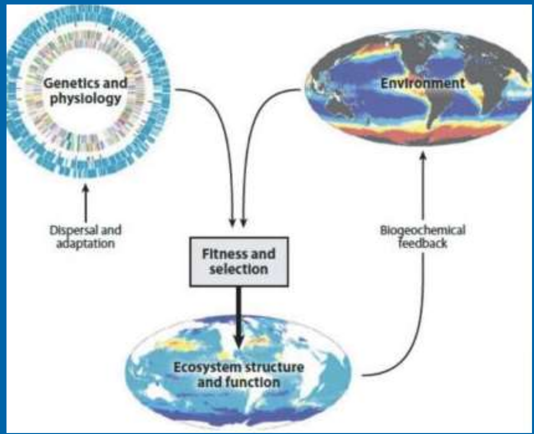
TECHNISCHE UNIVERSITÄT
BERGAKADEMIE FREIBERG
Die Ressourcenuniversität. Seit 1765.

Bioremediation and phytoremediation 2021/22

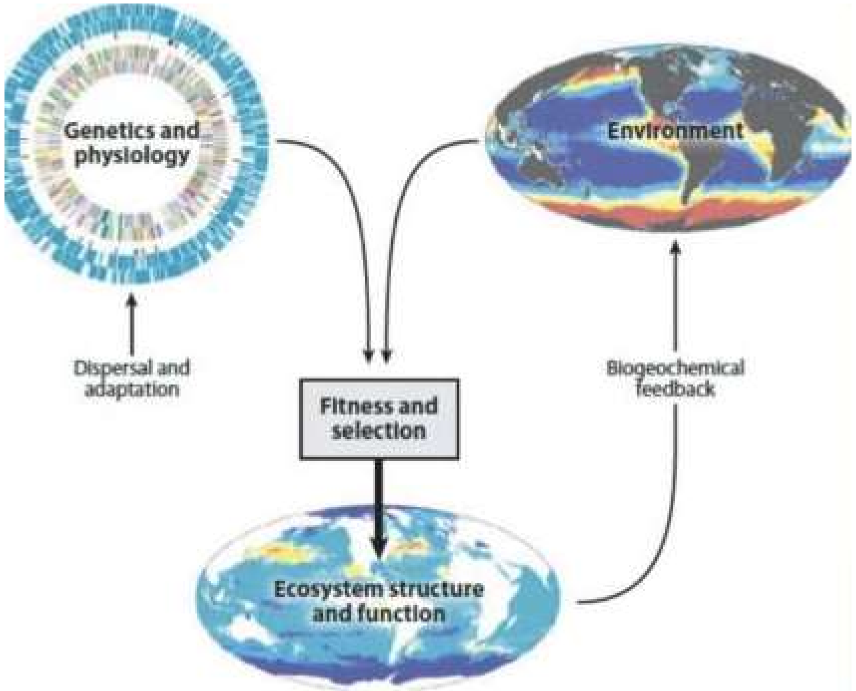
Topic 4: Microbial basics of bioremediation



Jun.-Prof. Dr. Sabrina Hedrich
TU Bergakademie Freiberg

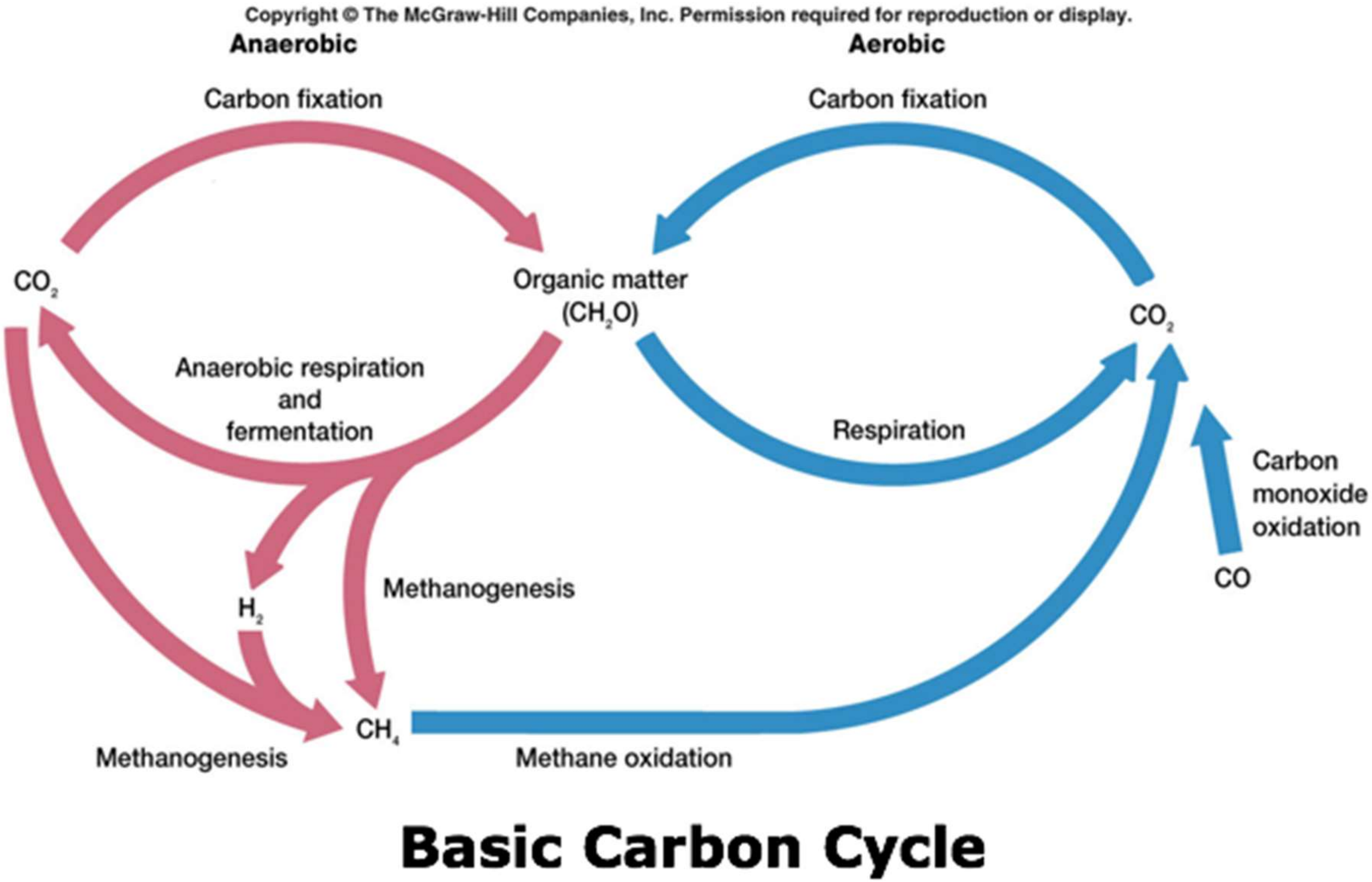
„Everything is everywhere but the environment selects“

(L.G.M. Baas Becking, 1934)

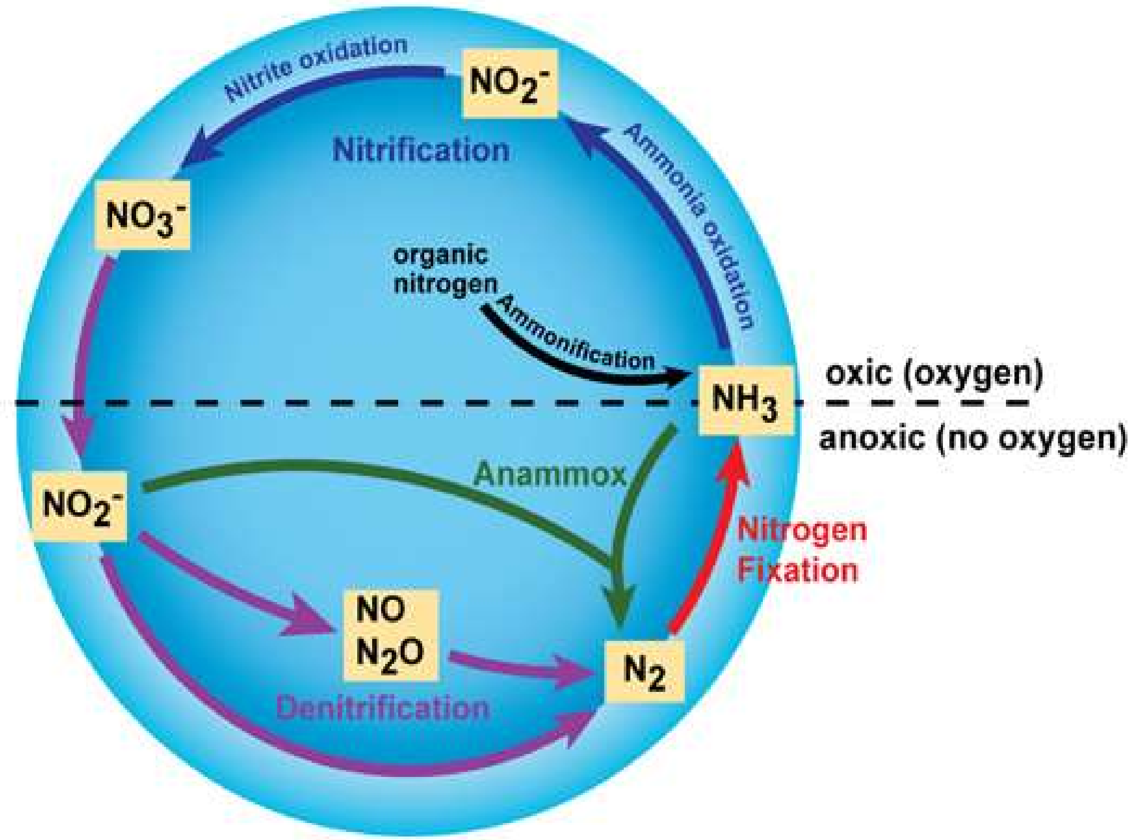


- bring together microbiology and geochemistry
- understand elemental cycling through microbial activity

