



STUDIECENTRUM VOOR KERNENERGIE  
CENTRE D'ETUDE DE L'ENERGIE NUCLEAIRE

# Geomicrobiology of waste repositories

Gas generation & removal  
Radionuclide migration & retention

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# Geomicrobiology in waste repositories

- Geochemical experimental set-ups were, are and will be influenced by microbiological activity.
  - E.g. Microbial  $H_2$  consumption &  $CH_4$  production in 'gas diffusion tests'
  - E.g. Microbial gas production in 'nitrate tests'
  - E.g. Biocorrosion and biodegradation of equipment & sensors
  - E.g. Microbial colonisation & biofilm development 'hampering *in situ* monitoring' and analysis of piezometers
  - ....
- Repository and host rock were, are and will never be sterile.

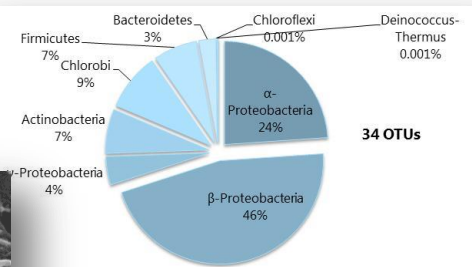
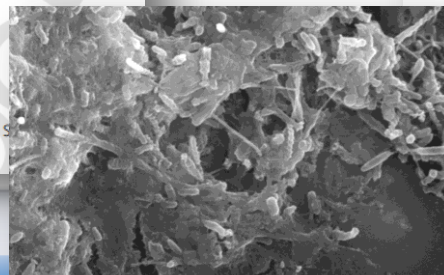
(And for safety it is irrelevant whether the microbes are introduced or indigenous.)

