



University of Naples "Federico II"

Marine Microbial Diversity

(Marine) Bioremediation

Bioremediation

Bioremediation is the use of biology to **degrade** or **immobilize** chemical compounds. It can be theoretically applied to the removal of any undesired compounds (organic or inorganic). Bioremediation has been used at a number of sites worldwide, including Europe, with varying degrees of success.

1972 - First commercial application: Sun Oil pipeline spill in Ambler, Pennsylvania

mid-1980s

Emphasis on **bioengineering organisms** for bioremediation. This technology did not live up to its initial promise

1990s

Emphasis switched to greater reliance on **natural microorganisms** and techniques to enhance their performance

Bioremediation: what is it?

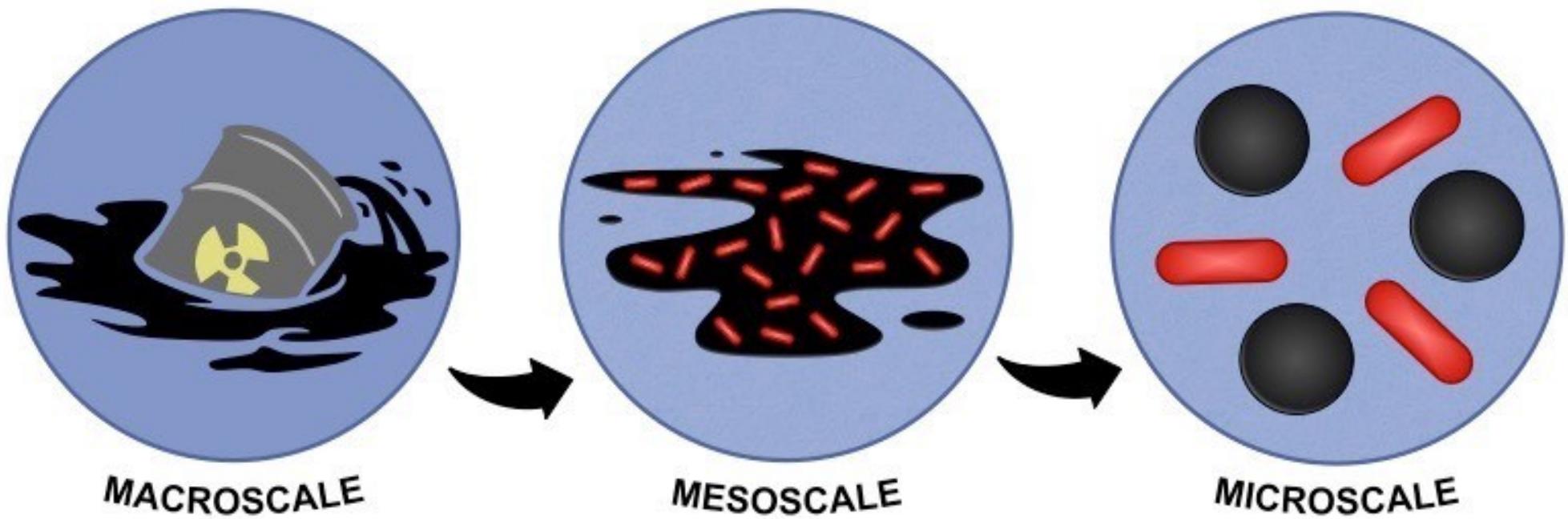
Generally speaking **bioremediations** refers to the use of biology (*sensu lato*) for the transformation or removal of unwanted compounds.

It refers to **a suite of different techniques** and approaches that can be classified in different ways depending on the focus of the classification approach.

Generally they are classified based on the location (*in situ* vs *ex situ*) or the approach (**intrinsic** vs **engineered**)



A multi-scale problem



Bioremediation: applications

Types of impact where bioremediation can be applied:

Petroleum hydrocarbons

Chlorinated solvents

Polynuclear aromatic hydrocarbons

Pesticides

TNT and a variety of other organic chemicals

Inorganic nitrogen (nitrate, ammonia)

Excessive nutrients and OM

Heavy metals (Pu, Np, Cr, As)

Radioactive metals (U, Th)

Naturally occurring vs man-made compounds

Persistent organic pollutants (POPs) are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes. Because of their persistence, POPs bioaccumulate with potential adverse impacts on human health and the environment.

Although some POPs arise naturally, for example volcanoes and various biosynthetic pathways, most are man-made via total synthesis.

The key difference is the concentrations and absolute quantities at which compounds derived from human activities occur in the environment.





POP's

(Persistent Organic Pollutants)
from Industry, Agriculture, Vessels, etc.

Flame retardants, fertilizers, industrial chemicals & other pollutants such as:

- PCBs
- PCDDs (Polychlorinated dibenzo-p-dioxins)
- PCDFs (Polychlorinated dibenzofurans)
- OCs (Organochlorine pesticides)
- PAHs (Polycyclic aromatic hydrocarbons)
- PBDEs (Polybrominated diphenyl ethers)
- APs (Alkylphenol)
- APnEOs (Alkylphenol ethoxylates)
- CPs (Chlorinated paraffins)
- PCNs (Polychlorinated naphthalenes)
- FOCs (Fluorinated organic compounds)

- Bitumen, Condensate, petroleum products
- Pesticides: from forestry, agriculture, industry
- Metals
- PPCPs (Pharmaceuticals & personal care products)
- Biological contaminants

Bioaccumulation from prey

TOXINS



Toxins concentrate as they move up the food chain and accumulate in whale tissue. Whales cannot expel toxins.

Toxin effects of increased mortality of prey

TOXINS



Smaller, fewer, and more toxic fish



Whales must eat 50% more fish to survive.

Environmental toxins result in poor fish reproduction (fewer fish hatch), lower lipids (fats) in each fish (each fish is less healthy), and greater fish mortality (more fish die). Whales need to eat up to 50% more fish to satisfy their nutritional requirements, and the fish they are eating are more toxic, resulting in a higher toxin load in whales.

Direct exposure to air and sea borne toxins via contact, breathing, and ingestion.

TOXINS

Exhaust fumes from vessels - including whale watching boats.

- CO_x (Carbon oxides)
- SO_x (Sulfur oxides)
- NO_x (Nitrogen oxides)
- PM (Particulate Matter)

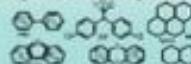
- HC (Hydrocarbons: unburned fuel; aromatics, alkanes, and alkenes; partially oxidized phenols and carbonyls)

Whales do not have an olfactory system or facial sinuses. They cannot detect or avoid engine exhaust, oil slicks or other toxins. Whales cannot prevent water-soluble toxins from entering their lungs.

Oil and chemical slicks on water surface

Bitumen
Tar balls,
and other
chemical
emulsions

Direct exposure to and ingestion of
chemicals, petroleum products



Adverse effects of toxins in marine mammals include: increased inflammation, toxicity; reduced reproductive, immune, and behavioural health, and increased mortality.

Bioremediation: ups

1. Bioremediation can be “natural process” and is therefore perceived by the public as an acceptable waste treatment process for contaminated material. Microbes able to degrade the contaminant increase in numbers when the contaminant is present.
2. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. The complete destruction of target pollutants is theoretically possible.
3. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.
4. Bioremediation can prove less expensive than other technologies that are used for cleanup of hazardous waste.

Bioremediation: downs

1. Bioremediation is generally **very costly, labor intensive, and time consuming** considering the extent of the impact considered.
2. Bioremediation is **limited to those compounds that are biodegradable**. Not all compounds are susceptible to rapid and complete degradation.
3. There are some concerns that the **byproducts of biodegradation** may be **more persistent or toxic** than the parent compound.
4. Biological processes are often **highly specific**.
5. It is difficult to extrapolate **from bench and pilot-scale studies to full-scale field operations**.
6. Bioremediation often takes **longer** than other treatment options, such as excavation and removal of soil or incineration.
7. **Regulatory uncertainty** remains regarding acceptable performance criteria for bioremediation.

Episodic vs chronic release



Bioremediation: *in situ* and *ex situ*

***In situ* bioremediation** means there is no need to excavate or remove soils or water in order to accomplish remediation. In situ biodegradation can involve the addition of microorganisms, oxygen and nutrients. In its intrinsic version is the most widely applied type of bioremediation, although has several drawbacks.

***Ex situ* bioremediation** requires excavation or removal of contaminated soil, sediments or waters (permanent or temporary) to facilitate microbial degradation. This technique is more expensive but it is able to overcome some of the disadvantage of in situ approaches.

Bioremediation: *intrinsic* vs *engineered*

Intrinsic bioremediation

This approach deals with stimulation of **indigenous or naturally occurring microbial populations** by feeding them nutrients and oxygen to increase their metabolic activity (**Biostimulation**). Chemotaxis is important to the study of insitu bioremediation because microbial organisms with chemotactic abilities can move into an area containing contaminants.

Engineered bioremediation

Involves the introduction of **external (exogenous) microorganisms** to enhance degradation (**Bioaugmentation**).

Basic Bioremediation Approaches

Natural attenuation – naturally occurring degradation of the pollutants by naturally occurring microbial populations ***not supported*** by human intervention

Biostimulation – Naturally occurring degradation of the pollutants by naturally occurring microbial population ***supported by the additions*** of nutrient, stimulants and surfactants

Bioaugmentation – Introduction of allochthonous engineered populations of microorganisms to enhance the degradation of the pollutants

Bioremediation: *intrinsic*

Natural attenuation is the process observed in the environment for which a contaminant concentration naturally decreases over time without human intervention.

This observation served as the basis for the development of intrinsic approaches that aim to stimulate the natural occurring (called also indigenous) microbial population capable of degrading the contaminant

This approach has several advantages over the engineered approach given the concern of releasing alloctonous organisms in the environment.

Microbial groups involved in degradation

Aerobic: Examples of aerobic bacteria recognized for their degradative abilities are *Pseudomonas*, *Alcaligenes*, *Sphingomonas*, *Rhodococcus*, and *Mycobacterium*. These microbes have often been reported to degrade pesticides and hydrocarbons, both alkanes and polyaromatic compounds. Many of these bacteria use the contaminant as the sole source of carbon and energy.

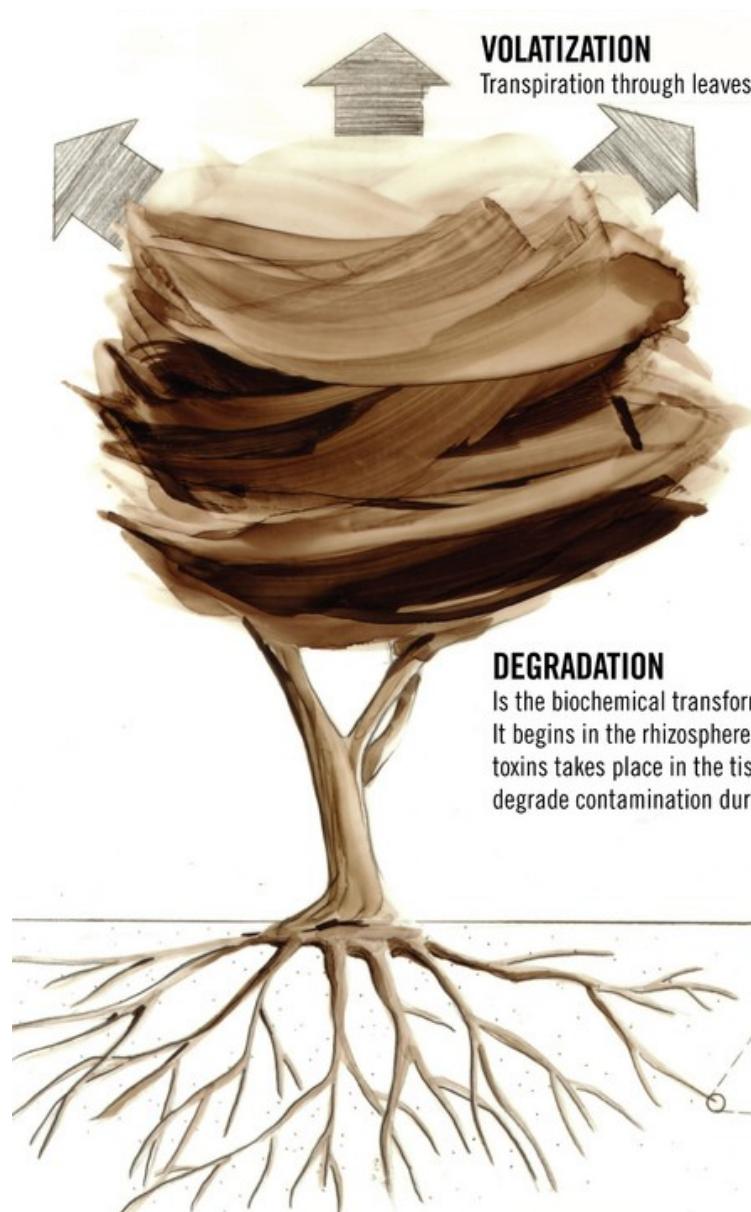
Anaerobic. Anaerobic bacteria are not as frequently used as aerobic bacteria. There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE) and chloroform.

Microbial groups involved in degradation

Ligninolytic fungi. Fungi such as the white rot fungus *Phanaerochaete chrysosporium* have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants

Methylotrophs. Aerobic bacteria that grow utilizing methane for carbon and energy. The initial enzyme in the pathway for aerobic degradation, methane monooxygenase, has a broad substrate range and is active against a wide range of compounds, including the chlorinated aliphatic trichloroethylene and 1, 2dichloroethane.

Bioremediation: not only microbes



DEGRADATION

Is the biochemical transformation of contaminants. It begins in the rhizosphere, then mineralization of toxins takes place in the tissues. Some plants may degrade contamination during this metabolism.

■ Released enzymes enhance the biochemical transformation of contaminants
Microbes (dots)

Rhizosphere is the area surrounding the roots. Released enzymes and drawing water encourages microbial activity. Microbes play a key role in the degradation of compounds.

CONTAINMENT

Is the use of trees that will uptake large quantities of water, you can hinder the travel of pollutants in the ground.

PHYTOREMEDIATION

Biostimulation

Biostimulation refers to the manipulation of the system to alter the conditions in order to favor microbial degradation.

This generally involves the addition of **oxygen**, **changes in pH**, **nutrients** and **trace elements** amendments, treatments to increase **bioavailability** and contaminant **surface accessibility**.

Biostimulation is the most widely used techniques due to the low potential for undesired side effects, (generally) known activity and fate of the used compounds and their relative low cost.

Bioaugmentation

Bioaugmentation refers to the addition of **exogenous microbial** groups to the contaminated substrate to supplement the indigenous populations.

These can be both **genetically engineered** or **non-engineered organisms**, and can be added both ***in situ*** and in ***ex situ*** decontamination facilities.

For this approach to be successful in the field, the seed microorganisms must be able to an efficient degrader, maintain genetic stability and viability during storage, survive in foreign and hostile environments, effectively compete with indigenous microorganisms, and have no undesired long-term ecological effects.

Since the 1970s, bioaugmentation has been proposed as an alternate strategy for the bioremediation of oil contaminated environments.

Bioaugmentation: ethics and ecology

Natural occurring population of degraders will generally respond to a contamination (especially episodic) by increasing rapidly in numbers both during natural attenuation and in the presence of bioremediation activities. Generally these population will revert to natural background abundances after the contaminant has been removed (keep in mind the concept of resilience and alternative stable state while thinking about this)

For exogenous organisms (both GMO and not) introduced during bioaugmentation it is difficult to predict their fate after the contaminant has been removed. They could, in theory, outcompete indigenous populations AFTER the contaminant has been removed and their long term ecological (and possibly economical) effects are difficult to predict.

Bioremediation: *in situ*

Aeration – Given that the majority of organics (hydrocarbons included) are degraded by **aerobic bacteria**, proper aeration of the substrate is important. This can be achieved directly by mechanical aeration or by air injection/bioventing/biosparging.