Microbial carbon cycle

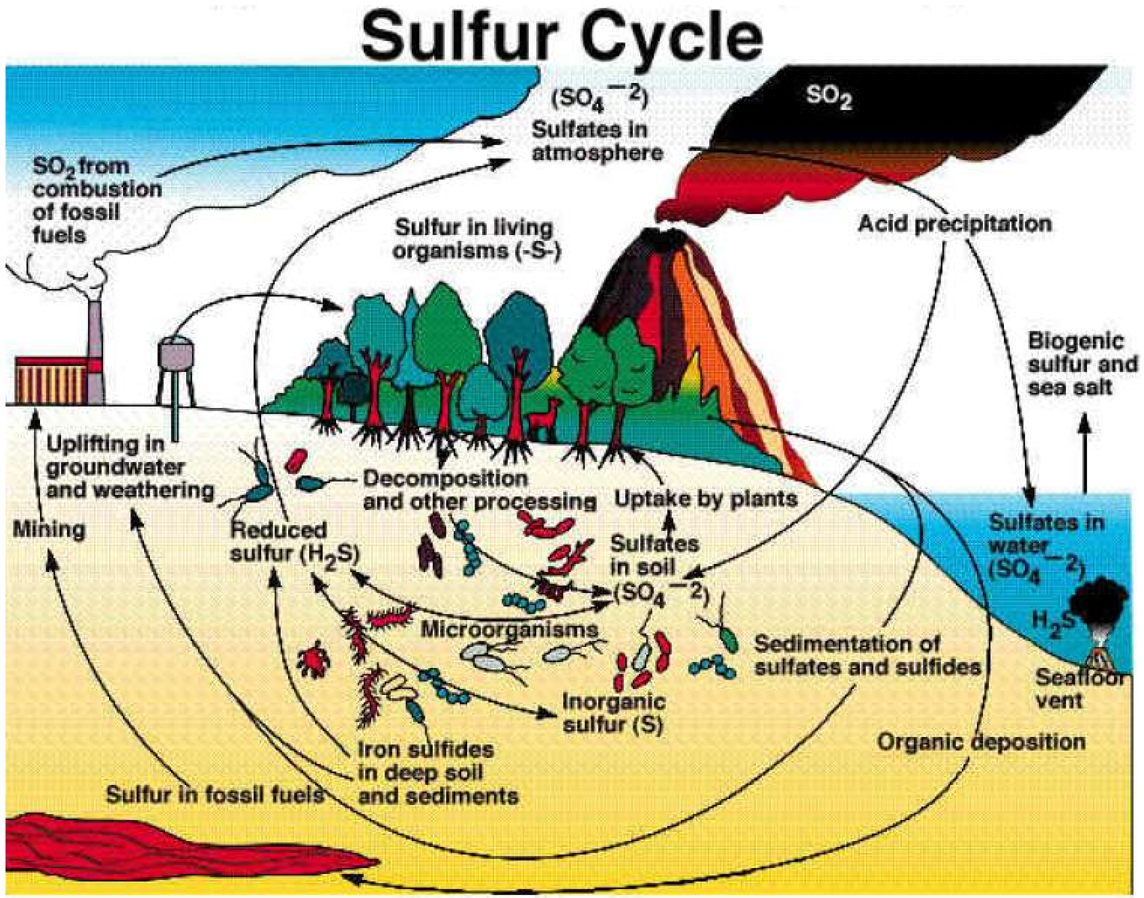


Microbial nitrogen cycle



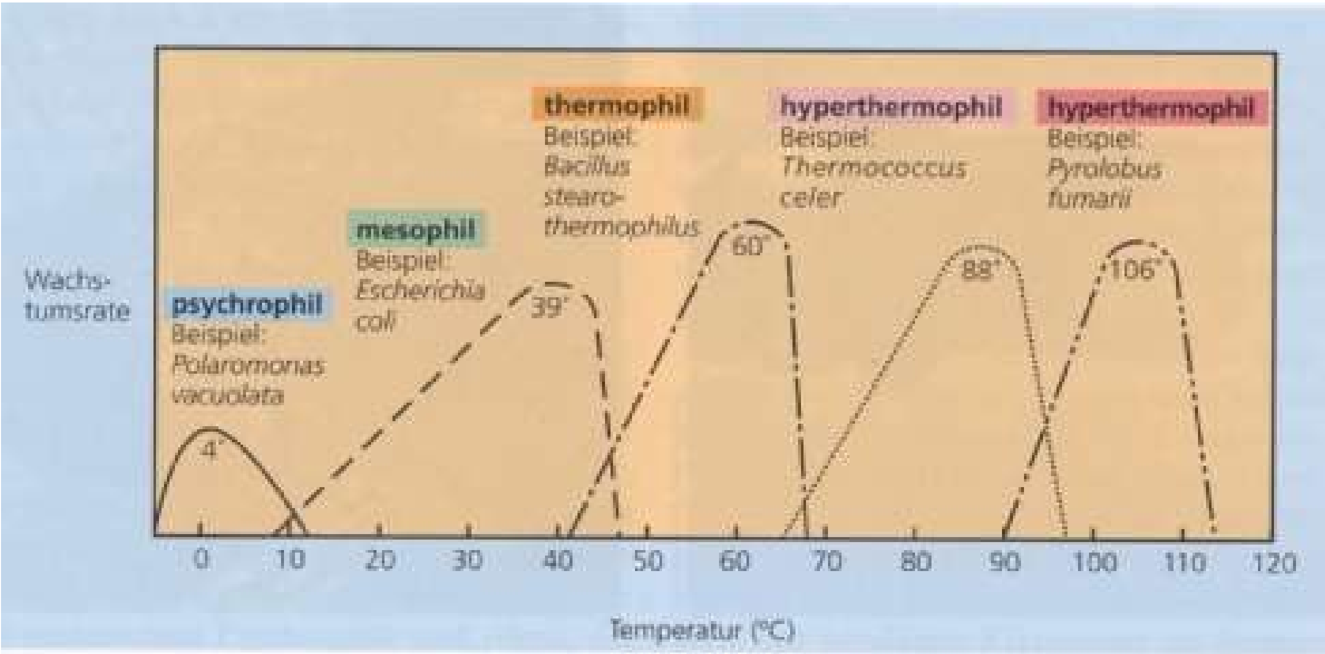
Sulfur reservoir	Metric tons sulfur
Atmosphere	
SO_2/H_2S	1.4×10^6
Ocean	
Biomass	1.5×10^8
Soluble inorganic ions (primarily SO_4^{2-})	1.2×10^{15}
Land	
Living biomass	8.5×10^9
Organic matter	1.6×10^{10}
Earth's crust[a]	1.8×10^{16}

R.Näveke, 1986: Environmental Microbiology
Ian L. Pepper, Charles P. Gerba, Terry J. Gentry, Raina M. Maier; Academic Press, 13.10.2011



Living condition	
Temperature range	
low (0-20°C)	psychrophile
medium (20-45°C)	mesophile
high(45-70°C)	thermophile
Extremely high (70-110°C)	extremely/hyper-thermophile
pH-range	
low (<pH 6)	acidophile
neutral (pH 6-7)	neutrophile
high (>pH 7)	alkalophile
pressure	
high	barophile
Osmotic pressure and water content	
high salt content	halophile
High sugar content	saccharophile
High content of osmotic compounds	osmophile

Relationship of temperature to growth rate of a typical psychrophile, a typical mesophile, typical thermophiles and two different hyperthermophiles