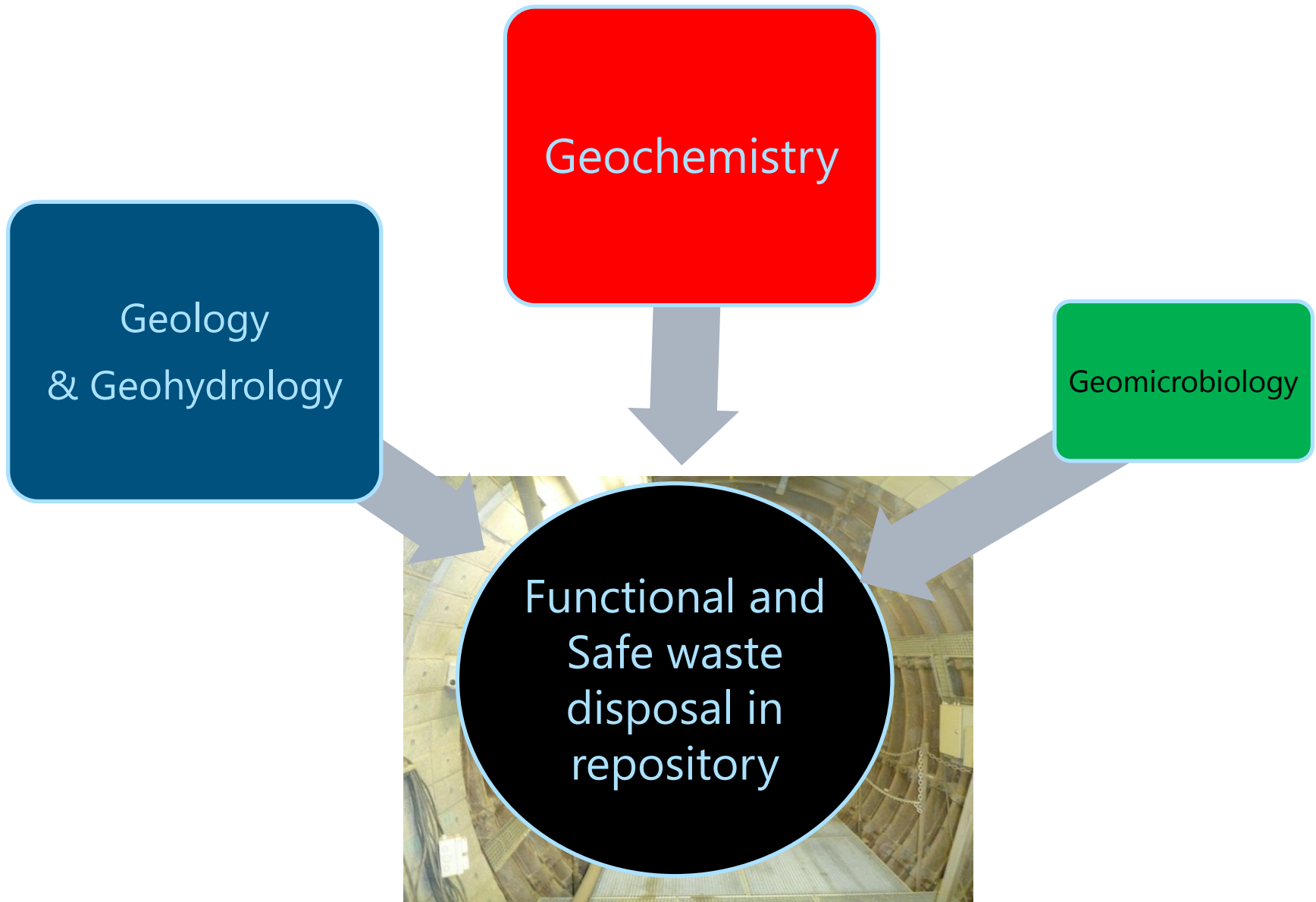
## RESEARCH ARTICLE

### Evidence and characteristics of a diverse and metabolically active microbial community in deep subsurface clay borehole water

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# Geomicrobiology in waste repositories

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## ● Problem

Microbes will be present and active,  
and will interact with waste, container, repository and host rock,  
during excavation, exploitation, and storage.



Microbiology **cannot be neglected** but has to be considered and taken into account in the experimentation and modelling, and the final design and operational procedures, to assure functional and safe waste storage.

## ● Need

- A better understanding of
  - the **microbial populations** present in waste, repository and host rock
  - the microbial **bioprocesses** that can occur **at in situ conditions**
  - the impact of those processes on the **water & surface geochemistry**
  - the **impact** of those geochemical changes on the waste, repository and host rock barrier function

## NUTRIENTS

- To Grow / Proliferate / Make biomass -

**C H N O P S** + trace elements

TIC & DOM



## MICROBIAL POPULATION

### HOW MANY

'Number' of cells

### WHO & WHAT DOING

'Type' of microbes  
= type of enzymes  
= type of **bioprocess**

### HOW FAST

'Speed' of enzymatic  
processes

## ENERGY

- To stay alive -

Electron donors  
(get oxidised)

Organics

H<sub>2</sub>

H<sub>2</sub>S

H<sub>2</sub>O

Metals & radionuclides

minerals



## ENERGY

- To stay alive -

Electron acceptor  
(get reduced)

