Microbes and their habitats – the Biofilm

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Microbes live where they find energy or food
this defines the groups of microbes

In the sediment – depending on the chemical characteristics

In the water – swimming forms and sessile forms of microbes

particles in water – sessile – conditions change

MICROBES
- take off
- go dormant
- encapsulate
- form ultra-microforms
- spores

but essentially they do not cease to exist

They are just not active - same as pathogens – they kill their host but then……

CONCLUSION: there are no sterile rock surfaces !!

Nutritional classification of microorganisms

All living organisms need sources of carbon, energy and electrons to carry out their metabolic activities. Bacteria have also been classified based on their method(s) used to obtain these three components - carbon, energy and electrons:

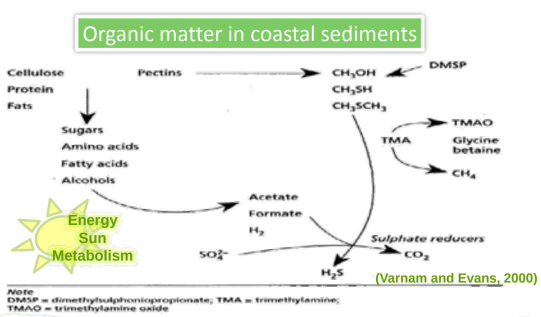
- •AUTOTROPHS obtain their **carbon from carbon dioxide**
- •HETEROTROPHS need **pre-made organic compounds** as carbon source
- •PHOTOTROPHS are organisms that utilize **light** as a source of energy
- •CHEMOTROPHS obtain energy by **oxidation of inorganic or organic** compounds.
- •ORGANOTROPHS obtain their **electrons from organic** compounds
- •LITHOTROPHS literally rock eaters) obtain **electrons from inorganic** compounds

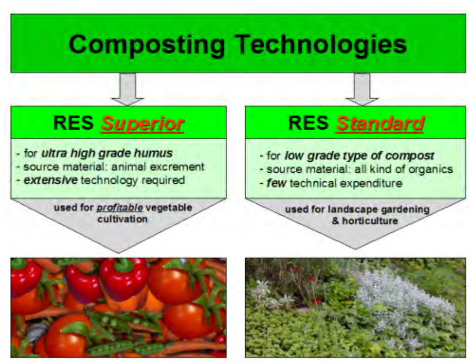
Nutritional classification of microorganisms

Major nutritional types of prokaryotes

Nutritional Type		Carbon Source	Electron Source	Examples
Photoautotrophic lithotrophs	Light	CO_2	Inorganic (H_2O or H_2S)	Cyanobacteria (e.g. *Oscillatoria*), some purple and green bacteria
Photoheterotrophic organotrophs	Light	Organic compounds	Organic compounds	Some purple (e.g. *Rhodobacter*) and green bacteria
Chemoautotrophic lithotrophs	**Rocks or minerals**	CO_2	Inorganic compounds	Bacteria (e.g. *Nitrosomonas*) and many archaea
Chemoheterotrophic organotrophs	Organic compounds	Organic compounds	Organic compounds	Most bacteria (e.g. *Escherichia coli*) some archaea