Brock, Lehrbuch Mikrobiologie

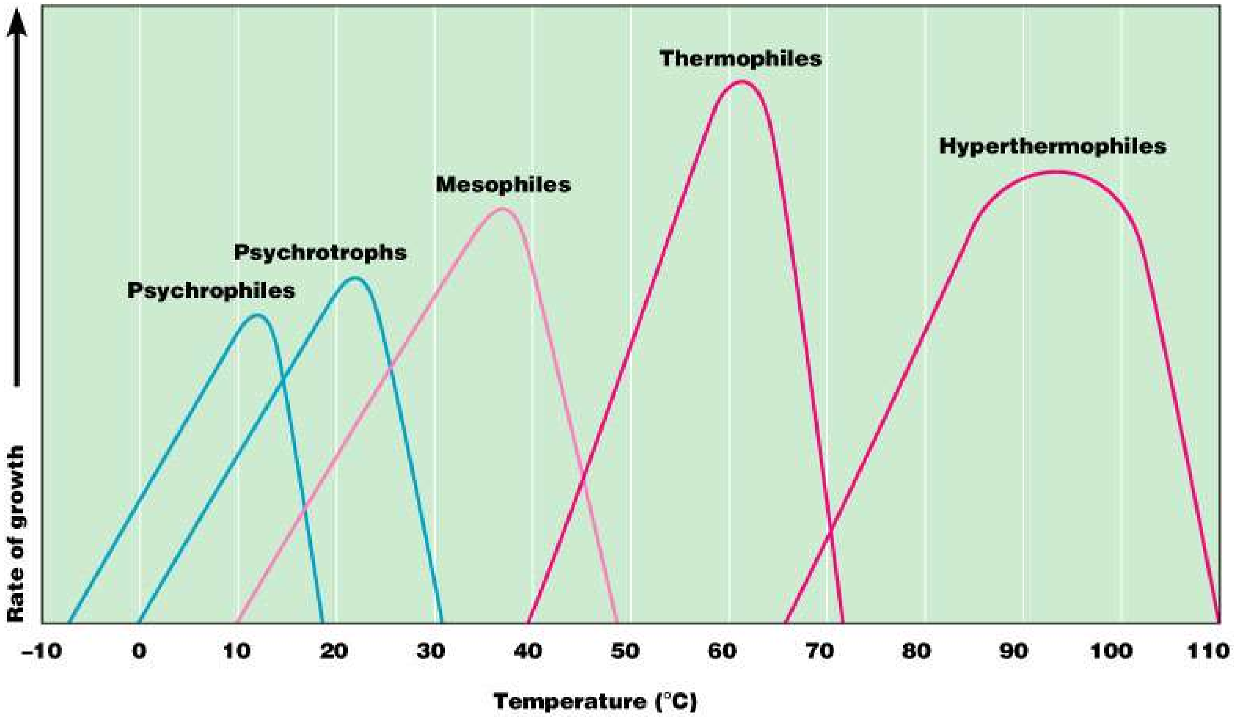
Definition

Psychrophile

- *psychrós (cold)*
- psychrotolerant

Thermophile

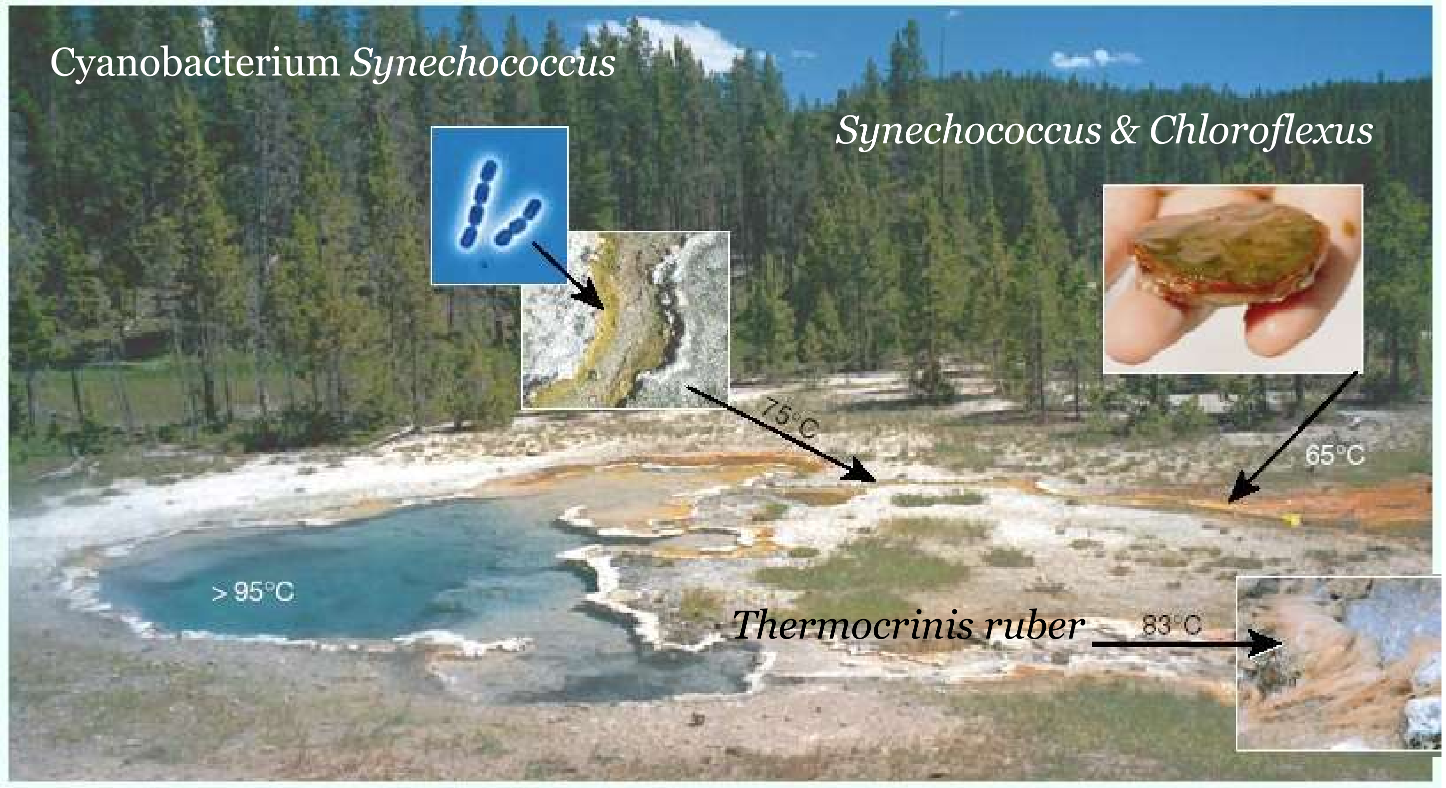
- *thermós (warm)*
- Thermotolerant



Up to which temperature is life possible?

- The sulfur-reducing, marine bacterium Pyrodictium occultum holds a temperature record of 113°C
- Evidence of life up to 120°C
- If liquid water is the only prerequisite for higher life: in deep-sea wells at 250°C
- Mean life expectancy of essential cellular components shrinks above 130°C to a few minutes or seconds

Octopus Spring, hot spring in Yellowstone National Park, USA



Rothschild & Mancinelli 2001

Polar Habitats

- 75-80% of the Earth's surface is constantly cold (<5 ° C)
- Permafrost 20% of the mainland surface
- Life in sea ice, permafrost, glaciers, Arctic and Antarctic rocks, icebergs
- MO active at -15 ° C
- Survival of MO in winter in polar systems to ensure colonization throughout the year
- Melting waters supply nutrients Microclimates for MO

Psychrophile – conditions for living

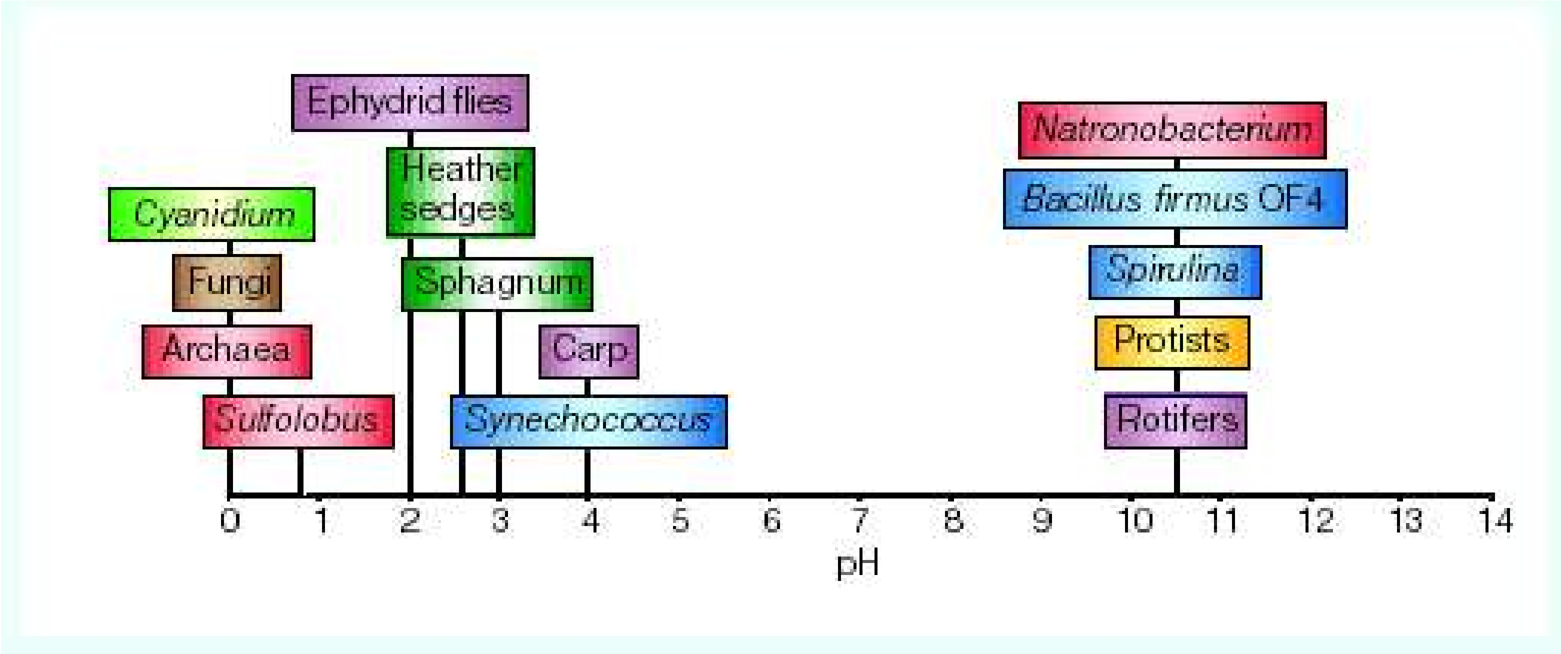
Temperature range: ~0°C – 20°C

- Optimum at ~15°C
- Cryophile ~-10°C

habitats

- Oceans (under the thermocline)
- Permafrost
- glaciers
- Antarctis
- Polar icecaps
- Snow fields, …

