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Microbes at work

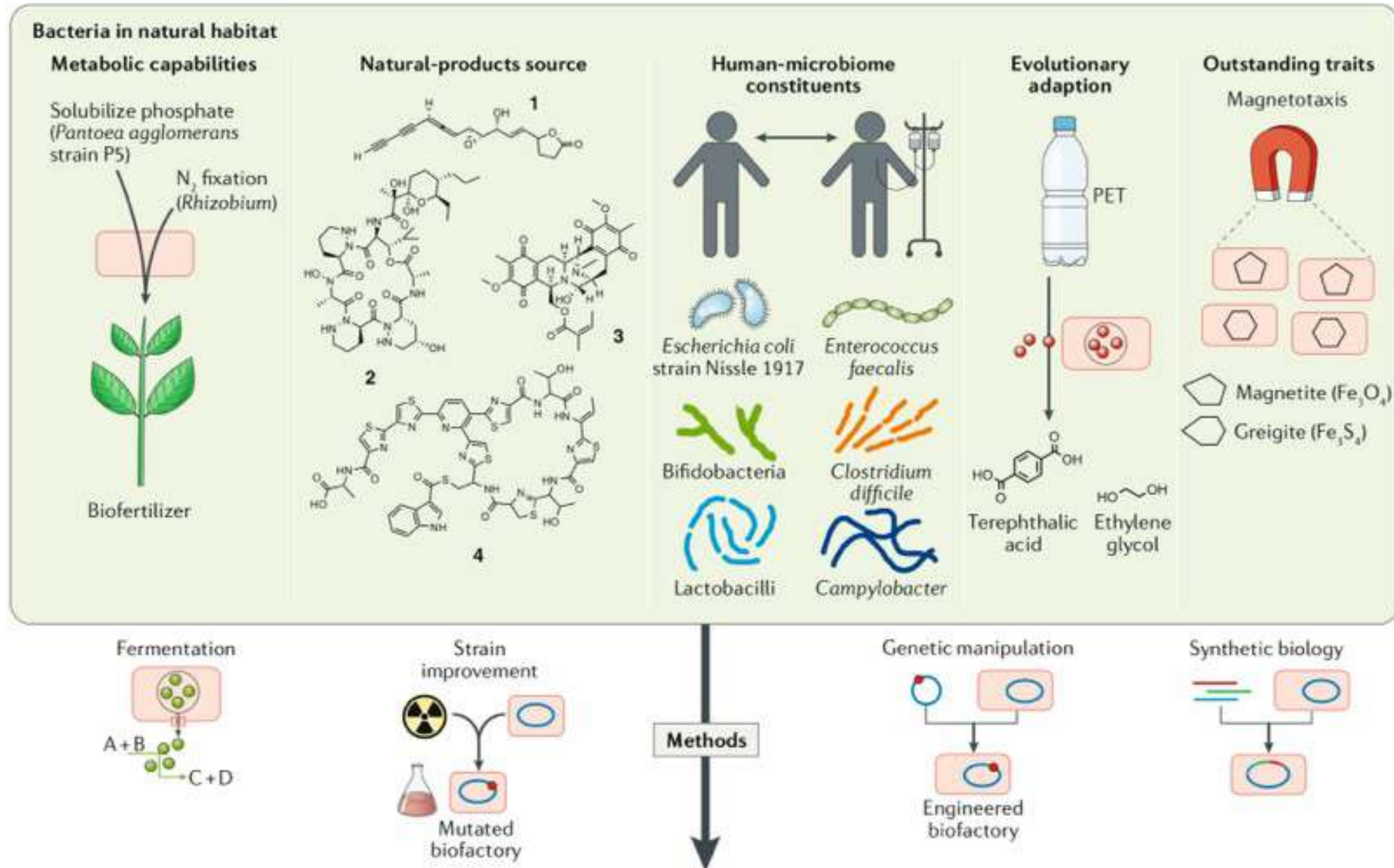
Microbes are biological factories

Pollution Fungi and Plants

Biofuels **Anthropogenic impact**
Bioremediation

The term **bioremediation** refers to the microbial cleanup of oil, toxic chemicals, or other environmental pollutants, usually by stimulating the activities of indigenous microorganisms in some way. These pollutants include both natural materials, such as petroleum products, and **xenobiotic** chemicals, synthetic chemicals not produced by organisms in nature.

Microbial factories, I



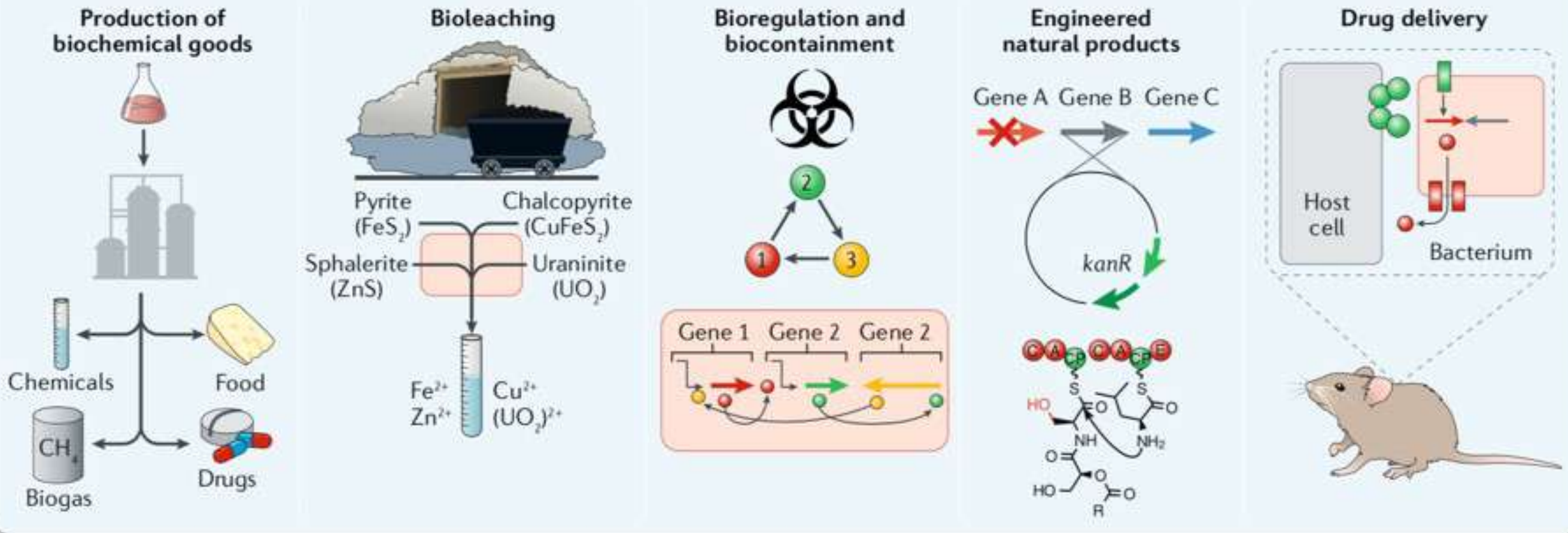
Natural-product structures: 1: cepacin A, 2: dentigerumycin, 3: renieramycin E, 4: lactocillin. PET, polyethylene terephthalate

- High metabolic diversity
- Metabolic flexibility —> tunable via genetic manipulation

Hug et al., 2020

Microbial factories, II

Application as microbial workhorses

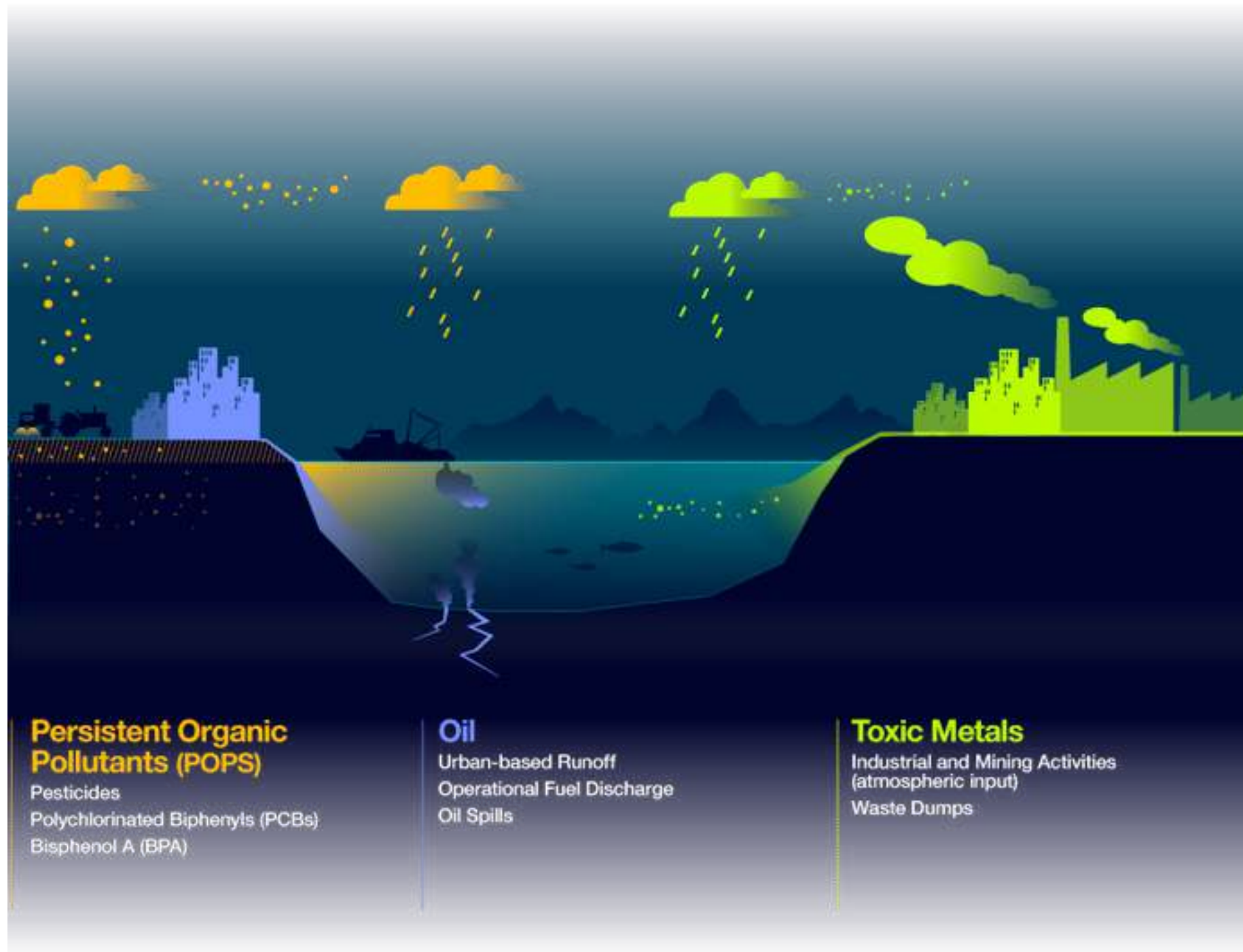


Hug et al., 2020

- Bacteria produce a multitude of enzymes and metabolites associated with the primary or secondary metabolism, which contribute to complex interactions and associations in their natural habitat
- A variety of methods are used to access the versatile capabilities of bacteria, including cultivation for biotransformation, strain-improvement techniques, genetic manipulation and synthetic biology to 'reprogram' their properties, leading to engineered bacteria as application-specific, fine-tuned microbial workhorses

Chemical pollution

<http://www.oceanhealthindex.org/methodology/components/chemical-pollution>



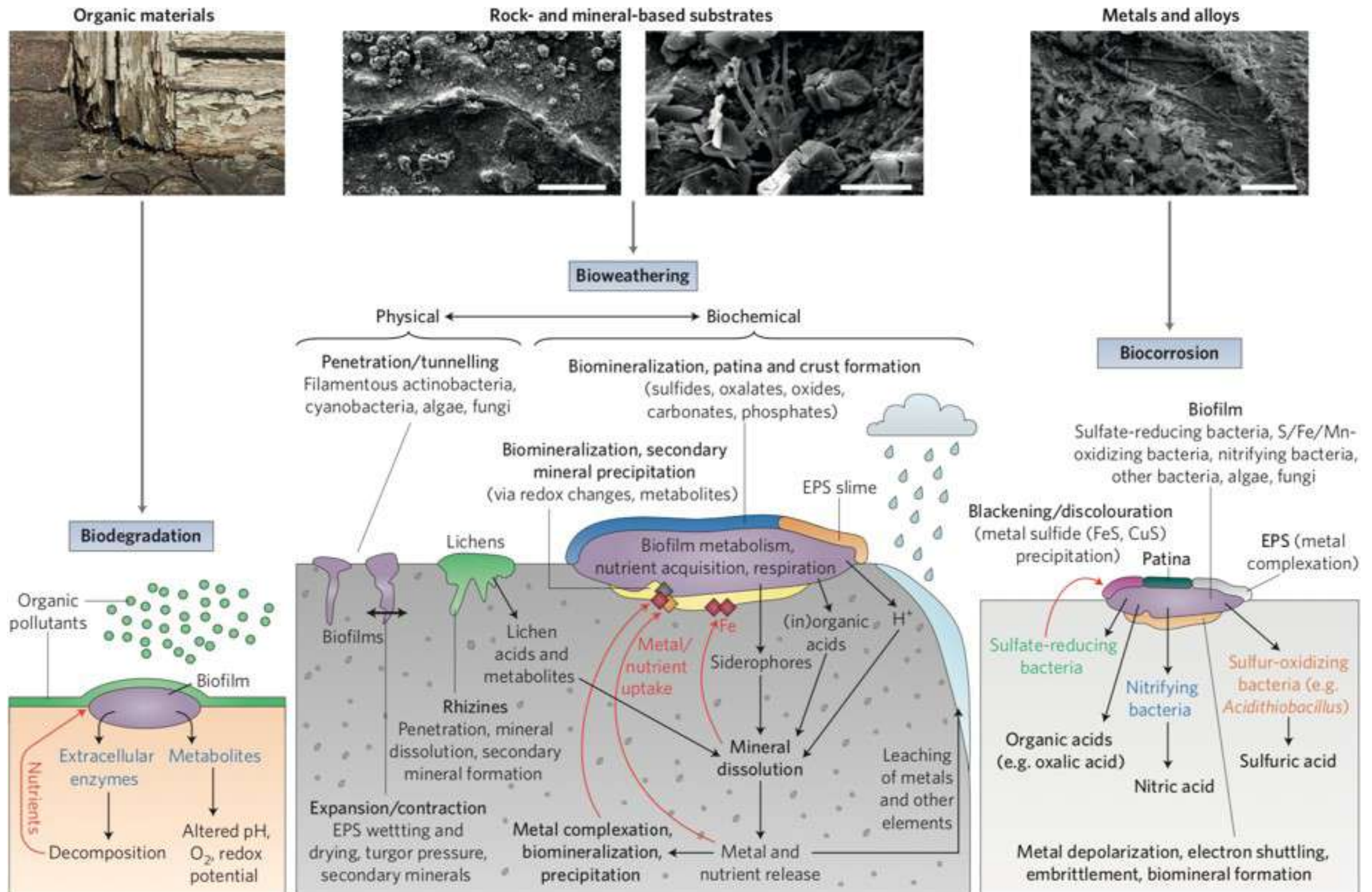
‘Chemical’ refers to a compound or substance that has been purified or manufactured by humans

More than 100,000 chemicals are used commercially (Daly 2006), and many enter the marine environment via atmospheric transport, runoff into waterways, or direct disposal into the ocean

Microbes at work: definitions, I

- **Bioweathering:** Biotic erosion, decay and deterioration of rocks and minerals
- **Biodegradation:** Breakdown of organic substrates by microorganisms
- **Biofouling:** Surface growth of microorganisms, and/or other organisms, which may or may not lead to alterations of the substratum
- **Biocorrosion:** Microbial deterioration of metal substrates
- **Biodeterioration:** Undesirable changes in the properties of a material caused by living organisms

Microbial influences on components of the built environment and human-made structures



Microbes at work: definitions, II

Biomineralization: The process by which bacteria **produce mineral phases**. Bacteria are known to form a variety of minerals, both inside and outside the cell

Biomineralization has applications in nanotechnology, wastewater treatment, bioremediation, and metal recovery

Biohydrometallurgy: A subdivision of hydrometallurgy. Microorganisms are used to produce the **leaching agents** (oxidants and/or acids) needed for **extraction of metals** from low-grade ores, tailings, or end-of-use wastes

Biohydrometallurgical methods require lower operating costs, have reduced environmental impact, and can make use of lower-grade ores or wastes – they are hence environmentally sustainable

Bioleaching: The **solubilization** of metal(s) from sulfidic ores or solid wastes into **aqueous solutions** using living microorganisms. The process is applied at a commercial scale to extract base metals (e.g., Cu, Co, and Ni)

Microbes at work: definitions, III

Biomining: Refers to technologies that utilize microorganisms to **extract** and **recover** metals from **ores** and **waste concentrates**.

This technology has been applied at industrial scale for processing sulfidic and uranium ores. Use of microorganisms in the extraction of metals from oxidized ores (e.g., laterites) and wastes under oxygen- limited growth conditions is also possible, so far mainly performed at laboratory scale and yet to be applied at the industrial scale

Biooxidation: The **extraction** of metals, mainly **gold** from ores, by **oxidizing the matrix** in which the metals are **embedded**.

Basically, the metals are made accessible for extraction in this process. Biooxidation is used to release gold in large-scale stirred tanks for further processing

Urban biomining: The use of microorganisms for **extracting** and **recovering** metals from **end-of-use consumer products**, electronic waste, electrical waste, and spent batteries

Genome information to support bioremediation strategies

Table 1 | **Examples of genomes available for microorganisms relevant to bioremediation**

Microorganism	Web site for genome documentation	Relevance to bioremediation
<i>Dehalococcoides ethanogenes</i>	http://www.tigr.org	Reductive dechlorination of chlorinated solvents to ethylene. The 16S rRNA gene sequence of <i>D. ethanogenes</i> is closely related to sequences that are enriched in subsurface environments in which chlorinated solvents are being degraded (see text).
<i>Geobacter sulfurreducens</i> , <i>Geobacter metallireducens</i>	http://www.tigr.org http://www.jgi.doe.gov	Anaerobic oxidation of aromatic hydrocarbons and reductive precipitation of uranium. 16S rRNA gene sequences closely related to known <i>Geobacter</i> species predominate during anaerobic <i>in situ</i> bioremediation of aromatic hydrocarbons and uranium.
<i>Rhodopseudomonas palustris</i>	http://www.jgi.doe.gov	Main organism for elucidating pathways of anaerobic metabolism of aromatic compounds, and regulation of this metabolism.
<i>Pseudomonas putida</i>	http://www.tigr.org	Metabolically versatile microorganism capable of aerobically degrading a wide variety of organic contaminants. Excellent organism for genetic engineering of bioremediation capabilities.
<i>Dechloromonas aromatica</i>	http://www.jgi.doe.gov	Representative of ubiquitous genus of perchlorate-reducing microorganisms and capable of the anaerobic oxidation of benzene coupled to nitrate reduction.
<i>Desulfitobacterium hafniense</i>	http://www.jgi.doe.gov	Reductive dechlorination of chlorinated solvents and phenols. <i>Desulfitobacterium</i> species are widespread in a variety of environments.
<i>Desulfovibrio vulgaris</i>	http://www.tigr.org	Shown to reductively precipitate uranium and chromium. An actual role in contaminated environments is yet to be demonstrated.
<i>Shewanella oneidensis</i>	http://www.tigr.org	A closely related <i>Shewanella</i> species was found to reduce U(VI) to U(IV) in culture, but <i>Shewanella</i> species have not been shown to be important in metal reduction in any sedimentary environments.
<i>Deinococcus radiodurans</i>	http://www.tigr.org	Highly resistant to radiation and so might be genetically engineered for bioremediation of highly radioactive environments.

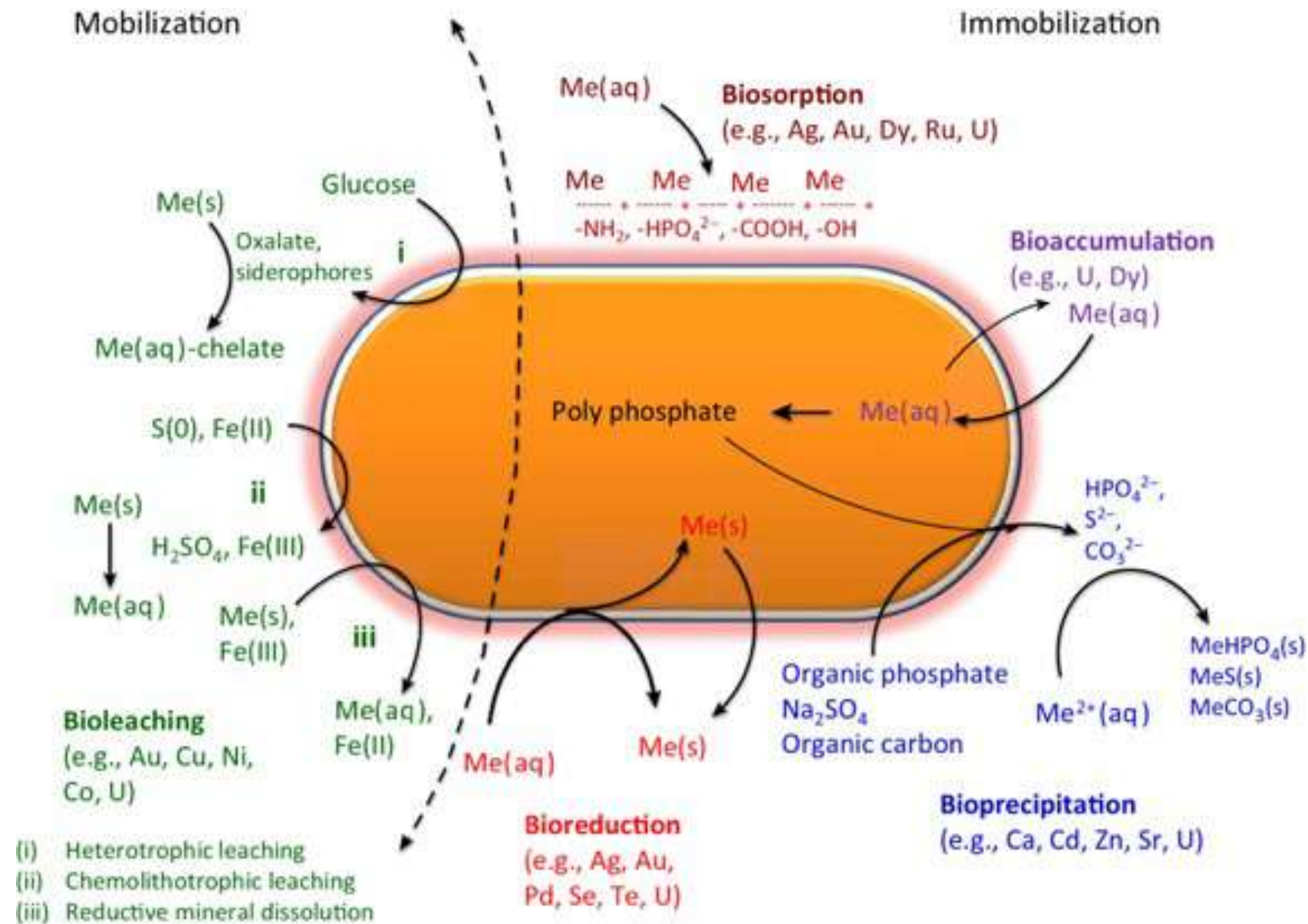
Present dogma for inorganic pollution

Major classes of inorganic pollutants are metals and radionuclides that cannot be destroyed, but only altered in chemical form. Often the extent of environmental pollution is so great that physical removal of the contaminated material is impossible. Thus, *containment* is the only real option, and a common goal in the bioremediation of inorganic pollutants is to change their mobility, making them less likely to move with groundwater and so contaminate surrounding environments.