Surfactants and dispersants – To increase the bioavailability and surface area accessible for microbial degradation a variate of surfactants and dispersant can be added to the substrate

Nutrient addition – C:N:P ratio in addition to the presence of trace nutrients are very important for the rapid degradation of the contaminant. **Nutrient, fertilizers, labile organic compounds** and **trace elements** can be added in order to achieve and maintain appropriate nutrient balance to support degradation

Bioaugmentation – Indigenous microbial species might not be equipped for the degradation of the contaminant and exogenous, either GEM or not, are added in order to increase degradation rates

Bioremediation: *ex situ*

Land farming: It is a simple technique in which contaminated soil is **excavated** and **spread over** a prepared bed and periodically tilled until pollutants are degraded.

Composting: Composting is a technique that involves **combining contaminated soil** with nonhazardous **organic amendants** such as manure or agricultural wastes.

Biopiles: Biopiles are a **hybrid of land farming and composting**. Essentially, engineered cells are constructed as aerated composted piles.

Bioreactors: **Slurry reactors or aqueous reactors** are used for *ex situ* treatment of contaminated soil and water pumped up from a contaminated plume. Bioremediation in reactors involves the processing of contaminated solid material e.g. soil, sediment, sludge or water through an engineered containment system.





Organics

Simple hydrocarbons and petroleum fuels - Degradability decreases as molecular weight and degree of branching increase

Aromatic hydrocarbons - One or two ring compounds degrade readily, higher molecular weight compounds less readily

Alcohols, esters - Nitrobenzenes and ethers degrade slowly

Chlorinated hydrocarbons - decreasing degradability within increasing chlorine substitution – highly chlorinated compounds like PCBs and chlorinated solvents do not appreciably degrade aerobically

Pesticides are not readily degraded

Table 1: Microorganisms having biodegradation potential for xenobiotics.

Organism	Toxic chemicals	Reference
<i>Pseudomonas spp</i>	Benzene, anthracene, hydrocarbons, PCBs	Kapley <i>et al.</i> , 1999; Cybulski <i>et al.</i> , 2003
<i>Alcaligenes spp</i>	Halogenated hydrocarbons, linear alkylbenzene sulfonates, polycyclic aromatics, PCBs	Lal and Khanna, 1996
<i>Arthrobacter spp</i>	Benzene, hydrocarbons, pentachlorophenol, phenoxyacetate, polycyclic aromatic	Jogdand, 1995
<i>Bacillus spp</i>	Aromatics, long chain alkanes, phenol, cresol	Cybulski <i>et al.</i> , 2003
<i>Corynebacterium spp</i>	Halogenated hydrocarbons, phenoxyacetates	Jogdand, 1995
<i>Flavobacterium spp</i>	Aromatics	Jogdand, 1995
<i>Azotobacter spp</i>	Naphthalene, biphenyl	Jogdand, 1995
<i>Rhodococcus spp</i>	Aromatics, branched hydrocarbons benzene, cycloparaffins	Dean-Ross <i>et al.</i> , 2002
<i>Mycobacterium spp</i>	Hydrocarbons	Sunggyu, 1995
<i>Nocardia spp</i>	Aromatics	Park <i>et al.</i> , 1998
<i>Methosinus sp</i>	Hydrocarbons, polycyclic hydrocarbons	Jogdand, 1995
<i>Met hanogens</i>	Phenoxyacetate, halogenated hydrocarbon diazinon	Jogdand, 1995
<i>Xanthomonas spp</i>	PCBs, formaldehyde	Jogdand, 1995
	PCBs, polycyclic aromatics, biphenyls	Ijah, 1998
		Jogdand, 1995

Heavy Metals

Heavy metals are commonly defined as chemical elements with an atomic mass greater than 22 and a density greater than 5g/mL. This definition includes 69 elements, of which 16 are synthetic. Some of these elements are extremely toxic to human beings, even at very low concentrations.

- 1) Essential and important for metabolism (Na, K, Mg, Ca, V, Mn, Fe, Co, Ni, Cu, Zn, Mo and W)
- 2) Toxic heavy metals (Hg, Cr, Pb, Cd, As, Sr, Ag, Si, Al, Tl), which have no biological function in higher organisms
- 3) Radionuclides (U, Rn, Th, Ra, Am, Tc), which are radioactive isotopes
- 4) Semi-metals or metalloids (B, Si, Ge, As, Sb, Te, Po, At, Se)

Table 2: Microbes utilize the heavy metals:

Microorganism	Elements	References
<i>Bacillus spp.</i>	Cu, Zn	Philip <i>et al.</i> , 2000; Gunasekaran <i>et al.</i> , 2003
<i>Pseudomonas aeruginosa</i>	U, Cu, Ni	Sar <i>et al.</i> , 1999; Sar and D'Souza, 2001
<i>Zooglea spp.</i>	Co, Ni, Cd	
<i>Citrobacter spp.</i>	Cd, U, Pb	Gunasekaran <i>et al.</i> , 2003
<i>Chlorella vulgaris</i>	Au, Cu, Ni, U, Pb, Hg, Zn	Yan and Viraraghavan, 2001;
<i>Aspergilus niger</i>	Cd, Zn Zn, Ag, Th, U	Gunasekaran <i>et al.</i> , 2003
<i>Pleurotus ostreatus</i>	Cd, Cu, Zn	Pearson, 1969; Gunasekaran <i>et al.</i> , 2003
<i>Rhizopus arrhizus</i>	Ag, Hg, P	Guibal <i>et al.</i> , 1995; Gunasekaran <i>et al.</i> , 2003
<i>Stereum hirsutum</i>	Cd, Pb, Ca	Favero <i>et al.</i> , 1991
<i>Phormidium valderium</i>	Cd, Co, Cu, Ni	Gunasekaran <i>et al.</i> , 2003
<i>Ganoderma applanatum</i>	Cd, Pb	Gabriel <i>et al.</i> , 1994 and 1996
<i>Volvariella</i>	Cu, Hg, Pb	Gabriel <i>et al.</i> , 1994 and 1996
	Zn, Pb, Cu	Gabriel <i>et al.</i> , 1994 and 1996 Purkayastha & Mitra 1992, Iyeradaman 0

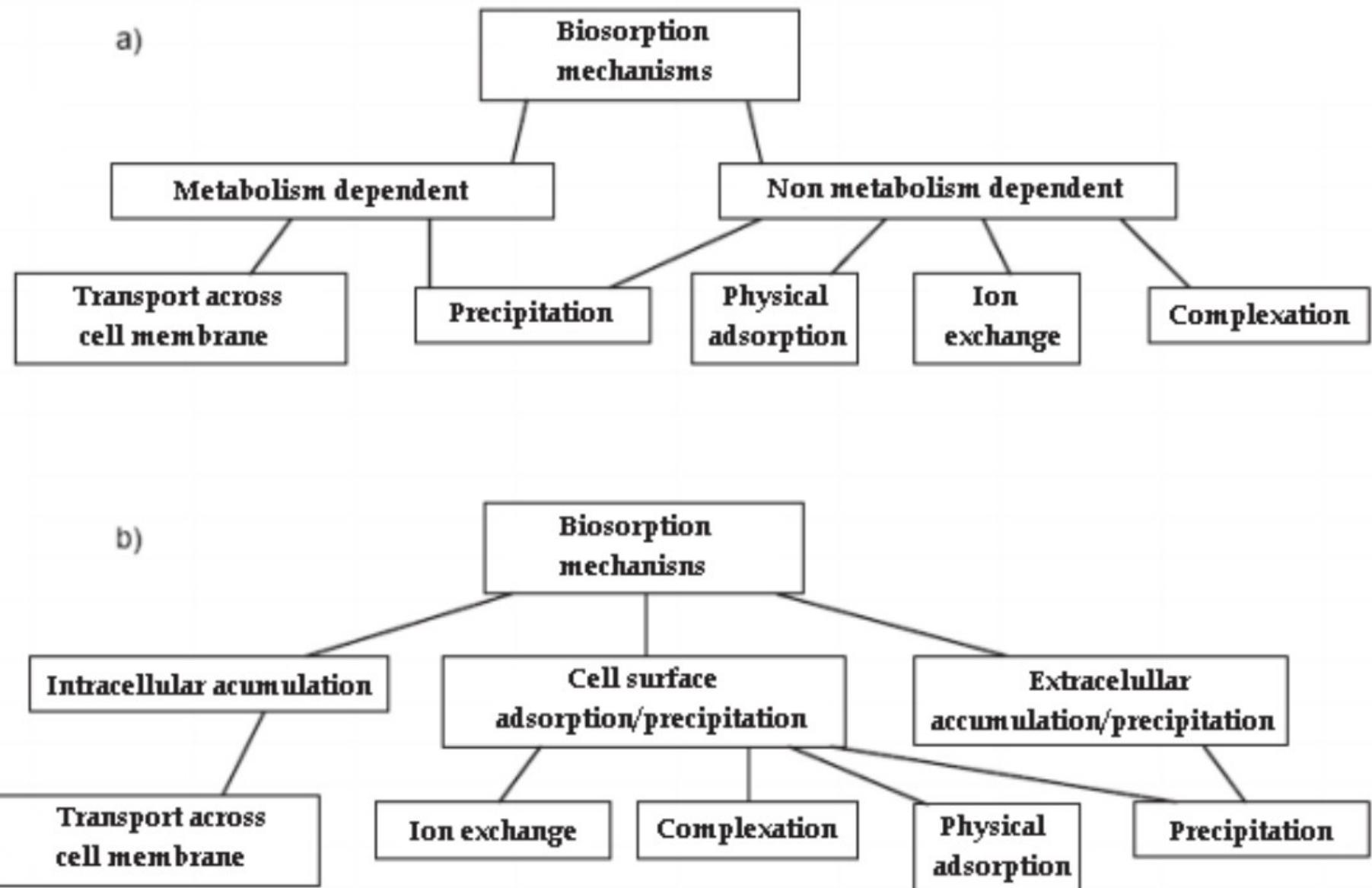


Fig. 2. Mechanisms of biosorption, a) classification according to dependence on cell metabolism, b) classification according to the location within the cell and the metal removed (Veglio & Beolchini, 1997).

Factors influencing bioremediation efforts

Contaminant concentrations: Directly influence microbial activity. Concentrations too high may have toxic effects. Low concentration may prevent induction of bacterial degradation enzymes

Contaminant bioavailability: Depends on the degree of sorption to solids or sequestration by molecules in the media

Site characteristics: Significant impact on effectiveness of bioremediation. Include pH, temperature, water content, nutrient availability, and redox potential.

Nutrients: Suitable amounts of trace nutrients are usually present, but nutrients can be added in a useable form to stimulate bioremediation.

Moisture content: Microbial growth requires an optimum presence of water in the environmental matrix (especially true for solids and certain chemicals)

Temperature: Directly affects the rate of microbial metabolism and consequently microbial activity in the environment.

The cost of cleaning

Different costs need to be take into account while considering bioremediation appraoches vs natural attenuation

Economic cost of bioremediation effort

Economic cost of the damage caused by the no-intervetion alternative

Economic cost of the post bioremediation dismantling

The cost of cleaning

Different costs need to be take into account while considering bioremediation appraoches vs natural attenuation

Economic cost of bioremediation effort

Economic cost of the damage caused by the no-intervetion alternative

Economic cost of the post bioremediation dismantling

Ecological cost of bioremediation effort

Ecological cost of the post bioremediation dismantling

Economic and ecological cost of long term effects of the bioremediation effort

The cost of cleaning

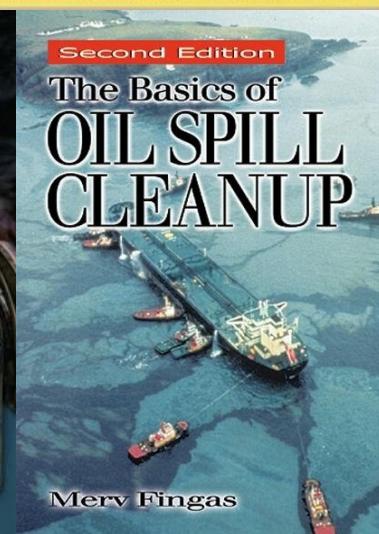
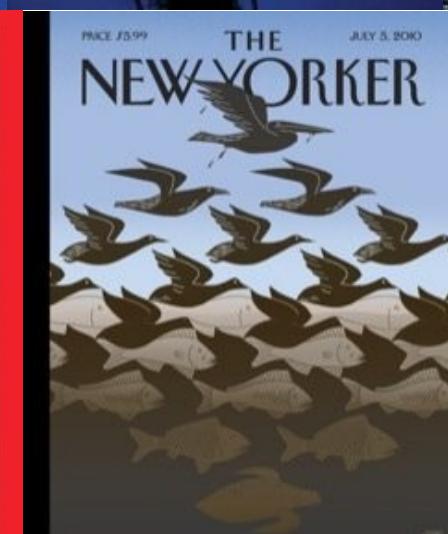
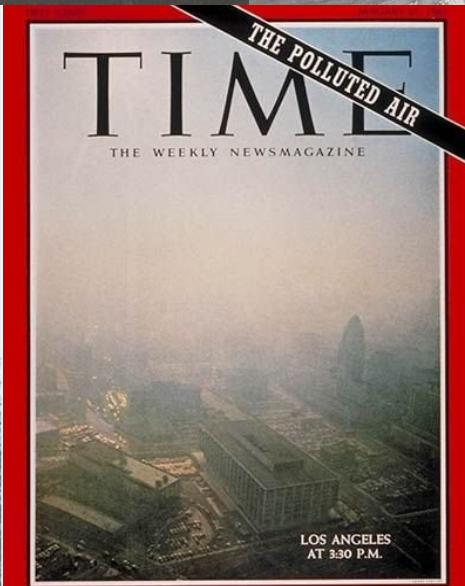
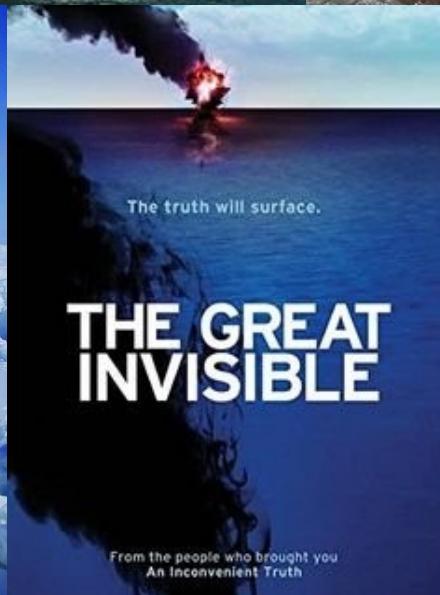
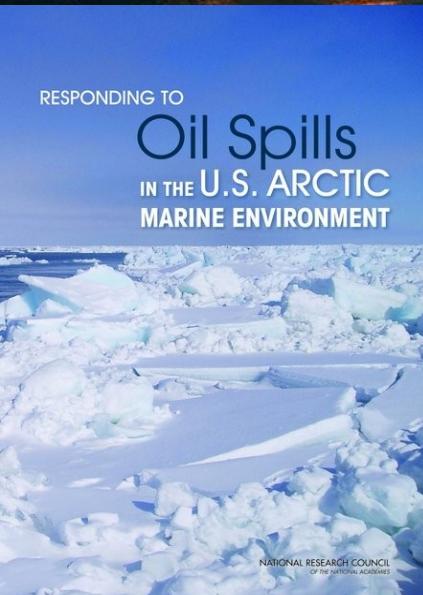
Table 2 Estimated costs of remediation strategies for hydrocarbon-polluted soils and sediments

Remediation strategy	Treating site	Cost (US \$/m ³) ^a
Biological		
Biostimulation	In situ	30–100
Bioaugmentation	In situ	30–100
Bioventing	In situ	79–970
Biopiles	Ex situ	130–260
Composting	Ex situ	630–757
Landfarming	Ex situ	30–70
Physicochemical		
Vapor extraction	In situ	405–1,485
Thermal desorption	Ex situ	44–252

^a Data from the US Federal Remediation Technologies Roundtable (2014).