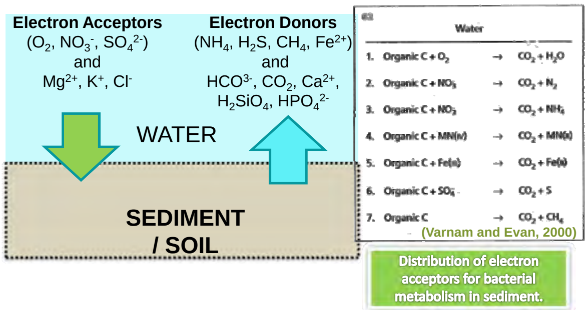
Anaerobic degradation-metabolism

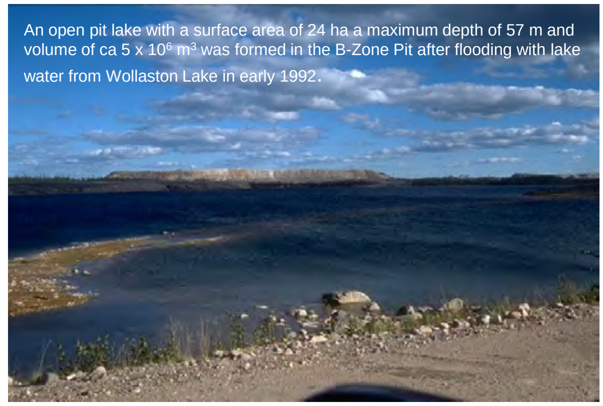




Without Microbes No compost – recalcitrant materials – slower

Electron acceptors and bacteria





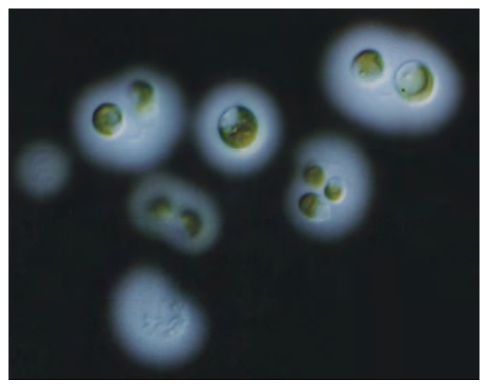
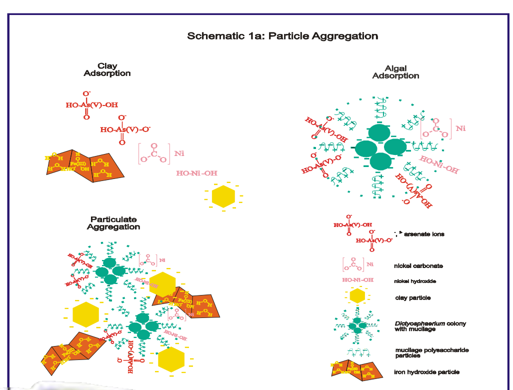


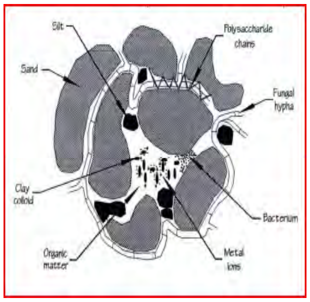
Table 1: Chemistry and concentration changes 1993-2001

Parameter	Mean Concentration (mg/L) 1993	2001	Decrease (-) / Increase (+)
$HCO3^-$	13.1	31.8	142%
Conductivity (µs/cm)	53.4	87.5	64%
Na	1.8	2.4	34%
Al	1	0.008	-99%
As	0.28	0.019	-93%
Cl	1.8	0.72	-59%
Fe	0.75	0.29	-61%
Ni	0.27	0.19	-31%
Ra^{226}-total (Bq/L)	0.143	0.018	-87%
U (total)	0.026	0.01	-62%



The structure of a soil particle

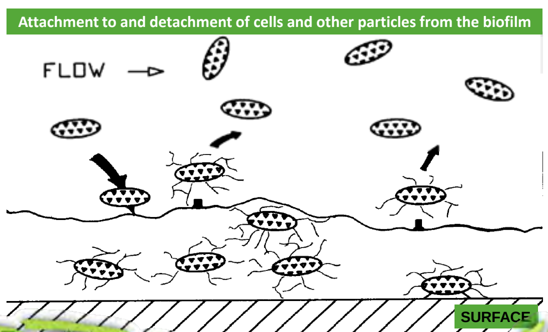
Ideal soil
25 % air, 25 % water
45% minerals and silica and 5 % organic matter
Fungi and bacteria hold the particle together



Rocks are the parent material of soils

Soil formation is part of the geologic cycle and soil characteristics are influenced **by parent material, climate, topography, weathering, and the amount of time** a particular soil has had to develop.

Biofilm formation



The basic structure of a biofilm

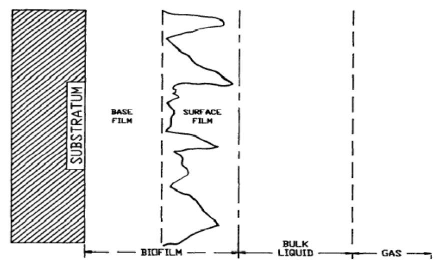


Figure 1.2 The biofilm system includes the following five compartments: (1) substratum, (2) base film, (3) surface film, (4) bulk liquid, and (5) gas. The base film and surface film constitute the biofilm.

Micro-Environments

or

Microbial habitats

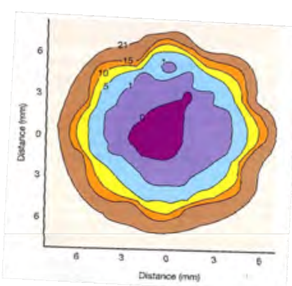
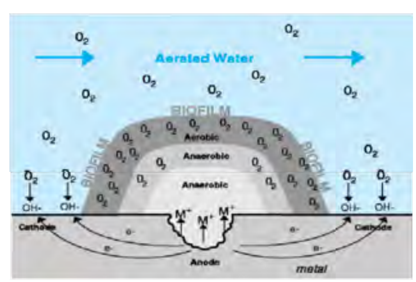


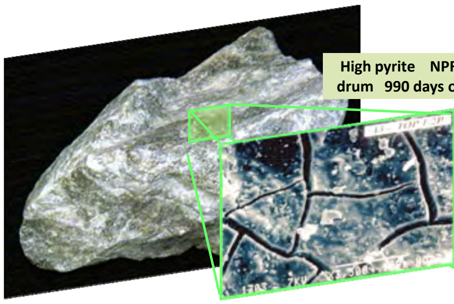
Figure 19.2 Contour map of O_2 concentrations in a soil particle The axes show the dimensions of the particle. The numbers on th contours are O_2 concentrations (in percent; air is 21% O_2). In terms o oxygen relationships for microorganisms, each zone can be consid ered a different microenvironment.