From soluble form to insoluble form: changing oxidation state of the element by microbial activity

Microbe-Metal interactions

Ahemad, 2019



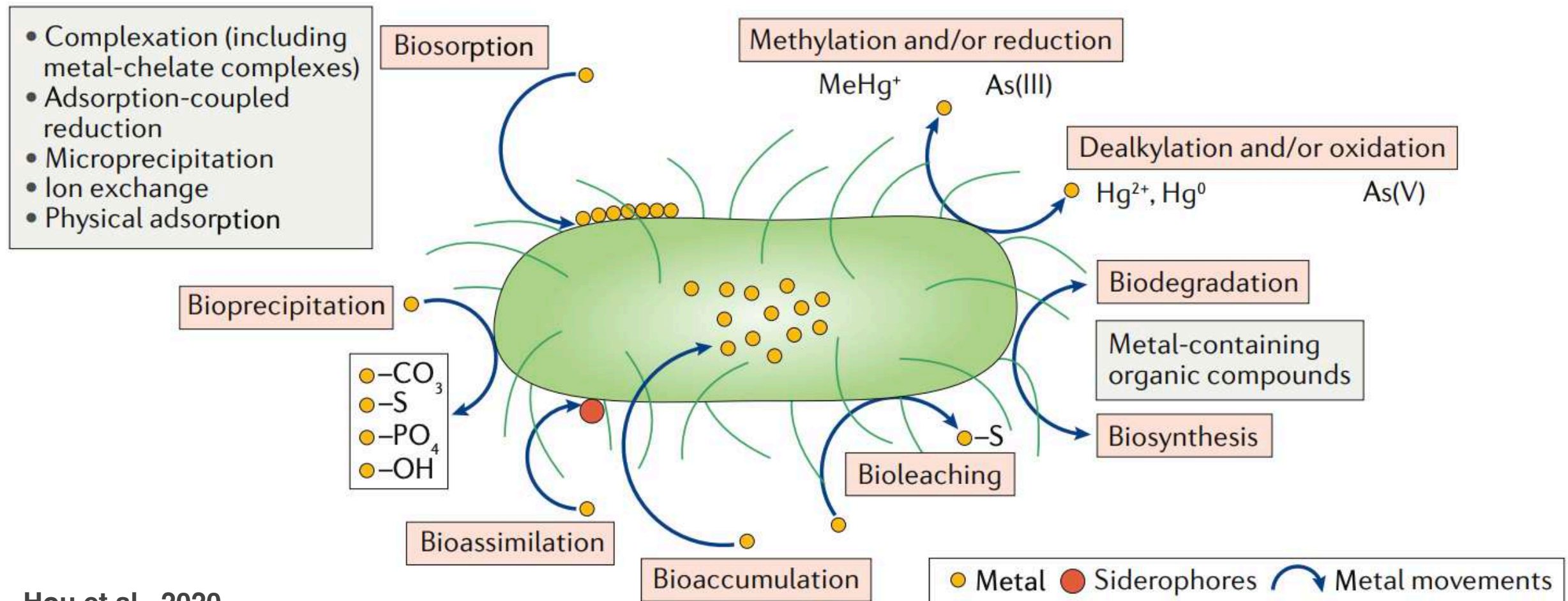
Trends in Biotechnology

Different mechanisms of metal solubilization and immobilization for biorecovery

In the bioleaching step (autotrophic and heterotrophic leaching of sulfidic ores, reductive dissolution of oxide ores) metals are released into aqueous solution through solubilization of ores or solid concentrates

Processes such as biosorption, bioaccumulation, bioprecipitation, and bioreduction enrich the dissolved metals of leachate streams or diffuse metals of wastewaters as solid precipitates for further metallurgical processing

Microbial bioremediation



Processes by which bacteria can mediate the removal or detoxification of heavy metal(loid)s from agricultural soil. Bacteria can interact with heavy metal(loid)s directly, accumulating them on the cell surface (biosorption). They can also reduce or oxidize metal(loid) species and synthesize or degrade metal-containing organic compounds via catalytic reactions (biosynthesis or biodegradation). Sulfur-oxidizing bacteria can release acids and dissolve metal-containing compounds for leaching of metals (bioleaching). Sulfate-reducing bacteria can precipitate metals by formation of low-mobility sulfides (bioprecipitation). Bacteria can also accumulate metals in the intracellular space by using proteins in their cellular processes (bioaccumulation). Bacteria assimilate metals via iron-assimilation pathways using siderophores (bioassimilation). CO_3 , carbonate CO_3^{2-} ; OH , hydroxyl OH^- ; PO_4 , phosphate PO_4^{3-} ; S , sulfide S^{2-}

Common microbe-metal interactions

Metal-sensing based on the presence of at least two out of three cysteine residues required for the cation binding proteins

Mines, ores, milling and tilling facilities

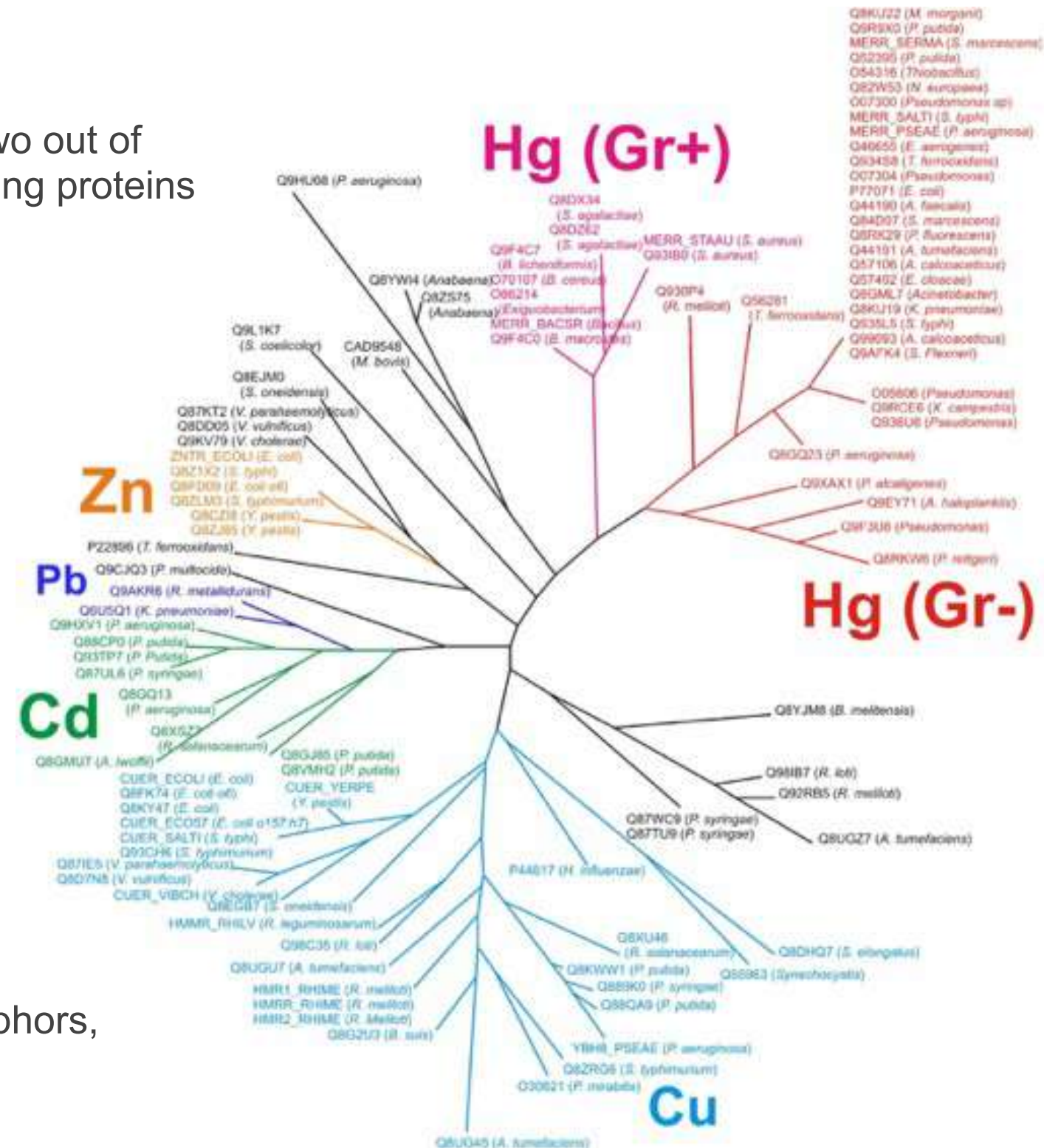
Increase the yield of extractions

Toxic wastes

REE, rare earth element

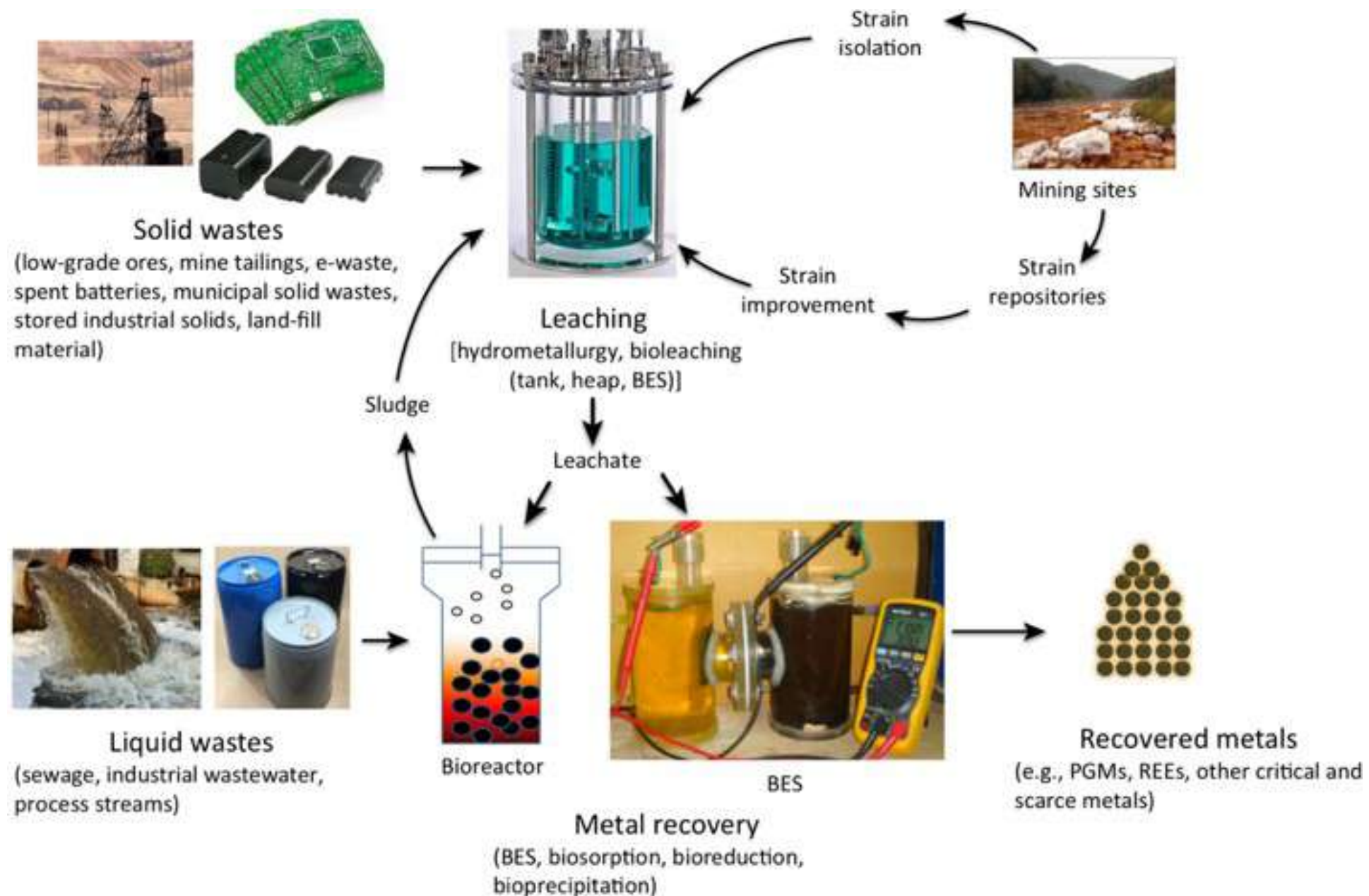
REEs are a group of 17 chemically similar metallic elements, including 15 lanthanides ($Z = 57$ to 71), scandium ($Z = 21$) and yttrium ($Z = 39$), which exhibit magnetism, fluorescence, and superconductivity

REEs are used in permanent magnets, lamp phosphors, rechargeable batteries, catalysts, and biomedical applications



Biomining

Abbreviations: PGM, platinum group metal;
REE, rare earth element.



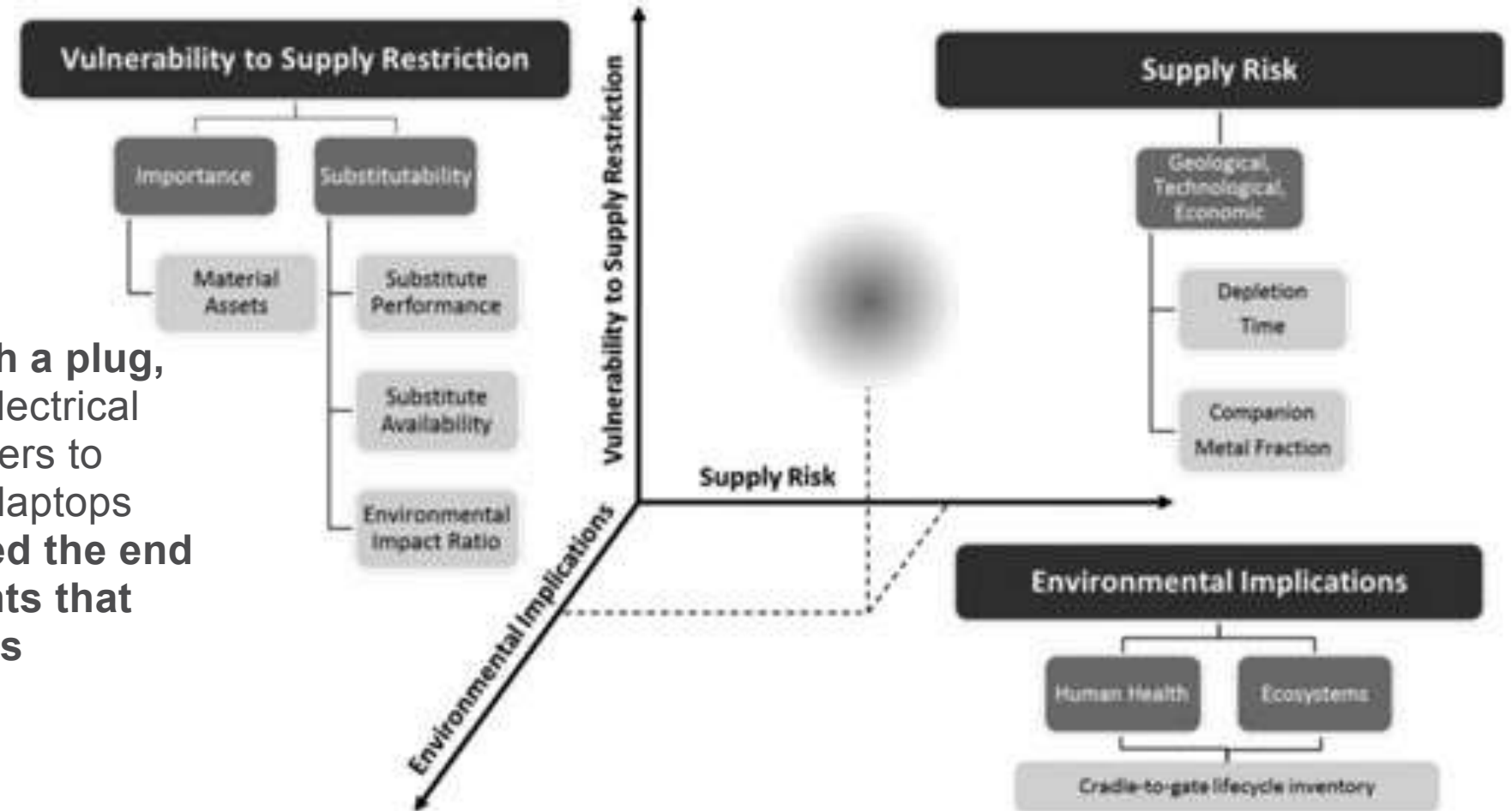
Nanchaiah et al., 2016

- Microorganisms in Biomining, Wastewater Treatment and Bioelectrochemical Systems (BES)
- Research is needed to explore novel microorganisms which can thrive in complex physicochemical conditions of waste streams and concentrate diffuse critical metals in a recoverable form
- Major challenges for metal recovery include low concentration of critical metals, low pH, co-existing metals and salts

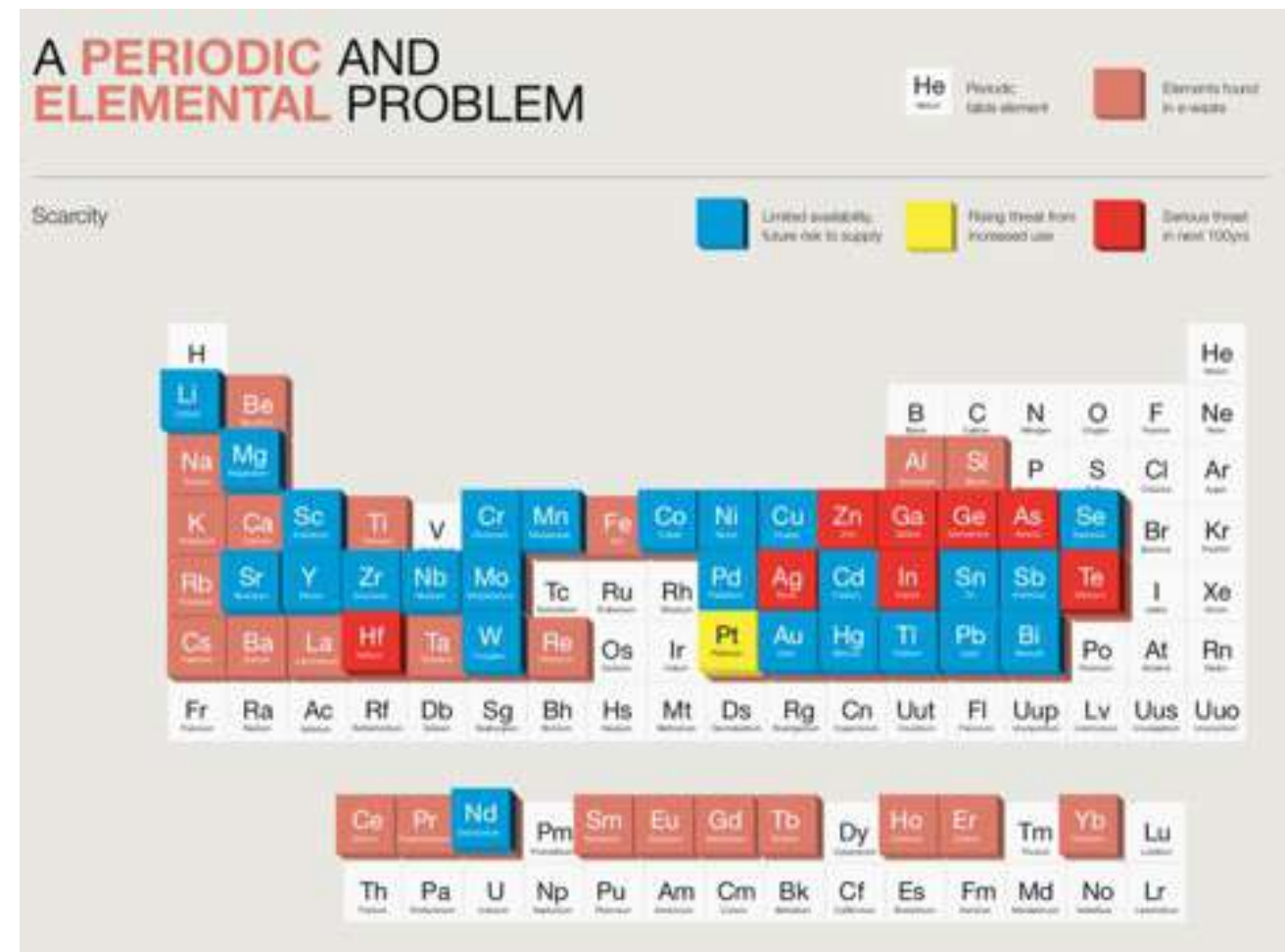
Microbial-metals interactions

E-waste is defined as anything with a plug, electric cord or battery (including electrical and electronic equipment) from toasters to toothbrushes, smartphones, fridges, laptops and LED televisions **that has reached the end of its life, as well as the components that make up these end- of-life products**

—> recycling



Graedel et al., 2013



World Economic Forum, 2019

Xu et al., 2023

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Anthropogenic Impact of terrestrial subsurface biosphere