BP DWH Spill – 61 Billion USD



Hydrocarbons spills

Petroleum is a **natural resource** confined in large deposits in the Earth crust.

Hydrocarbons are made of **organic matter** (i.e. marine organisms) that have been buried under variable pressure-temperature in anoxic conditions for millions of years.

Two events set the stage for the Age of Oil: The first was in 1846, when Abraham Gesner invented **kerosene making from coal and petroleum** practical raw materials for lighting fuel. The second was in 1859, when Edwin Drake invented the first **modern drilling process for deep oil wells**.

Oil spills became an international concern in **1967**, when ~120,000 tons of crude oil was released by the Torrey Canyon supertanker into the English Channel. This **first large-scale oil spill** forced UNO's International Maritime Organization to create in 1973 the International Convention for the Prevention of Pollution from Ships with the aim of designing emergency protocols and strategies toward oil spills.

Spill / Tanker	Location	Date	Tonnes of crude oil (thousands) ^[a]	Barrels (thousands)	US Gallons (thousands)	References
Kuwaiti Oil Fires ^{[dubious – discuss][b]}	Kuwait	January 16, 1991 – November 6, 1991	136,000	1,000,000	42,000,000	[6][7]
Kuwaiti Oil Lakes ^[c]	Kuwait	January 1991 – November 1991	3,409–6,818	25,000–50,000	1,050,000–2,100,000	[8][9][10]
Lakeview Gusher	Kern County, California, USA	March 14, 1910 – September 1911	1,200	9,000	378,000	[11]
Gulf War oil spill ^[d]	Kuwait, Iraq, and the Persian Gulf	January 19, 1991 – January 28, 1991	818–1,091	6,000–8,000	252,000–336,000	[9][13][14]
Deepwater Horizon	United States, Gulf of Mexico	April 20, 2010 – July 15, 2010	560–585	4,100–4,900	172,000–180,800	[15][16][17][18][19]
Ixtoc I	Mexico, Gulf of Mexico	June 3, 1979 – March 23, 1980	454–480	3,329–3,520	139,818–147,840	[20][21][22]
Atlantic Empress / Aegean Captain	Trinidad and Tobago	July 19, 1979	287	2,105	88,396	[23][24][25]
Fergana Valley	Uzbekistan	March 2, 1992	285	2,090	87,780	[26]
Nowruz Field Platform	Iran, Persian Gulf	February 4, 1983	260	1,900	80,000	[27]
ABT Summer	Angola, 700 nmi (1,300 km; 810 mi) offshore	May 28, 1991	260	1,907	80,080	[23]
Castillo de Bellver	South Africa, Saldanha Bay	August 6, 1983	252	1,848	77,616	[23]
Amoco Cadiz	France, Brittany	March 16, 1978	223	1,635	68,684	[23][26][26][28][29]
Taylor Energy	United States, Gulf of Mexico	September 23, 2004 – Present	210–490	1,500–3,500	63,000–147,000	[30]

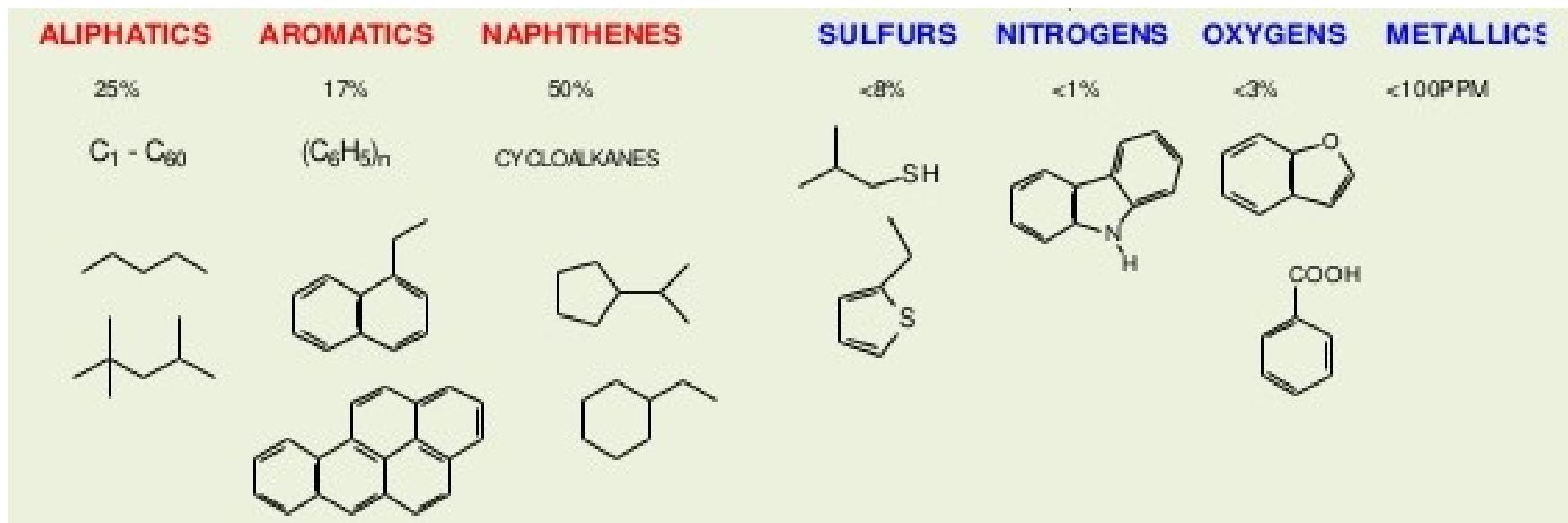
Hydrocarbons chemistry

Petroleum is mainly composed by three distinct hydrocarbon fractions.

Paraffin is usually the most abundant fraction and contains linear and branched aliphatic hydrocarbons.

Naphthenes are alicyclic hydrocarbons composed by one or more saturated rings with or without lateral aliphatic branches.

The **aromatic fraction** is composed by hydrocarbons containing at least one aromatic ring.



Hydrocarbons chemistry

Each petroleum fraction is usually composed by hundreds of different hydrocarbon molecules rather than a defined composition. Thus, fractions are dissimilar in terms of volatility, **bioavailability**, **toxicity**, **degradability**, and **persistence**.

The most common petroleum hydrocarbons include aliphatic, branched, and cycloaliphatic alkanes, as well as monocyclic and polycyclic aromatic hydrocarbons (PAHs). PAHs include naphthalene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo[a]anthracene, and benzo[a]pyrene.

Unbranched intermediate **C5–C11 chain-length n-alkanes** are the major hydrocarbon constituents of petroleum.

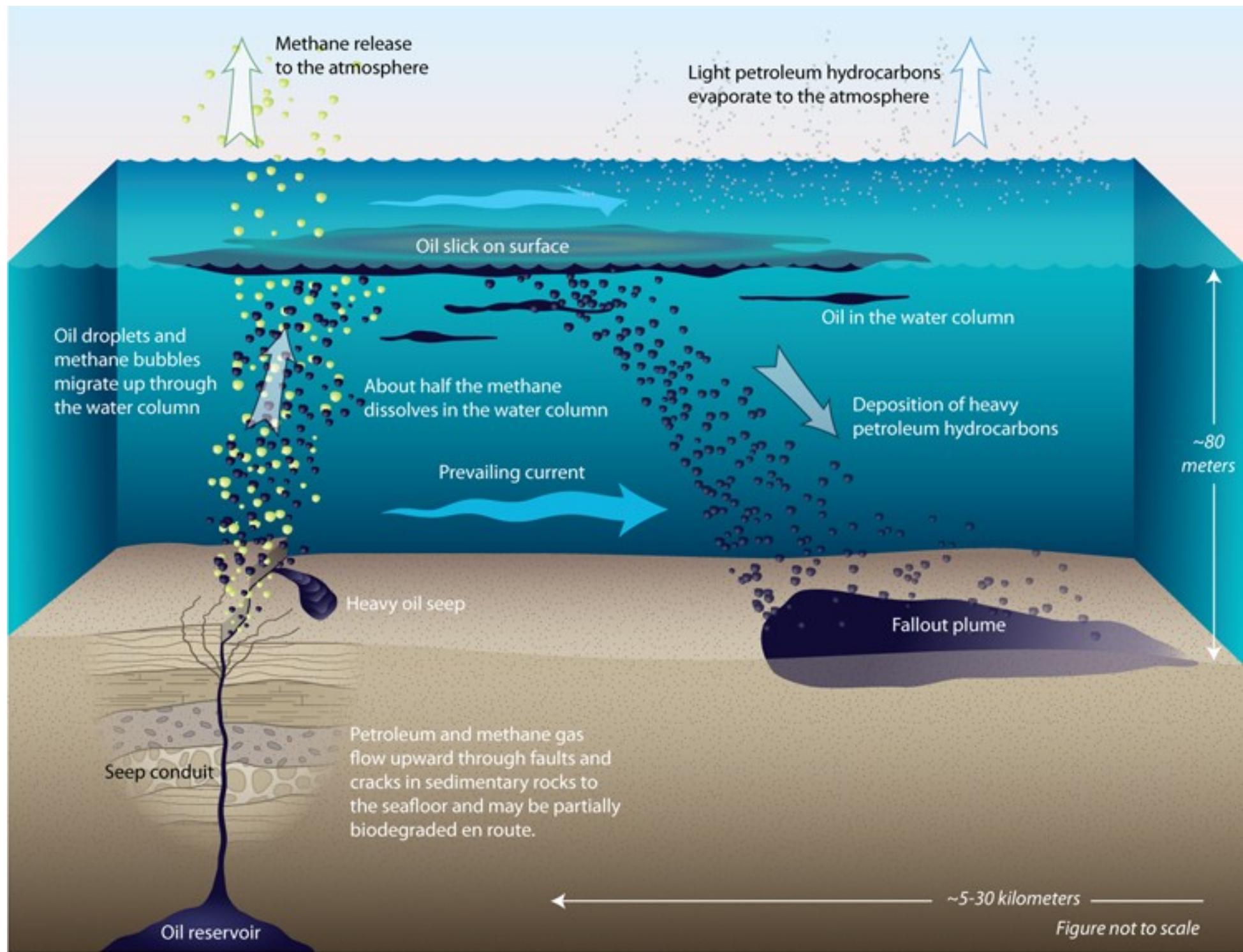
Hydrocarbons in the environment

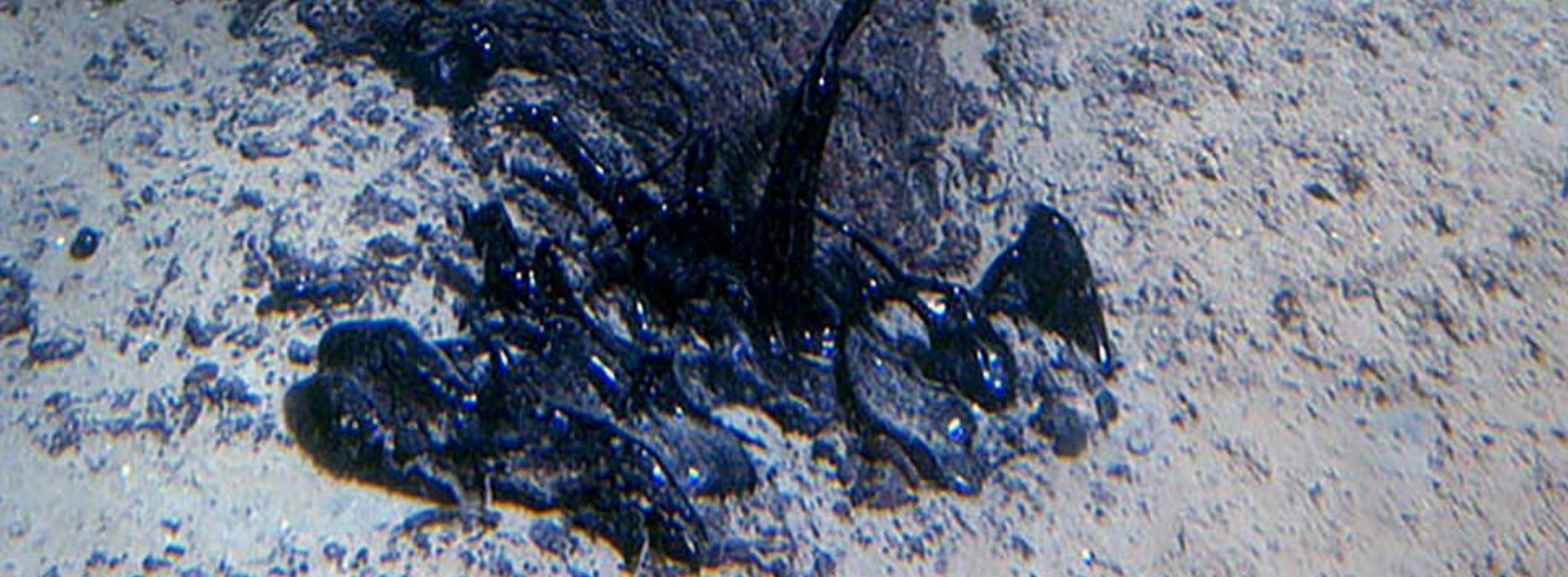
Once petroleum hydrocarbons reach an environment, damage can be the result of several causes.

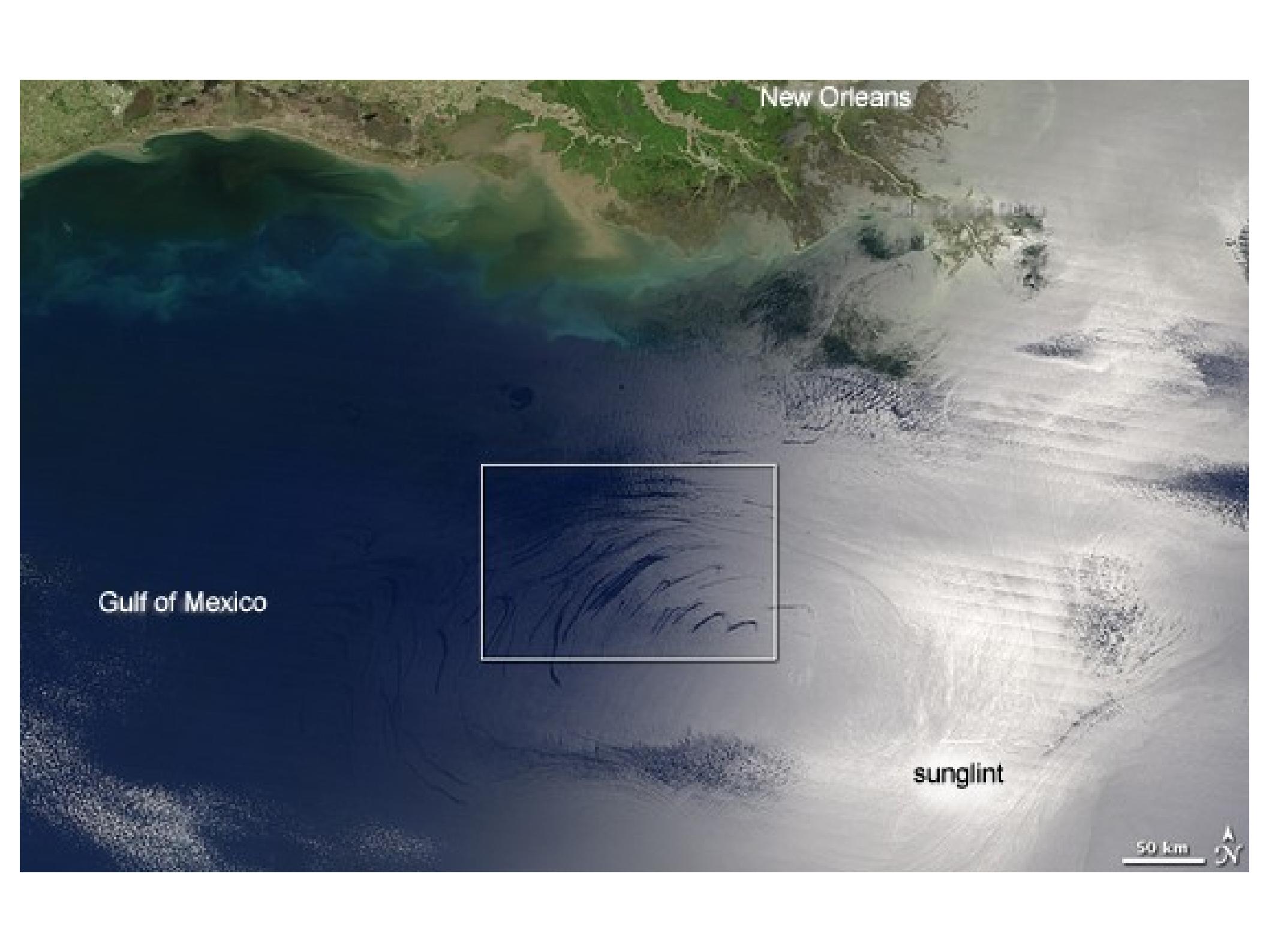
Primary biological impact is due to the **blocking effect** of oil layer to **water, nutrients, oxygen**, and **light access**. **Cytotoxic** and **mutagenic** effects of hydrocarbons are behind the long-term pollution consequences. A more bioavailable toxic compound not only shows increased noxious effects but also has higher accessibility for biodegradation. In contrast, strongly adsorbed fraction is less toxic but more recalcitrant.