Biofilms and Corrosion



From: Borenstein, S.B., Microbiologically Influenced Corrosion Handbook, Industrial Press Inc., New York (1994).

Mineral Surfaces



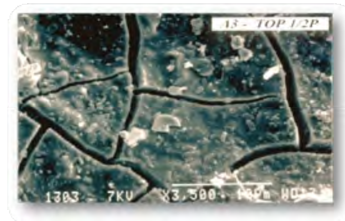
High pyrite drum

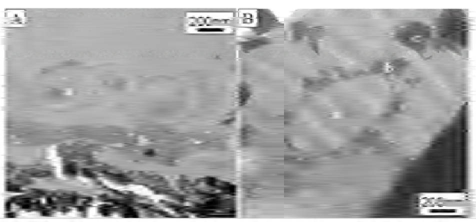
NPR throughout 990 days of weathering

Biofilm – additons of NPR

BIOFILMS

MICROBIAL COLONIES ENCASED IN AN ADHESIVE, USUALLY POLYSACCHARIDE MATERIAL, AND ATTACHED TO A SURFACE.





- **Figure 4.** Stained thin sections of the biofilms formed in the pH 2.2 and 7.2 system after 73 days (A and B, respectively). (A). The biofilm was composed of cells (a), very fine precipitates trapped in an ecopolymer-like matrix (b) and detrital fragments from the rock surface (c). (B): cell (a) surrounded by secondary precipitates trapped in extracellular material (b) and detrital fragments from the rock surface (c).

Geomicrobiology – pre-biofilms

1838

- **EHRENBERG** – *Gallionella ferruginea* with ochreous deposits of bog iron.

1887

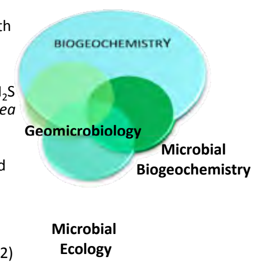
- **WINOGRADSKY** – *Beggiatoa* oxidation H_2S to elemental sulfur; *Leptothrix ochracea* oxidation of $FeCO_3$ to ferric oxid.

1919

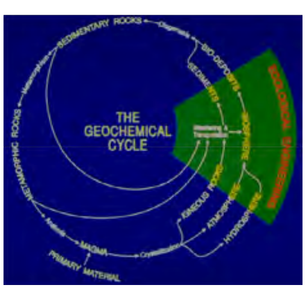
- **HARDER** –microbial iron oxidation and precipitation in iron sedimentary deposits.

…

- *STUTZER* (1911); **VERNADSKY** (1908-1922)



Where does bio-mineralization take place?
Answer: in sediments



The major problem is the natural weathering processes which releases minerals to the biosphere – both aquatic and terrestrial

Mining increases the surface area available for weathering therefore introduces imbalances in the biosphere

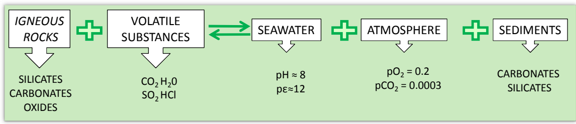
Weathering /hydro-chemical cycles

- Chemical Weathering is the major process controlling the global hydro-chemical cycle of elements.
- Water is the reactant and the transporting agent of dissolved and particulate components from land to sea.

Geochemical Cycles

CLASSICAL GEOCHEMICAL MATERIAL BALANCE (GOLDSCHMIDT, 1933)

- H^+ balance → interaction between of igneous rocks with volatile substances.



Weathering

Climate is the main driver of the weathering process – *"the production of metals from the rocks"* - either as:

ECONOMIC RESOURCE

OR

CONTAMINATION

?