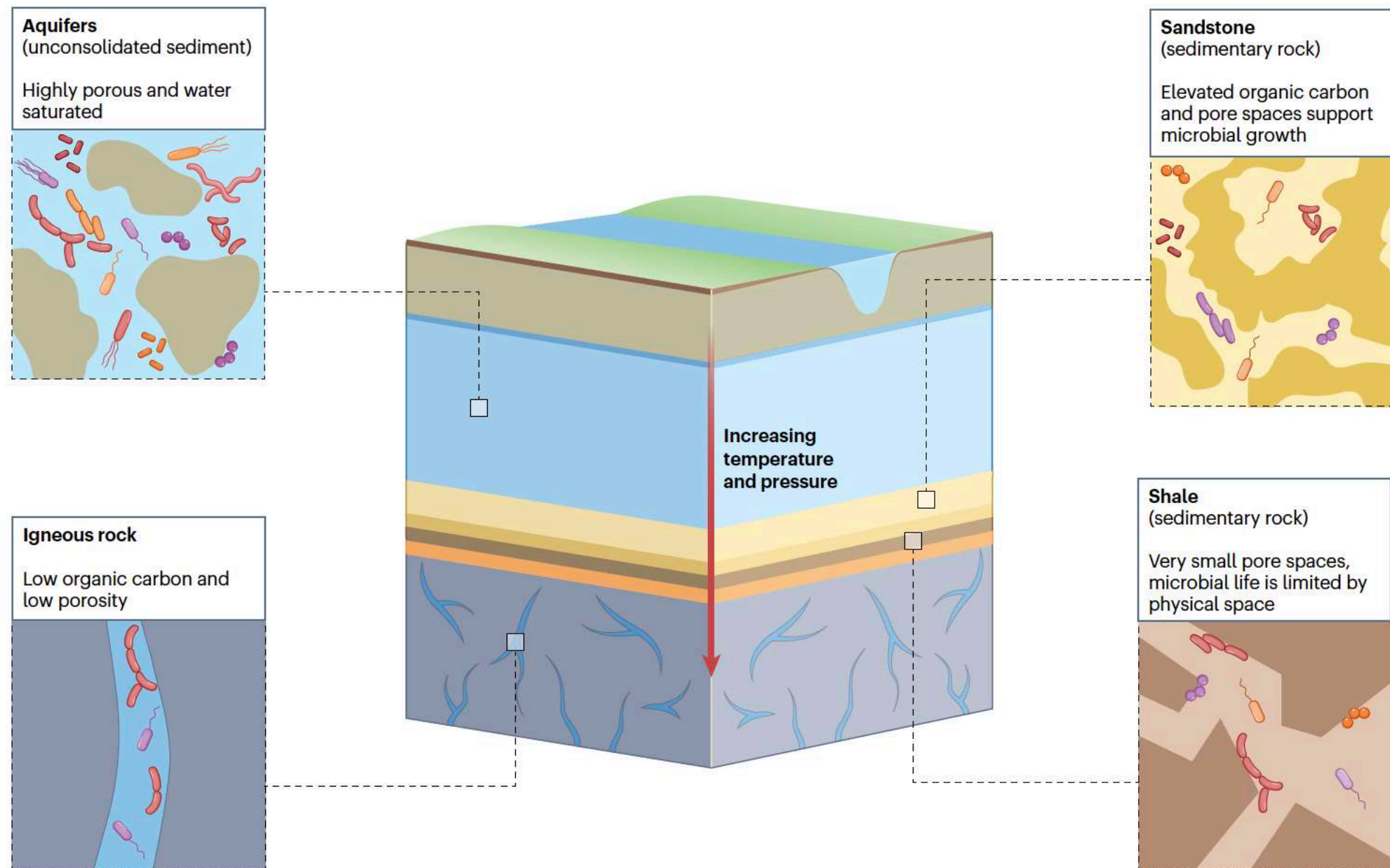
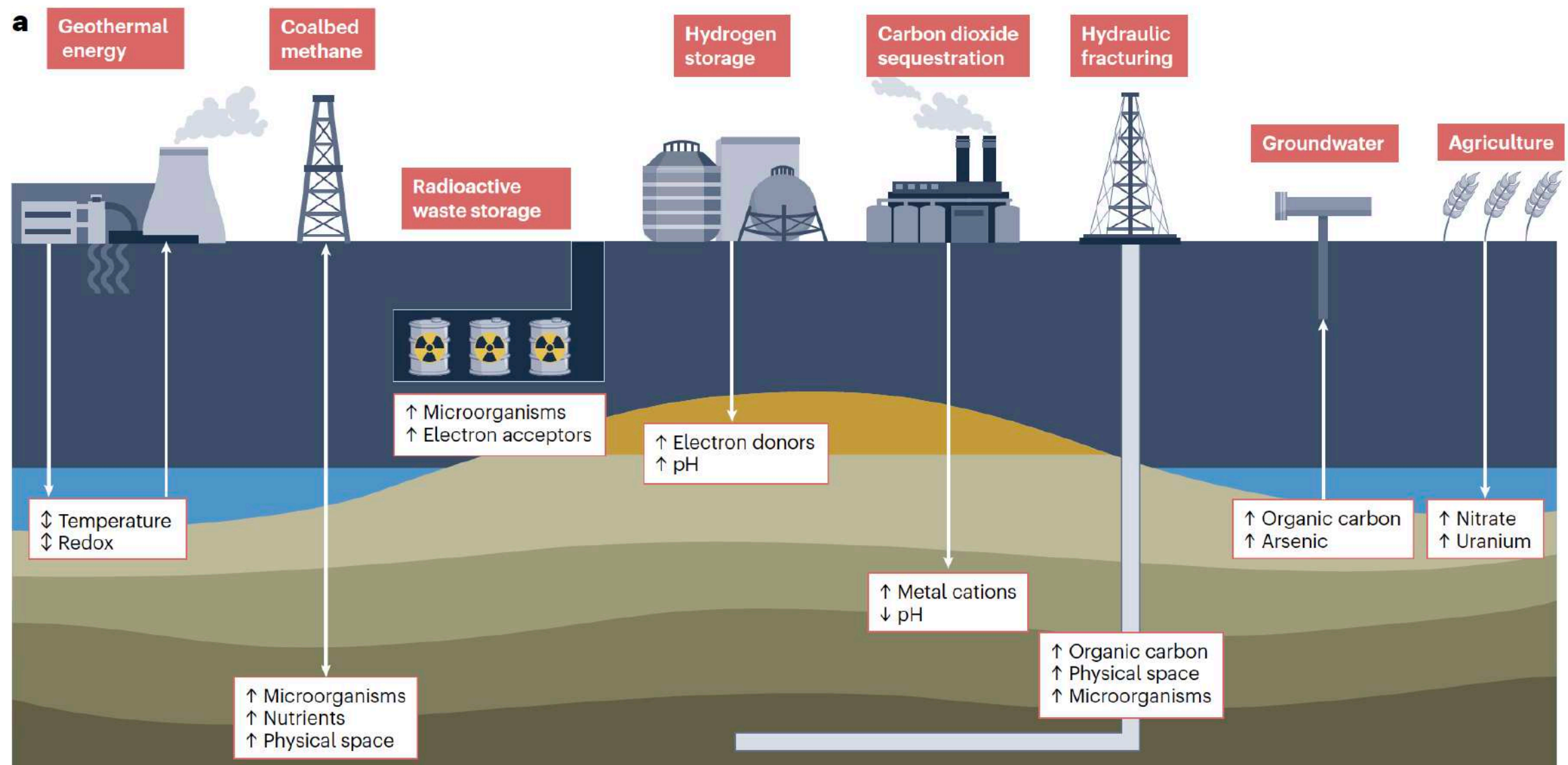


Fig.1 | Physicochemical and microbial features of the subsurface biosphere. The subsurface is composed of many layers of differing lithology, chemistries, porosities and native microorganisms. For example, aquifers tend to be shallower in the subsurface and are saturated with water, have higher levels of dissolved oxygen and carbon and host more diverse microbial communities. Sedimentary

layers, such as sandstone and shales, have varying but elevated levels of organic carbon compared to igneous rock, but porosity and microbial diversity and composition vary. Throughout the subsurface, temperature, pressure and rock compaction increase with depth.

Terrestrial subsurface biosphere: a microbial universe

Subsurface conditions limit most multicellular eukaryotic life, these environments are dominated by microorganisms that can survive under strong environmental pressures, such as elevated temperature and pressure, and lack of oxygen, light and a steady supply of key nutrients

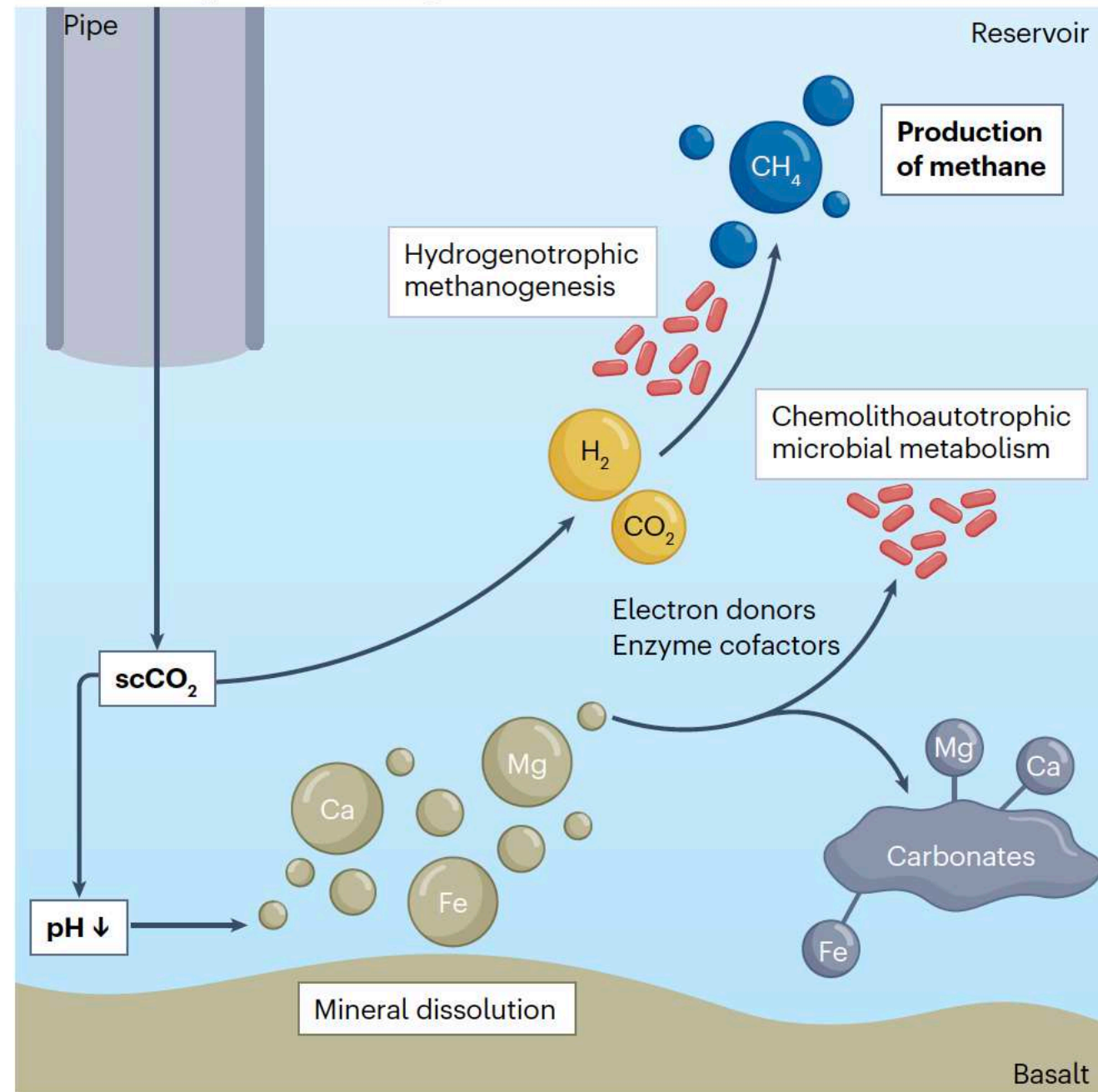




Ray Troll

Microbial effects on subsurface waste storage, I

a Carbon capture and storage

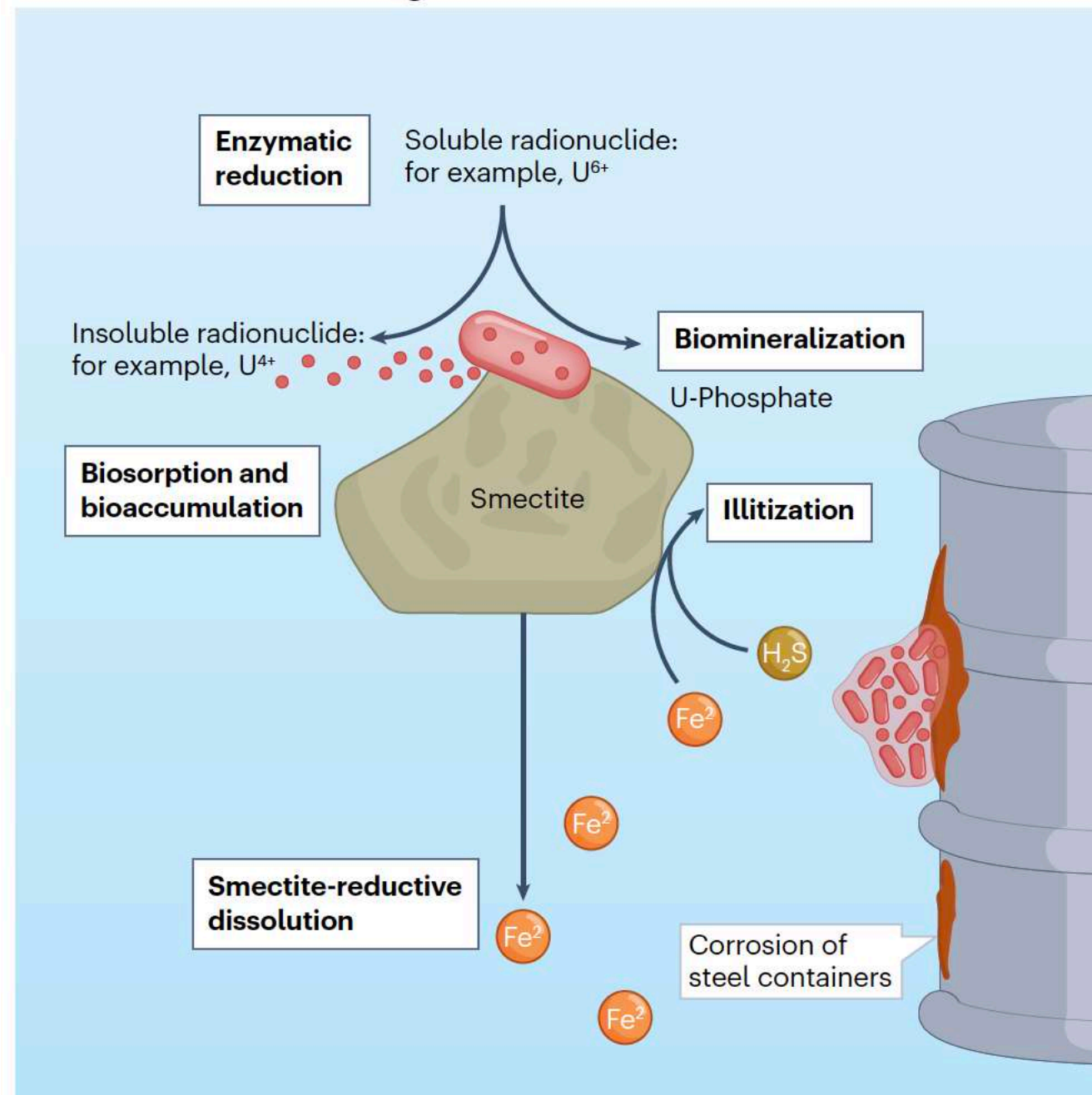


In carbon capture and storage systems, the injection of carbon dioxide (CO_2) into subsurface reservoirs can cause rapid decreases in local pH, directly affecting microbial activity and composition.

While some studies have noted the potential for the added CO_2 to stimulate hydrogenotrophic methanogenesis, the increasingly acidic conditions can drive mineral dissolution in basaltic formations, liberating metal cations such as calcium (Ca), magnesium (Mg) and iron (Fe) that could support microbial activity either as enzyme cofactors or electron donors for chemolithoautotrophic metabolisms

Microbial effects on subsurface waste storage, II

b Radioactive waste storage



Microbial metabolism interacts with engineered systems for radioactive waste storage through diverse mechanisms.

Microorganisms can directly alter the mobility of soluble and insoluble radionuclides through redox reactions, biomineralization pathways, and biosorption and intracellular bioaccumulation.

Mineral phases (for example, smectite and bentonite) used during the construction of these engineered systems can also be altered by microbial activity, potentially leading to altered physical behaviours.

Finally, microbially stimulated corrosion of steel storage canisters is a common concern, similar to many other engineered subsurface systems

Nuclear energy and nuclear waste

With **nuclear energy providing 10% of the world's power** from over **400 active power reactors** understanding the risks associated with the long-term storage of the resulting waste is crucial

Nuclear energy, although not emitting greenhouse gases, generates about **30 tonnes of high-level waste per reactor annually**

Among the radionuclides present in radioactive **waste**, **selenium** (^{79}Se isotope, half-life: 3.3×10^5 years), **uranium** (^{238}U isotope, half-life: 4.5×10^9 years), and **curium** (^{247}Cm isotope, half-life: 1.6×10^7 years; ^{248}Cm isotope, half-life: 3.5×10^6 years) are common components of the spent nuclear fuel

For these types of waste, the proposed safest options for **storage until toxicity decreases to natural levels are deep geological repositories, or multi-barrier systems placed below ground at 500–1,000 m depths that encapsulate containers of radioactive waste**

Specifically, these systems are built with a **combination of engineered and natural barriers (rock, salt and clay) and designed to contain hazardous radioactive materials for thousands of years**, so that there is **no obligation to actively maintain the facility in future generations**

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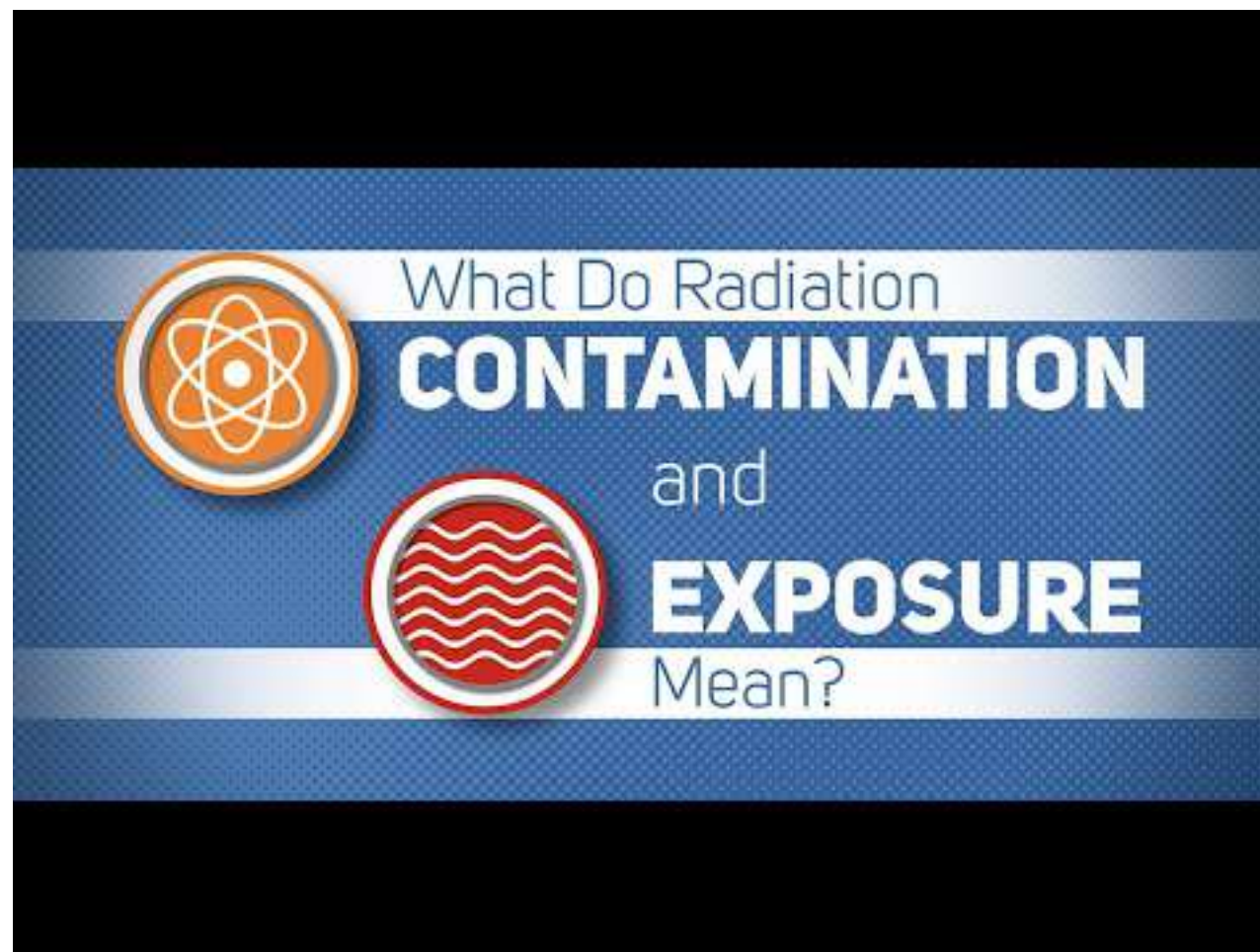
Radioactive Substances

Radioactive pollution is the result of released radionuclides in the environment. A radionuclide is an atom with an unstable nucleus which has excessive energy. While breaking down through radioactive decay it emits gamma rays

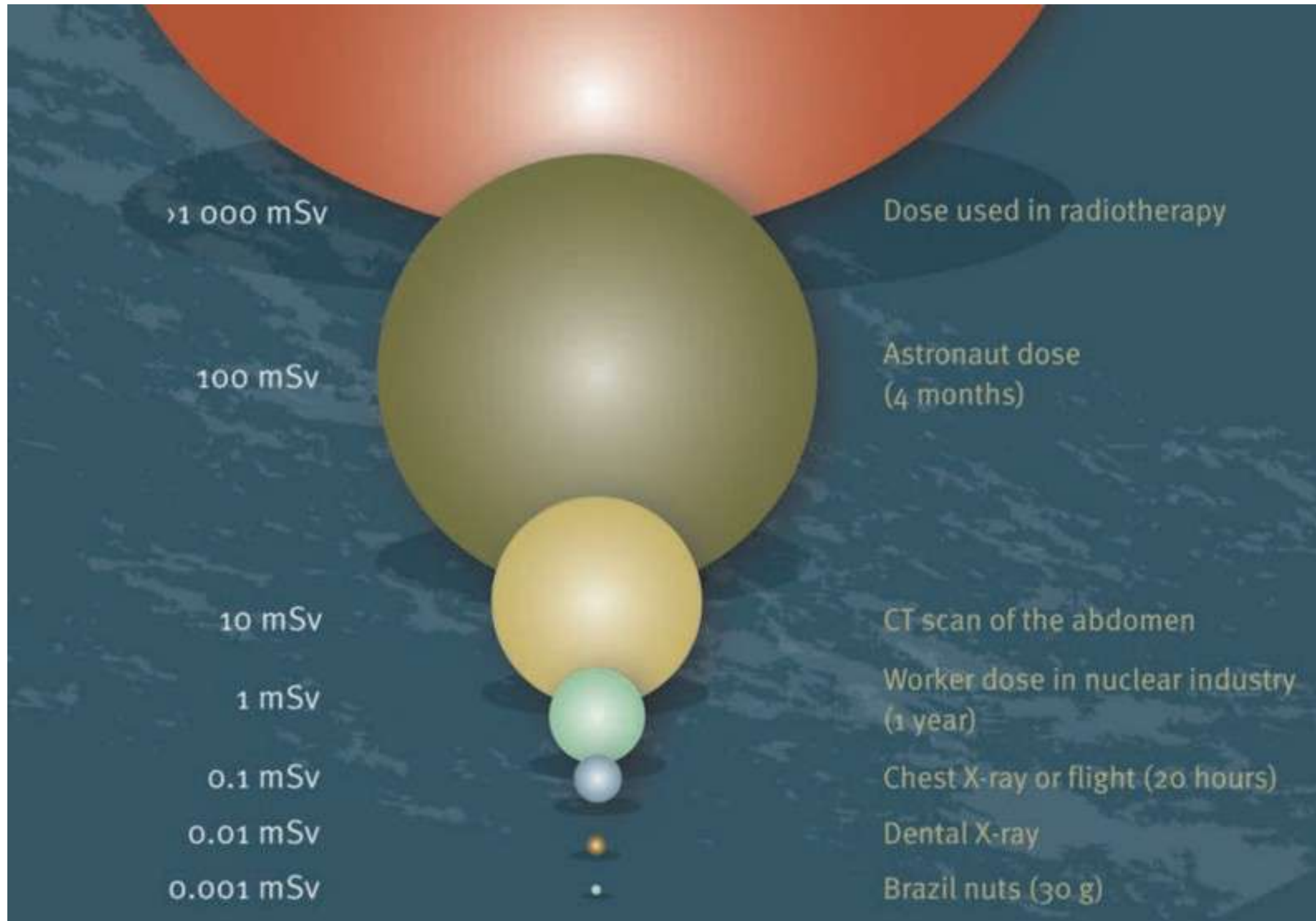
According to the UN Atlas of the Oceans, the main sources of radionuclides released into the marine environment include nuclear weapon testing's, fallout from accidents such as the Chernobyl accident in 1986, foundering of nuclear submarines, dumping of nuclear waste into the deep ocean, and discharges from nuclear power plants and nuclear reprocessing plants (<http://www.oceansatlas.org/servlet/CDSServlet?status=ND0xOTE4MiY2PWWuJjMzPSomMzc9a29z>).

Other sources are oil extraction, phosphate rock processing and radioactive transport at sea (Greenpeace 1998). Ocean currents can transport radionuclides over large distances. Radioactive waste can be in gas, liquid or solid form and may remain radioactive from a few hours to hundreds of thousands of years (<http://www.epa.gov/radiation/docs/radwaste/index.html>).