Two hydrocarbon fractions are present in solid particles (i.e., soil, sediment). The fraction that remains **irreversibly adsorbed** to particles is considered non-toxic and non-biodegradable because it is not bioavailable. The **reversibly bound** portion able to desorb and diffuse into water phase constitutes the bioavailable fraction.









New Orleans

Gulf of Mexico

sunglint

50 km 

Microbial degradation of hydrocarbons

Main hydrocarbon degraders are prokaryotes, filamentous fungi, and yeasts.

Bacteria can get access to hydrophobic substrates by reducing surface tension or by direct contact with hydrophobic droplet. Surface tension reduction can be achieved by the secretion of **surfactants**, molecules that disperse hydrocarbons into small droplets. **Biosurfactants** can be **glycolipids, phospholipids, lipopeptides, lipoproteins, fatty acids, neutral lipids, polymeric lipids, and high molecular weight biopolymers**.

Direct attachment to hydrocarbon droplet interface can be achieved by increasing the hydrophobicity of cell surface. This includes the synthesis of **adhesion structures** like proteins, lipopolysaccharides, mycolic acids, and other hydrophobic exopolymers.

Microorganisms possess evolved mechanisms to activate hydrocarbons, generating metabolic intermediates that funnel to central metabolic pathways. By oxidizing these substrates, microorganisms can take advantage in nutrient-limited niches.

Microbial degradation of hydrocarbons

Addition of one or **two hydroxyl groups** to the hydrocarbon skeleton seems to be the ubiquitous first step during aerobic catabolism.

The key enzymes in hydrocarbon degradation pathways are oxygenases, which catalyze the **addition of molecular oxygen** to the substrate.

Dioxygenases catalyze the addition of two hydroxyl groups, whereas **monooxygenases** catalyze introduction of one atom of oxygen into the hydrocarbon.

During **anaerobic degradation**, activation is achieved coupling CO₂ or fumarate to hydrocarbons, and sulfate and nitrate are used as terminal electron acceptors.

Anaerobic degradation of alkanes occurs at **lower rates** compared with aerobic microbial catabolism

alkB-like genes

The initial aerobic alkane degradation step is oxidation via an alkane oxygenase, carried out by either a **multimeric monooxygenase** or a **cytochrome P450 monooxygenase**.

These catalytic systems normally contain at their active site iron–sulfur, di-iron, heme, and copper.

One of the primary multimeric monooxygenase is the *alkB* systems, involved in short chain alkane degradtion.

The *alkB*-like genes are widely distributed in *Proteobacteria* and *Actinobacteria*, including environmental, opportunistic, and pathogenic strains. Strains able to degrade intermediate C 5 –C 11 and long C12 n-alkanes generally possess AlkB-like monooxygenases.

pMMOs and *Cyp153* genes

Two additional hydroxylation systems include **methane monooxygenases** (MMOs) found primarily in methanotrophic bacteria and **cytochrome P450** protein superfamily.

The pMMOs have a mono- or di-copper center and hydroxylate only C1–C5 substrates such as alkanes, halogenated alkanes, and alkenes and sMMOs possess a [2Fe-2S] catalytic center and utilize FAD and NADH as cofactors and oxidize C1–C8 chain-length alkanes, halogenated alkanes, alkenes, and cycloalkanes.

Cytochrome P450 comprises several oxidase systems in Bacteria, Archaea, and Eukarya. Soluble cytochromes P450 involved in alkane oxidation.

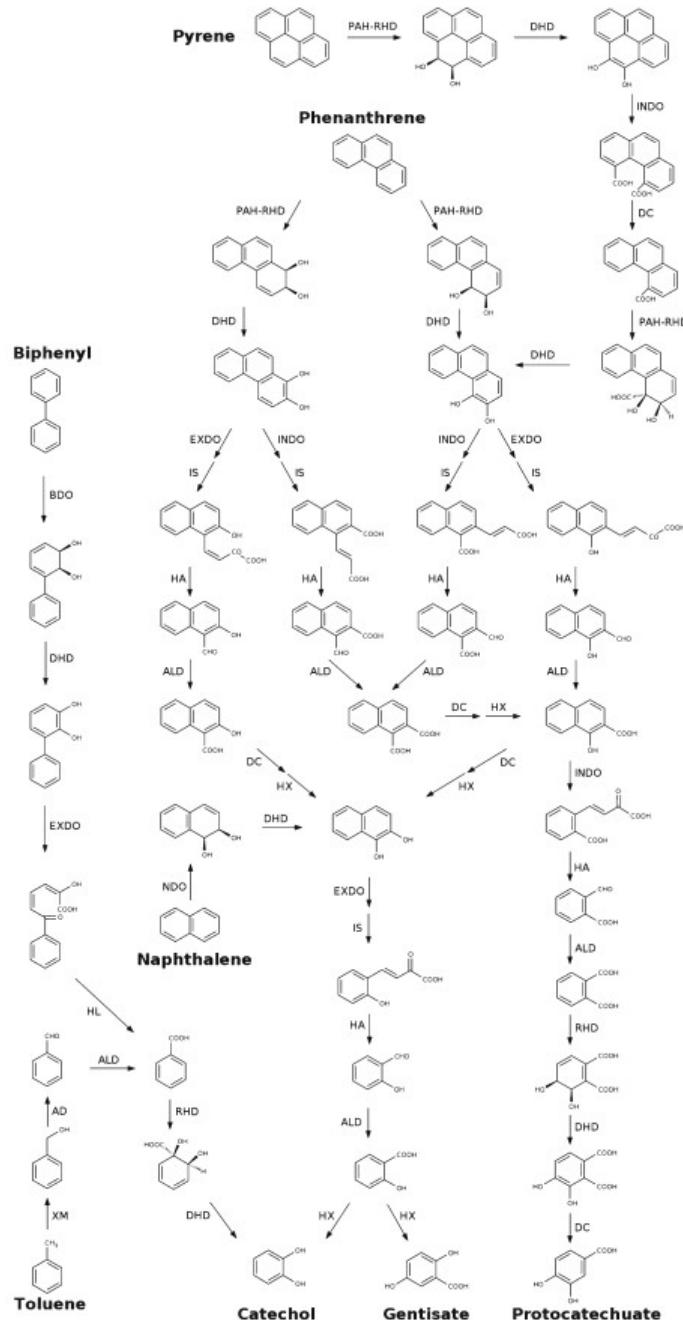
RDH, xyl, nah, bph genes

Degradation of **aromatic hydrocarbons** such as biphenyl, naphthalene, phenanthrene, and pyrene involves an initial dioxygenation reaction by a **ring hydroxylating dioxygenase (RHD)**.

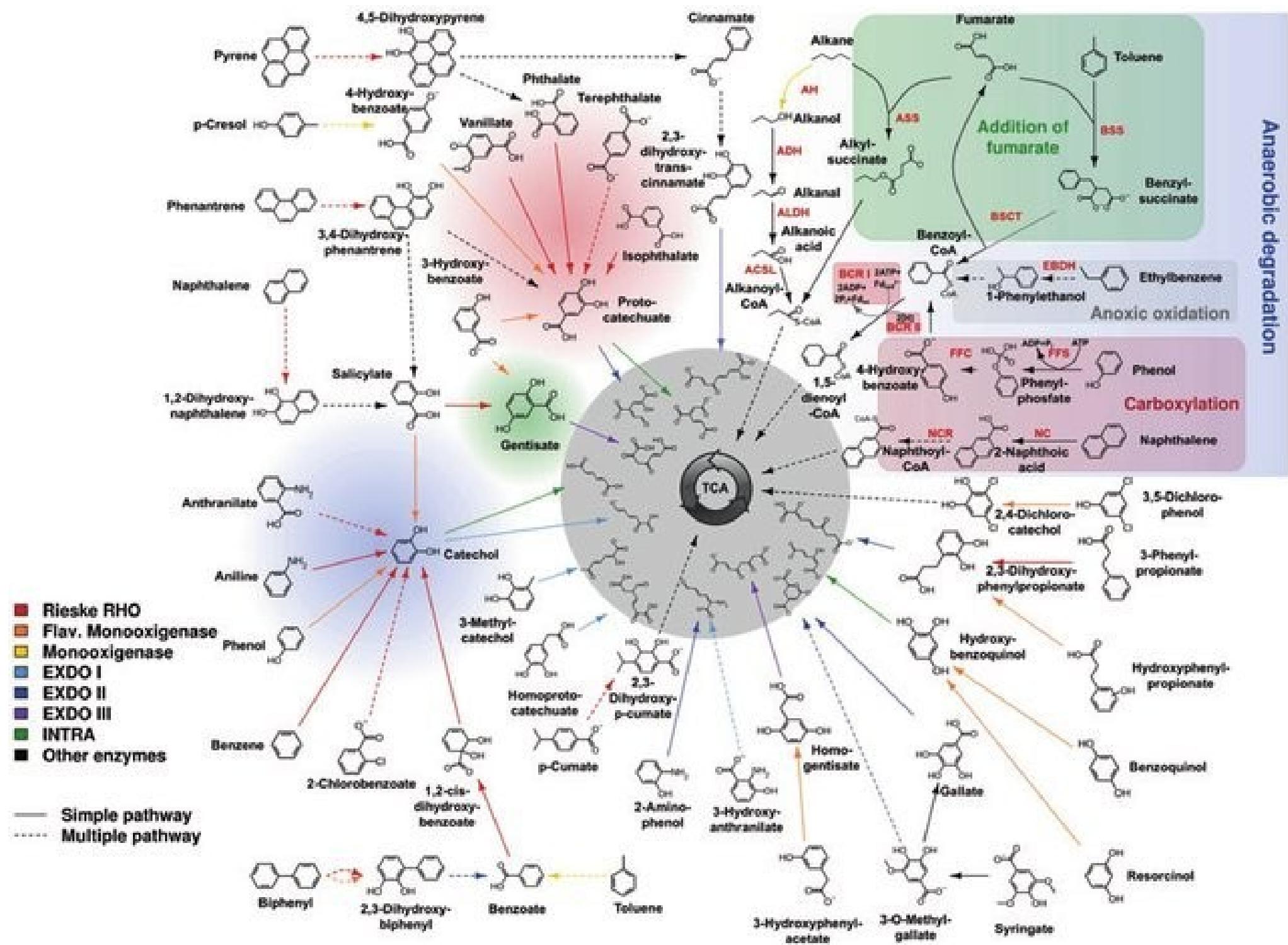
Low substrate specificity seems to be a common feature in bacteria that degrade several PAHs.

The low substrate specificity allows RHDs to oxidize related compounds usually found together in nature, but with different conversion rates.

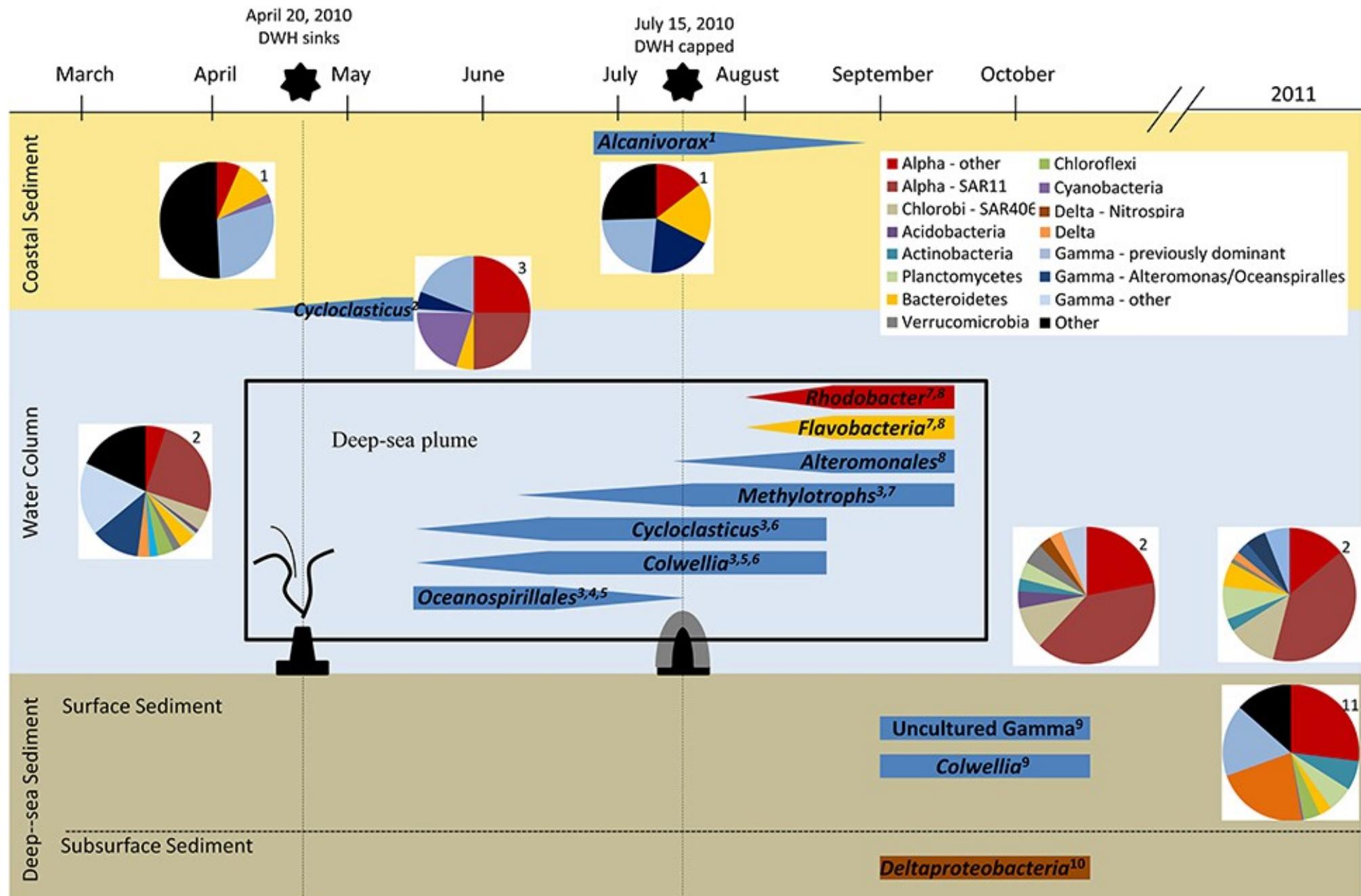
Benzene, toluene, ethylbenzene, and xylenes (BTEX) are main components of the volatile organic fraction from crude oil. For toluene degradation alone, five aerobic pathways and one anaerobic route have been reported



Anaerobic degradation



Naturally occurring degraders



Obligate Hydrocarbonoclastic Bacteria

The ecology of a special group of **ubiquitous marine hydrocarbon-degrading bacteria** offers a model of microbial dynamics after oil spills.

