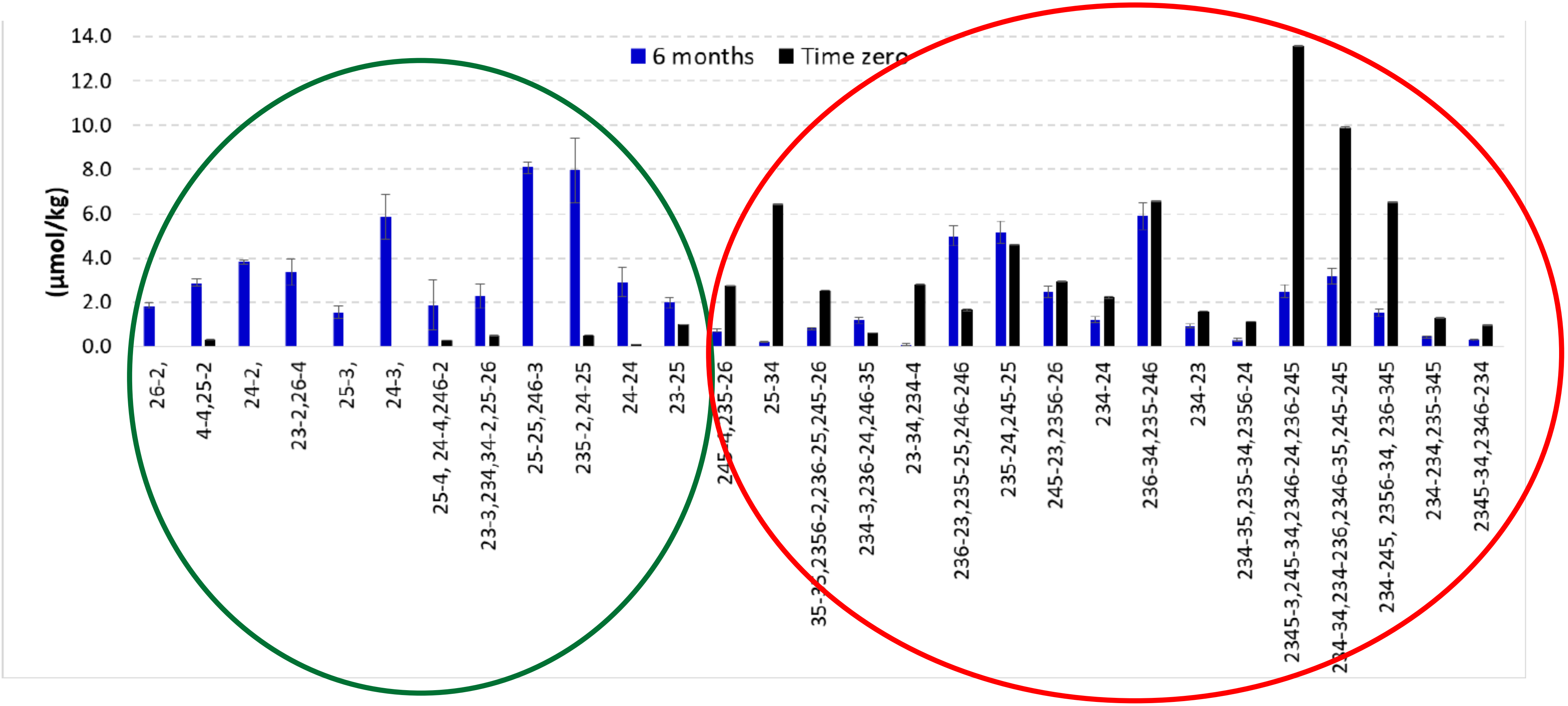


Reductive dechlorination of PCBs adsorbed to different MPs:

- ✓ PCB dechlorination faster on MPs than on sediment
- ✓ Dechlorination of sorbed PCBs: PP < PE < PET < PVC = PS

3. PCB biotransformation

The same dechlorination pattern (highly chlorinated congeners which are depleted and low chlorinated ones which accumulate) has been observed on all MPs. Dechlorination pattern (PE is shown as example):



Conclusions

- Different types of MPs can be **rapidly colonized** by a **dehalogenating marine microbial community**. A biofilm **maturation** follows, in which the microbial community richness and organization may change depending of the MP type.
- The **biofilm community composition is significantly affected by the type of polymer** and not by the presence of sorbed PCBs; however, the presence of PCBs increases the relative abundance of **Dehalococcoidia**, i.e., of the organohalide respiring Chloroflexi members, on the MPs.
- Complex mixtures of **PCBs sorbed on MPs can undergo microbial reductive conversion** into into less chlorinated products by marine biofilms.

The microbial colonization of contaminated MPs sunken in anaerobic sediments could thus change the toxicity and/or bioavailability of the sorbed PCB mixture.

Study currently under in progress in juvenile turbot (*Scophthalmus maximus*) at Universitade Nova de Lisboa (Poster Mariaelena D'ambrosio et al., > 222597)