<https://www.cdc.gov/radiation-emergencies/causes/index.html>

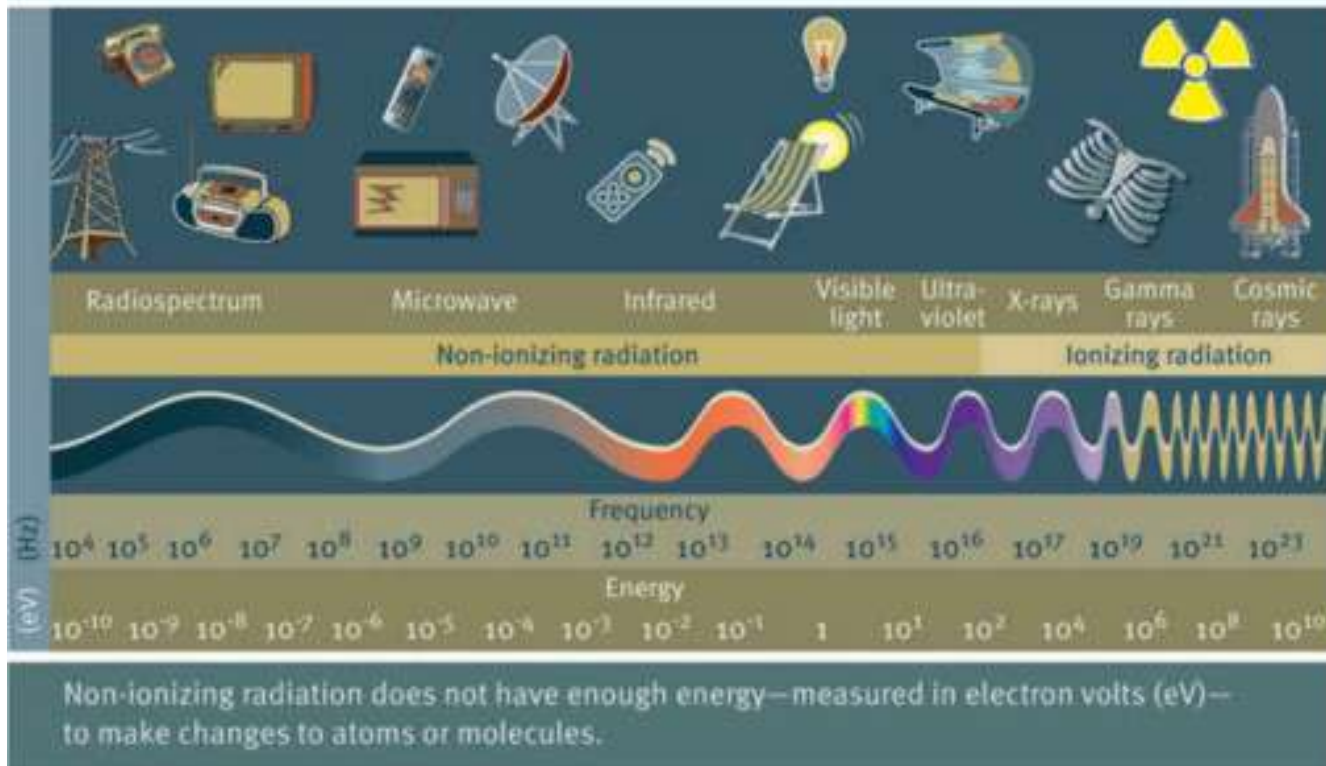


Radioactivity: dose range



To compare absorbed doses of different types of radiation, they need to be weighted for their potential to cause certain types of biological damage. This weighted dose is called the equivalent dose, which is evaluated in units called sieverts (Sv), named after the Swedish scientist Rolf Sievert

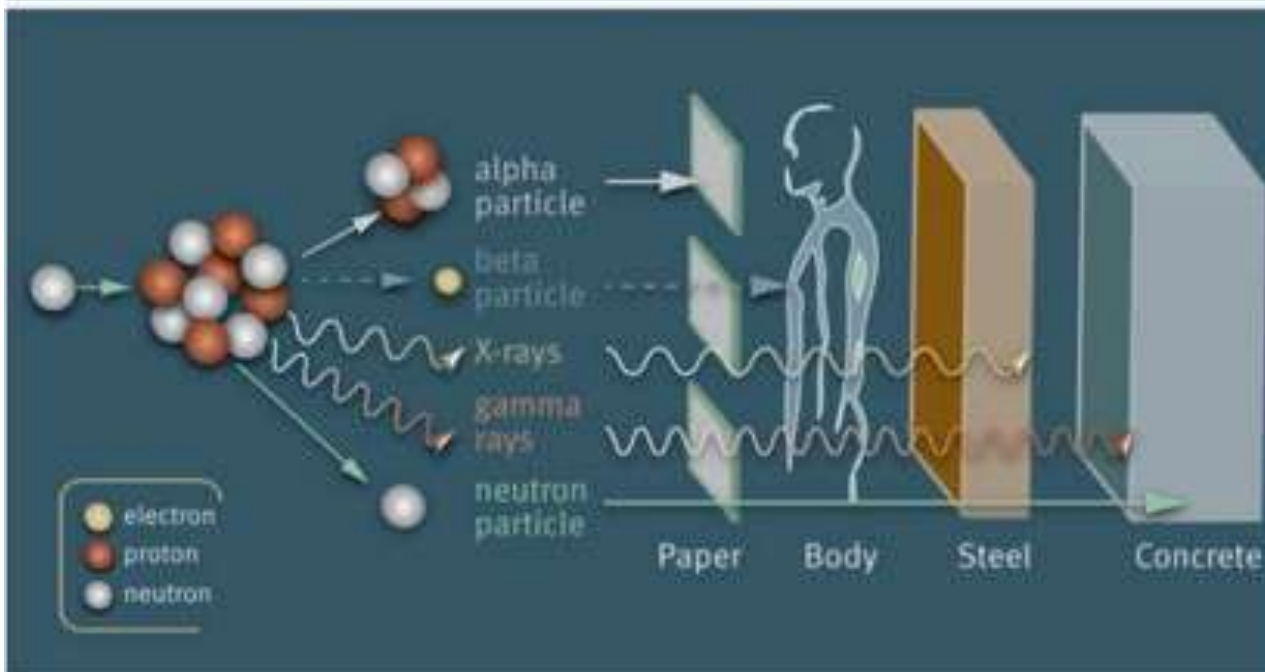
Examples of different applications using radiation



Ionizing radiations

Ionizing radiation has enough energy to liberate electrons from an atom, thereby leaving the atom charged, whereas **non-ionizing radiation**, such as radio waves, visible light or ultra-violet radiation, **does not**

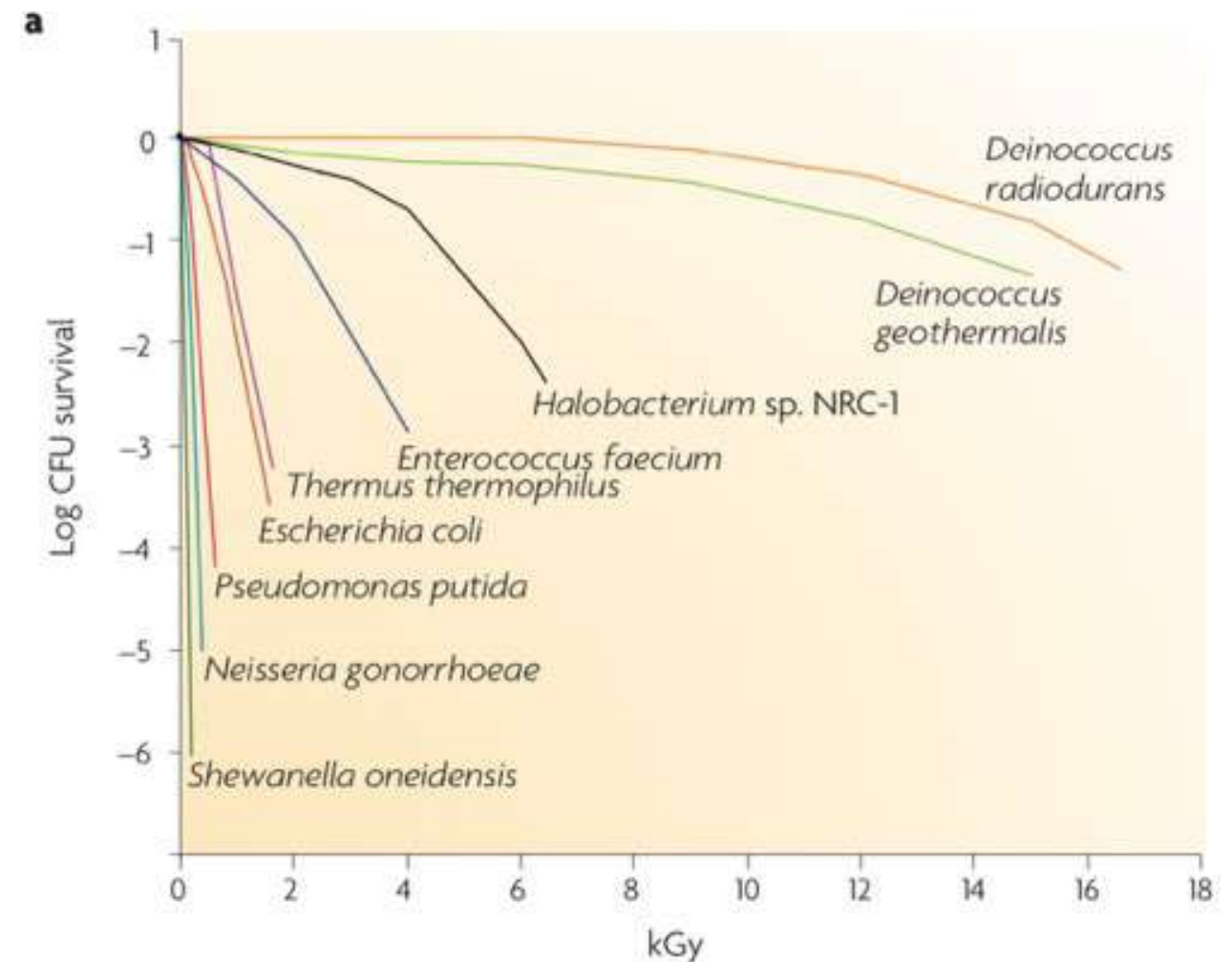
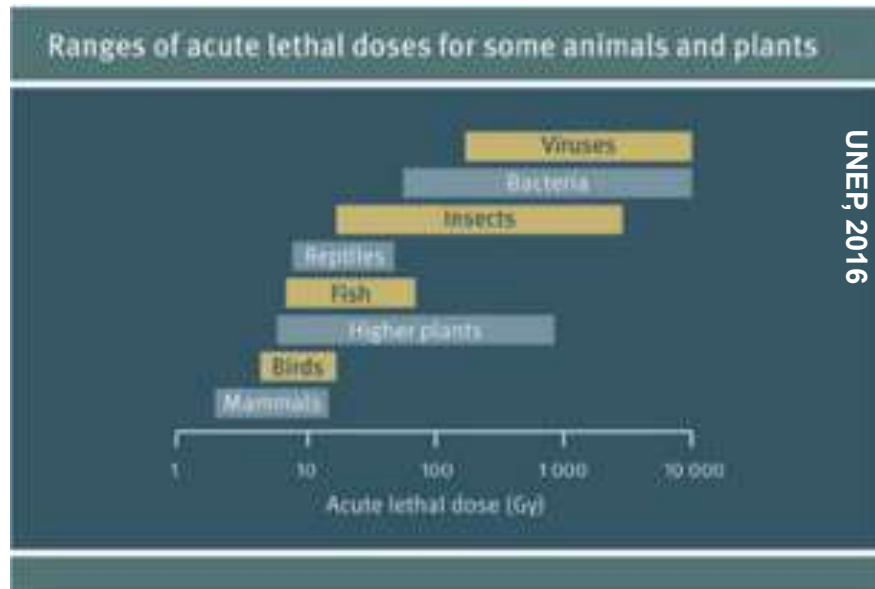
Penetrating power of different types of radiation



Worldwide distribution of radiation exposure



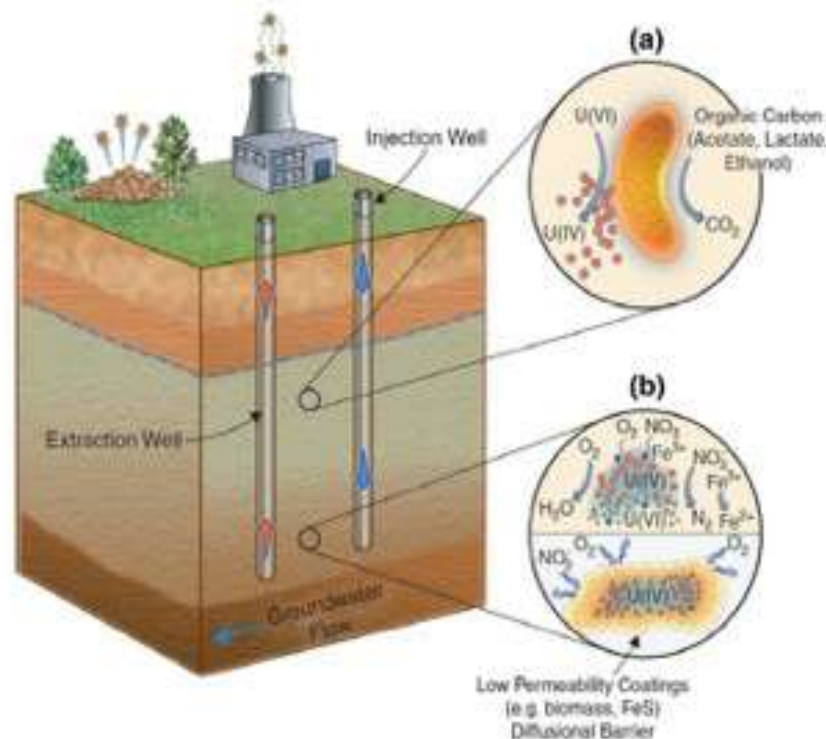
Cell-repair systems support microbial survival



The amount of radiation energy absorbed per kilogram of tissue is called the absorbed dose and is expressed in units called grays (Gy) named after the English physicist and pioneer in radiation biology, Harold Gray

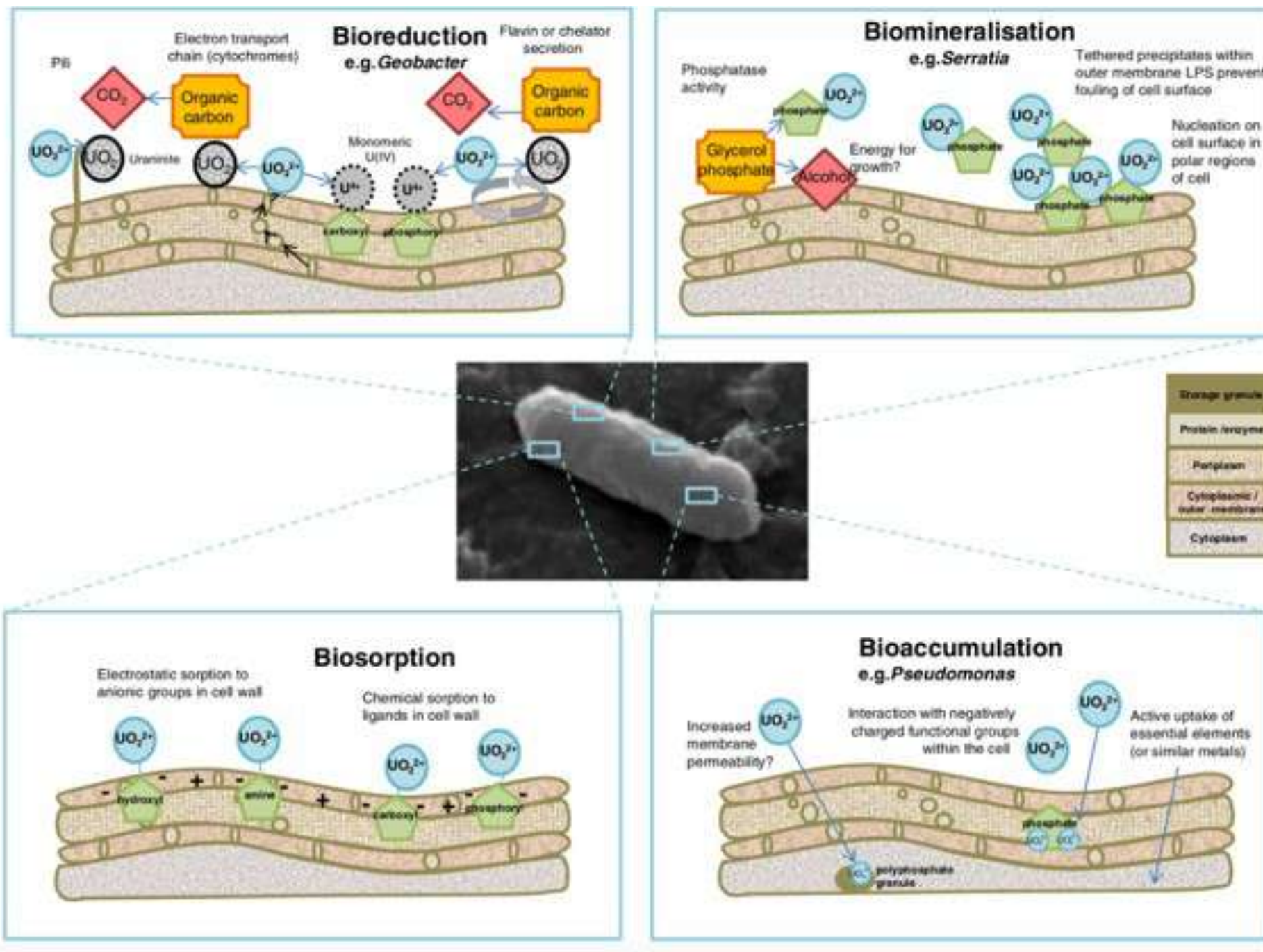
Daly, 2009

Newsome et al., 2014



- Ionizing radiation (IR) survival curves for whole-genome sequenced strains that encode a similar repertoire of DNA-repair proteins (DNA damage)
- Bioremediation: reduction of U⁶⁺(soluble) to U⁴⁺(insoluble), coupling the oxidation of organic matter and H₂

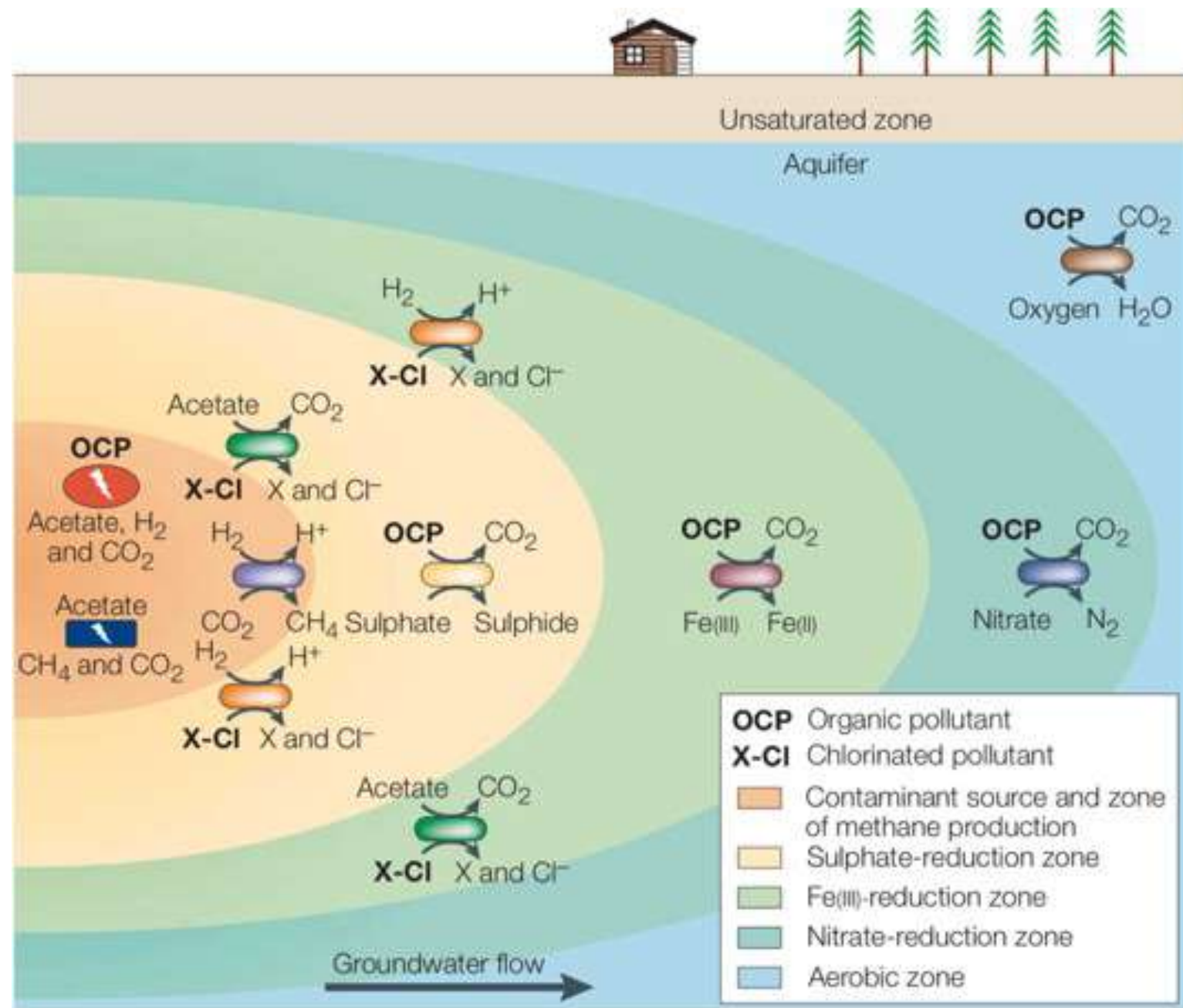
Microbes-Uranium interactions



- Decades of nuclear activities have left a legacy of environmental contamination
- Elevated concentrations of uranium and other radionuclides are present in mining and milling (mancinatura, fresatura) areas, at sites where uranium ore was processed, and where uranium was enriched
- This contamination potentially represents an uncontrolled source of radiation, and therefore regulatory bodies may require it to be remediated to acceptable levels