Obligate hydrocarbonoclastic bacteria (OHCB) are an unusual ecological group that plays a key role in biodegradation of petroleum hydrocarbons in marine environments.

OHCB are able to grow on a reduced spectrum of hydrocarbons.

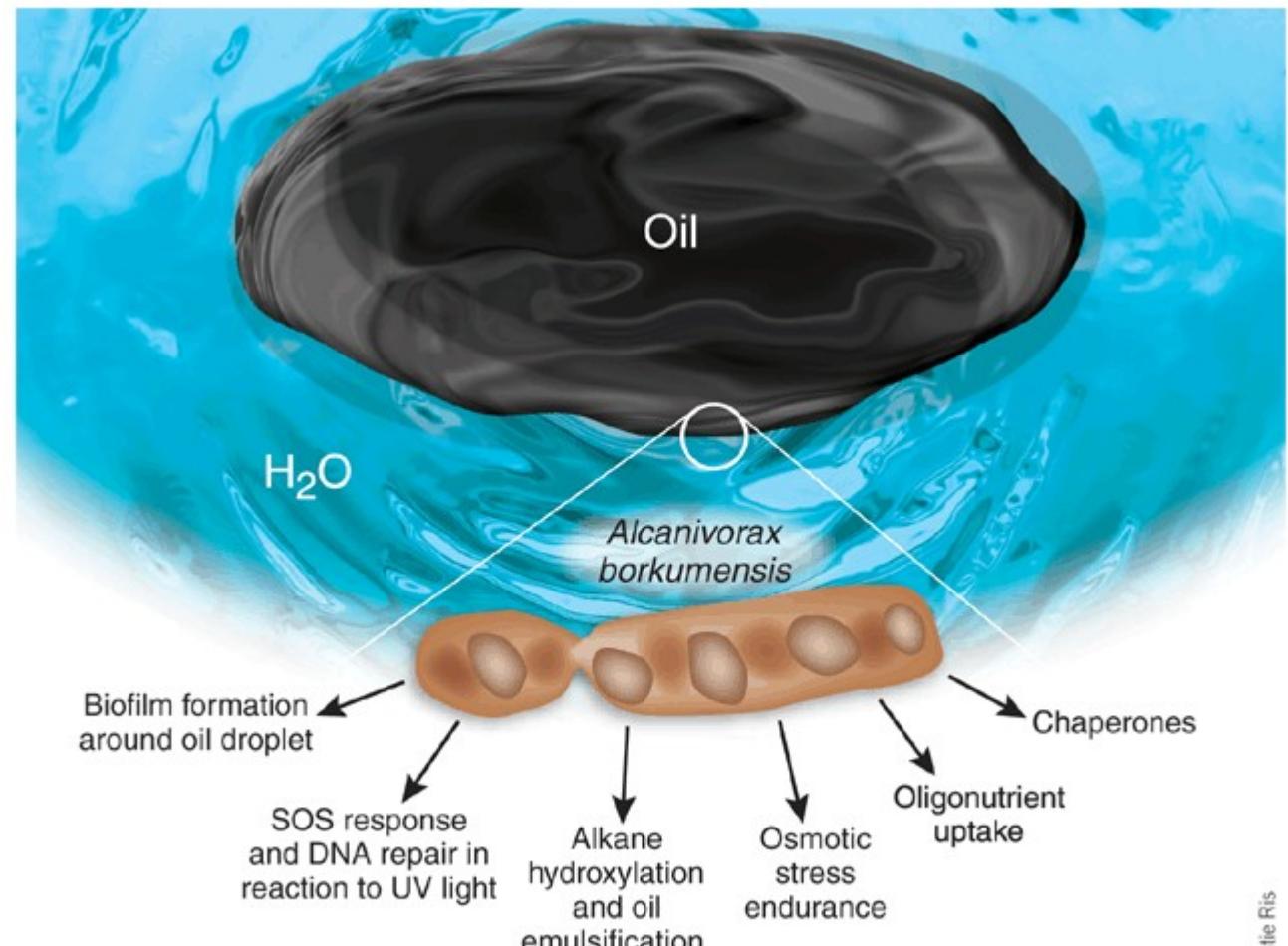
In non-polluted marine environments, OHCB are found in **extremely low numbers**, but after an oil pollution event, members of this group undergo a **bloom**. OHCB growth is favored in hydrocarbon-polluted seawater because they can breakdown substrates that are useless for most bacteria.

The model OHCB is ***Alcanivorax borkumensis*** SK2, able to degrade n-alkanes of chain-length up to C32, long-chain isoprenoids, phytane, pristane, and alkyl-aromatic hydrocarbons and to synthesize **biosurfactants** and **exopolysaccharides** probably involved in **biofilm formation**.

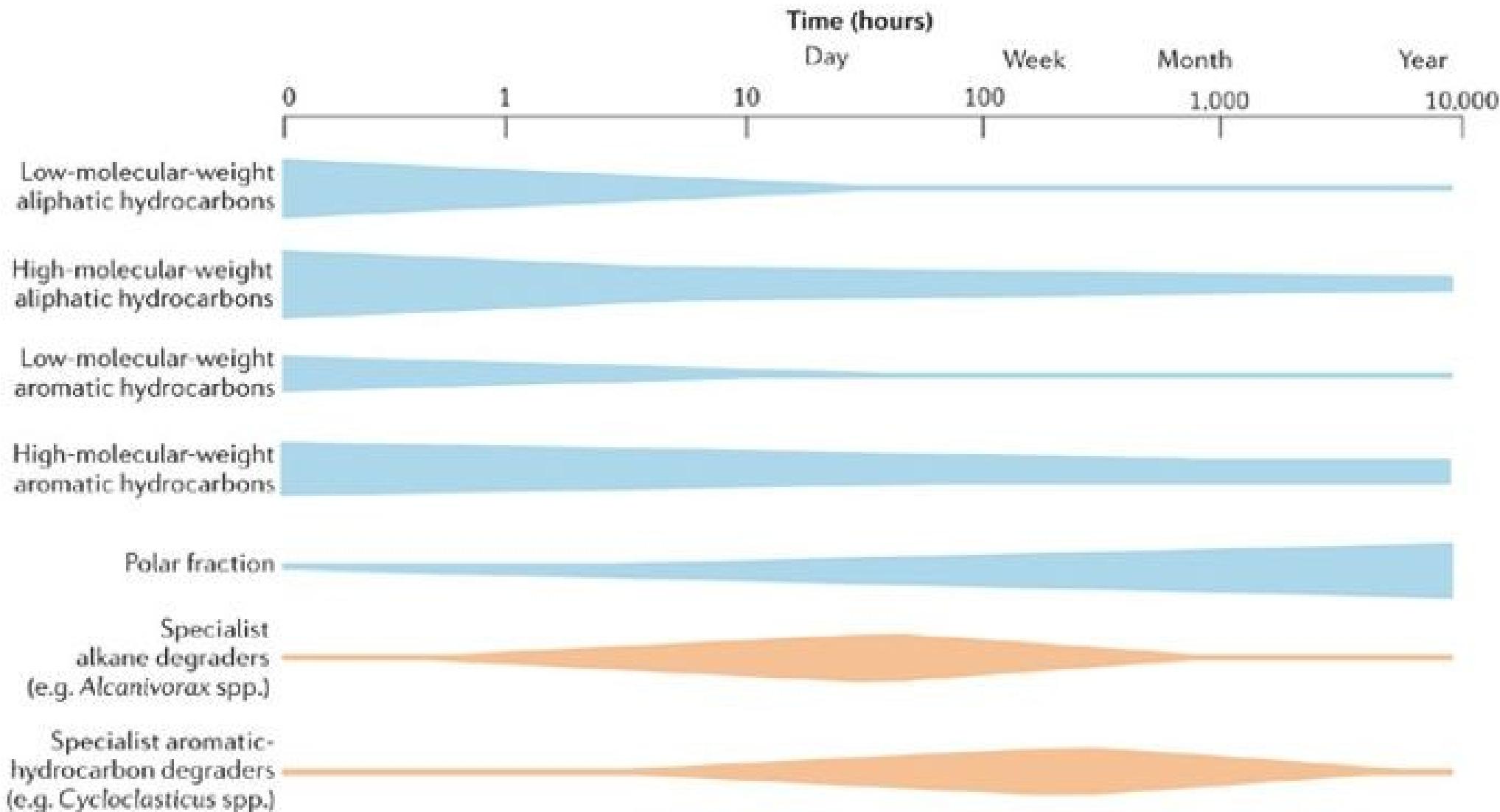
Alcanivorax borkumensis

A. borkumensis genome revealed **multiple systems for hydrocarbon degradation** pathways, i.e., two alkane hydroxylase systems, AlkB1 and AlkB2, and three P450 cytochromes.

The **absence** of genes belonging to **glucose breakdown** pathways highlights its amazing metabolic specialization.



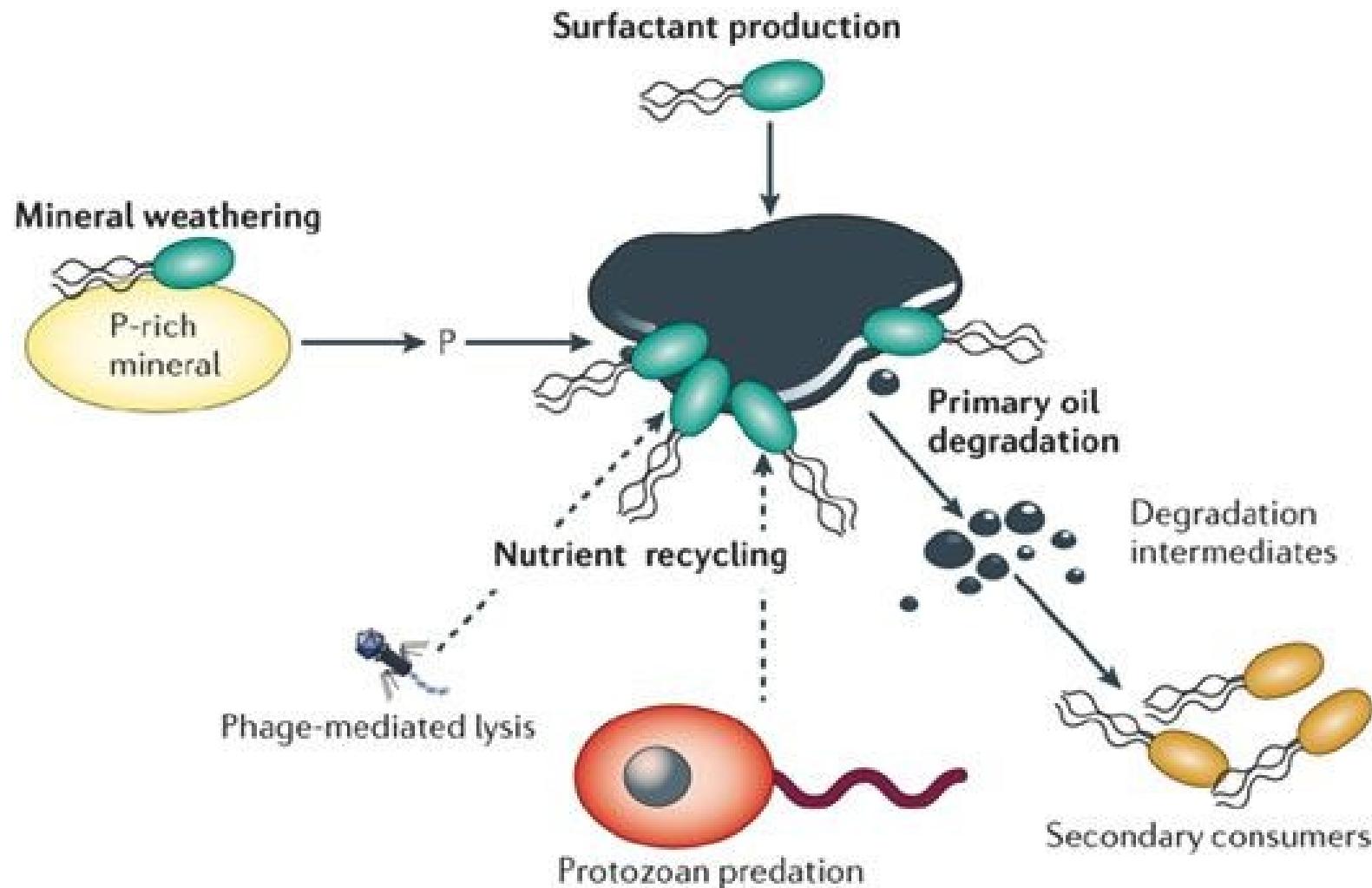
Community Response



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Head et al., 2006 NatRevMicrobiol

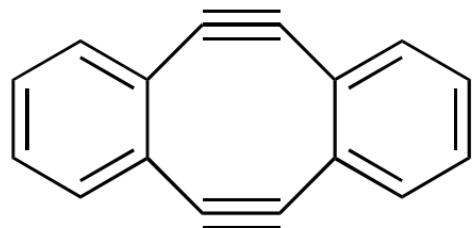
Community Response



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Enriching/Isolating oil degrading microbes