pH limits of life

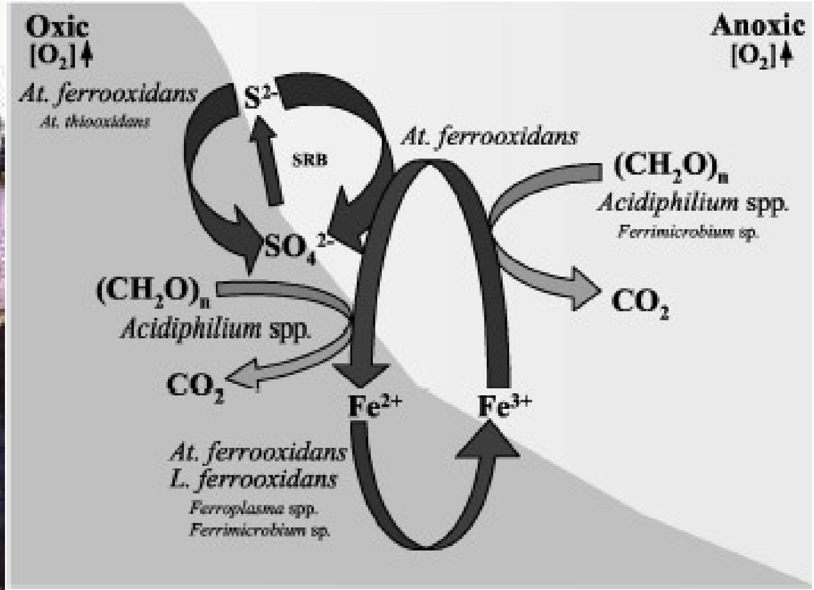


Archaea Bacteria, Algae, Protists, Fungi, Plants, Animals

Rothschild & Mancinelli 2001

Acidic habitats

Rio Tinto, Spain: 3-20 g/L Fe, pH ~ 2



Zettler et al. 2002; González-Toril et al. 2003

Acidic habitats

BGR, Referat Geomikrobiologie; TZ
Botswana

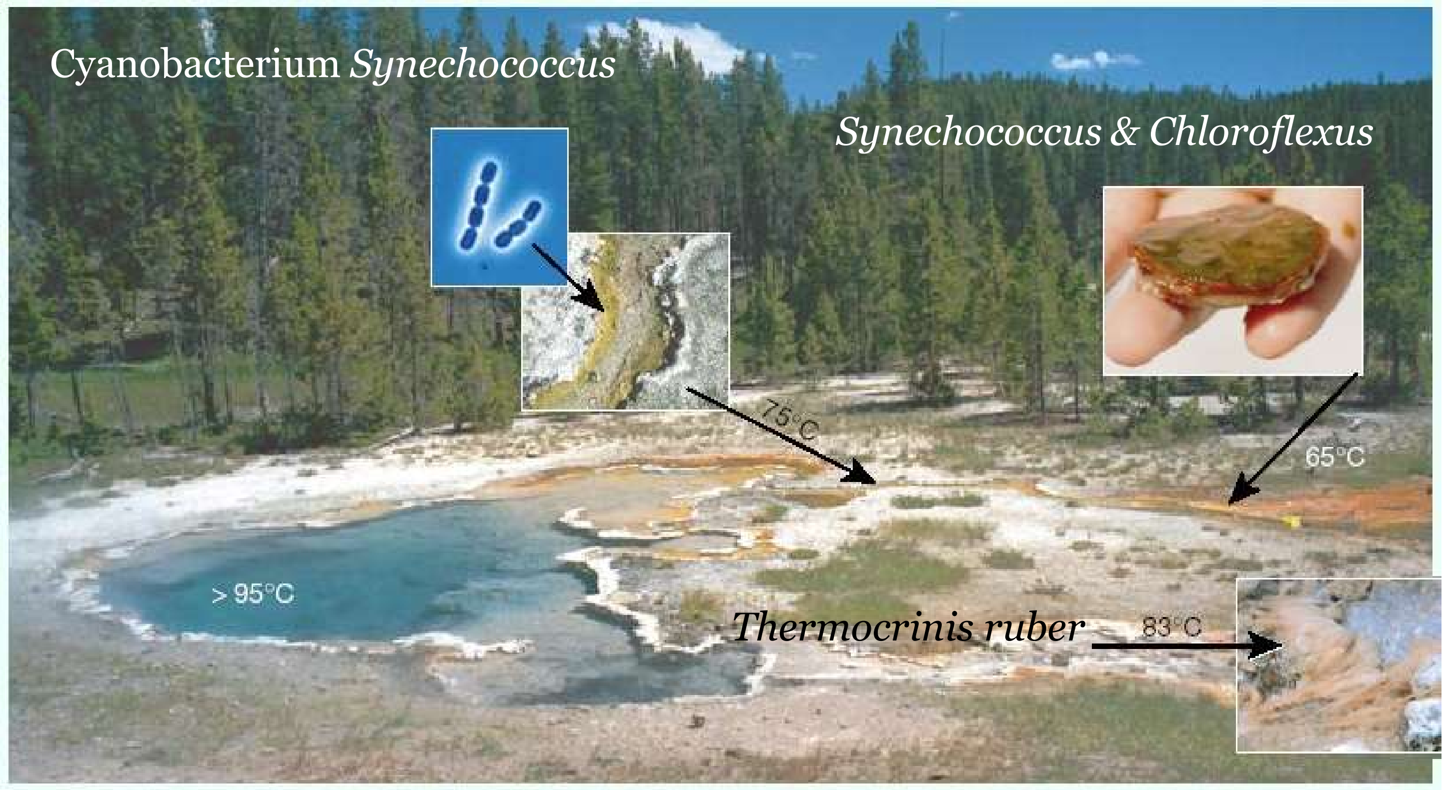
Hypersaline and alkaline habitats

- Large salt lake and Dead Sea neutral pH and salinity> 20%
- Many saline waters are poor in Ca^{2+} and Mg^{2+} → increased carbonate content→ alkaline pH
- Lake Natron in the Kenyan Rift Valley (salinity> 30%, pH> 12, high T)

Salar de Uyuni , Bolivia

Lake Natron, Tanzania

Octopus Spring, alkaline (pH 8.8–8.3) hot spring in Yellowstone National Park, USA



Rothschild & Mancinelli 2001

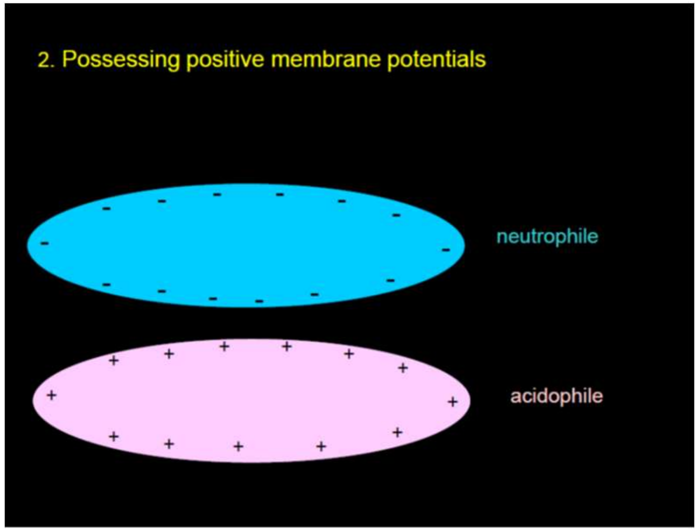
Adaptation of extremophilic microorganisms

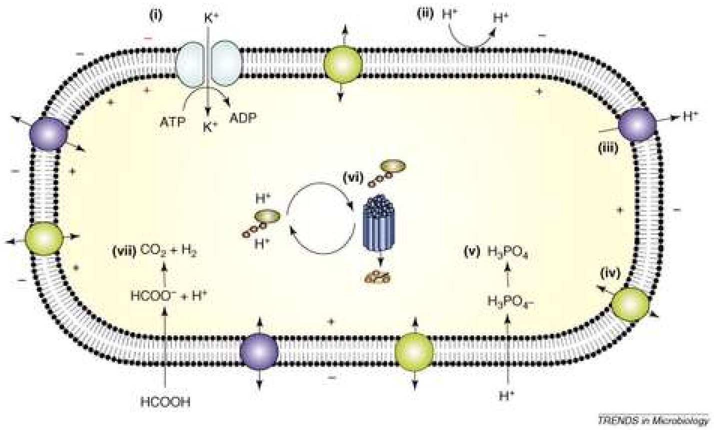
- Thermophiles: reduction of membrane fluidity (total fatty acids)
- Psychrophiles: increase in membrane fluidity (unspecified fatty acids)
- Acidophiles: positively charged cell surface, modified proteins,

  metal efflux systems
- Alkaliphiles: Negatively charged cell wall polymers
- Halophiles: increase in osmolarity in the cytoplasm