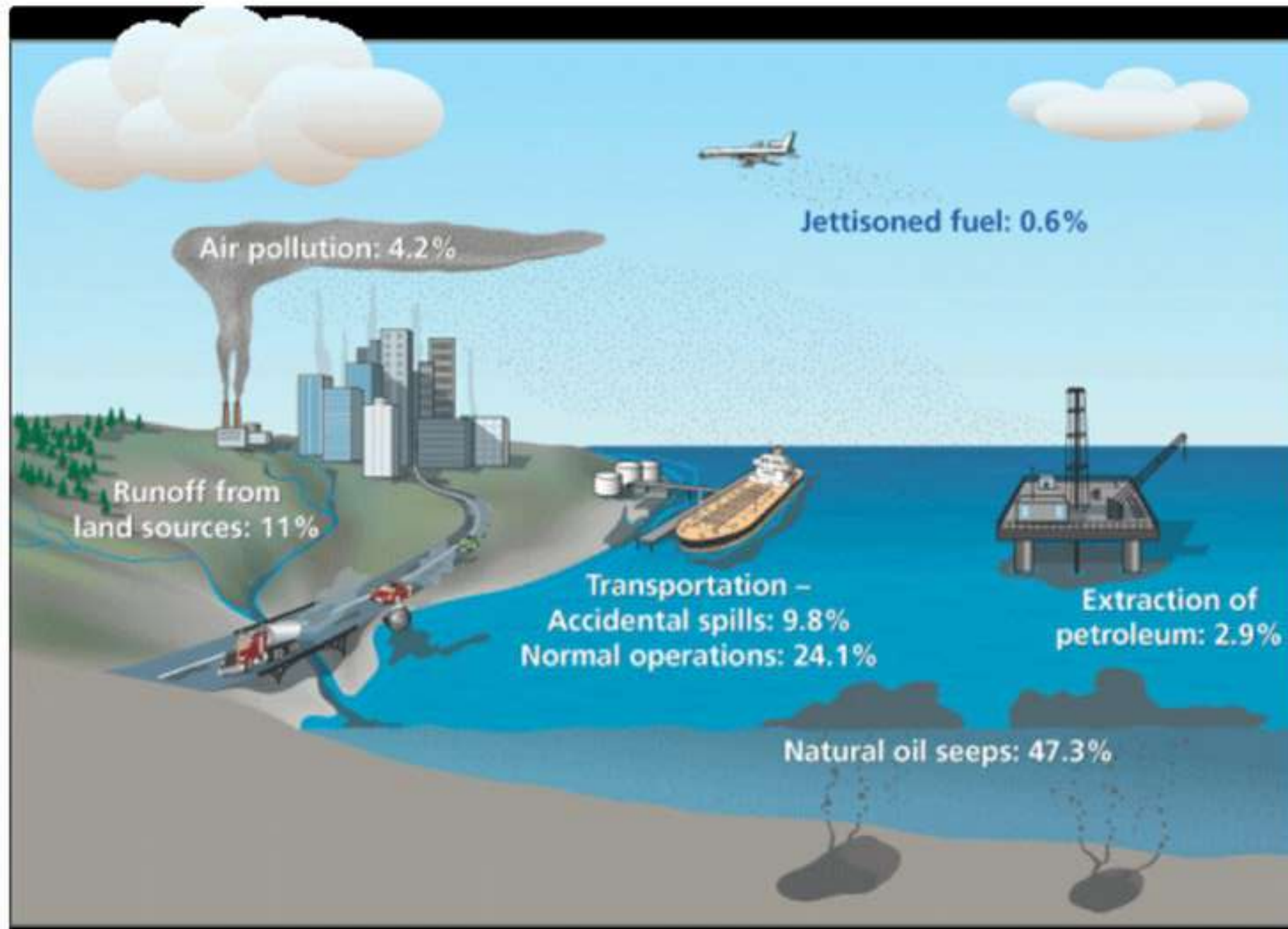
Bioremediation reactions for oxidizable, organic contaminants and chlorinated solvents in contaminated aquifers

Lovely, 2013



- At the source of contamination, such as the leachate emanating from a landfill, methane production often predominates —> microorganisms convert organic contaminants to simpler molecules, such as acetate and hydrogen, which methane-producing microorganisms convert to methane
- In other zones, organic contaminants are oxidized to carbon dioxide with the reduction of sulphate, Fe (III), nitrate or oxygen
- Chlorinated contaminants, which are not easily oxidized, undergo reductive dechlorination in the methanogenic, sulphate-reduction or Fe (III)-reduction zones

Hydrocarbon sources in the environment



Hydrocarbon-degrading microbes

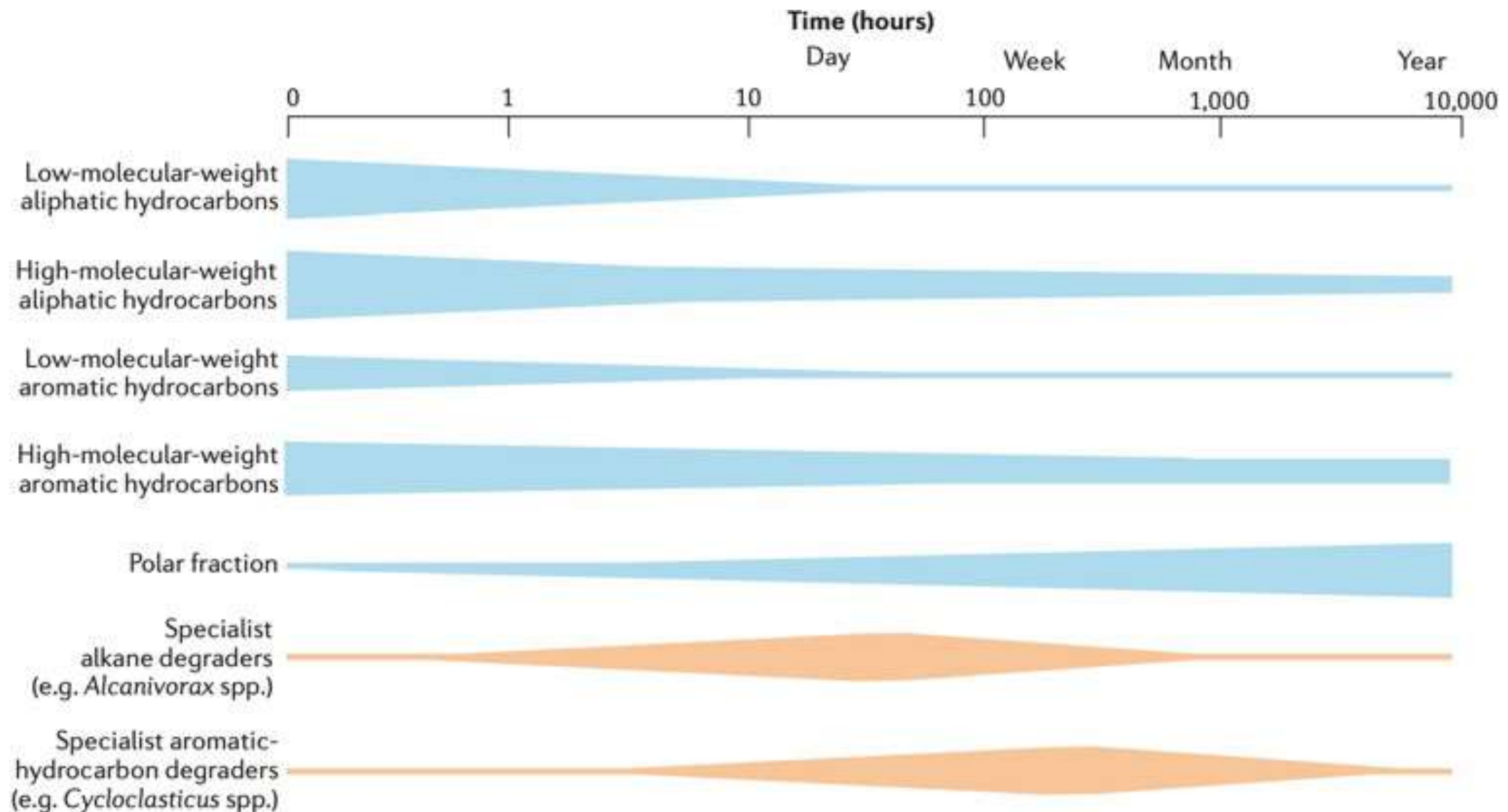
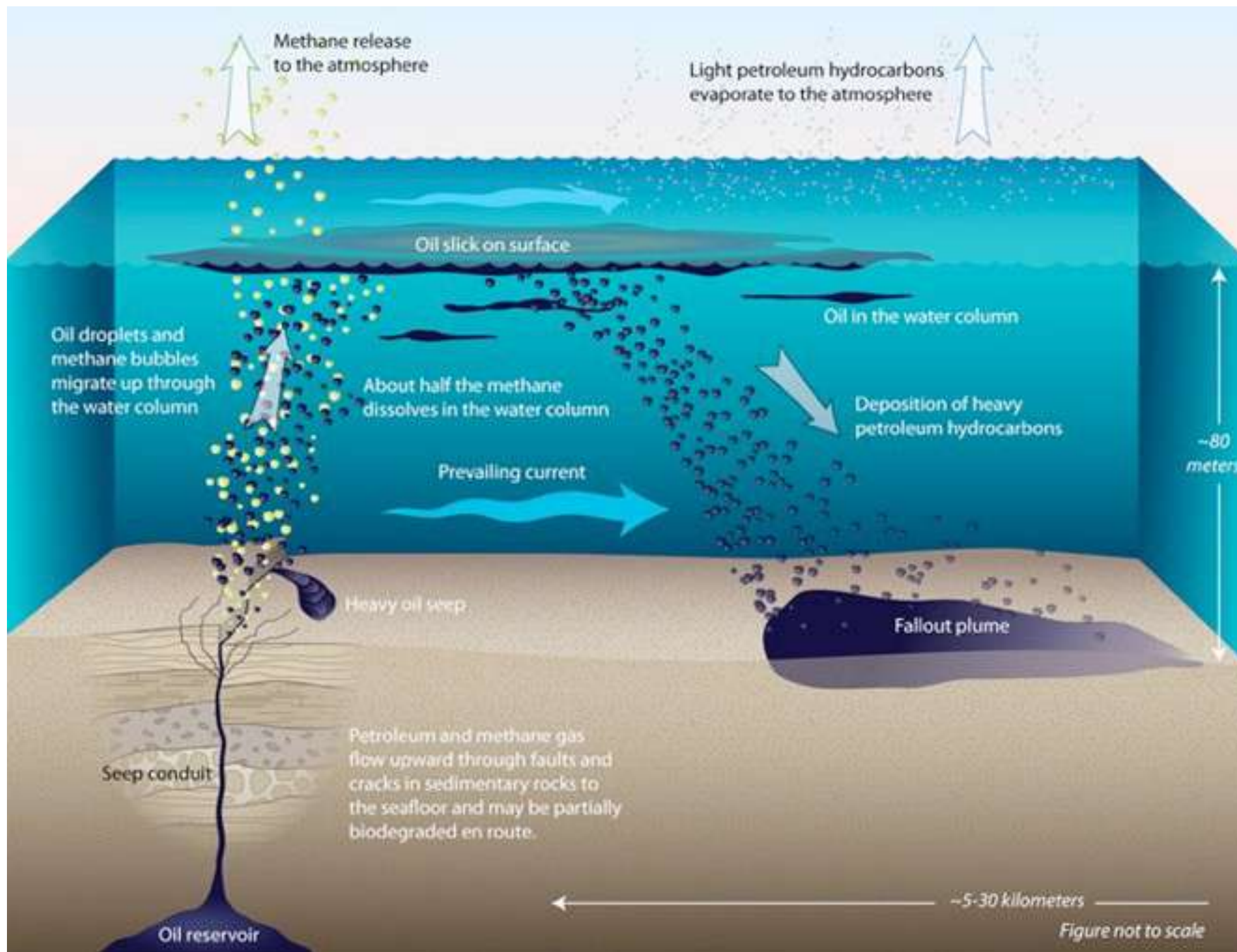


Figure 3 | **Changes in the composition of spilled oil and corresponding changes in the abundance of key organisms.** This schematic diagram represents general changes that have been observed in several studies. Slight variations are likely, both in the specific organisms that are involved and in the extent of biodegradation of different crude oils, which have a range of physical and chemical properties that affect their fate in the environment.

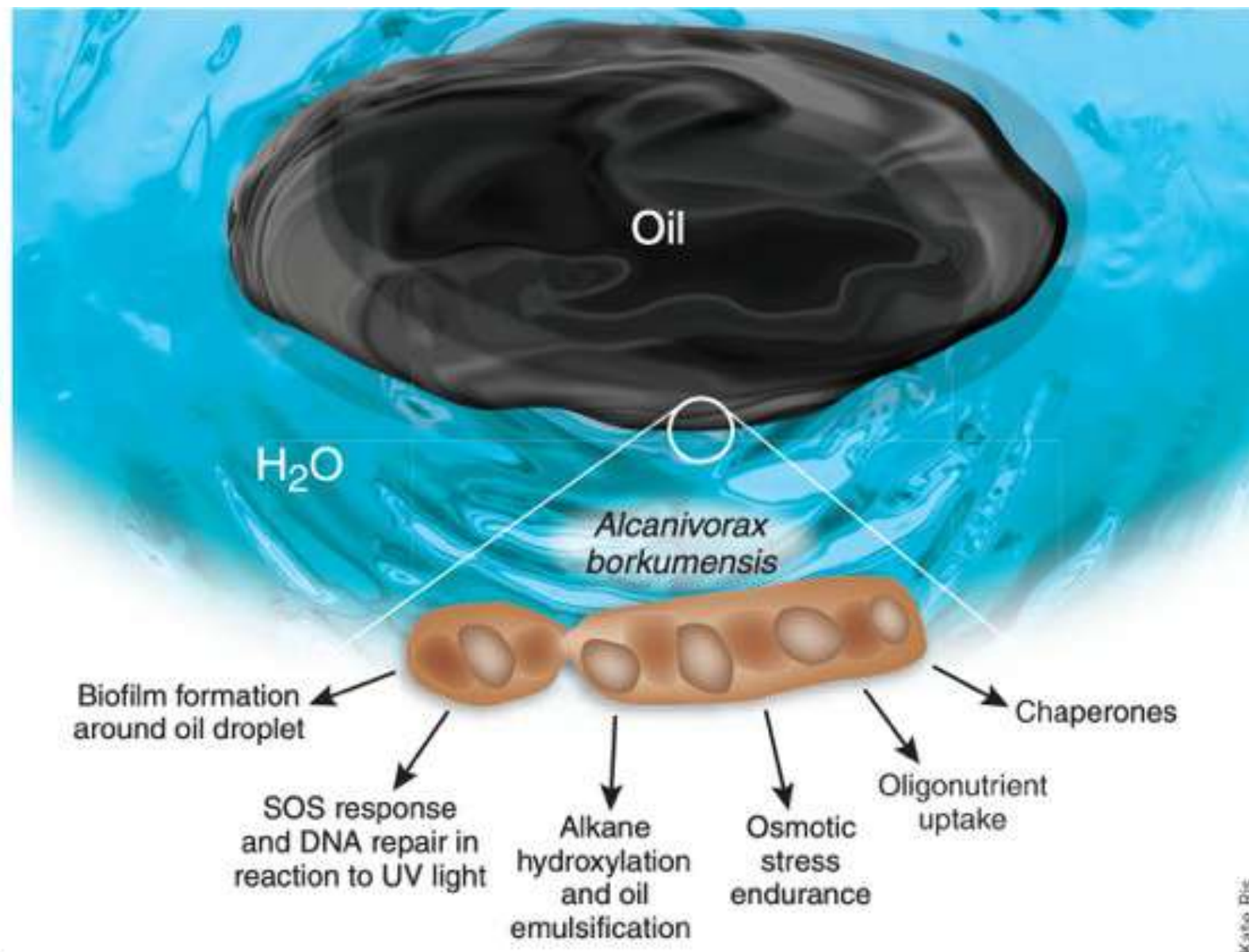
Fate of oil

Illustration by Jack Cook, Woods Hole
Oceanographic Institution



Route traveled by oil leaving the subseafloor reservoir as it travels through the water column to the surface and ultimately sinks and falls out in a plume shape onto the seafloor where it remains in the sediment

Microbial oil-degradation and survival mechanisms



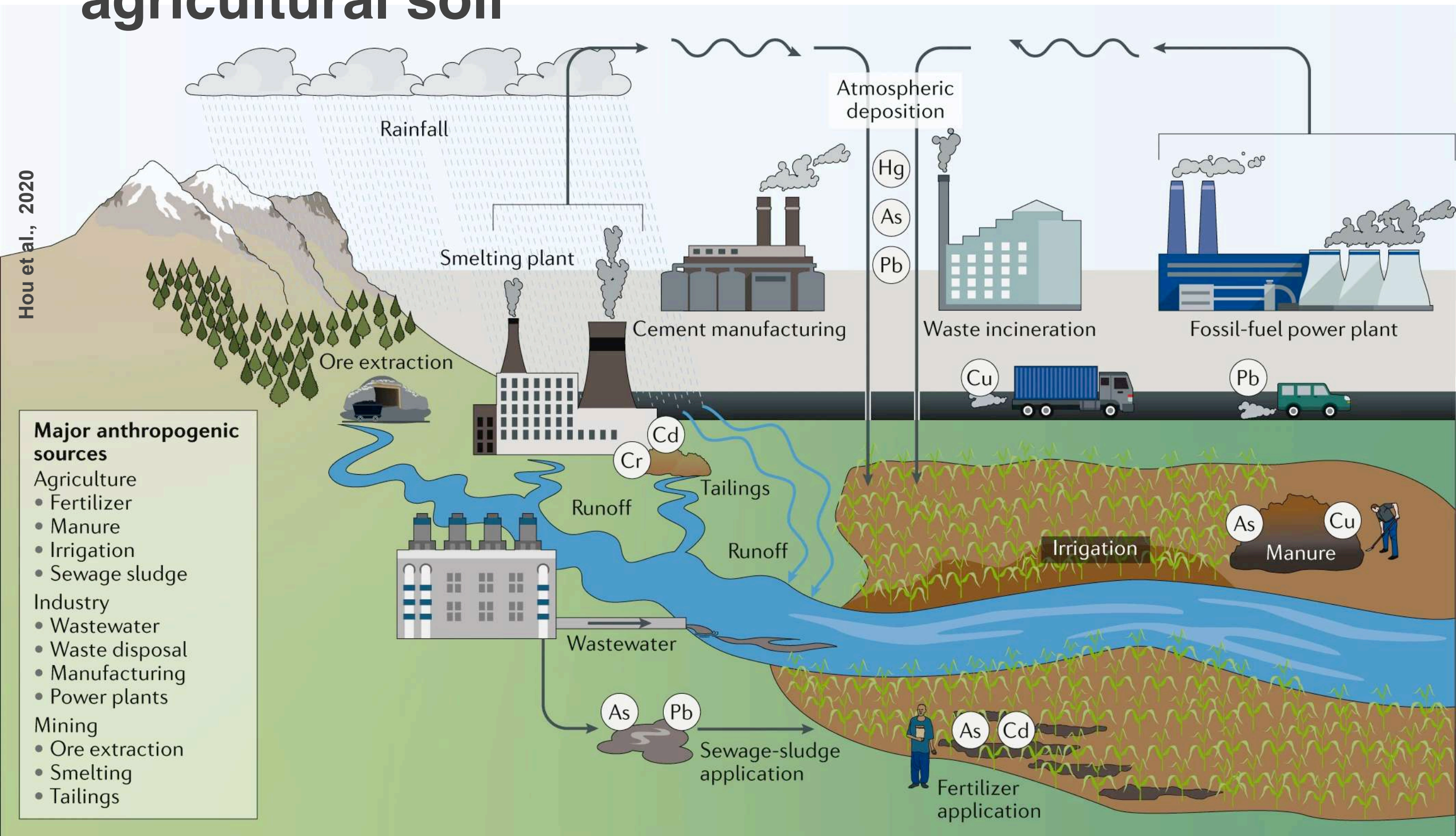
Both crude petroleum and refined oil contain a complex mixture of organic molecules, including a considerable fraction of linear, cyclic and branched aliphatic hydrocarbons

During oil spills, the various components are deposited on shorelines, sink in the seabed or remain in suspension in seawater

The niches for *Alcanivorax borkumensis* are probably located at the aerobic, alkane-rich areas of shorelines and the oxygen-rich layers of the oil-polluted sea surface

A. borkumensis cannot tackle contaminated anaerobic sediments, aromatic hydrocarbons or the heavier oil fractions

Sources of heavy metal(loid)s pollution in agricultural soil



Major anthropogenic sources can be classified into three categories: agricultural, industrial and mining

Brownfield sites

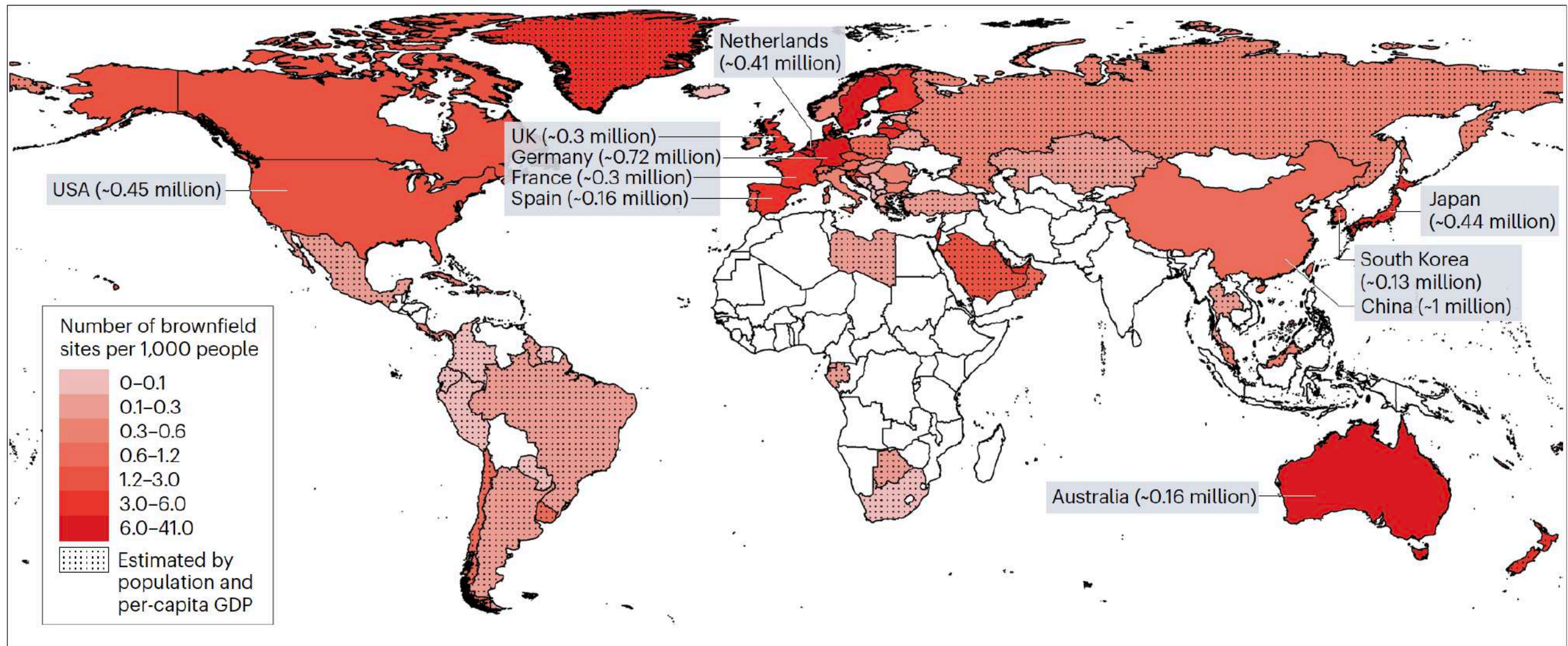
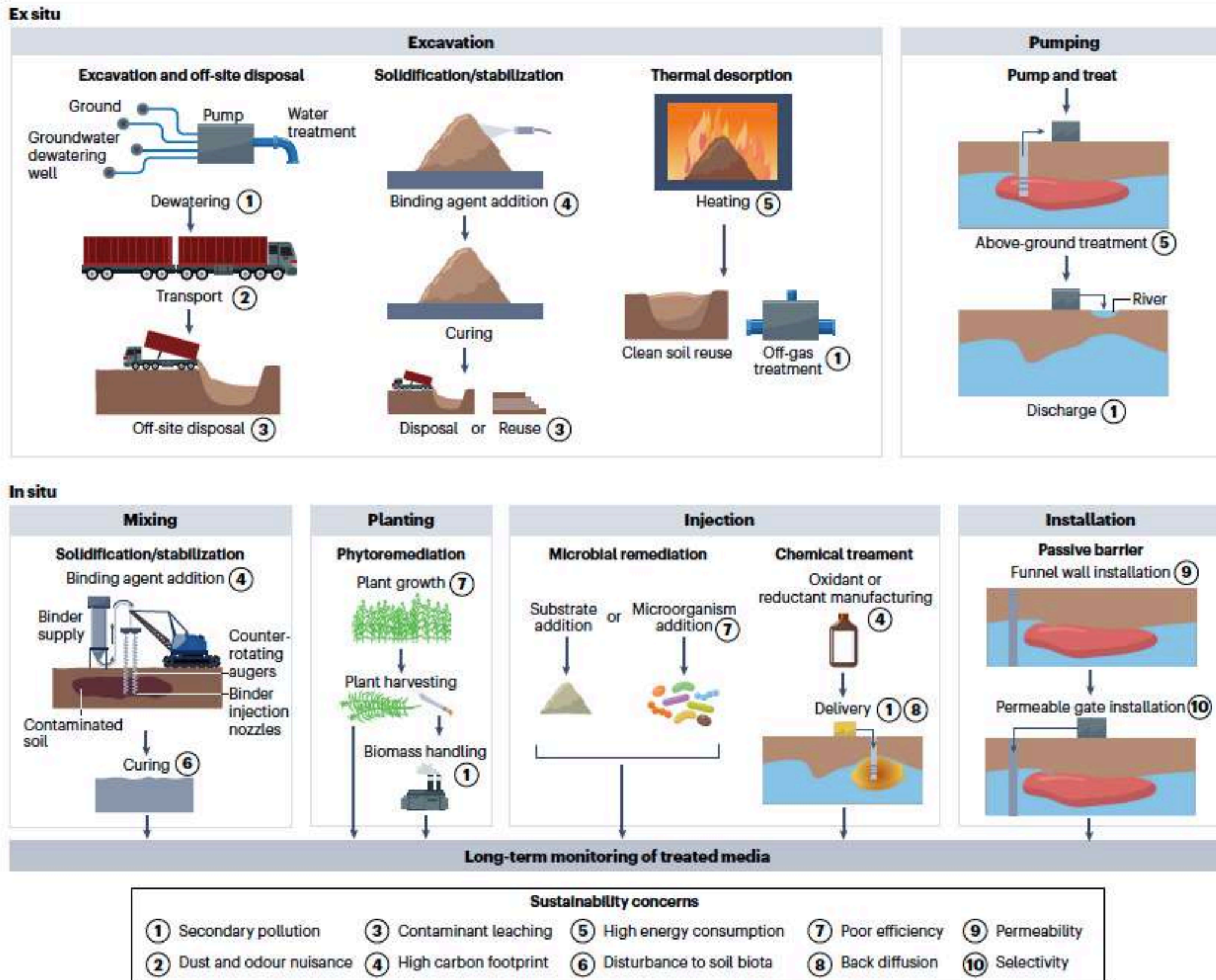


Fig. 1 | Global distribution of brownfield sites. The number of brownfield sites per 1,000 people is shaded at the country level. Countries with literature data are solid, and estimates for other countries derived using population and per-capita GDP data are hatched. The countries with the largest number of brownfields are

labelled, with the number of sites in parentheses. Number of contaminated sites is estimated to exceed 5 million globally. Remediation and redevelopment of these sites is needed to ensure future sustainable development.