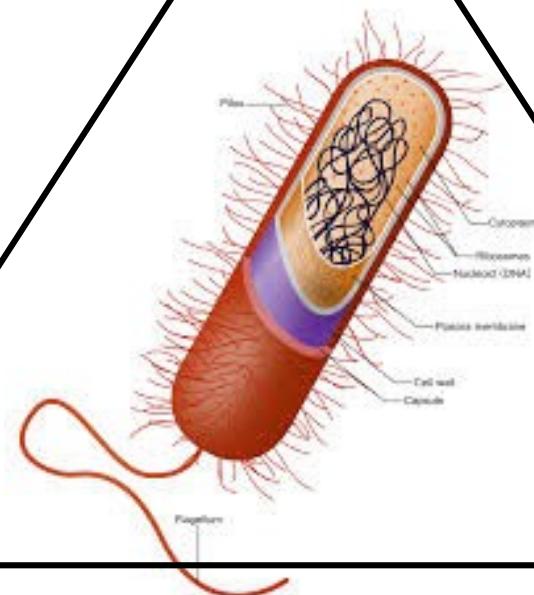


Hydrocarbons

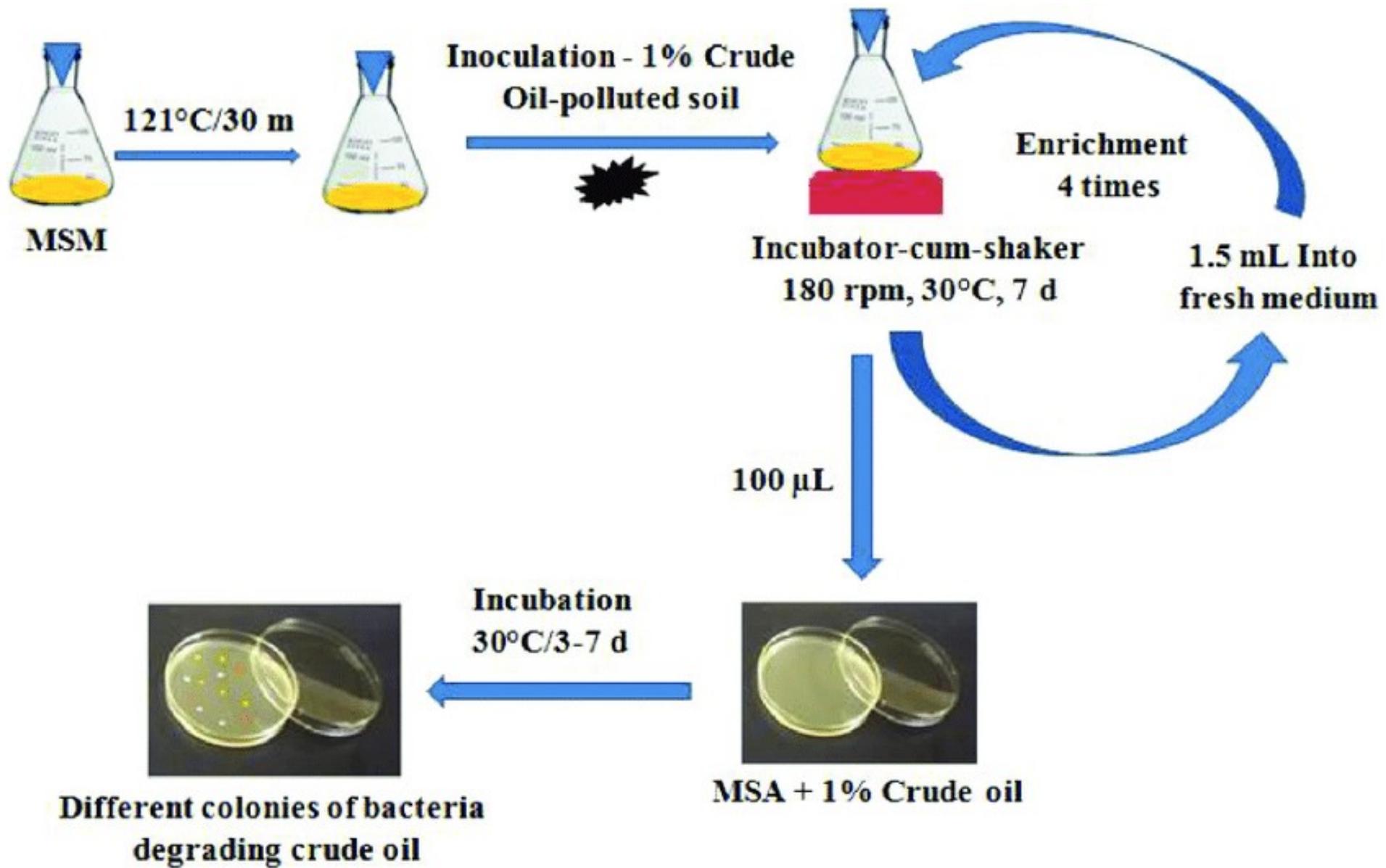
Carbon source

e⁻ donor

e⁻ acceptor



Enriching/Isolating oil degrading microbes



Basic Bioremediation Approaches

Natural attenuation – naturally occurring degradation of the pollutants by naturally occurring microbial populations ***not supported*** by human intervention

Biostimulation – Naturally occurring degradation of the pollutants by naturally occurring microbial population ***supported by the additions*** of nutrient, stimulants and surfactants

Bioaugmentation – Introduction of allochthonous engineered populations of microorganisms to enhance the degradation of the pollutants

Basic Bioremediation Approaches

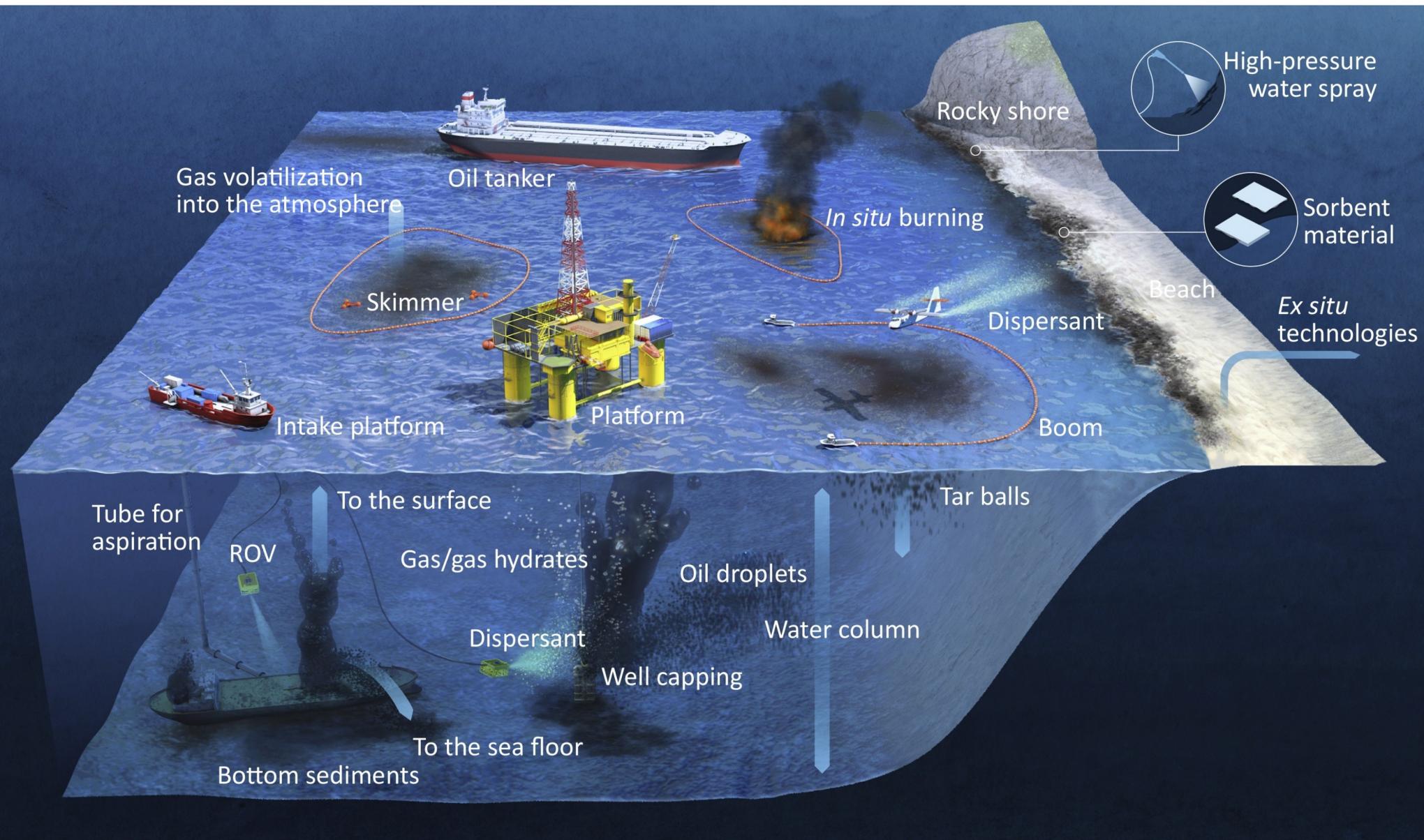
Natural attenuation – naturally occurring degradation of the pollutants by naturally occurring microbial populations *not supported* by human intervention

Biostimulation – Naturally occurring degradation of the pollutants by naturally occurring microbial population *supported by the additions* of nutrient, stimulants and surfactants

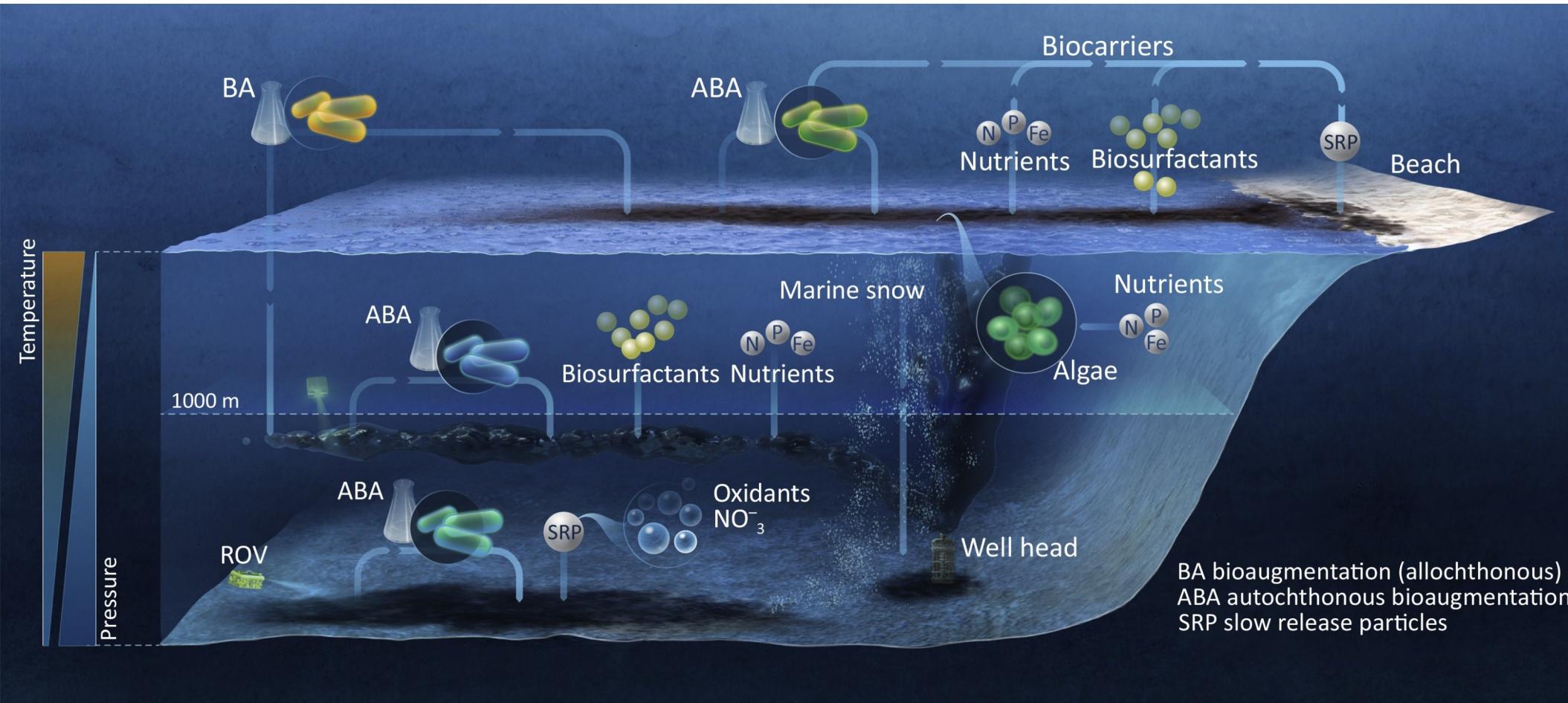
Autochthonous Bioaugmentation – Introduction of autochthonous populations of microorganisms isolated from the site and selected for improved degradation of the pollutants

Bioaugmentation – Introduction of allochthonous engineered populations of microorganisms to enhance the degradation of the pollutants