Year 2000

MICROBIAL WEATHERING

Pyrite ⇨ *A. ferrooxidans* ⇨ ARD/AMD

$$2\,FeS_2 + 7\,O_2 + 2\,H_2O \rightarrow 2\,Fe^{2+} + 4\,SO_4 + 4\,H^+$$
$$4\,Fe^{2+} + O_2 + 4\,H^+ \rightarrow 4\,Fe^{3+} + 2\,H_2O$$
$$4\,Fe^{3+} + 12\,H_2O \rightarrow 4\,Fe(OH)_3 + 12\,H^+$$
$$FeS_2 + 14\,Fe^{3+} + 8\,H_2O \rightarrow 15\,Fe^{2+} + 2\,SO_4^{2-} + 16\,H^+$$

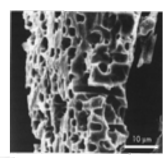


Figure 5.16 SEM image showing extensive pyrite dissolution after only 43 days of oxidation by A. ferrooxidans. (From Mustin et al., 1992. Reproduced with permission from the American Society for Microbiology.)

Source: Konhauser, K. (2007). Introduction to geomicrobiology. Blackwell Publishing

Biologically-induced bio-mineralization

Minerals are formed as byproduct of the cell's metabolic activity or through its interactions with the surrounding aqueous environment.

✓Microbial nucleation and growth

✓Silicates ✓Carbonates ✓Phosphates ✓Sulfates ✓Sulfide minerals

✓Manganese Oxides
- a) Hydrothermal manganese deposits
- b) Ferromanganese deposits
- c) Desert varnish

✓Iron Hydroxides
- a) Passive iron mineralization
- b) Chemo-heterotrophic iron mineralization
- c) Photoautotrophic iron mineralization
- e) Hydrothermal ferric hydroxide deposits
- d) Formation of iron oxides

Ferromanganese nodules

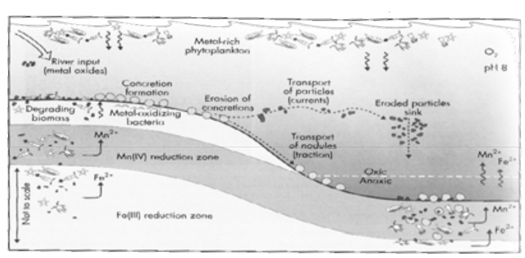


Figure 4.10 Model showing how ferromanganese nodules might form in certain lake sediments. (Adapted from Dean et al., 1981.)

Source: Konhauser, K. (2007). Introduction to geomicrobiology. Blackwell Publishing

Biologically -controlled bio-mineralization

Completely regulated by the organism, allowing the organism to precipitate minerals that serve some physiological purpose.

- ✓Magnetite
- ✓Greigite
- ✓Calcite
- ✓Amorphous Silica

Fossilization - Pyritization model

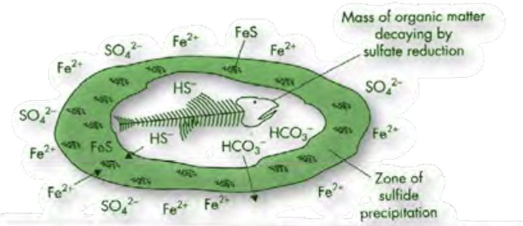
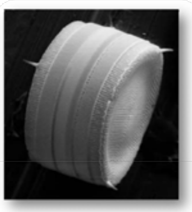


Figure 4.36 Model of organic matter pyritization. Its oxidation by SRB releases HS⁻ into the pore waters immediately surrounding the degrading biomass. Those pore waters, however, must have sufficiently high Fe^{2+} so that FeS (and ultimately FeS_2) precipitation is confined to the decay site, instead of HS⁻ diffusing away into the surrounding sediments. (Adapted from Raiswell, 1997.)

Source: Konhauser, K. (2007). Introduction to geomicrobiology. Blackwell Publishing

Biologically-induced bio-mineralization



Bio-mineralization in Diatoms (Bio-silica)
\http://www.biologie.uni-regensburg.de/Biochemie/Sumper/startseite.html

Bio-mineralization of Pyrite (Ammonite Fossil) Alden,Erie Co., NY
http://www.nysm.nysed.gov/nysam/recentaq/18917.htm

Amorphous Silica

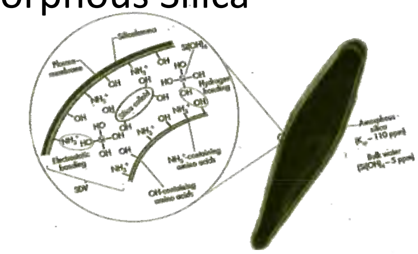


Figure 4.30 Representation of how diatoms form their siliceous shells. Despite living in undersaturated solutions with respect to amorphous silica, diatoms actively extract $Si(OH)_4$ from solution and pump it into an intracellular silica deposition vesicle (SDV) that lines the inside of the plasma membrane. There, the concentration of silica is increased to supersaturation. The SDV also contains hydroxyl and cationic amino-containing amino acids that react with colloidal silica, and thus facilitate the subsequent nucleation and mineralization stages. In this regard, the SDV acts as a template, for silicification.