- **Brownfield sites:** industrial sites that were once at the heart of industrialized urban centres are increasingly abandoned
- Toxic heavy metals and volatile organic compounds are released from piled solid wastes, leaking pipelines, broken storage tanks and wastewater ponds, causing the contamination of adjacent soil, water and air

Brownfield remediation and redevelopment strategies



Fungi

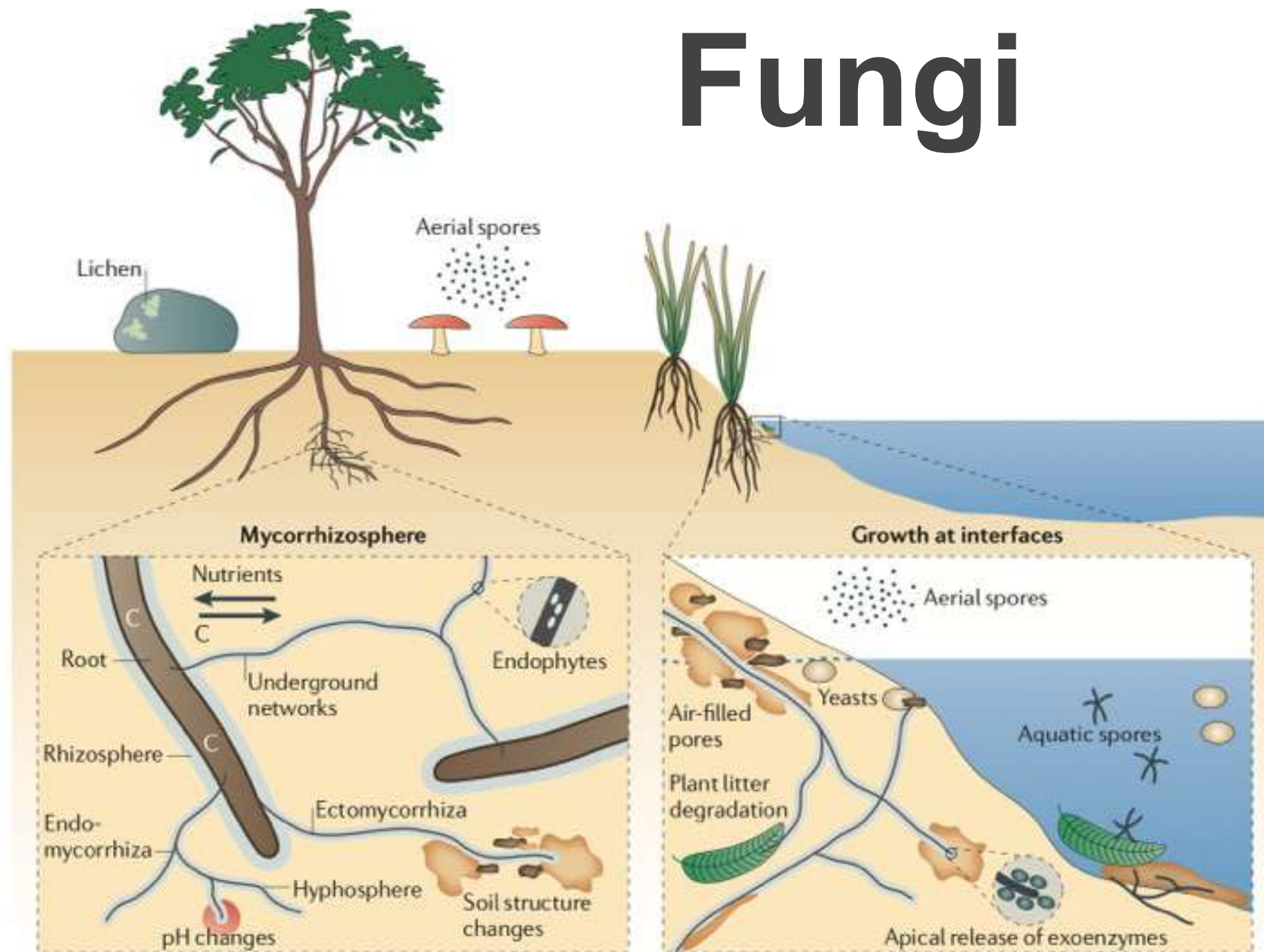


Figure 1 | **Typical habitats of terrestrial and aquatic fungi, and some of their ecological features.** Fungi in the environment can exist as terrestrial and aquatic saprobes, yeasts, symbiotic partners of lichens, and mycorrhizal symbionts associated with plant roots. They can form ectomycorrhizal and endomycorrhizal associations and can influence soil structure (by enmeshment of particles), soil chemistry (by excretion of acids, for example) and plant growth (through the mobilization and provision of nutrients in exchange for photosynthetic assimilates). Saprotrophic fungi adapted to water-unsaturated soil or aqueous environments can propagate through spores adapted to dispersal through the atmosphere or water, respectively. Growing hyphae explore food sources by deploying extracellular enzymes (exoenzymes).

Fungi-based bioremediation applications

	Phylum or subphylum	Organic chemicals degraded	Major ecological characteristics
Basal fungal lineages	Microsporidia		Obligate parasites of animals
	Kickxellomycotina (2)	PAHs	Saprobies, and parasites of animals and fungi
	Zoopagomycotina		Parasites of nematodes, protozoa and fungi
	Entomophthoromycotina (2)	PAHs	Parasites of insects
	Blastocladiomycota		Saprobies, and parasites of plants and animals; aquatic and terrestrial
	Mucoromycotina (16)	Benzoquinoline, biphenyl, PAHs, pesticides, synthetic dyes and TNT	Saprobies, parasites or ectomycorrhizal symbionts
	Neocallimastigomycota		Gut symbionts of ruminant herbivores
	Chytridiomycota (2)	PAHs	Saprobies, and parasites of plants and animals; fresh water and wet soil
	Glomeromycota	PAHs and pesticides	Arbuscular mycorrhizal symbionts
Dikarya	Ascomycota (88) Pezizomycotina (57)	Alkanes, alkylbenzenes, biphenyl, chlorophenols, coal tar oil, crude oil, diesel, EDCs, fragrances, PAHs, PCDDs, pesticides, synthetic dyes, TNT and toluene	Saprobies, pathogens of plants and animals, and symbiotes of algae (lichens), plants (ectomycorrhizae, ercoid mycorrhizae and endophytes) and insects; terrestrial and aquatic
	Saccharomycotina (9)	Alkanes, alkylbenzenes, biphenyl, crude oil, EDCs, PAHs and TNT	
	Other ascomycetes (22)	Alkanes, diesel, coal tar oil, crude oil, MTBE, PAHs, pesticides, RDX, toluene and synthetic dyes	
	Basidiomycota (53) Agaricomycotina (50)	Alkanes, BTEX compounds, chloroaliphatics, lignols and phenols, crude oil, coal tar, EDCs, PAHs, PCBs, PCDDs, PCDFs, personal care product ingredients, pesticides, pharmaceutical drugs, RDX, synthetic dyes, synthetic polymers, TNT and other nitroaromatics	Saprobies, ectomycorrhizal symbionts, pathogens of plants and animals, and parasites of other fungi; terrestrial and aquatic
	Pucciniomycotina (3)	Cresols, crude oil, dibenzothiophene, PAHs and RDX	

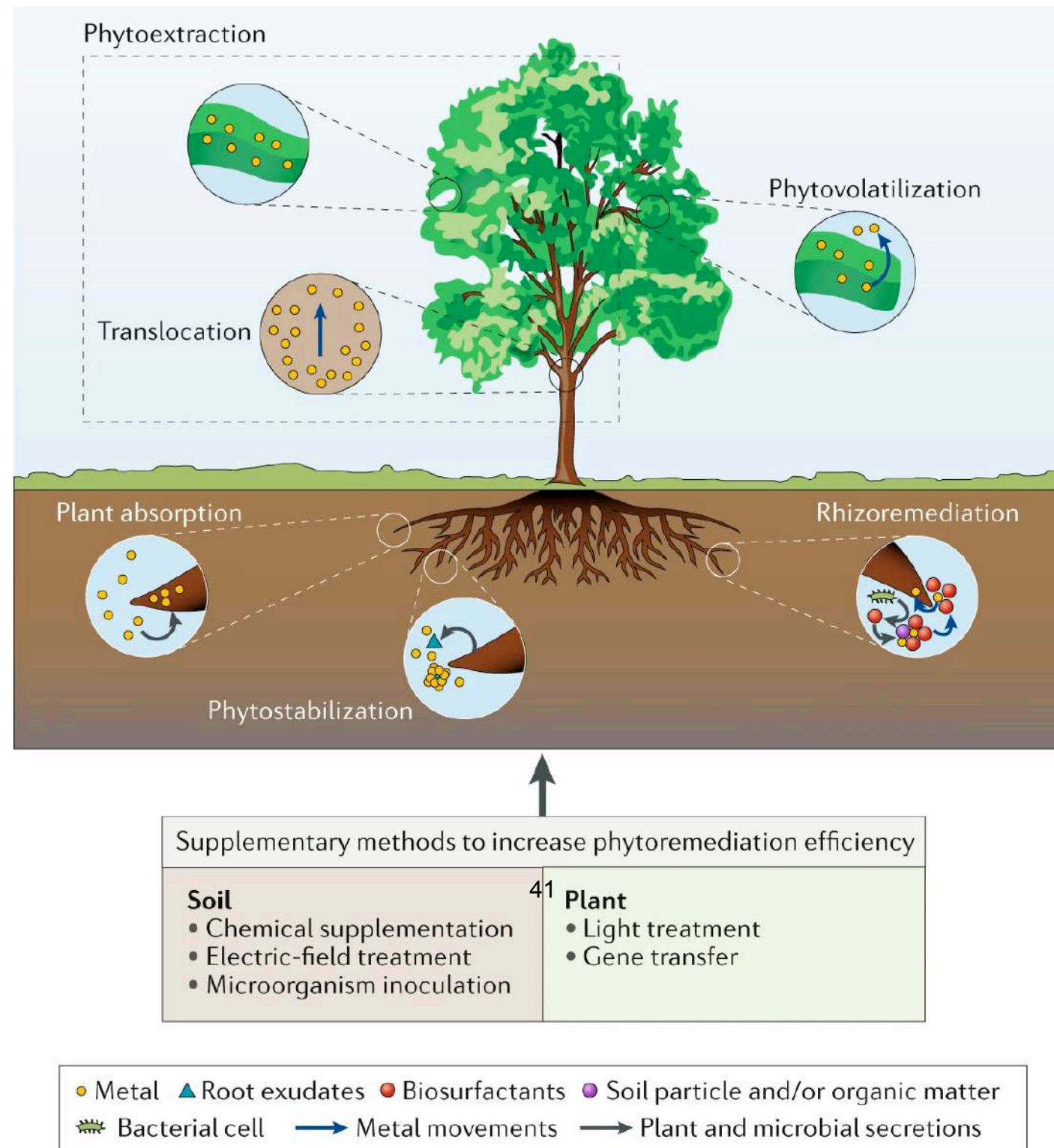
Harms et al., 2011

Phytoremediation: microbes-plant- fungi interactions

- **Phytoremediation** basically refers to **use of plants and associated soil microbes to reduce concentrations or toxic effects of contaminants in environment**
- Phytoremediation is widely accepted as a cost-effective environmental restoration technology
- Phytoremediation is **limited to root-zone of plants**
- **Limited application when concentrations are toxic to plants**
- Different processes such as *in situ* stabilization or degradation and removal (i.e., volatilization or extraction) of contaminants

Technology	Action on Contaminants	Main Type of Contaminants	Vegetation
Phytostabilization	Retained <i>in situ</i>	Organics and metals	Cover maintained
Phytodegradation	Attenuated <i>in situ</i>	Organics	Cover maintained
Phytovolatilization	Removed	Organics and metals	Cover maintained
Phytoextraction	Removed	Metals	Harvested repeatedly
Table 1: Comparison between phytoremediation technologies			

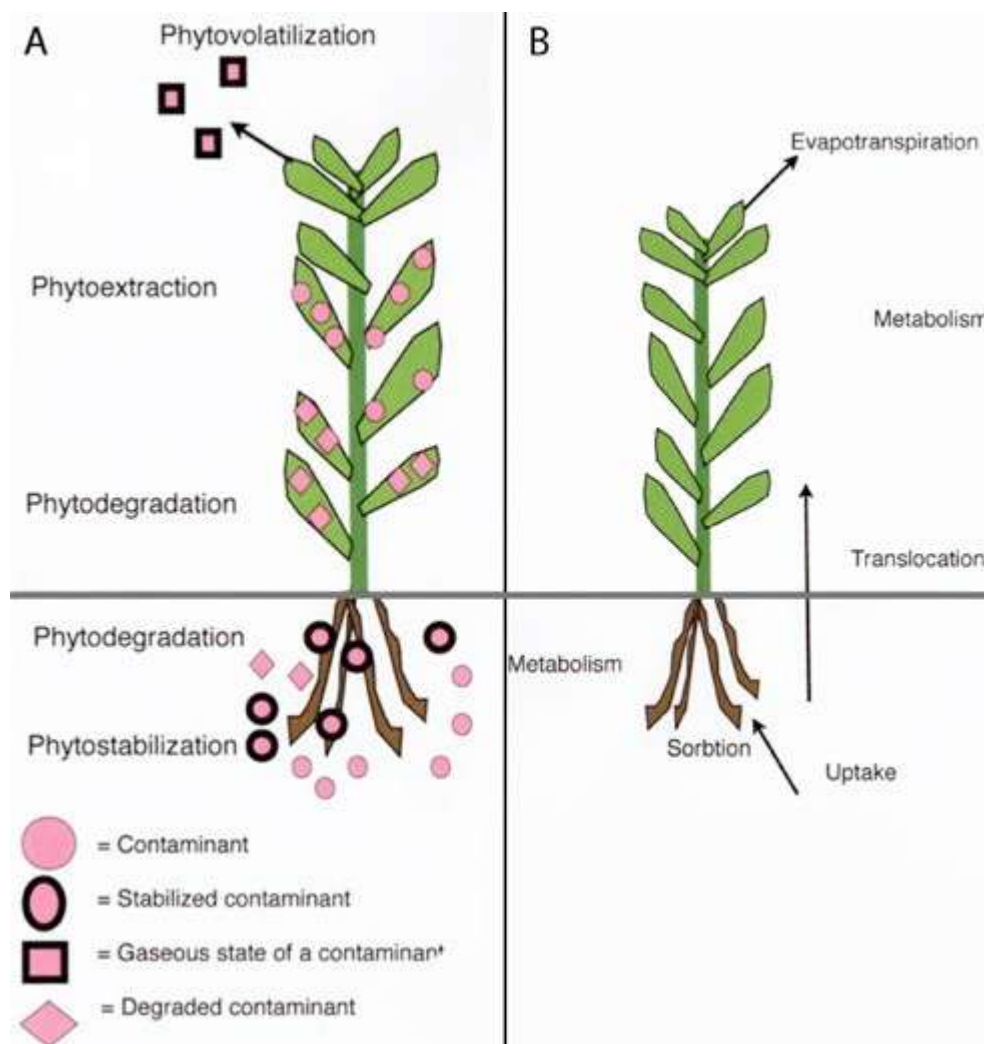
Phytoremediation



Hou et al., 2020

Natural methods of removing or detoxifying soil metal(loid)s, and supplementary methods to increase phytoremediation efficiency

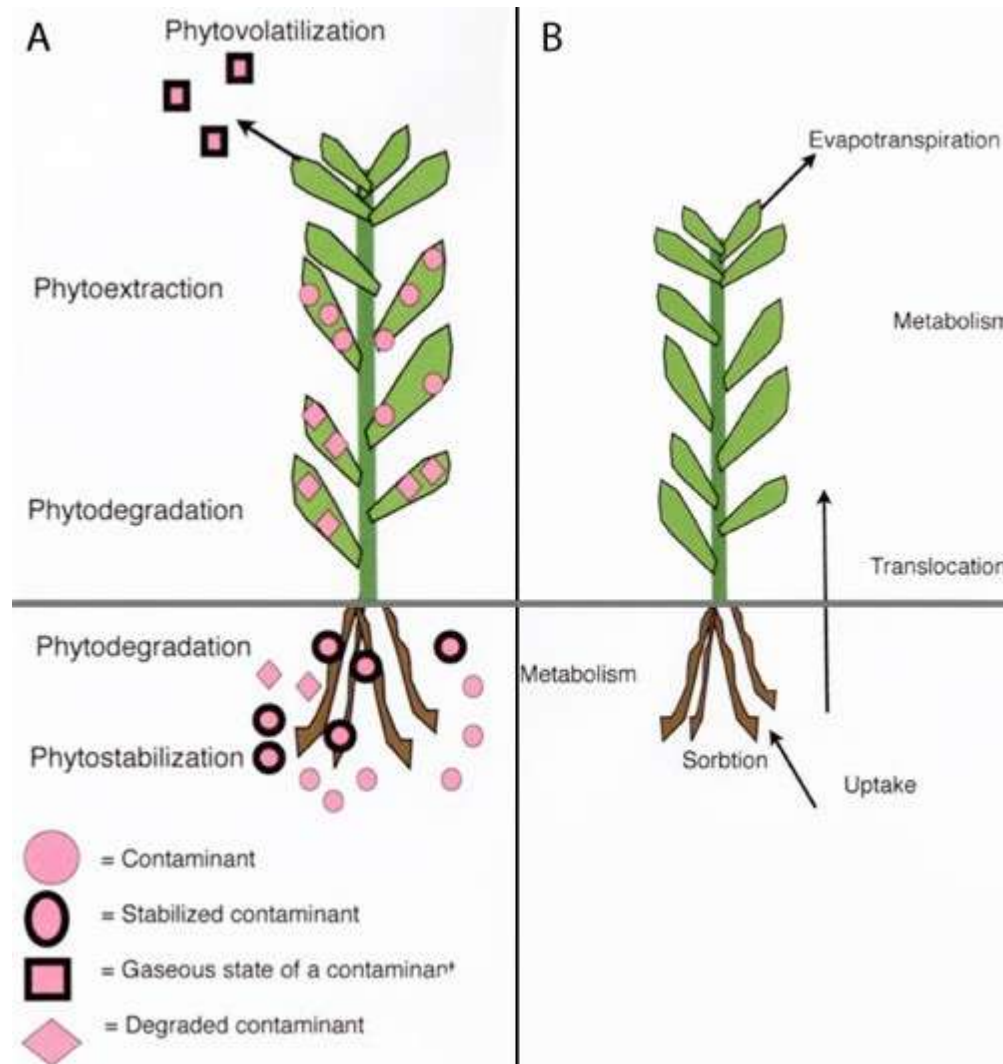
Phytostabilization



Phytostabilization aims to retain contaminants in the soil and prevent further dispersal

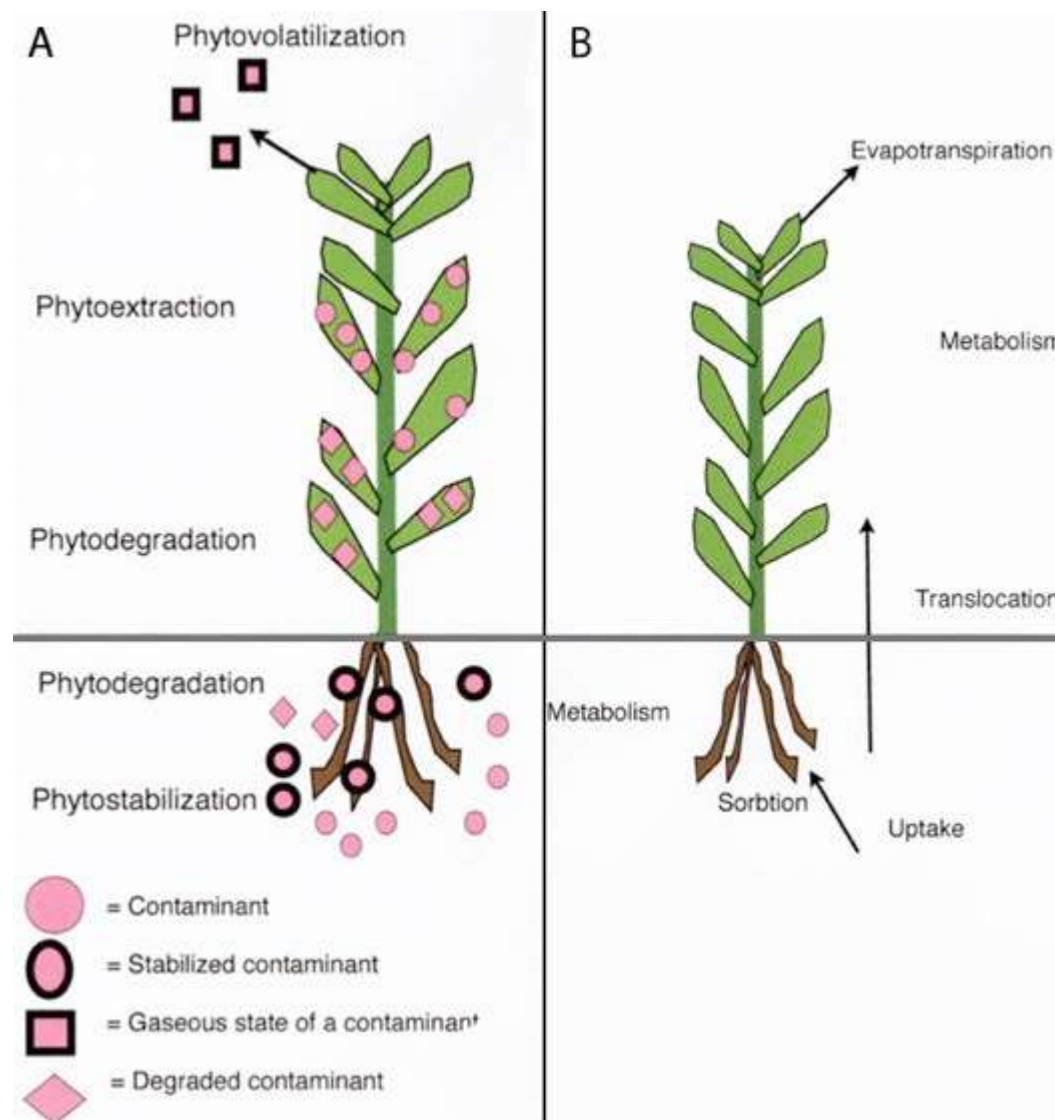
Contaminants can be stabilized in roots or within rhizosphere → revegetation of mine tailings is a common practice to prevent further dispersal of contaminants

