

Microbes at work

Microbes are biological factories Fungi and Plants Biofuels 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



• Metabolic flexibility—> tunable via genetic manipulation

Hug et al., 2020

Microbial factories, II



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



'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



Gadd, 2017

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 microrganisms 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
Dehalococcoides ethanogenes	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).
Geobacter sulfurreducens, Geobacter metallireducens	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.
Rhodopseudomonas palustris	http://www.jgi.doe.gov	Main organism for elucidating pathways of anaerobic metabolism of aromatic compounds, and regulation of this metabolism.
Pseudomonas putida	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.
Dechloromonas aromatica	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.
Desulfitobacterium hafniense	http://www.jgi.doe.gov	Reductive dechlorination of chlorinated solvents and phenols. <i>Desulfitobacterium</i> species are widespread in a variety of environments.
Desulfovibrio vulgaris	http://www.tigr.org	Shown to reductively precipitate uranium and chromium. An actual role in contaminated environments is yet to be demonstrated.
Shewanella oneidensis	http://www.tigr.org	A closely related Shewanella species was found to reduce U(vI) to U(IV) in culture, but Shewanella species have not been shown to be important in metal reduction in any sedimentary environments.
Deinococcus radiodurans	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



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). CO3, carbonate CO3 2– ; OH, hydroxyl OH– ; PO4, phosphate PO4 3– ; S, sulfide S2–

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



• Microorganisms in Biomining, Wastewater Treatment and Bioelectrochemical Systems (BES)

Nancharaiah et al., 2016

- 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

Ē

rare earth element

Biocorrosion

Humans have been producing metallic iron (Fe0) for only a few thousand years. Thus, corrosion processes associated with microorganisms probably evolved for reasons other than utilizing Fe0 as an energy source а

Microbial corrosion										
Various industries					Marine environments		Medical	Space		
Power plants	Fuel systems	Water utilities	Oil and gas	Rebar and concrete	Heat exchangers	Nuclear waste storage	Offshore assets	Ships and port facilities	Dental devices	Manned spacecraft

2023 Xu et al., 3



I Key reactions and diversity of microorganisms involved in metal corrosion.

a, Key reactions associated with microbial corrosion of ferrous metals. Each of these reactions has been shown to be thermodynamically favourable under conditions related to corrosion.

-> Cu, Ni, Al, Zn, Ti

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

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





Cell-repair systems support microbial survival



- 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 methaneproducing 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, sulphatereduction 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



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 oxygenrich 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



In situ







Fungi-based bioremediation applications

	Phylum or subphylum	Organic chemicals degraded	Major ecological characteristics
<u> </u>	Microsporidia		Obligate parasites of animals
-	Kickxellomycotina (2)	PAHs	Saprobes, and parasites of animals and fungi
	Zoopagomycotina		Parasites of nematodes, protozoa and fungi
age	Entomophthoromycotina (2)	PAHs	Parasites of insects
al line	Blastocladiomycota		Saprobes, and parasites of plants and animals; aquatic and terrestrial
fung	Mucoromycotina (16)	Benzoquinoline, biphenyl, PAHs, pesticides, synthetic dyes and TNT	Saprobes, parasites or ectomycorrhizal symbionts
asa	Neocallimastigomycota		Gut symbionts of ruminant herbivores
	Chytridiomycota (2)	PAHs	Saprobes, and parasites of plants and animals; fresh water and wet soil
-	Glomeromycota	PAHs and pesticides	Arbuscular mycorrhizal symbionts
arya	Pezizomycotina (57) Saccharomycotina (9) Other ascomycetes (22)	Alkanes, alkylbenzenes, biphenyl, chlorophenols, coal tar oil, crude oil, diesel, EDCs, fragrances, PAHs, PCDDs, pesticides, synthetic dyes, TNT and toluene Alkanes, alkylbenzenes, biphenyl, crude oil, EDCs, PAHs and TNT Alkanes, diesel, coal tar oil, crude oil, MTBE, PAHs, pesticides, RDX, toluene and synthetic dyes	Saprobes, pathogens of plants and animals, and symbiotes of algae (lichens), plants (ectomycorrhizae, ercoid mycorrhizae and endophytes) and insects; terrestrial and aquatic
Dik	Basiodiomycota (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	Saprobes, ectomycorrhizal symbionts, pathogens of plants and animals, and parasites of other fungi; terrestrial and aquatic
	Pucciniomycotina (3)	Cresols, crude oil, dibenzothiophene, PAHs and RDX	

Phytoremediation: microbesplant- 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 in situ	Organics and metals	Cover maintained
Phytodegradation	Attenuated in situ	Organics	Cover maintained
Phytovolatilization	Removed	Organics and metals	Cover maintained
Phytoextraction	Removed	Metals	Harvested repeatedly

Greipsson, S. (2011) Phytoremediation. Nature Education Knowledge 3(10):7

Phytoremediation



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

Greipsson, S. (2011) Phytoremediation. Nature Education Knowledge 3(10):7

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, <u>detoxification</u>, and/or destruction of the contaminant physically or chemically, meaning that the contaminant undergoes stabilization, solidification, immobilization, incineration or destruction

Greipsson, S. (2011) Phytoremediation. Nature Education Knowledge 3(10):7

Biofuel production, I



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

 \mathbf{b}_{\parallel} Photosynthetic organisms, such as microalgae and cyanobacteria, can harness energy from sunlight to reduce CO₂ and convert it to liquid fuels.

Biofuel production, II



 c_1 A broad range of lithoautotrophs can fix CO₂ to produce fuels with reducing power from electrons or electrochemically generated electron shuttles, such as H₂ 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 (CH₃OH) 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



b Enzymatic degradation



Plastic degradation conditions

			Weeks	mineral medium		
Polypropylene	Pseudomonas Vibrio Aspergillus niger	Plastic dumping site	Mineral medium (B7) with 0.05% glucose and 0.05% sodium lactate at 30°C	175 days incubation at neutral pH and 30°C	60% weight loss	Cacciari et al., 1993
	Bacillus flexus	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
	Bacillus cereus	Mangrove sediments	Mineral salt medium at 29°C	40 days incubation at 33°C, 150 rpm	12% weight loss	Auta et al., 2017
	Sporosarcina globispora				11% weight loss	
	Bacillus sp.	Municipal compost waste	Minimal media at 37°C	15 days incubation at 37°C, 120 rpm	10–12% weight loss	Jain et al. (2018)
Polystyrene	Pseudomonas 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
	Bacillus sp.				23.7% weight loss	
	Pseudomonas aeruginosa	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
	Pseudomonas putida 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
	Rhodococcus ruber Enterobacter sp. Citrobacter sedlakii Alcaligenes sp. Brevundimonas diminuta	Soil samples	NB medium at 35°C, 120 rpm for 10–14 days	8 weeks incubation at 35°C in synthetic medium	0.8% weight loss	Mor and Sivan, 2008
	Exiguobacterium sp. strain YT2	Degraded plastic waste	MSM with e-plastic film at 30°C, 150 rpm for 2 weeks	30 days incubation at 30°C, 150 rpm in mineral medium	12.4% weight loss	Sekhar et al., 2016

Bacteria can utilise (degrade) plastic

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 !!!

Business as Usual Scenario



Continuum of size classes

- Decarbonisation
- Diverse plastic structures
- Societal education