Source: Konhauser, K. (2007). Introduction to geomicrobiology. Blackwell Publishing

BIOLOGICALLY-CONTROLLED BIO-MINERALIZATION

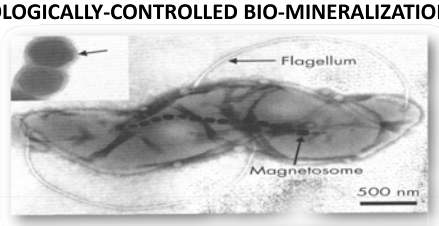
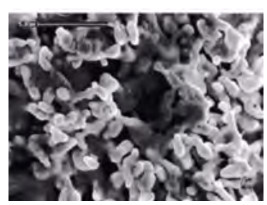


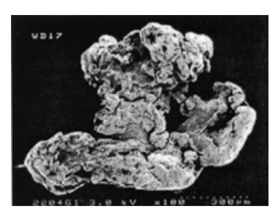
Figure 4.25 TEM image of a magnetotactic bacterium, designated strain MV-4, grown in pure culture. Cells of this strain produce a single chain of magnetite crystals that longitudinally traverse the cell. Inset shows close-up of the magnetosome membrane (arrow) that surrounds each individual particle. (Courtesy of Dennis Bazylinski.)

Source: Konhauser, K. (2007). Introduction to geomicrobiology. Blackwell Publishing



Scanning electron image of a gold-encrusted Biofilm on a gold flake panned from soil

Scanning electron image of a Gold flake panned from soil Overlaying the Tomakin Park Gold mine

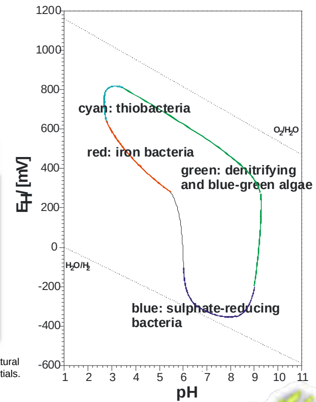


DEFINING LIMITS

Eh/pH diagram – Baas Becking
Total measurements: 6200
Organisms: 2100
Environments: 4100

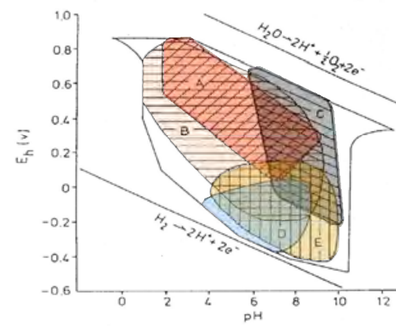
pH / Eh Range

- THIOBACTERIA → 1.00-9.92/ +855 to -190
- IRON BACTERIA → 2.00-8.90/ +850 to +60
- DENITRIFIERS → 6.20-10.20/+665 to -220
- BLUE-GREEN ALGAE → 6.15-9.78/+7 to-293
- SULPHATE REDUCERS → 4.15-9.92/+115 to -450



Baas Becking, Kaplan and Moore (1960). "Limits of the natural environment in terms of pH and Oxidation-reduction potentials. The journal of Geology, Vol.68, N°3, 243-284pp

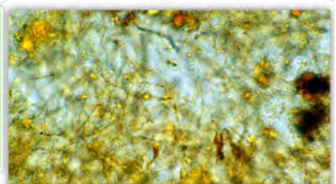
Environmental Limits of Eh and pH for aquatic Bacteria



A, "iron bacteria";
B, thiobacteria;
C, denitrifying bacteria;
D, facultative and anaerobic heterotrophic bacteria and methanogens;
E, sulfate-reducing bacteria

Iron Bacteria

Gallionella ferruginea
- Promotes iron corrosion
- But also removes ferrous iron from



Photograph by Eleanor Robbins, U.S. Geological Survey (http://www.discoverlife.org/nh/tx/Bacteria/images/bacteria.jpg.html)

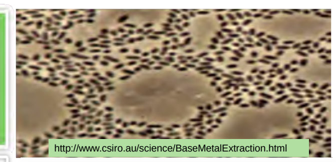
- aerobic
- Micro-aerophilic.
- Chemo-litho-autotrophic.
- HABITAT: oligotrophic ferrous iron-bearing waters (optimally Eh +200 to +300 mV.)

IMPORTANT:
forms large masses of ferri-hydrite in water bodies and water supply systems. This is probably one of the major culprits for the dirty water samples.