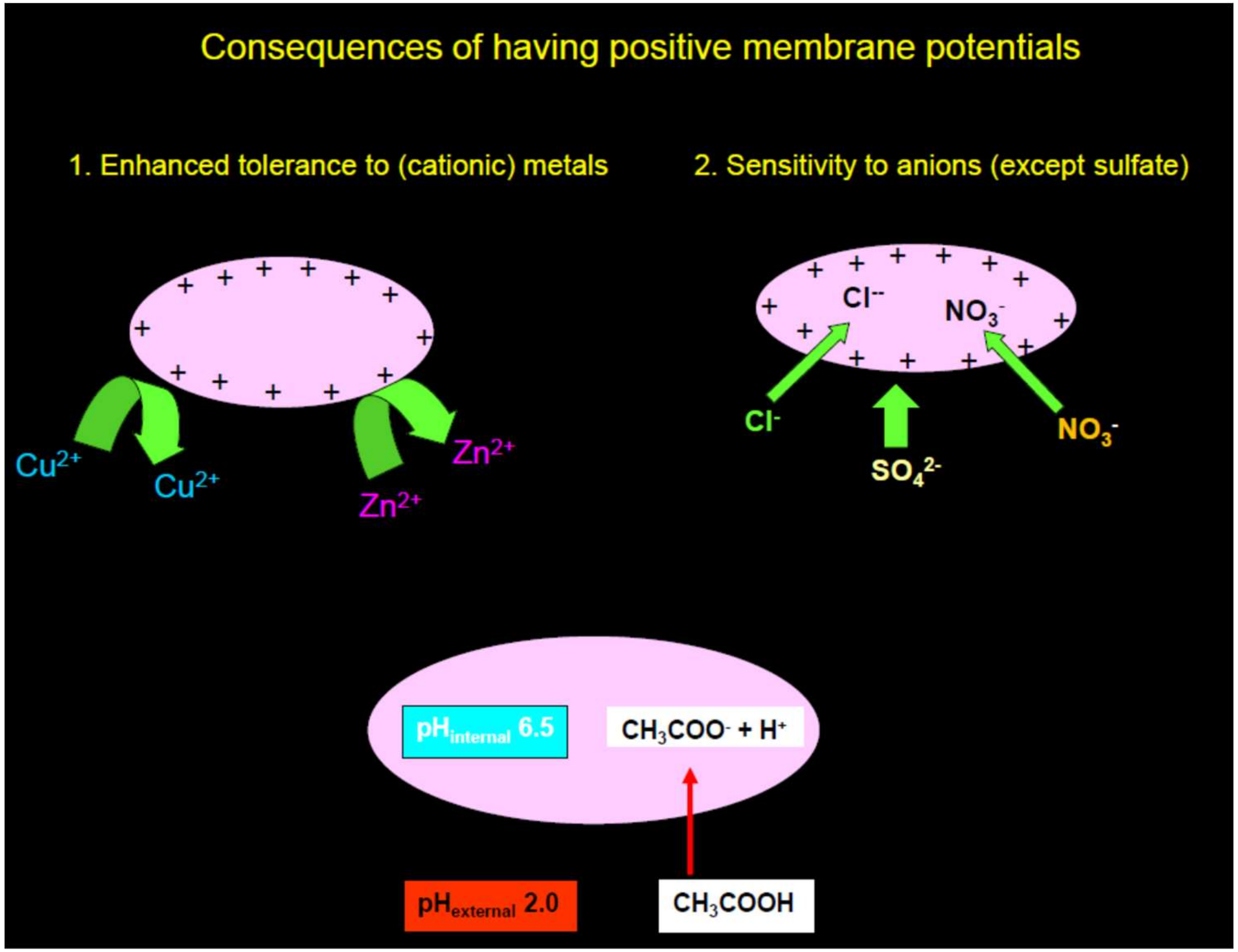
Acidophiles

- positive membrane potential
- highly-impermeable cell membrane
- proton pumps actively carry H^+ out of the cell to keep the cytoplasm neutral





Acidophiles



Metal resistance of acidophilic bacteria

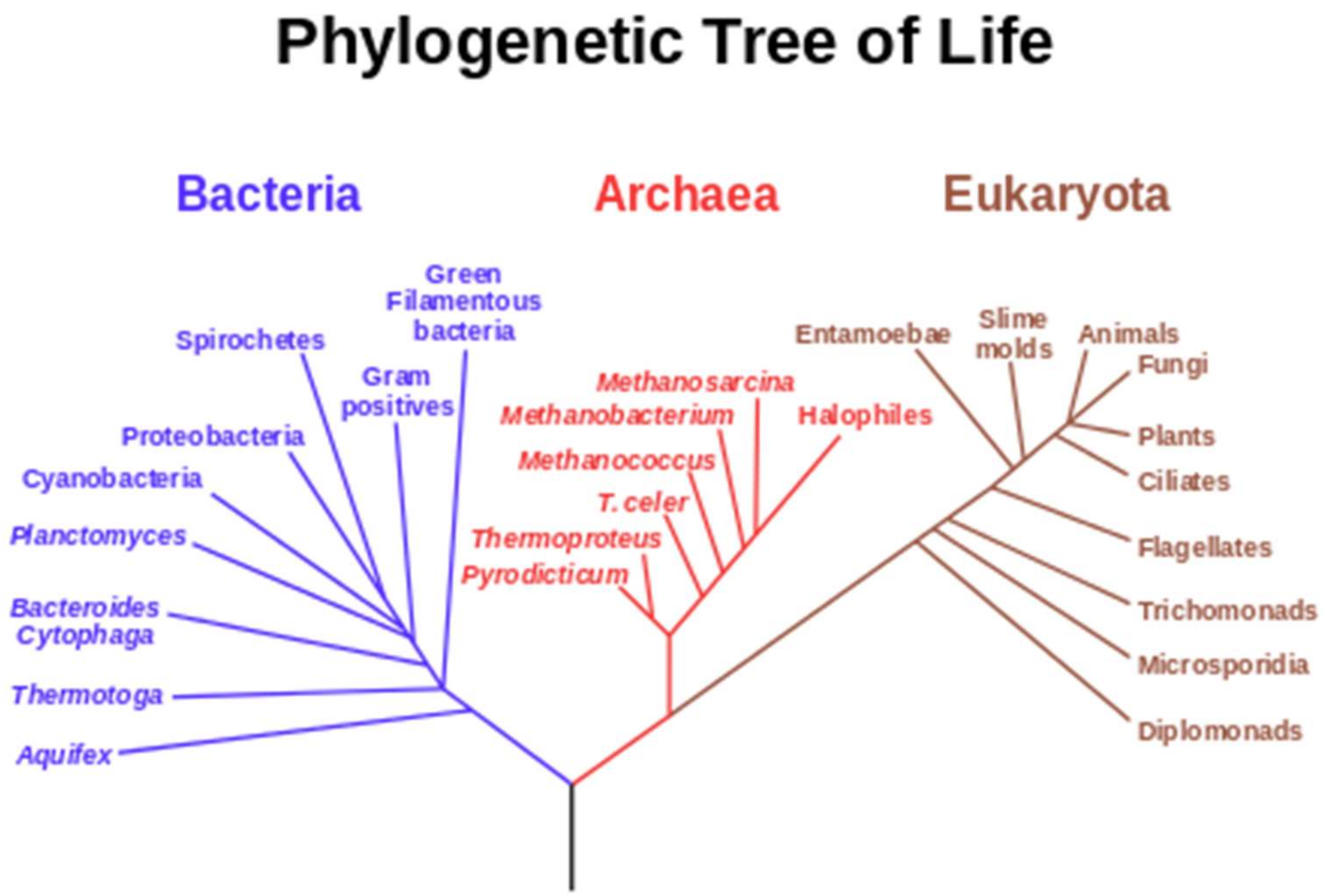
Acidithiobacillus ferrooxidans tolerates high metal contents in solution:

- 12 g/L U_3O_8
- 72 g/L Ni^{2+}
- 120 g/L Zn^{2+}
- 160 g/L Fe^{2+}

Acidithiobacillus ferrooxidans
(30.000fach)

Rawlings, 2005

Phylogenetic tree of life based on DNA sequence comparison of ribosomal RNA (Ribonucleic acid)



Woese (1987, 1990)