Oil spill response



Hydrocarbon decontamination during spills

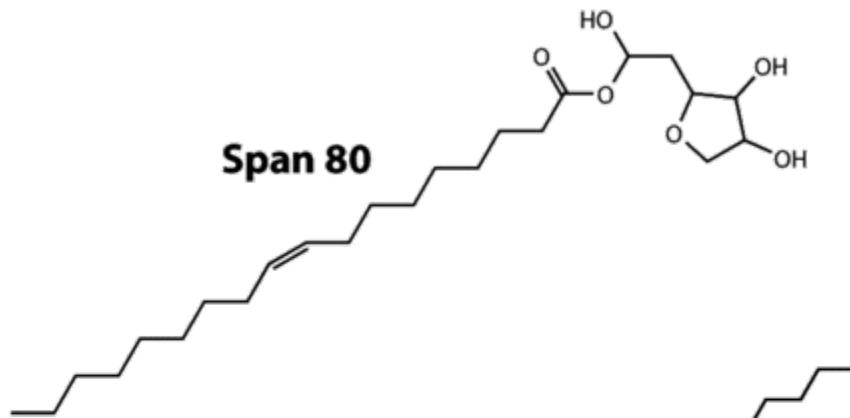


Trends in Biotechnology

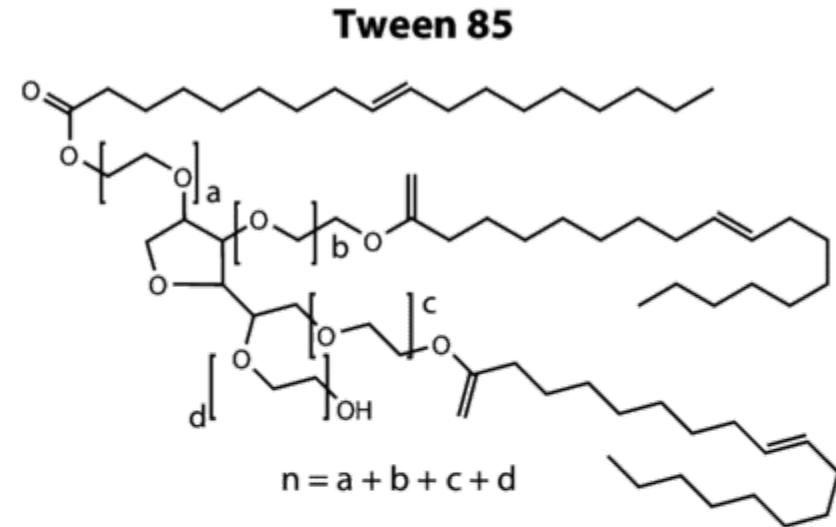


Unexpected effects of dispersant

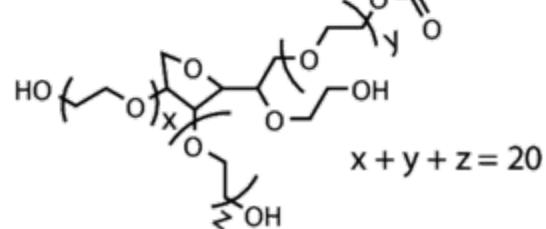
Span 80



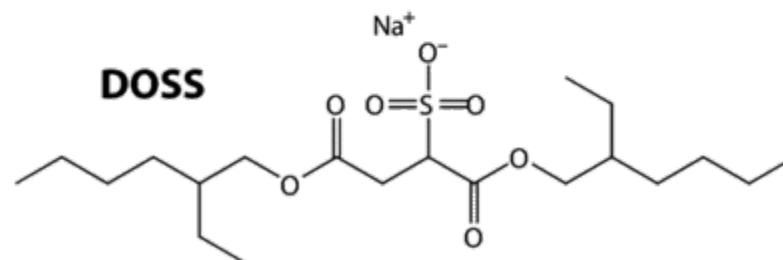
Tween 85



Tween 80

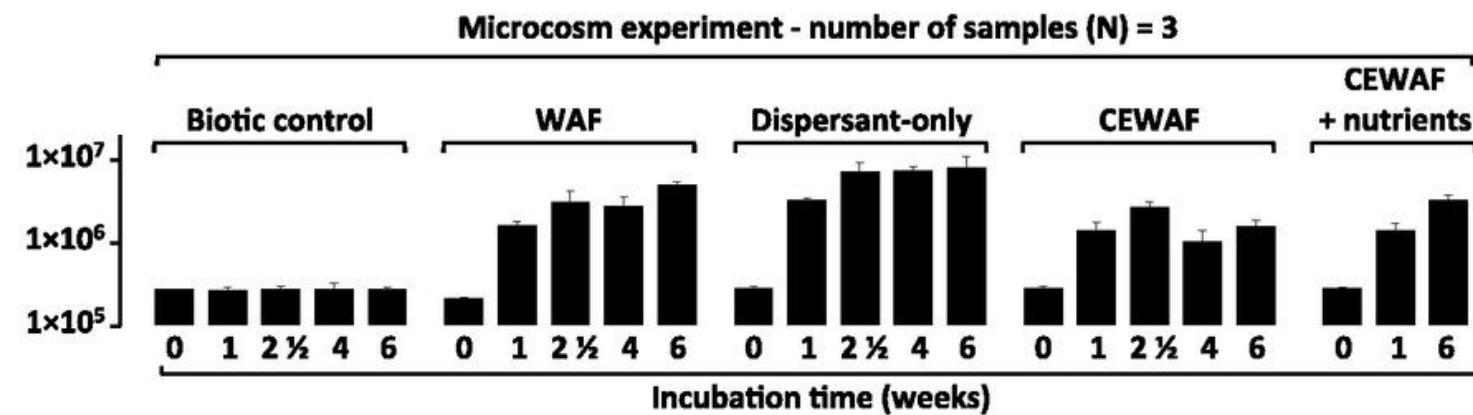


DOSS



Unexpected effects of dispersant

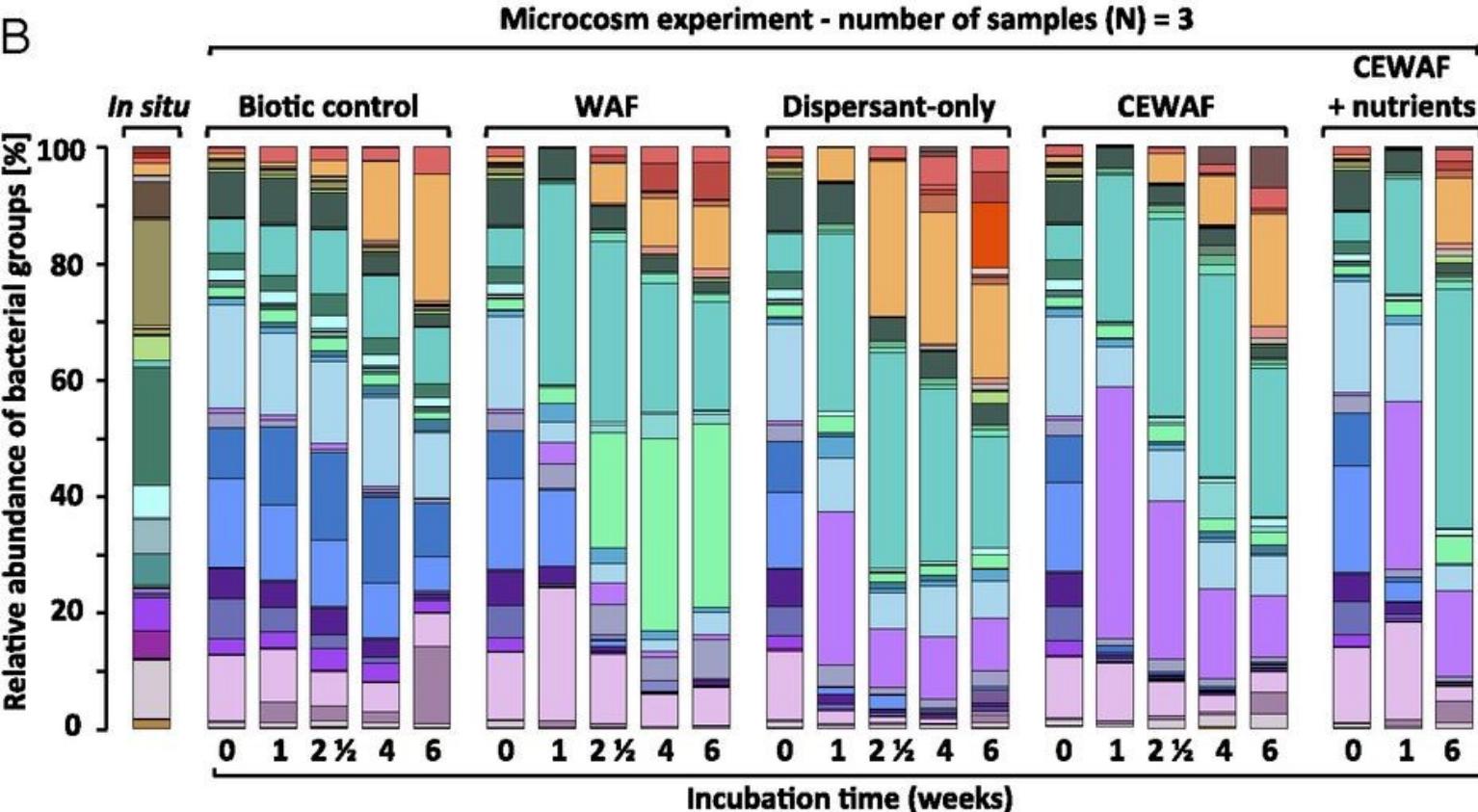
A



Taxonomic identification

<i>Flavobacteria</i>	<i>Acidobacteria</i>
	<i>Actinobacteria</i>
	<i>Crocinitomix</i>
	<i>Owenweeksia</i>
	<i>Bizonia</i>
	<i>Flavobacteriaceae</i>
	<i>Psychroserpens</i>
	<i>Kordia</i>
	<i>Ulvibacter</i>
	<i>Balneola</i>
	<i>Other Bacteriodetes</i>
	<i>Chloroflexi / SAR202</i>
	<i>Deferribacteres / SAR406</i>
	<i>Phyllobacteriaceae</i>
	<i>Rhodobacteraceae</i>
	<i>SAR11</i>
	<i>Other Alphaproteobacteria</i>
	<i>Bdellovibrionaceae</i>
	<i>Marinobacter</i>
	<i>SAR92</i>
	<i>Teredinibacter</i>
	<i>Other Alteromonadaceae</i>
	<i>Uncult. Alteromonadaceae</i>
<i>Proteobacteria</i>	<i>α</i>
	<i>Colwellia</i>
	<i>Alkanivorax</i>
	<i>Amphritea</i>
	<i>Balnearia</i>
	<i>Oceaniserpentilla</i>
	<i>Oceanospirillaceae</i>
	<i>Oleiphilus</i>
	<i>Oceanospirillales</i>
	<i>Cycloclasticus</i>
	<i>Methylophaga</i>
	<i>Other Gammaproteobacteria</i>
	<i>Verrucimicrobia</i>

B



Case study: Exxon Valdez oil spill

Exxon Valdez oil spill

During the supertanker Exxon Valdez oil spill (EVOS) incident in 1989, ~37,000 crude oil tons was released into the Prince William Sound in Alaska.



Exxon Valdez oil spill

The oleophilic fertilizer Inipol EAP22 and the slow-release fertilizer Customblen 28-8-0 were selected. 48 tons of nitrogen-containing fertilizers was applied between 1989 and 1991



Exxon Valdez oil spill

Fertilizer addition **enhanced crude oil degradation** by the indigenous microorganisms. Speeding up biodegradation gave rise to hydrocarbon losses in field as high as 1.2 %/day.

Sediment analyses carried out during **1989 indicated that 25–30 % of hydrocarbons on shorelines were removed** within the first days to weeks. Nonetheless, after the more readily degradable components were depleted, degradation rates decreased even when fertilizer was reapplied.

Population dynamics of oil-degrading bacteria gave some insights into microbial response. Oil-degrading bacteria represented **about 1–10 %** of the heterotrophic bacterial community just **after the oil spill** in 1989. However, late that year, **oil-degrading bacteria reached about 40%** of the heterotrophic community in oiled shoreline pore waters.

After massive fertilizer amendments during summer 1990, oildegrading bacteria **returned to background levels**.

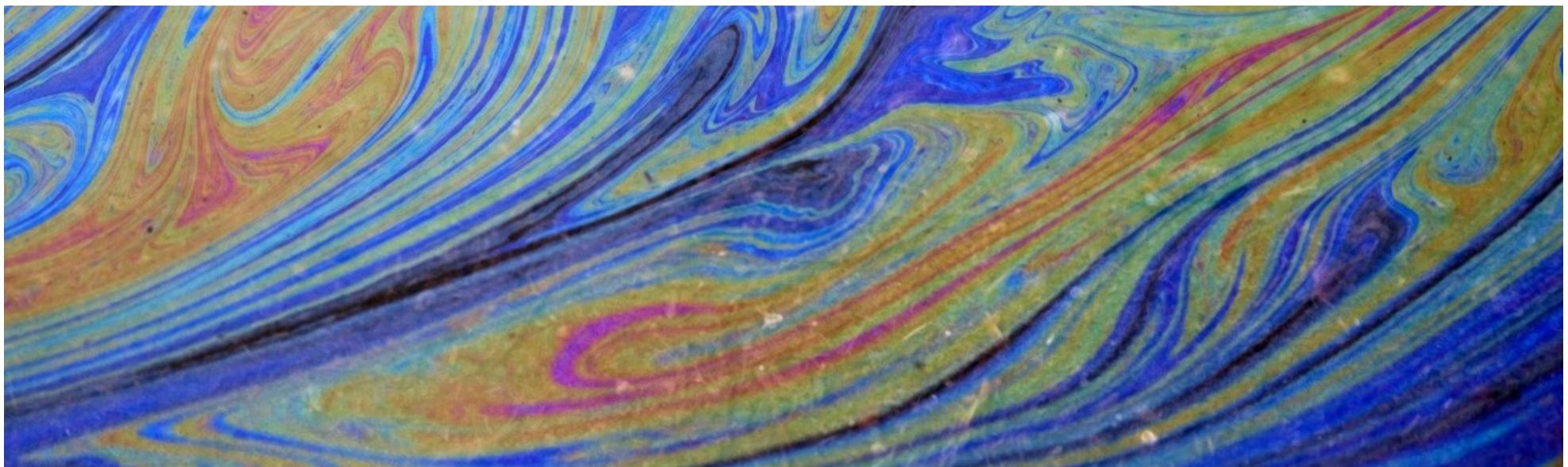


Exxon Valdez oil spill

Monitoring biodegradation after the EVOS accident left lessons about the importance of nutrients balance for bioremediation.

Appropriate nitrogen-containing fertilizer amounts showed to be critical.

Oxygen, phosphorous, hydrocarbon bioavailability, and oil age were less important



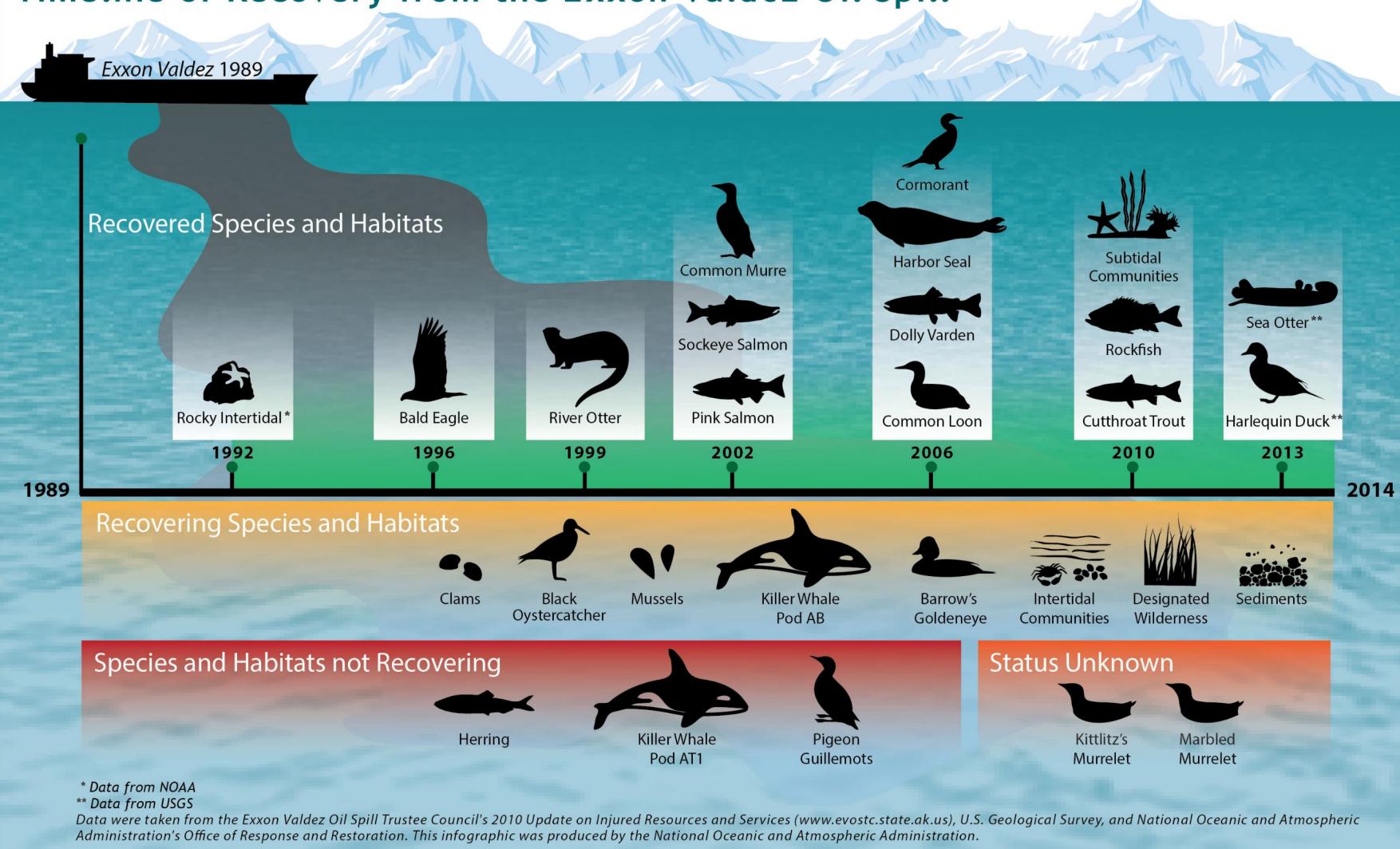
Exxon Valdez oil spill

25 YEARS
LATER

The tanker Exxon Valdez spilled almost 11 million gallons of oil into Alaska's Prince William Sound on March 24, 1989, injuring 28 types of animals, plants, and marine habitats. How long has it taken them to recover from this spill? Twenty-five years later, which ones have not yet recovered?

Here is a timeline showing when natural resources were declared officially "recovered," though actual recovery could have occurred earlier than this official designation from the Exxon Valdez Oil Spill Trustee Council.