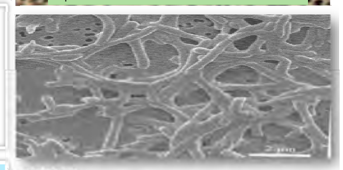
REFERENCES
Bergey's Manual of Determinative Bacteriology. 1994. Ninth Edition. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams. (eds.). Williams and Wilkins publishers.
Environmental Microbiology ,2000, ASM , Varnam A.H. and Evans M.G

Acidithiobacillus ferrooxidans

- metabolizes iron and sulfur
- sulfuric acid generation (AMD)
- bioleaching



http://www.csiro.au/science/BaseMetalExtraction.html



- Fe^{2+} oxidation to Fe^{3+}
- Chemo-lithoautotrophic
- **HABITAT:** pyrite deposits (pH:1.8-2.5)

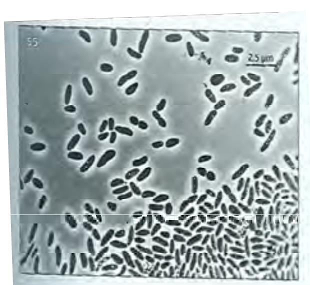
AMD - generation

REFERENCES
Environmental Microbiology ,2000, ASM , Varnam A.H. and Evans M.G
Bergey's Manual of Determinative Bacteriology. 1994. Ninth Edition. Holt, J.G., N.R. Krieg, P.H.A. Sneath, J.T. Staley, and S.T. Williams. (eds.). Williams and Wilkins publishers.

Sulphate reducing-bacteria

- assimilate oxygen from sulfate compounds and reduce them to sulfides

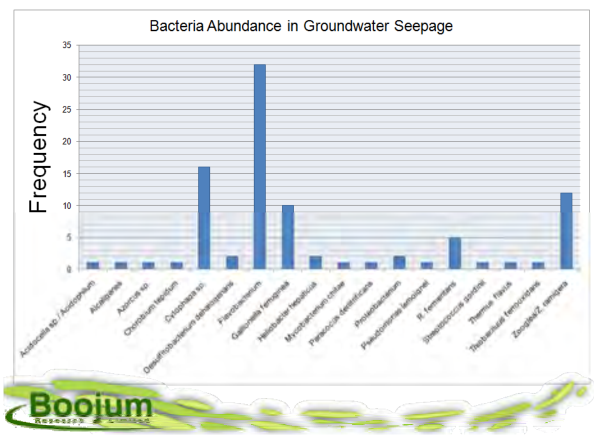
- anaerobic /photoautotrophic
- Rotten egg odor (H_2S)
- Purple and green sulphur bacteria
- Corrosion
- **HABITAT:** oligotrophic/aquatic environments with high organic matter content.

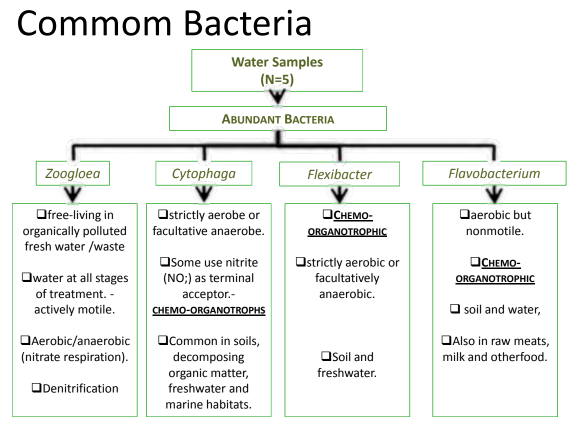


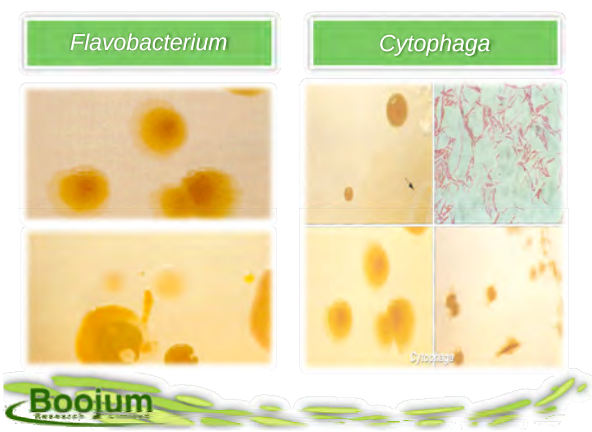
55 Purple sulphur bacteria *Chromatium salexigens*. Reproduced by courtesy of Prof. P. Caumette, University of Bordeaux.