Classification of metabolism

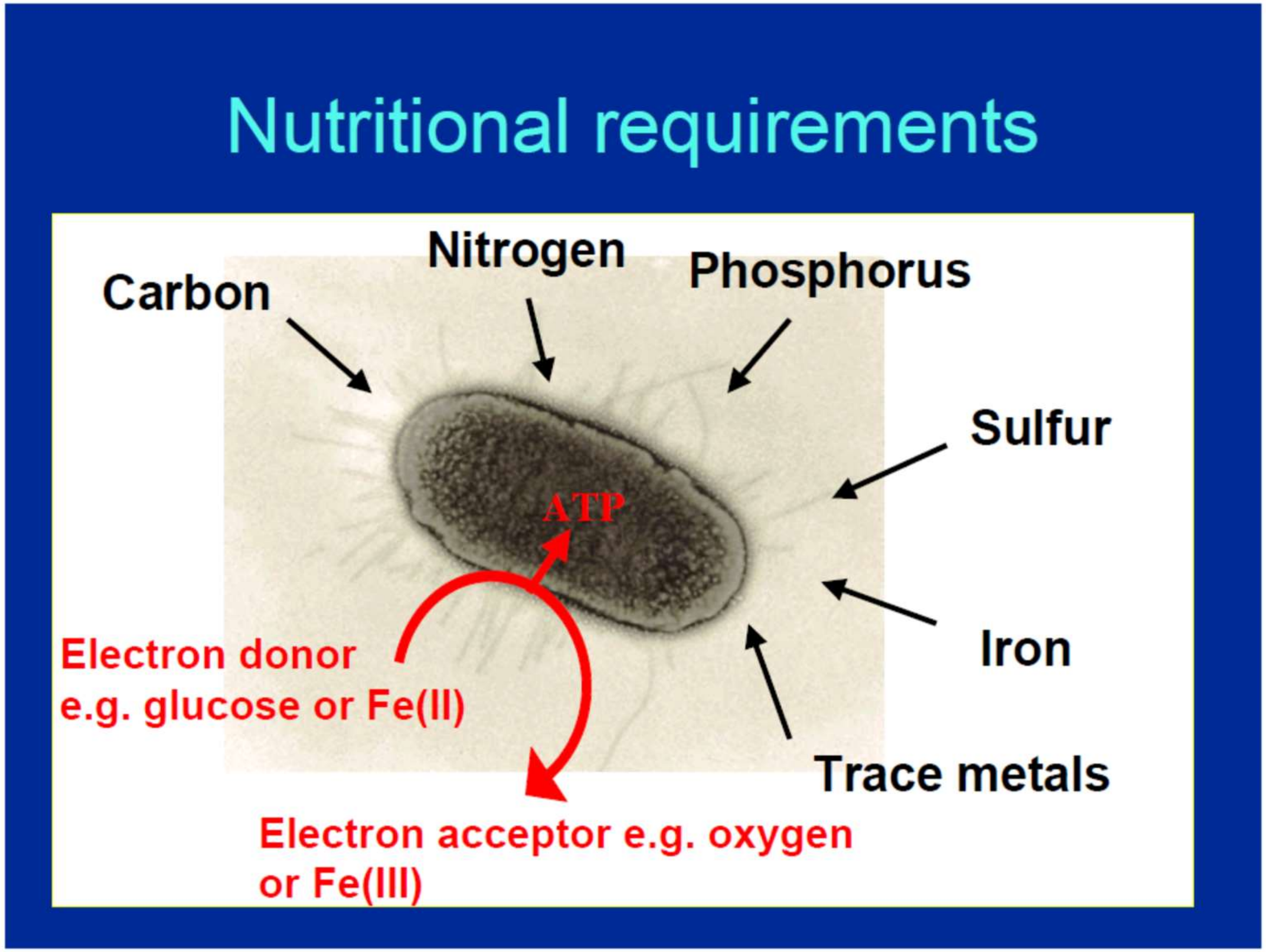
Important for the classification of the metabolism are energy, electron donor and C-source:

Energy: phototrophic (use of sunlight)
chemotrophic (use of chemically bound energy, e.g., sugar)

Electron donor: organotrophic (organic electron donor, e.g., sugar)
lithotroph (inorganic electron donor, e.g., Fe (II), sulfide, H_2)

C-source: *autotrophic* (CO_2 fixation, e.g., in green plants, bacteria)
heterotrophic (organic C-source, e.g., sugar)

Humans always *chemo-organoheterotrophs*,
green plants are *photolithoautotrophically*
bacteria and archaea contain almost all possible combinations

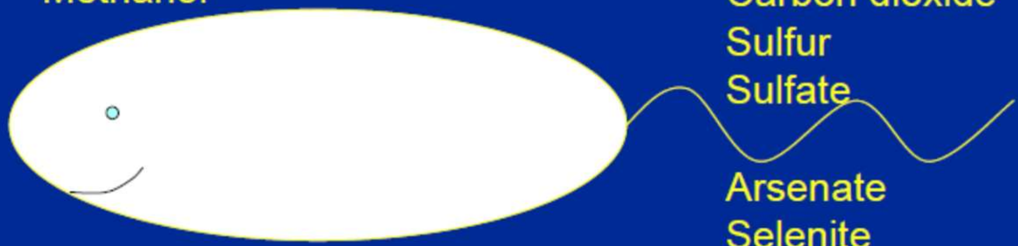

Nutritional requirements
Carbon
Nitrogen
Phosphorus
Sulfur
Iron
Trace metals
ATP
Electron donor
e.g. glucose or Fe(II)
Electron acceptor e.g. oxygen
or Fe(III)

Energy metabolism (electron flow)

Fuels (EDIBLES!!) electrons → **Oxidants (BREATH-ABLES !!)**

Fuels (EDIBLES!!)

- SUNLIGHT
- ORGANICS
  - Glucose
  - Ethanol
  - Formaldehyde
  - Methanol
- Hydrogen
- Ammonia
- Hydrogen sulfide
- Sulfur
- Iron: Fe(II)
- Manganese
- Carbon monoxide
- Arsenite



Oxidants (BREATH-ABLES !!)

- ORGANICS
  - fumarate, DMSO
  - TMAO
- Carbon dioxide
- Sulfur
- Sulfate
- Arsenate
- Selenite
- Iron: Fe(III)
- Manganese
- Nitrate
- Oxygen

Chemoorganotrophy

Examples of chemoorganotrophic organisms and chemoorganotrophic nutrient cycling:

- animals, humans, most bacteria:

$C_6H_{12}O_6 + 6\ O_2 \rightarrow 6\ CO_2 + 6\ H_2O$

Chemolithotrophy

Examples of chemolithotrophic organisms and chemolithotrophic nutrient conversion:

- Hydrogenotrophic bacteria e.g. genus *Ralstonia*:

$2\ H_2 + O_2 \rightarrow 2\ H_2O$

-Bacteria of the species *Acidithiobacillus thiooxidans*:

$S^0 + 2\ O_2 \rightarrow H_2SO_4$

- Sulfate-reducing bacteria e.g. genus *Desulfovibrio*:

$4\ H_2 + H_2SO_4 \rightarrow H_2S + 4\ H_2O$

The microbiology of bioremediation

Microbially-catalysed bioremediation can be carried out using extremely low pH solutions (lixiviants) under strongly oxidising conditions

Consequentially:

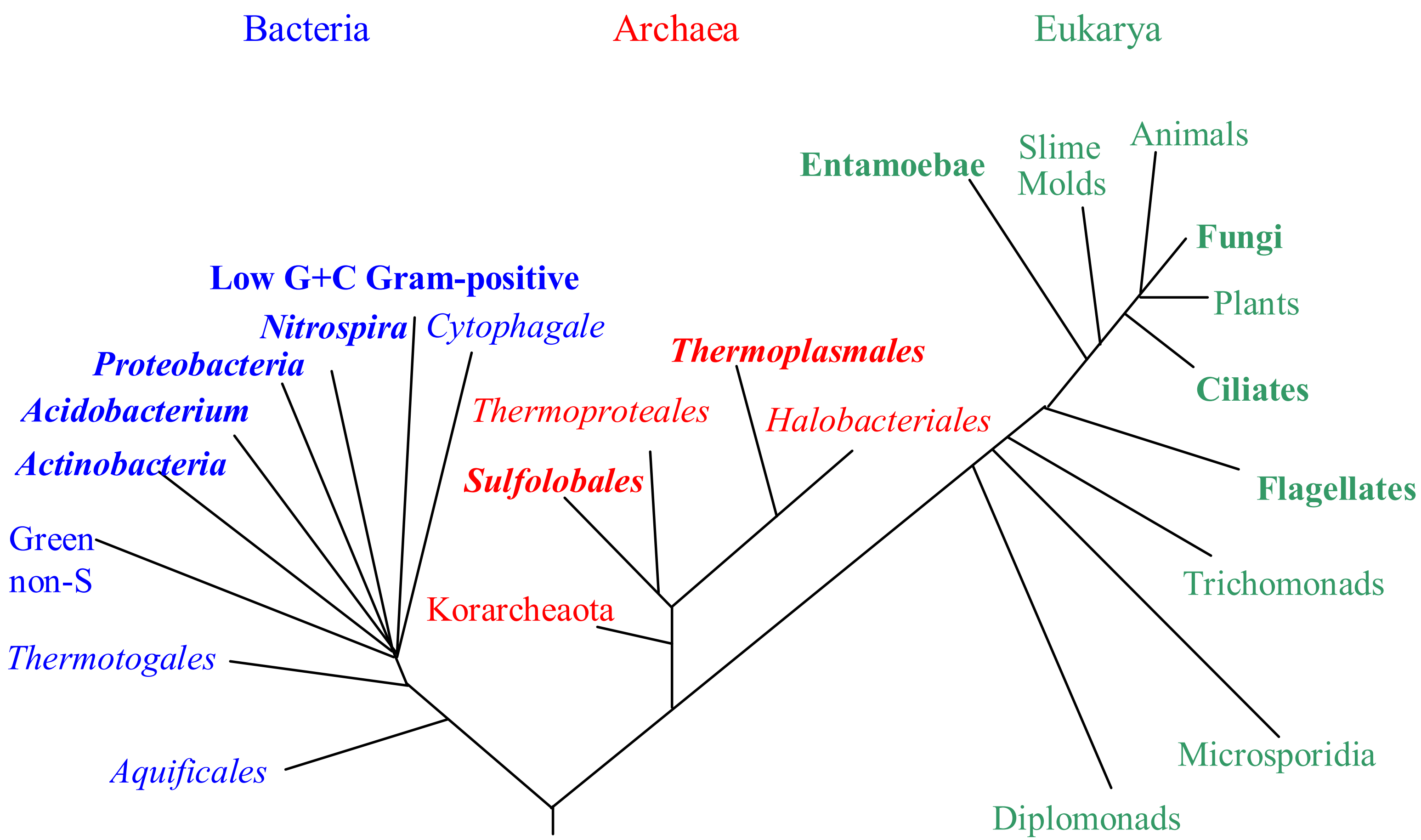
1. Sulfate and metals/metalloids present in waste water can be transformed