Timeline of Recovery from the *Exxon Valdez* Oil Spill



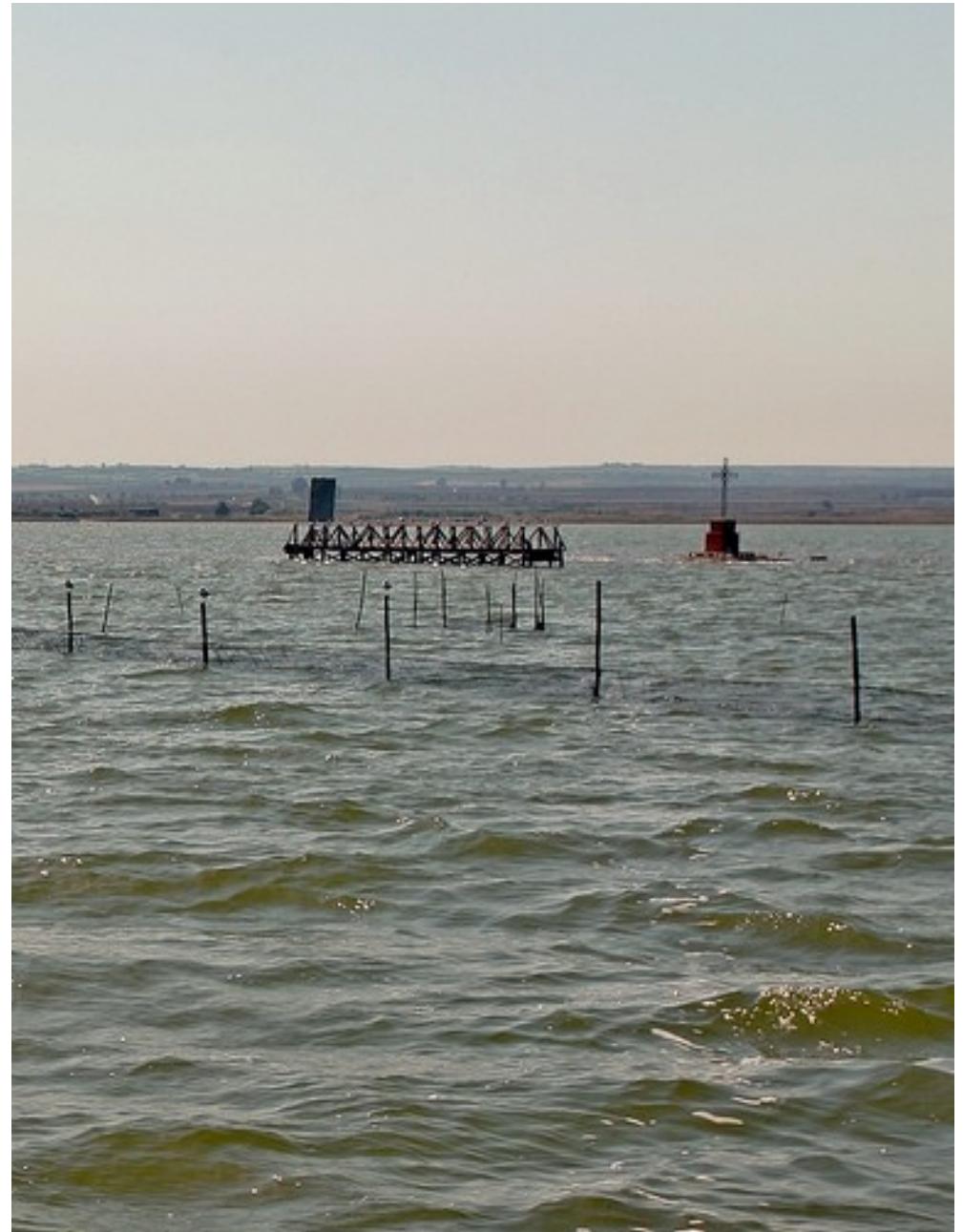
Case study: High organic load sediments

Bioremediation of high organic loads in lagoons

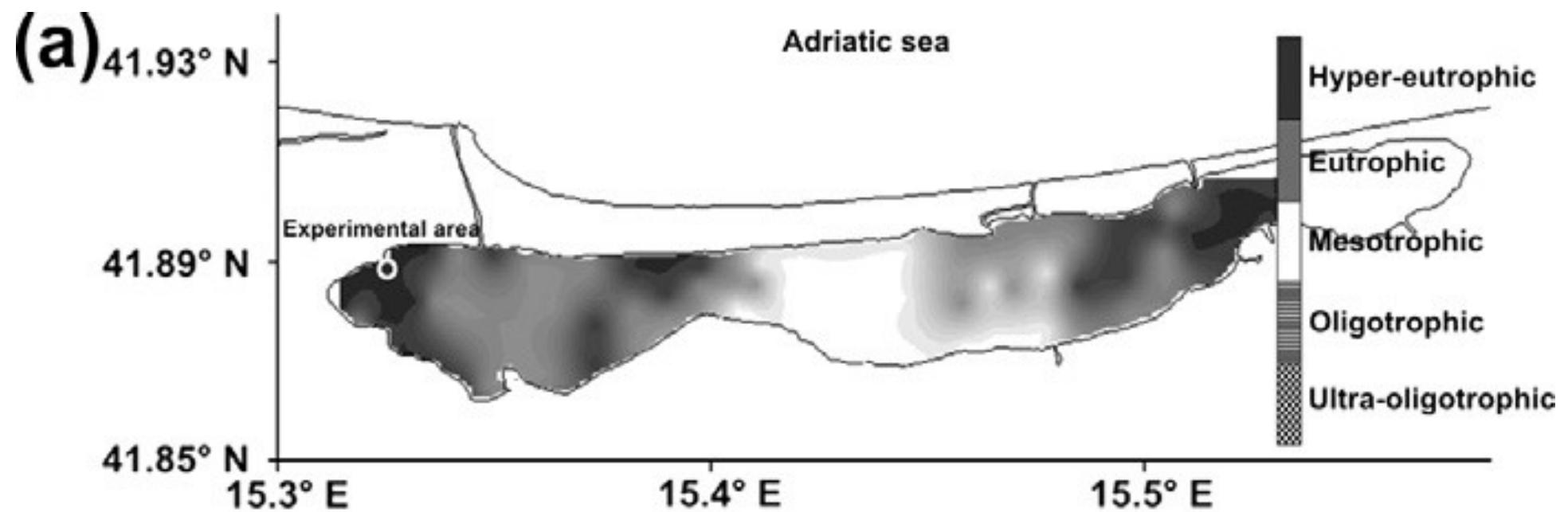
Lagoons are often affected by eutrophication phenomena, due to their shallow nature, high productivity, weak hydrodynamism and anthropic exploitation.

Some of the ecological consequences are:

(i) a shift in the biochemical composition of the organic substrates; (ii) changes in the structure of the resident communities; and (iii) oxygen depletion, which can lead to mass mortality of marine organisms

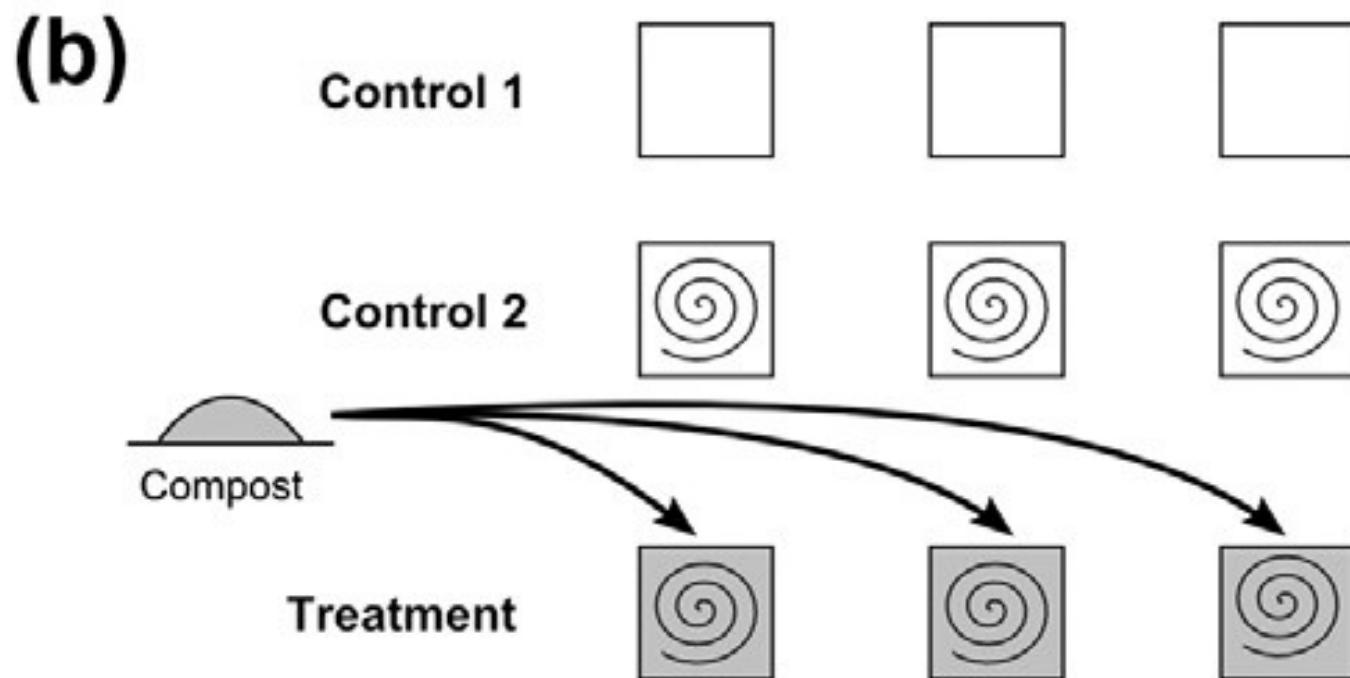


A matter of nutrient imbalance and C:N ratio

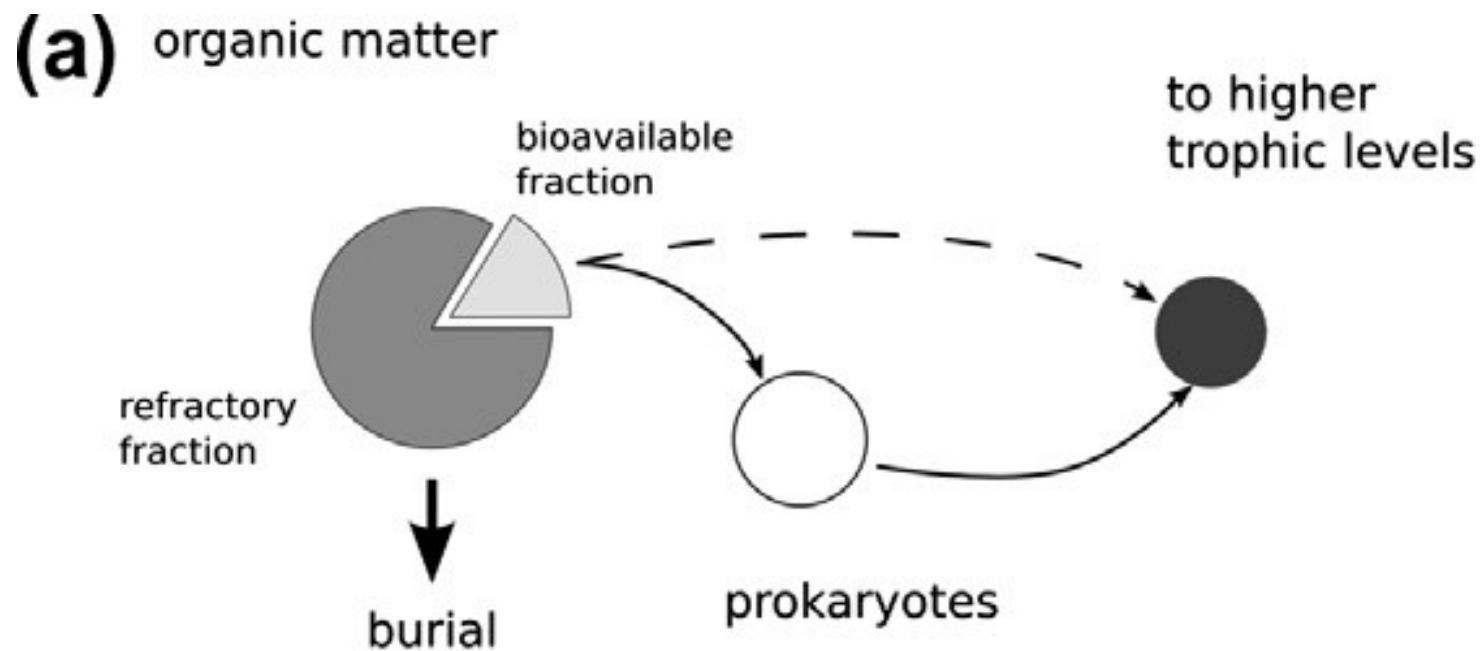


Stimulating Microbial Degradation of OM

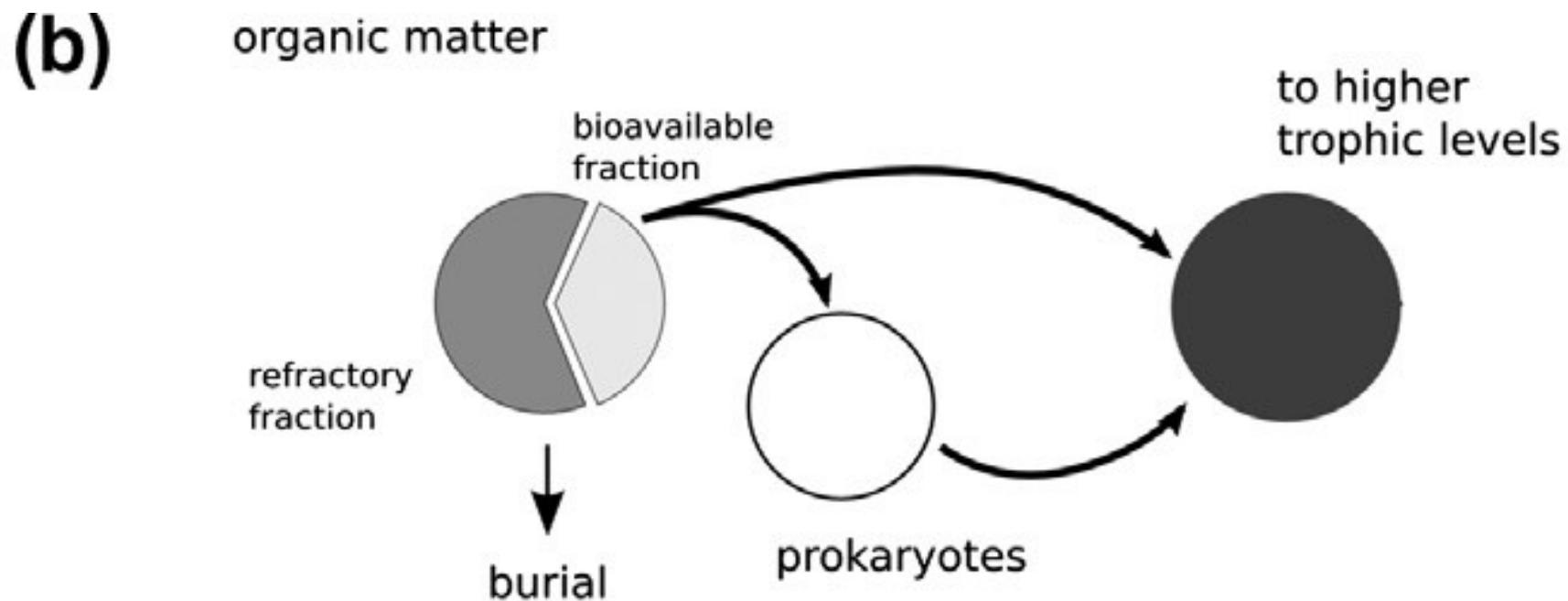
The addition of labile organic-rich compounds to the sediments should enhance the microbial degradative activity, and the potential OM flow through the microbial loop, also providing quality OM to heterotrophs.



Priming Effects



Priming Effects



Priming Effects

Priming or a "Priming Effect" is said to occur when something that is added to soil or compost affects the rate of decomposition occurring on the organic matter (OM), either positively or negatively.

Organic matter is made up mostly of carbon and nitrogen, so adding a substrate containing certain ratios of these nutrients to soil may affect the microbes that are mineralizing OM.

Fertilizers, plant litter, detritus, and carbohydrate exudates from living roots, can potentially positively or negatively prime OM decomposition.

Similarly, **terrigenous inputs** from land can stimulate priming effects in transition and coastal environments, temporarily increasing Om degradation rates.

This week read

Mapelli, F., Scoma, A., Michoud, G., Aulenta, F., Boon, N., Borin, S., et al. (2017). **Biotechnologies for Marine Oil Spill Cleanup: Indissoluble Ties with Microorganisms.** Trends in Biotechnology 35, 860–870. doi:10.1016/j.tibtech.2017.04.003.

For the curious:

Hazen, T. C., Dubinsky, E. A., DeSantis, T. Z., Andersen, G. L., Piceno, Y. M., Singh, N., et al. (2010). **Deep-Sea Oil Plume Enriches Indigenous Oil-Degrading Bacteria.** Science 330, 204–208. doi:10.1126/science.1195979.

Kleindienst, S., Seidel, M., Ziervogel, K., Grim, S., Loftis, K., Harrison, S., et al. (2015). **Chemical dispersants can suppress the activity of natural oil-degrading microorganisms.** Proceedings of the National Academy of Sciences 112, 14900–14905. doi:10.1073/pnas.1507380112.