Phytodegradation



Phytodegradation involves degradation of organic contaminants directly, through release of enzymes from roots, or through metabolic activities within plant tissues —> organic contaminants are taken up by roots and metabolized in plant tissues to less toxic substances

Phytovolatilization



Phytovolatilization involves uptake of contaminants by plant roots and its conversion to a gaseous state, and release into atmosphere —> driven by evapotranspiration of plants

Phytoextraction

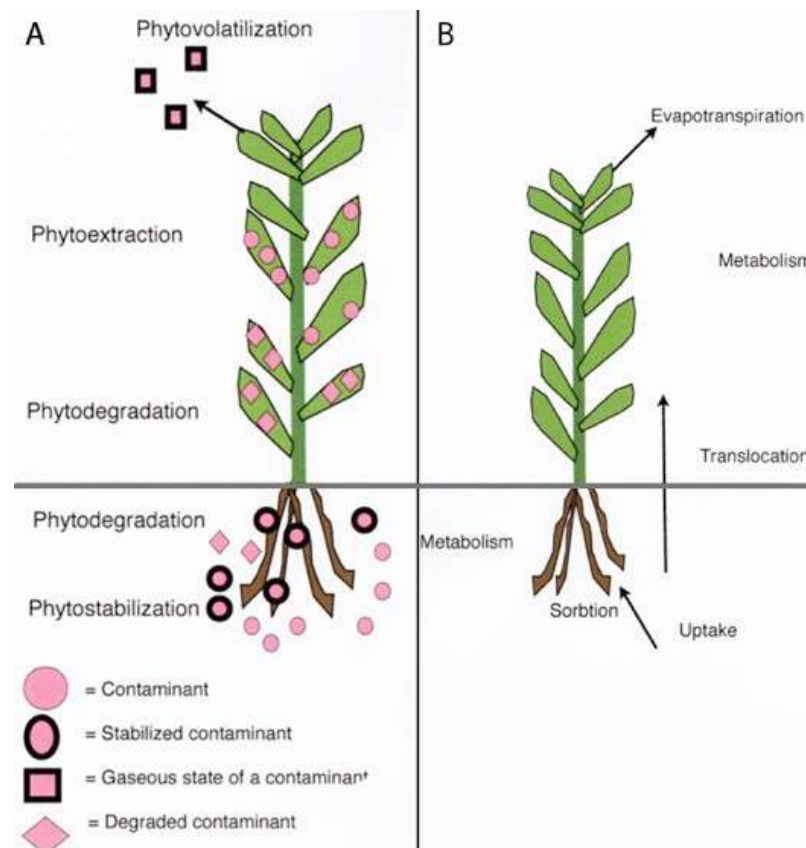
- **Phytoextraction** uses ability of plants to accumulate contaminants in the aboveground, harvestable biomass

This process involves repeated harvesting of biomass in order to lower the concentration of contaminants in soil

Phytoextraction is either a continuous process (using metal hyperaccumulating plants, or fast growing plants), or an induced process (using chemicals to increase bioavailability of metals in soil)

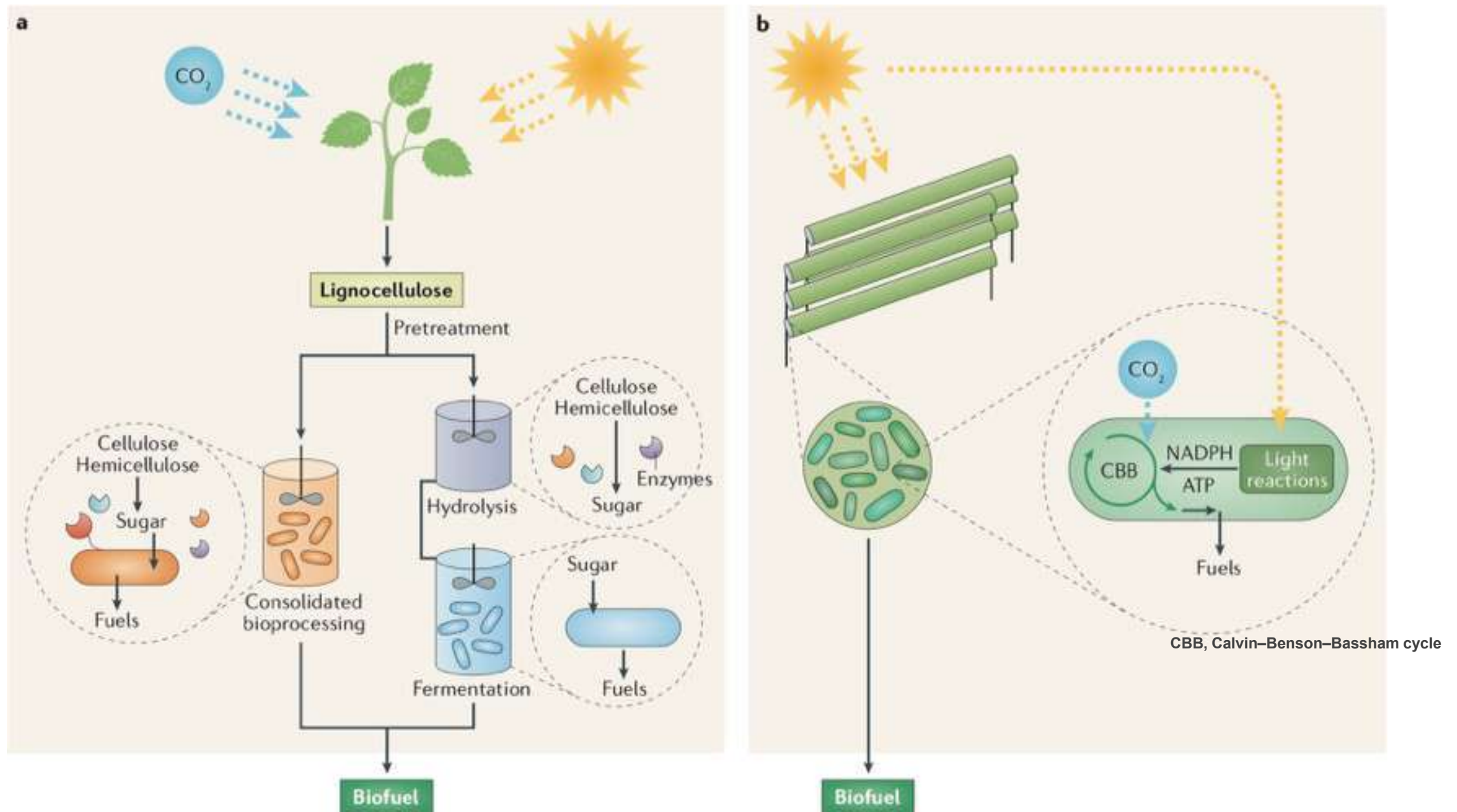
Continuous phytoextraction is based on ability of certain plants to gradually accumulate contaminants (mainly metals) into their biomass → ***ex situ***

Conventional *ex situ* methods applied to remediate polluted soils include excavation, [detoxification](#), and/or destruction of the contaminant physically or chemically, meaning that the contaminant undergoes stabilization, solidification, immobilization, incineration or destruction



Biofuel production, I

Liao et al., 2016

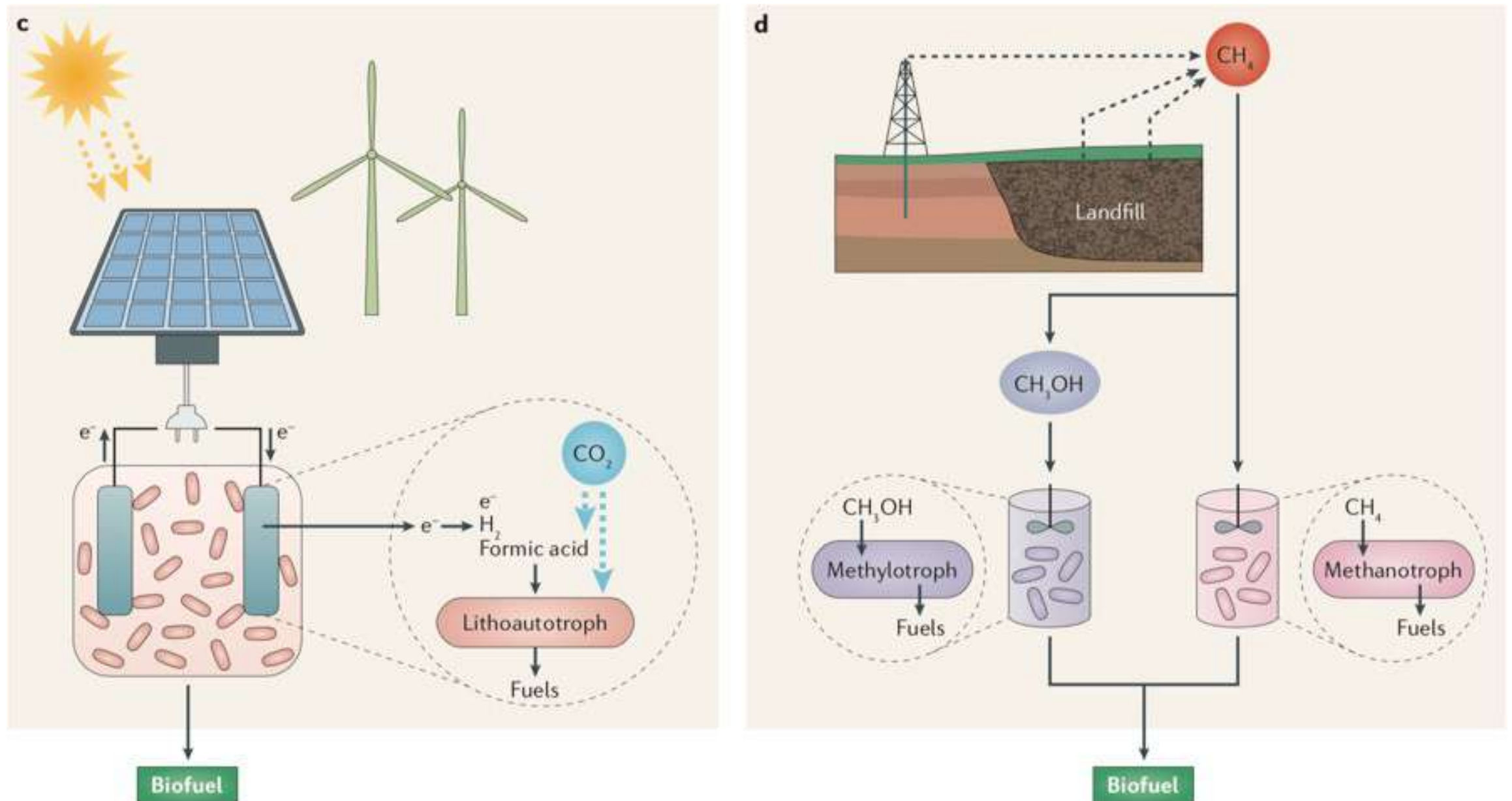


a | Biomass can be converted to fuels through hydrolysis followed by fermentation, or through consolidated bioprocessing, which combines the two processes in one reactor

b | Photosynthetic organisms, such as microalgae and cyanobacteria, can harness energy from sunlight to reduce CO_2 and convert it to liquid fuels.

Biofuel production, II

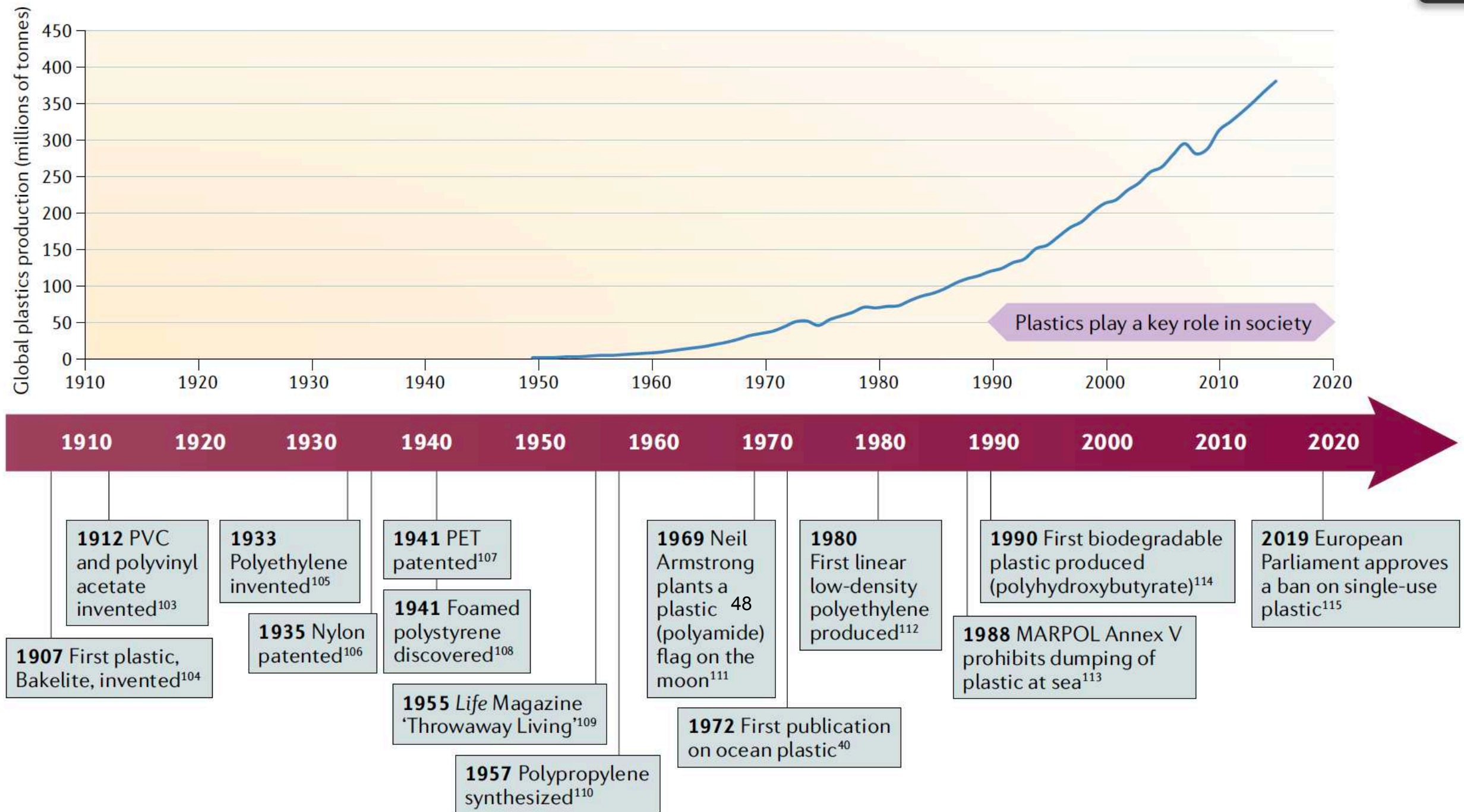
Liao et al., 2016



c | A broad range of lithoautotrophs can fix CO_2 to produce fuels with reducing power from electrons or electrochemically generated electron shuttles, such as H_2 and formic acid

d | Low-throughput methane from landfill or natural gas wells that is otherwise flared can be used directly by methanotrophs to produce fuels, or it can be converted to methanol (CH_3OH) and can then be utilized by methylotrophs for fuel production

Plastic from discovery to pollution



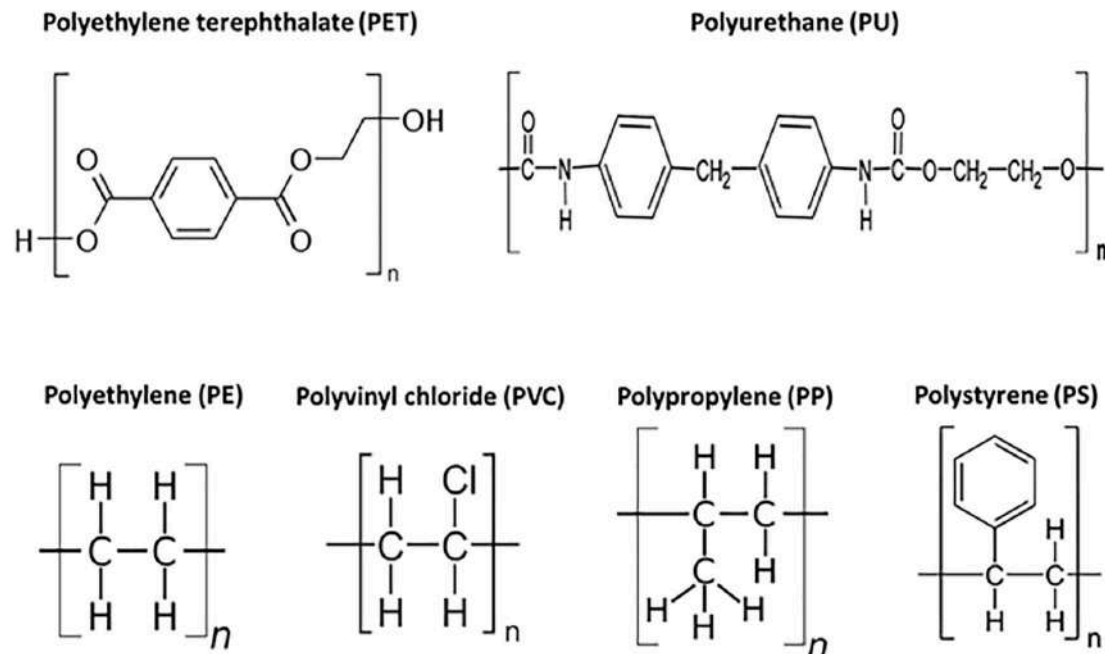
From biomass to petroleum-derived (petro-)plastics

Polymer	Density (g/L)	Crystallinity (%)	Life span (years)
PET	1.35	0–50	450
LDPE	0.91–0.93	50	10–600
HDPE	0.94–0.97	70	> 600
PS	1.03–1.09	0	50–80
PP	0.90–0.91	50	10–600
PVC	1.35–1.45	0	50–150

PET, Polyethylene terephthalate; LDPE, Low density polyethylene; HDPE, High density polyethylene; PS, Polystyrene; PP, Polypropylene; PVC, Polyvinyl chloride.

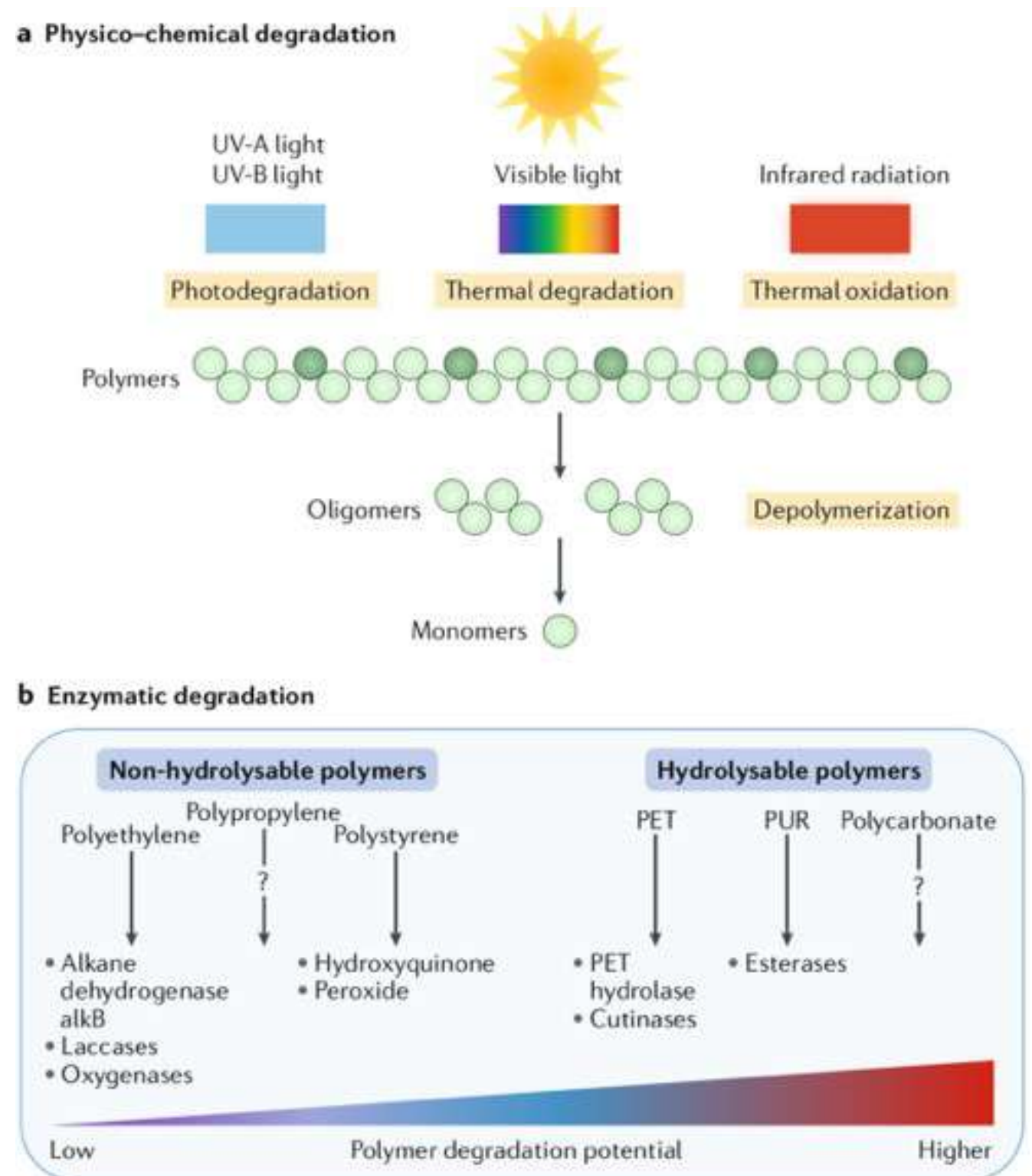
TABLE 1. Selected properties of major synthetic thermoplastic polymers ([Ojeda, 2013](#)).