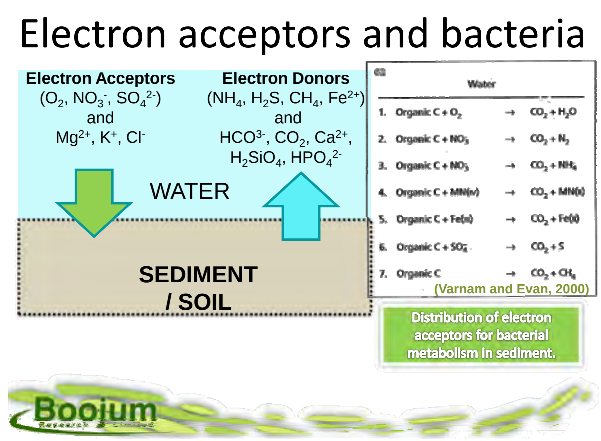
REFERENCES
Environmental Microbiology ,2000, ASM , Varnam A.H. and Evans M.G
Source: Konhauser, K. (2007). Introduction to geomicrobiology. Blackwell Publishing

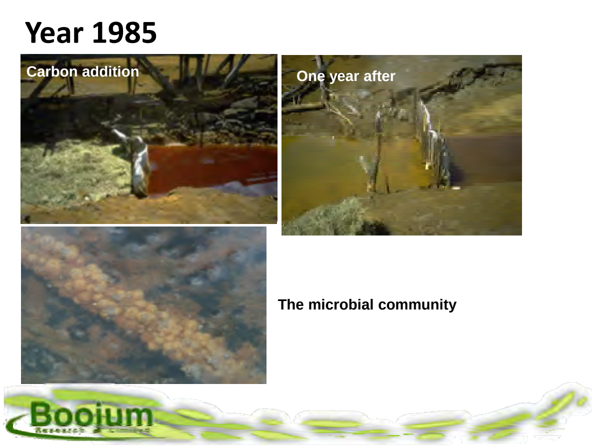
BACTERIAL CONSORTIA IN ACID MINE GROUNDWATER SEEPAGE

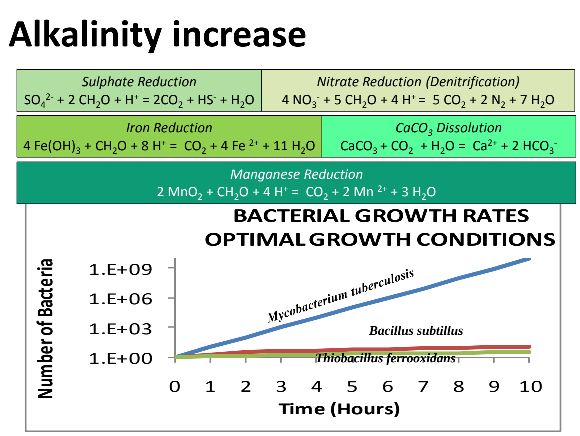

Bacteria Abundance in Groundwater Seepage
Frequency
Boojum


Commom Bacteria
Water Samples (N=5)
Abundant Bacteria
Zoogloea
Cytophaga
Flexibacter
Flavobacterium
free-living in organically polluted fresh water /waste
water at all stages of treatment. - actively motile.
Aerobic/anaerobic (nitrate respiration).
Denitrification
strictly aerobe or facultative anaerobe.
Some use nitrite (NO_2) as terminal acceptor.- CHEMO-ORGANOTROPHS
Common in soils, decomposing organic matter, freshwater and marine habitats.
CHEMO-ORGANOTROPHIC
strictly aerobic or facultatively anaerobic.
Soil and freshwater.
aerobic but nonmotile.
CHEMO-ORGANOTROPHIC
soil and water,
Also in raw meats, milk and otherfood.


Flavobacterium
Cytophaga
Boojum


Electron acceptors and bacteria
Electron Acceptors
(O_2, NO_3^-, SO_4^{2-}) and Mg^{2+}, K^+, Cl^-
Electron Donors
(NH_4, H_2S, CH_4, Fe^{2+}) and HCO^{3-}, CO_2, Ca^{2+}, H_2SiO_4, HPO_4^{2-}
WATER
SEDIMENT / SOIL
Water
1. Organic C + O_2 → $CO_2 + H_2O$
2. Organic C + NO_3^- → $CO_2 + N_2$
3. Organic C + NO_3^- → $CO_2 + NH_4$
4. Organic C + MN(iv) → CO_2 + MN(ii)
5. Organic C + Fe(iii) → CO_2 + Fe(ii)
6. Organic C + SO_4^- → CO_2 + S
7. Organic C → $CO_2 + CH_4$
(Varnam and Evan, 2000)
Distribution of electron acceptors for bacterial metabolism in sediment.
Boojum