2. The extreme acidity means that most metals are soluble/bioavailable

- elevated concentrations of Al, transition metals (Fe, Mn, Cu, Zn…), metalloids (As…), Groups I & II metals (Mg, K), and SO_4
- mineral leach liquors characterised by high osmotic potentials

selects for extremophilic prokaryotes: (thermo)acidophiles

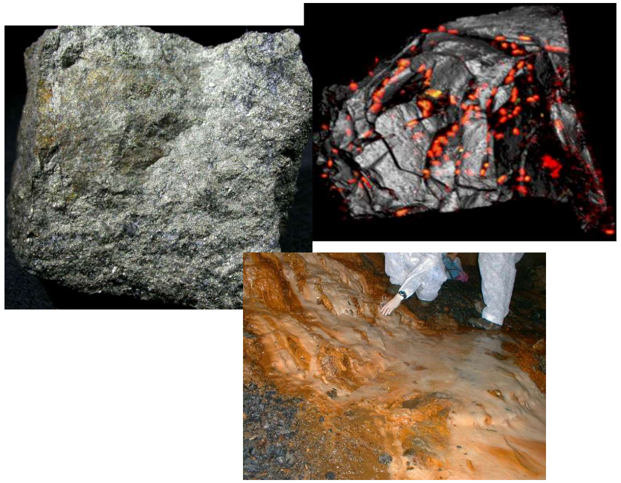
Acidophilic microorganisms are predominantly prokaryotic (bacteria and archaea)



(ii)) Where can acidophiles be found?

Occurrence of acidophilic bacteria

- biofilm on mineral surface



- macroscopic growths in streams

- microbial stalactites and snottites

- planktonic phase of acidic water bodies, streams and pit lakes

Richmond mine – Iron mountain

- polymetallic deposit in California
- lowest reported pH (< 0)
- studied for many years by Jill Banfield´s group

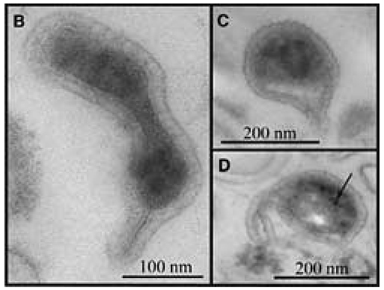
(biofilms, OMICS techniques, isolation,…)

- most diverse habitat in terms of physicochemical conditions and microbiology

(Fe/S-metabolism, mesophilic vs. thermophilic, Archaea, Eukarya,…)

- discovery of several new acidophilic microorganisms

ARMAN- Archaeal Richmond Mine Acidophilic Nanoorganisms



Nordstrom et al., 2000; Edwards et al., 1999; Tyson et al., 2004; Bond et al., 2000; Baker et al., 2006

Other important mine sites

Parys Mountain copper mine, UK
• moderate temperature
• anoxic underground lake
• dominated by *Acidithiobacillus ferrivorans*
• mesophilic Fe-oxidizers
• Archaea

Coupland and Johnson, 2004

Moderate acidic to circumneutral mine sites

Wheal Jane tin mine, Cornwall
• moderate acidophiles/ acid-tolerant organisms (*Halothiobacillus neapolitanus*)

Hallberg & Johnson, 2003

Acidic streams

Rio Tinto (Spain)

- ~ 100km long river in Spain
- iron is the dominant substrate
- macroscopic growths
- iron- and sulfur-cycling communities
- high diversity of eukaryotes
- „Mars-analogon“

Amils et al.; Gonzalez-Toril et al., 2003; Aguilera, 2013

Cantareras (Spain)

- *At. ferrivorans* and heterotrophs
- algaea

Rowe et al., 2007

Dyffryn Adda (UK)

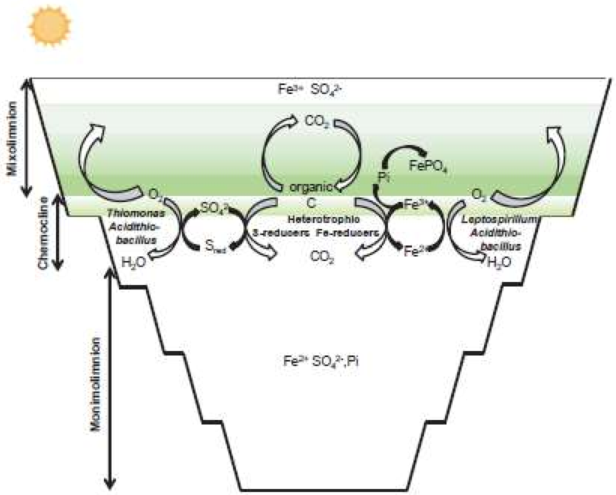
- draining Parys Mountain
- macroscopic growth of iron-oxidizer ‚*Ferrovum myxofaciens*‘

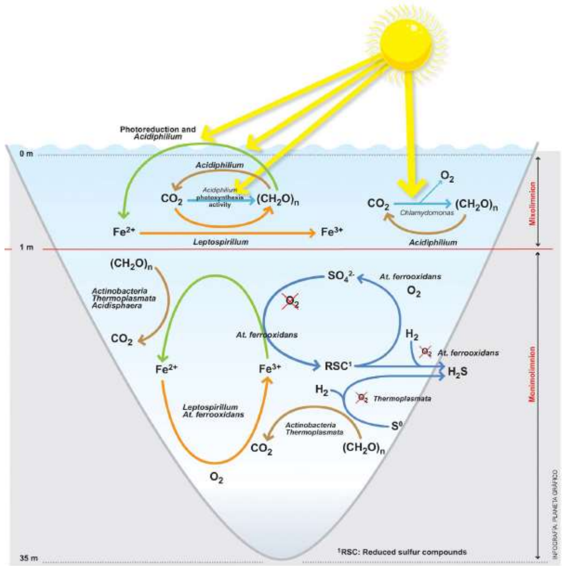
Kay et al., 2013

Acidic pit lakes

- result of opencast mining of coal or metals
- most intensively studied in Spain and Germany

- pit lakes in Germany pH 2-4
- rich in iron and sulfate (minor amounts of other toxic metals)
- nutrient concentration (P, N) is low





- geothermal areas will contain more moderate and extreme thermophilic acidophiles

- consider the pH of the environmental samples (moderate acidophiles pH 3-5; extreme acidophiles pH <3; hyper-acidophiles pH <1)

- abandoned mine sites are often excellent for sourcing acidophiles that are metal-tolerant and are able to oxidise sulfide minerals

- sample low pH, oxygen-depleted environments if anaerobic are required

Acidophiles are highly diverse, metabolically

- Energy source: chemical or solar
- Carbon source: organic or inorganic (or both)
- Electron donors: ferrous iron, reduced sulfur, organic substrates, hydrogen
- Electron acceptors: oxygen, ferric iron, oxidized sulfur

Chemolithotrophy (the ability to use energy derived from oxidizing inorganic chemicals) is widespread amongst acidophiles

Iron-/sulfur-oxidizing acidophiles

$S^0 \rightarrow SO_4^{2-}$

- first acidophile to be discovered:
  Acidithiobacillus thiooxidans (formerly *Thiobacillus thiooxidans)*

$Fe^{2+} \rightarrow Fe^{3+}$

- most widely studied iron-oxidizing acidophile:
  Acidithiobacillus ferrooxidans (formerly *Thiobacillus ferrooxidans)*

Iron-oxidizing acidophiles may be:

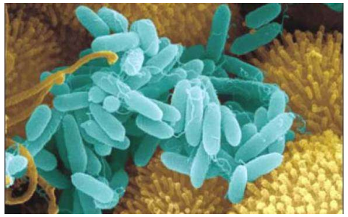
	C-source	Appropriate supply
1. Autotrophic (e.g. *Acidithiobacillus* spp.)	CO_2	air CO_2-enriched air
2. Obligately heterotrophic (e.g. *Ferrimicrobium* sp.)	organic C	yeast extract (0.02% w/v)
3. Facultatively autotrophic (e.g. *Sulfobacillus* spp.)	CO_2 organic C*	air yeast extract

*generally superior growth

Iron as energy source for bacteria

Acidophiles

- best studied, because Fe^{2+} stable at pH <5
- bacteria and archea
- autotrophic, mixotrophic, heterotrophic