- Degradation of conventional plastics is a combination of physical, chemical and biological interactions
- Biological knowledge from cultured strains and consortia in the laboratory and most from terrestrial environments
- In aquatic environments: floating plastic debris is subjected to different types of degradation induced by sunlight



Plastic: fossil-fuel derived material

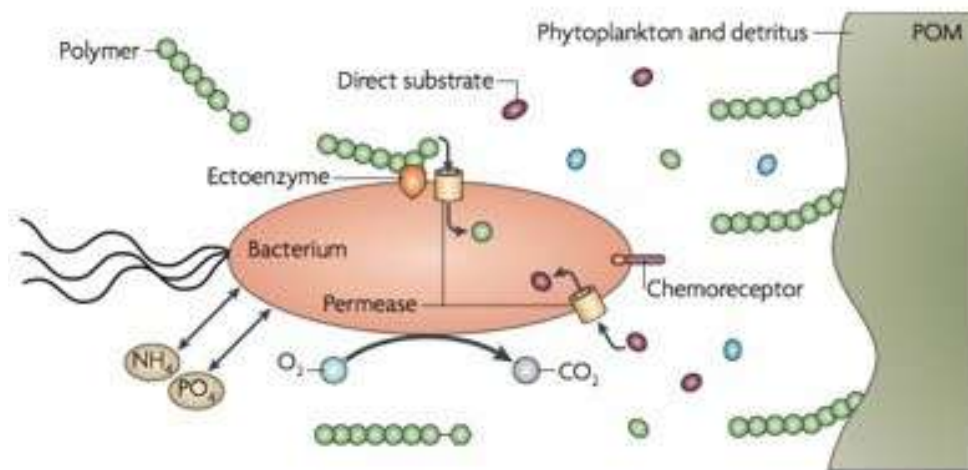
- The **visible spectrum leads to heating and thermal degradation**, whereas ultraviolet (UV) light leads to **photodegradation** of the **polymers into monomers** through bond scission, and **infrared radiation** can result in **thermal oxidation** of polymer chains
- Biological pathways of polymer degradation include the **mechanical action of organisms that grow** in cracks and crevices of the polymer surface (not shown), but also **enzymatic processes** that can hydrolyse the polymer into oligomers and ultimately monomers
- Polyethylene, polypropylene and expanded polystyrene contain very stable backbones and are difficult to degrade, whereas polyethylene terephthalate (PET), polyurethane (PUR) and polycarbonate are more susceptible to hydrolysis and to enzymes
- Enzymes that can hydrolyse polypropylene and polycarbonate have not yet been reported



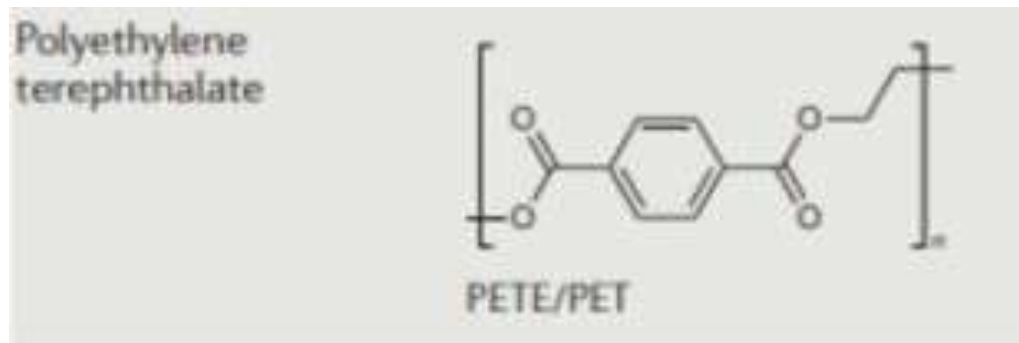
Plastic degradation conditions

Polypropylene	<i>Pseudomonas</i> <i>Vibrio</i> <i>Aspergillus niger</i>	Plastic dumping site	weeks Mineral medium (B7) with 0.05% glucose and 0.05% sodium lactate at 30°C	mineral medium 175 days incubation at neutral pH and 30°C	60% weight loss	Cacciari et al., 1993
	<i>Bacillus flexus</i>	Plastic dumping site	Minimal media with 0.25% glucose at 37°C	365 days incubation at neutral pH and at 35–37°C, 180 rpm	2.5% weight loss	Arkatkar et al., 2010
	<i>Bacillus cereus</i>	Mangrove sediments	Mineral salt medium at 29°C	40 days incubation at 33°C, 150 rpm	12% weight loss	Auta et al., 2017
	<i>Sporosarcina globispora</i> <i>Bacillus</i> sp.	Municipal compost waste	Minimal media at 37°C	15 days incubation at 37°C, 120 rpm	11% weight loss 10–12% weight loss	Jain et al. (2018)
Polystyrene	<i>Pseudomonas</i> sp.	Soil samples from plastic dump yard	Mineral medium with 0.85% NaCl and HIPS film at 30°C, 150 rpm	30 days incubation at 30°C	> 10% weight loss	Mohan et al., 2016
	<i>Bacillus</i> sp.				23.7% weight loss	
	<i>Pseudomonas aeruginosa</i>	Degraded polymer nanocomp-osite	NB medium at 30°C for 24 h	28 days incubation at 30°C in MSM	9.9% degradation at 10 and 25% PS: PLA composites	Shimpi et al., 2012
	<i>Pseudomonas putida</i> CA-3	Industrial bioreactor isolate	E2 mineral medium with 67 mg nitrogen/l and 9.5 mg/l styrene oil at 30°C, 200 rpm for 24 h	48 h of fermentation at 30°C, 500 rpm	A single pyrolysis run and four fermentation runs resulted in the conversion of 64 g of polystyrene to 6.4 g of PHA	Ward et al., 2006
	<i>Curvularia</i> sp.	Soil samples	Sabouraud's broth at 25°C for 13 days	9 weeks incubation at 25°C in Sabouraud's agar embedded with Ecoflex	Microscopic examination showed adherence and penetrance to the polymer	Motta et al., 2009
	<i>Rhodococcus ruber</i> <i>Enterobacter</i> sp. <i>Citrobacter sedlakii</i> <i>Alcaligenes</i> sp. <i>Brevundimonas diminuta</i> <i>Exiguobacterium</i> sp. strain YT2	Soil samples Degraded plastic waste	NB medium at 35°C, 120 rpm for 10–14 days MSM with e-plastic film at 30°C, 150 rpm for 2 weeks	8 weeks incubation at 35°C in synthetic medium 30 days incubation at 30°C, 150 rpm in mineral medium	0.8% weight loss 12.4% weight loss	Mor and Sivan, 2008 Sekhar et al., 2016

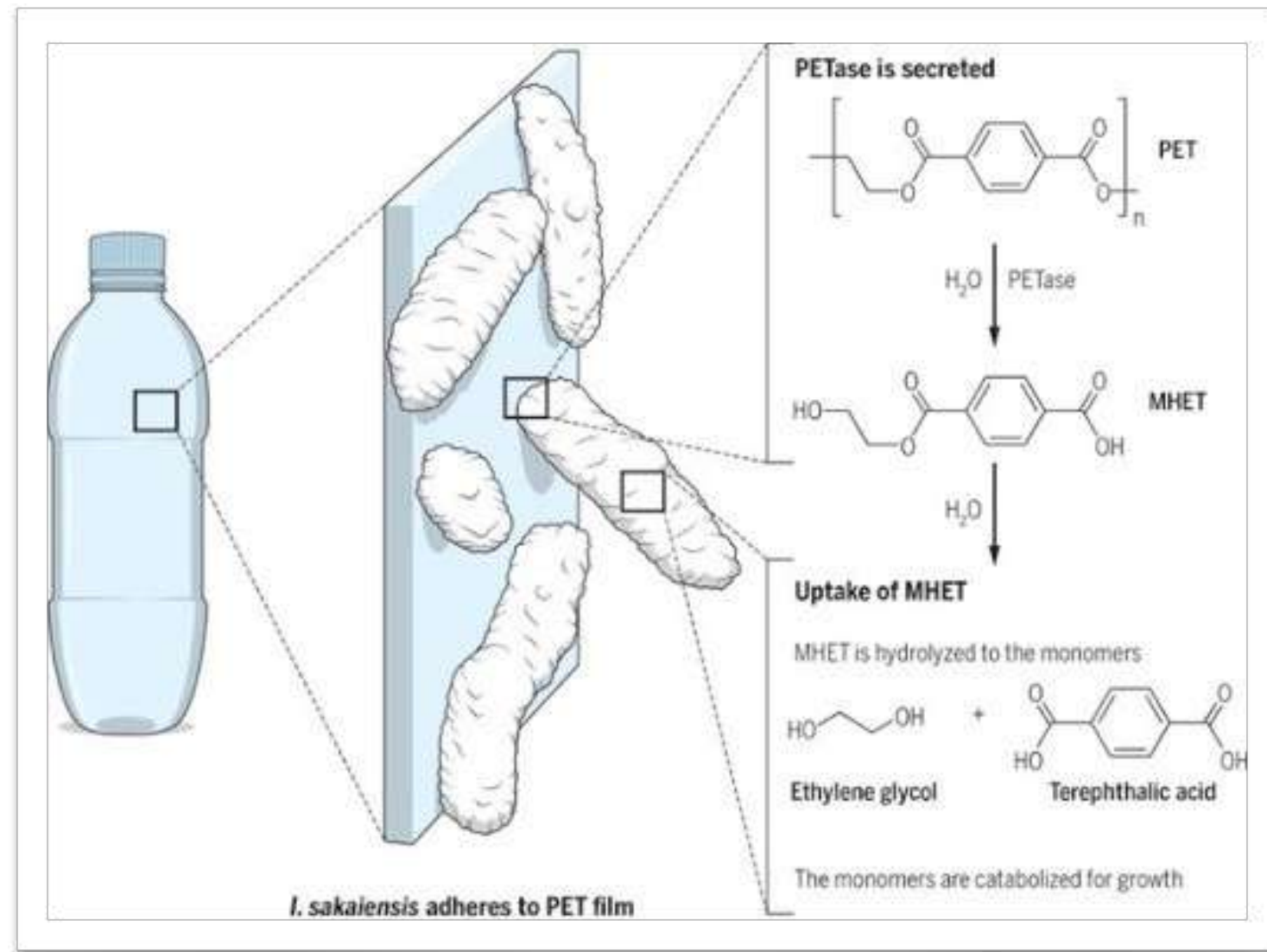
Bacteria can utilise (degrade) plastic



Azam and Malfatti, 2007 Nature Reviews Microbiology 10:782



Ideonella sakaiensis



- Microbes are master recyclers
- *I. sakaiensis* use PET for growing
- *Ideonella sakaiensis* 201-F6, is able to depolymerize PET polymers and utilize the terephthalate subunits as a carbon and energy source for metabolism and growth , at 30°C 80 days 50% weight loss
- No trace left !!!

State-of-the-art microbial technologies for sustainable production and degradation of bio-based plastics, I

Polyethylene (PE), polypropylene (PP), polyvinyl chloride (PVC) and polyethylene terephthalate (PET)—are now being produced from **renewable biomass**

The **precursors** for these plastics are obtained from biological feedstocks like **sugars, glycerol** and **lignocellulosic** biomass through **chemical and/ or biological conversion methods**

These produced **precursors** are then subjected to **polymerization** to create the final plastic products using conventional chemical processes

Fermentative production typically accompanies cell growth, whereas whole-cell biocatalysis can also utilize inactivated or resting cells

In vitro conversion employs purified enzymes for the conversion process

State-of-the-art microbial technologies for sustainable production and degradation of bio-based plastics, II

Conventionally, **PE, PP and PVC** are produced by the radical **polymerization of ethylene, propylene and vinyl chloride**, respectively. These monomers are usually **derived from petroleum or natural gas**. With the aim to produce them from renewable resources, **bio-based ethylene can be prepared by dehydrating bio-ethanol, which itself is produced by microbial fermentation of sugars derived from biomass**

Bio-based propylene and vinyl chloride can then be obtained from this bio-based ethylene through conventional chemical conversion methods

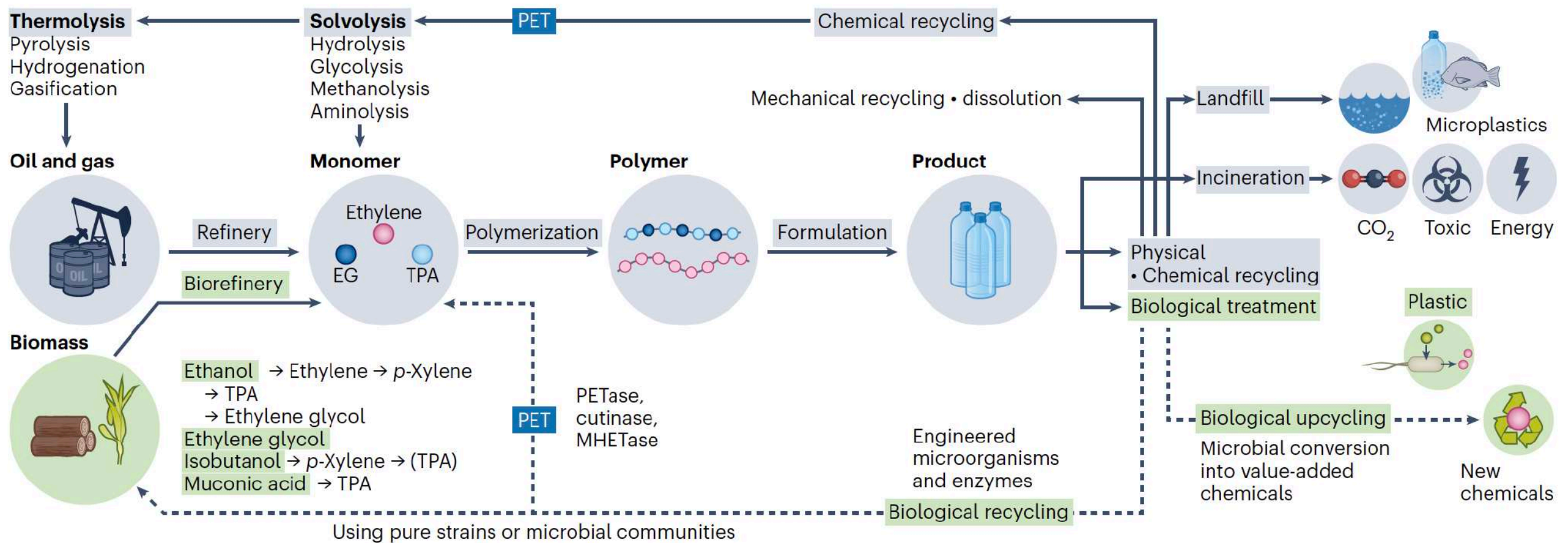
State-of-the-art microbial technologies for sustainable production and degradation of bio-based plastics, III

Bio-ethanol fermentation is a well-established process used for producing alcoholic beverages and biofuels. Among several ethanol-producing microorganisms, *Saccharomyces cerevisiae* and *Zymomonas mobilis* are most commonly employed in industrial settings due to their high ethanol yields—up to 90% of the theoretical maximum when glucose is used as a substrate

Commercial **bio-ethanol production** typically relies on **sugar and starch-rich biomass like cereal grains, sugar cane and sugar beets**, which are known as **first-generation feedstocks**. Recent research has focused on using **non-food lignocellulosic feedstocks (second generation) such as wood, agricultural residues and pulp wastes** as well as **algae-derived feedstocks (third generation)** to address concerns regarding food consumption competition and terrestrial land requirements

State-of-the-art microbial technologies for sustainable production and degradation of bio-based plastics, I

a PE and PET

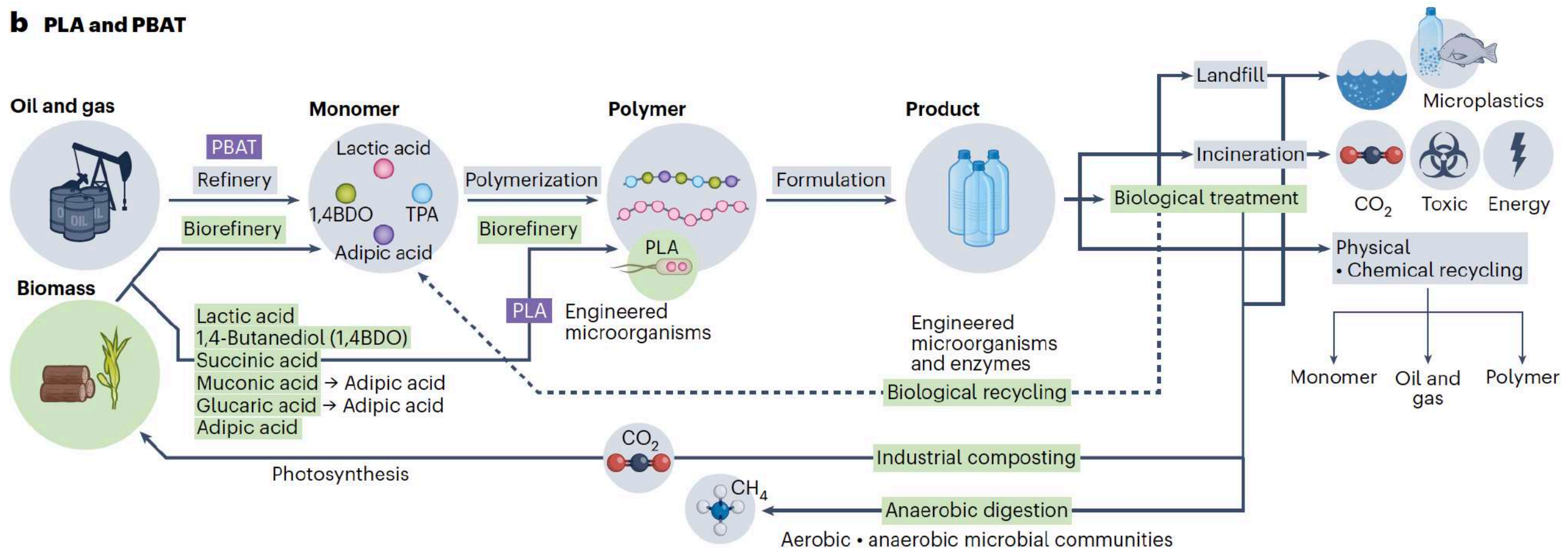


Choi et al., 2023

The dotted arrows represent technologies that are still in research and development

State-of-the-art microbial technologies for sustainable production and degradation of bio-based plastics, II

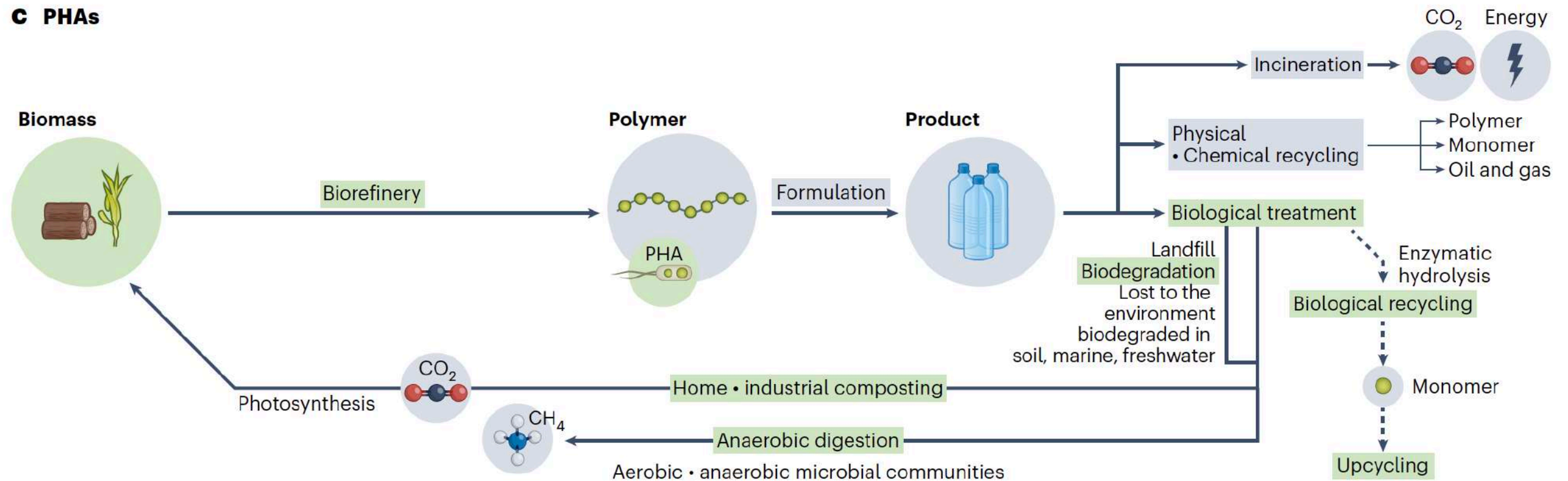
b PLA and PBAT



The dotted arrows represent technologies that are still in research and development

State-of-the-art microbial technologies for sustainable production and degradation of bio-based plastics, III

C PHAs



The dotted arrows represent technologies that are still in research and development

Business as Usual Scenario

Geyer et al., 2017

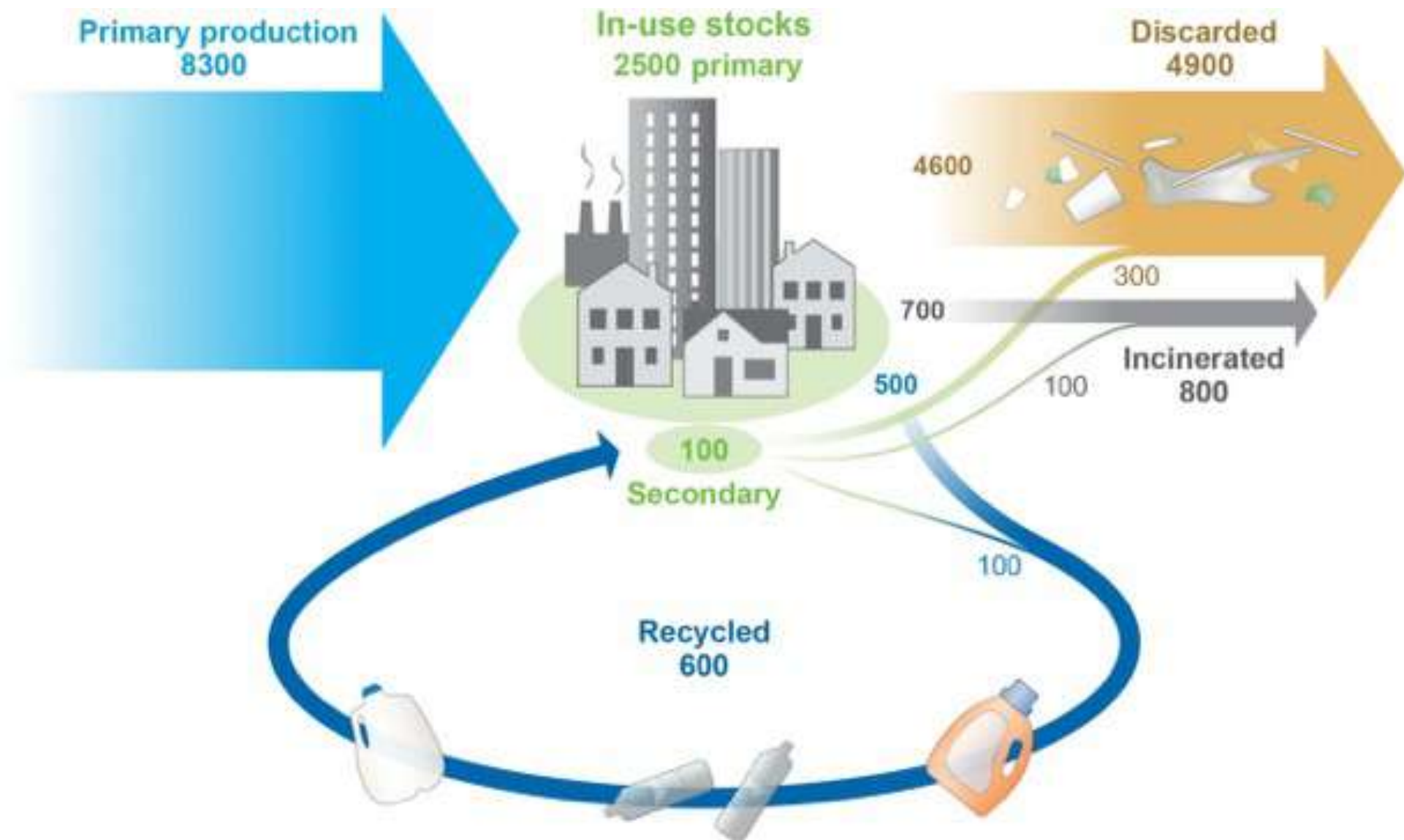


Fig. 2. Global production, use, and fate of polymer resins, synthetic fibers, and additives (1950 to 2015; in million metric tons).

- Continuum of size classes
- Diverse plastic structures
- Decarbonisation
- Societal education