Year 1985
Carbon addition
One year after
The microbial community
Boojum


Alkalinity increase
Sulphate Reduction
$SO_4^{2-} + 2 CH_2O + H^+ = 2CO_2 + HS^- + H_2O$
Nitrate Reduction (Denitrification)
$4 NO_3^- + 5 CH_2O + 4 H^+ = 5 CO_2 + 2 N_2 + 7 H_2O$
Iron Reduction
$4 Fe(OH)_3 + CH_2O + 8 H^+ = CO_2 + 4 Fe^{2+} + 11 H_2O$
$CaCO_3$ Dissolution
$CaCO_3 + CO_2 + H_2O = Ca^{2+} + 2 HCO_3^-$
Manganese Reduction
$2 MnO_2 + CH_2O + 4 H^+ = CO_2 + 2 Mn^{2+} + 3 H_2O$
BACTERIAL GROWTH RATES
OPTIMAL GROWTH CONDITIONS
Number of Bacteria
1.E+09
1.E+06
1.E+03
1.E+00
Mycobacterium tuberculosis
Bacillus subtillus
Thiobacillus ferrooxidans
0 1 2 3 4 5 6 7 8 9 10
Time (Hours)

CARBON ADDITION

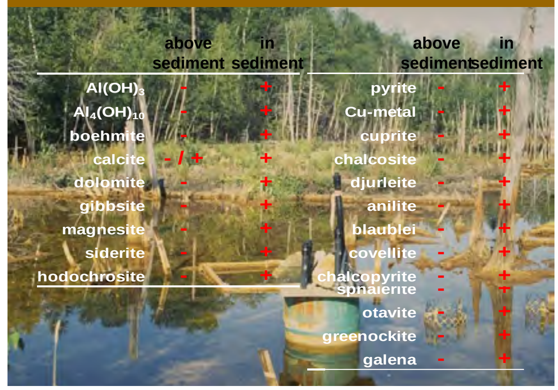
Surface pH 2.3
Bottom pH 5.2
Conductivity 11,000 µS/cm

March 1991

June 1991

July 1992

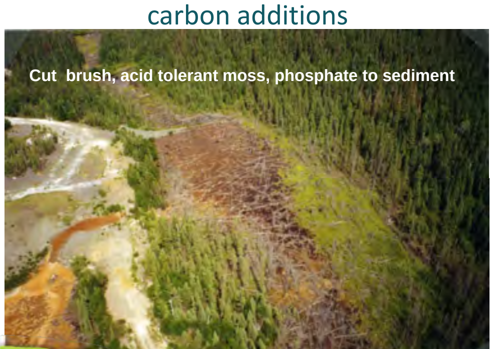
SEDIMENTS : BIOMINERALIZATION
Conversion of Metals into Stable Forms



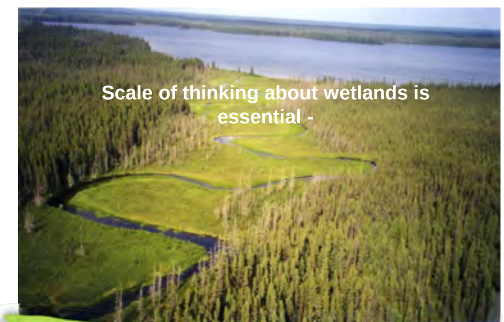
	above sediment	in sediment
$Al(OH)_3$	-	+
$Al_4(OH)_{10}$	-	+
boehmite	-	+
calcite	- / +	+
dolomite	-	+
gibbsite	-	+
magnesite	-	+
siderite	-	+
hodochrosite	-	+

	above sediment	in sediment
pyrite	-	+
Cu-metal	-	+
cuprite	-	+
chalcosite	-	+
djurleite	-	+
anilite	-	+
blaublei	-	+
covellite	-	+
chalcopyrite	-	+
sphalerite	-	+
otavite	-	+
greenockite	-	+
galena	-	+

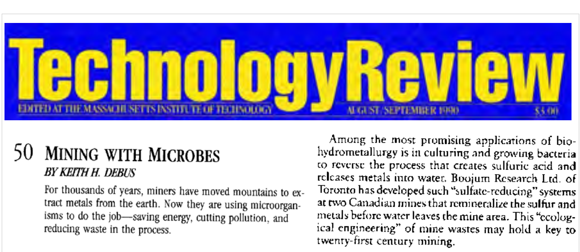
Ecological engineering – effects of NPR and carbon additions



Floating cover removes oxygen below



MIT Technology Review 1990



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50 MINING WITH MICROBES

BY KEITH H. DEBUS

For thousands of years, miners have moved mountains to extract metals from the earth. Now they are using microorganisms to do the job—saving energy, cutting pollution, and reducing waste in the process.

Among the most promising applications of biohydrometallurgy is in culturing and growing bacteria to reverse the process that creates sulfuric acid and releases metals into water. Boojum Research Ltd. of Toronto has developed such "sulfate-reducing" systems at two Canadian mines that remineralize the sulfur and metals before water leaves the mine area. This "ecological engineering" of mine wastes may hold a key to twenty-first century mining.