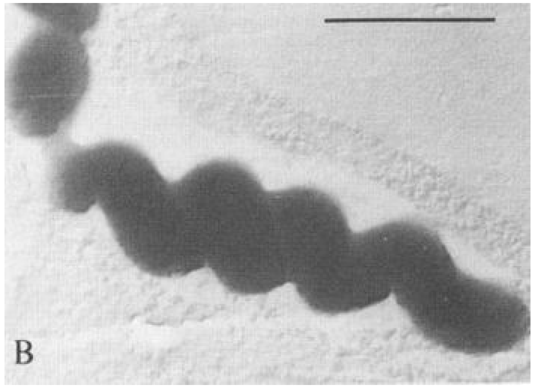


Acidithiobacillus ferrooxidans

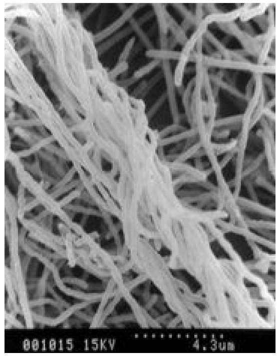
$Fe^{2+} + O_2 \rightarrow Fe^{3+} + H_2O$ (autotrophic)

$Fe^{3+} + S^0 + 4H_2O \rightarrow Fe^{2+} + 8H^+ + SO_4^{2-}$ (anaerobic conditions)

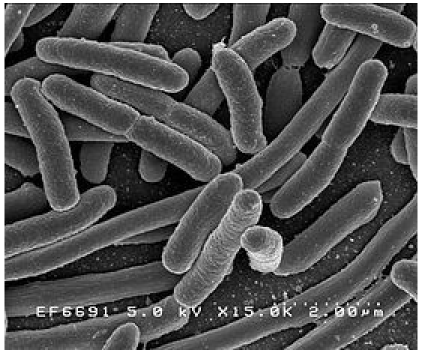
Iron-metabolising microorganisms



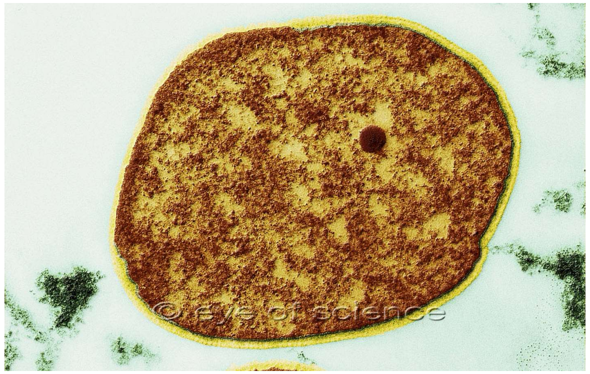
Leptospirillum ferrooxidans



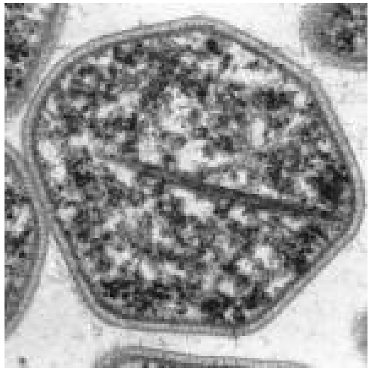
Ferrimicrobium acidophilum



Sulfobacillus thermosulfidooxidans



Sulfolobus acidocaldarius

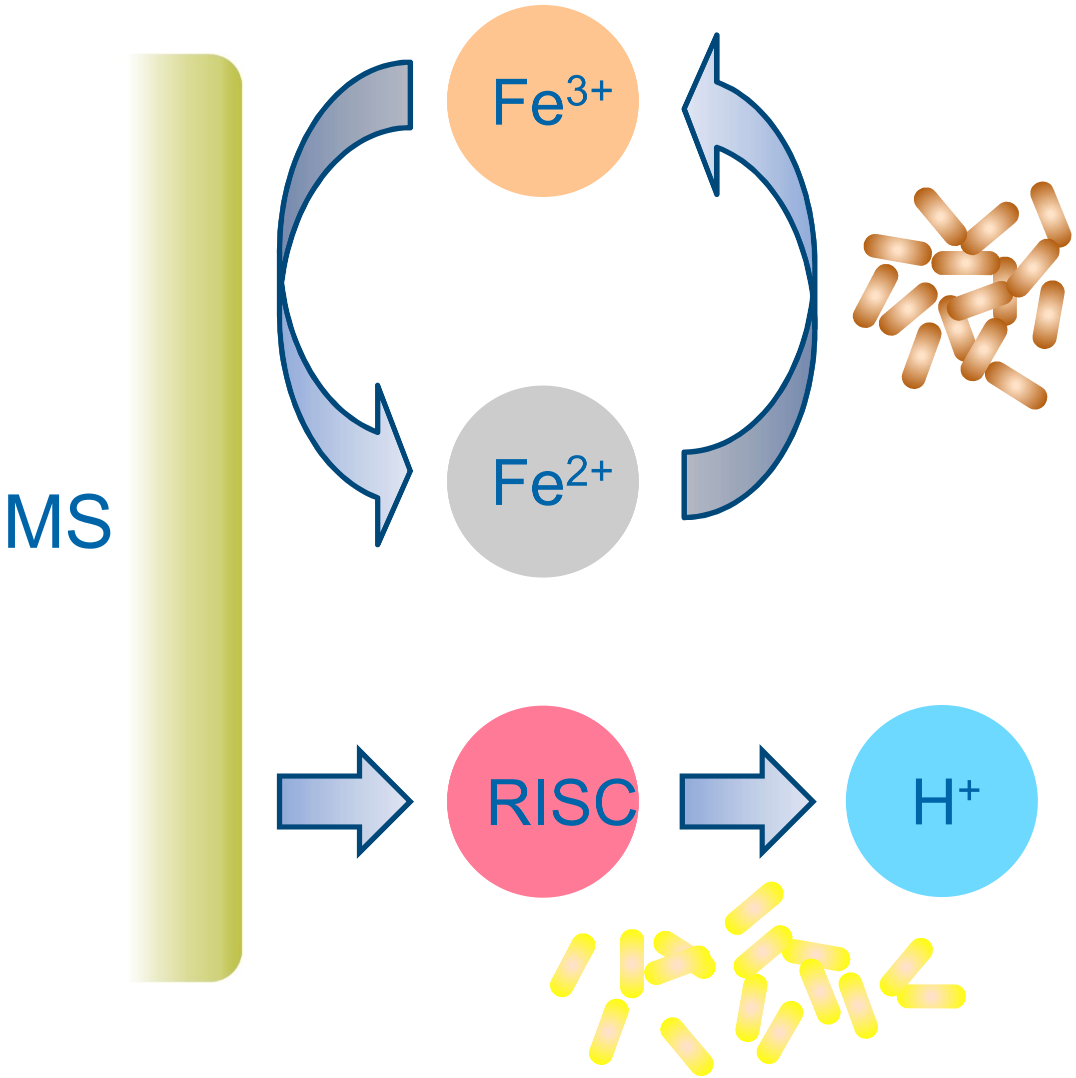


Acidianus brierleyi

However, bioremediation environments are necessarily non-sterile, and axenic cultures are never encountered

- consortia are more robust, and generally more efficient, than pure cultures
- acidophiles interact with each other in these environments, mostly in synergistic ways, though negative interactions have also been reported

Microbial nutrition and growth



KEY TRAITS OF BIOREMEDIATION MICROORGANISMS

Iron (and sulphur) oxidation

Sulfate reduction

Autotrophy/ Heterotrophy

- Modest nutrient requirements (small quantities of NPK)

Acidotrophy

Aerobes and Anaerobes

- Aerobes- Use molecular oxygen as terminal electron acceptor
- Anaerobes – Thrive under oxygen-limited conditions

Tolerance of physico-chemical conditions

- [metal]
- [anions]
- Mechanical shear (reactors)

IMPORTANT BIOREMEDIATION ACIDOPHILES

		Thermal classification[a]
Iron-oxidiser		
	Leptospirillum ferrooxidans	Meso
	L. ferriphilum	Mod Thermo
	Ferrovum myxofaciens	Meso
Sulfur-oxidisers		
	Acidithiobacillus thiooxidans	Meso
	At. caldus	Mod Thermo
	Metallosphaera spp.	Ext Thermo
	Sulfolobus spp.	
Sulfate-reducing microorganisms		
	Desulfotomaculum spp.	Meso
	Desulfosporosinus spp.	
Heterotrophic acidophiles		
	Acidocella sp.	Meso
	Acidiphilium spp.	
	Alicyclobacillus spp.	
	Thermoplasma spp.	Mod Thermo
Obligate anaerobes		
	Stygiolobus azoricus	Ext Thermo
	Acidilobus aceticus	

[a]Meso, mesophiles ($T_{optimum}$ < 40°C); Mod Thermo, moderate thermophiles ($T_{optimum}$ 40-60°C); Ext Thermo, extreme thermophiles ($T_{optimum}$ >60°C);

Question: what species of microorganisms are actually found in bioremediation operations?

- It depends very much on the engineering system being used and the water chemistry

    - wetlands
    - stirred tanks
    - Oxygen supply
    - pH
    - metal concentration
    - substrate availability

Thank you for your attention!

Glück auf!