Oxygen

N-compounds

S-compounds

C-compounds

Metals & radionuclides

minerals



## Physical environmental conditions

Radiation  
Gy

Temperature  
T

Space  
Porosity

Water/dryness  
Aw

Salinity  
IC

Alkalinity/Acidity  
pH

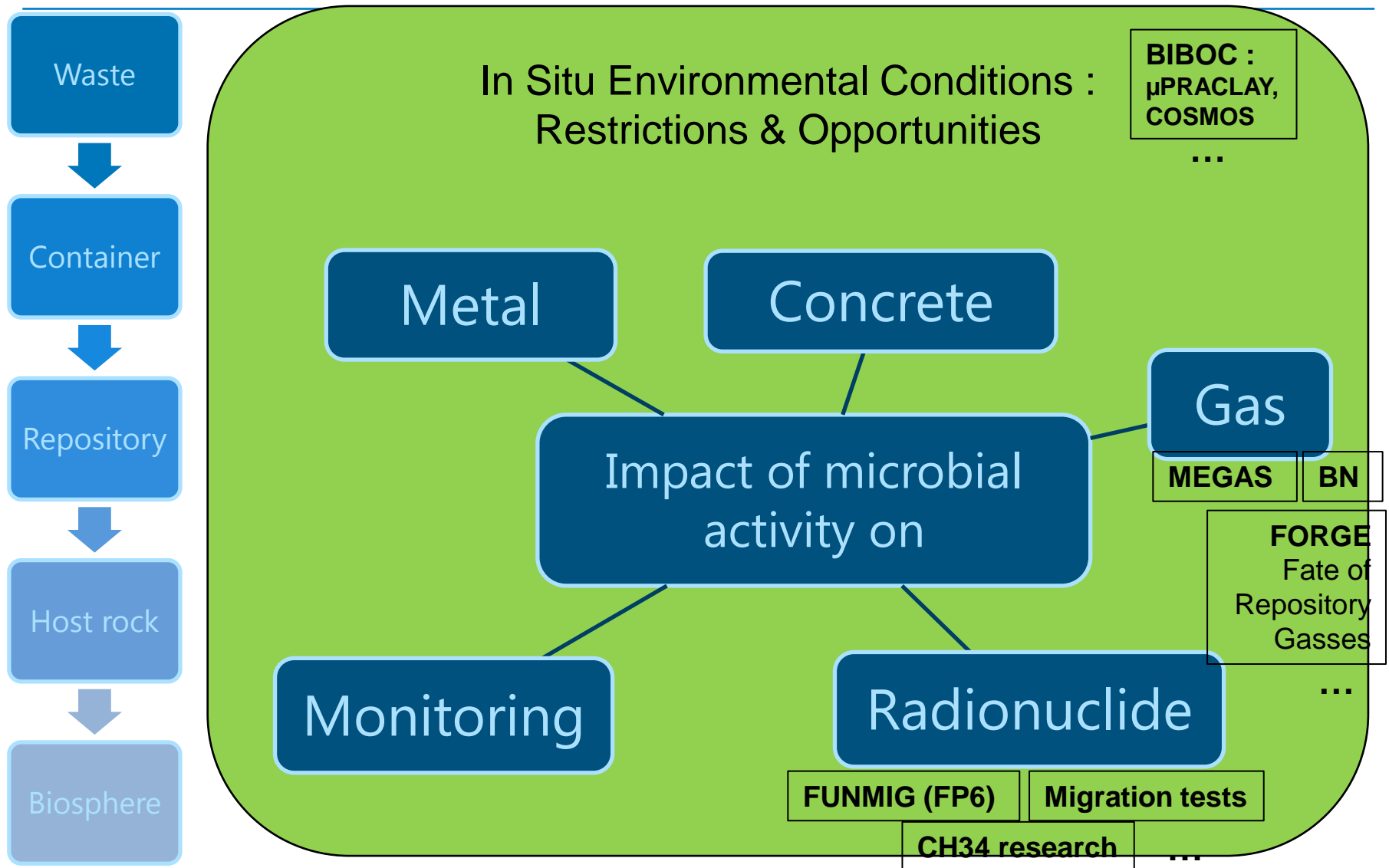
Surfaces  
carriers



excavation & installation

exploitation

storage



- Nitrogen cyclus :
    - nitrate from waste  $\rightarrow$   $\text{N}_2\text{O}$ ,  $\text{NO}$ ,  $\text{N}_2$  (NRB, Nitrate E-acceptor, only when anoxic)
  - Sulphur cyclus :
    - clay  $\rightarrow$  pyrite  $\rightarrow$  sulphate (gips) (during excavation, when oxic)
    - sulphate (gips)  $\rightarrow$   $\text{H}_2\text{S}$  (SRB, sulphate E-acceptor, only when anoxic)
  - Carbon cyclus :
    - organic acids  $\rightarrow$   $\text{CO}_2$  (Hetrotrophs & organic E-donor, oxic & anoxic)
    - $\text{CO}_2$   $\rightarrow$  methane  $\text{CH}_4$  (methanogens,  $\text{CO}_2$  E-acceptor, anoxic)
- $\rightarrow$  Production of reduced gas species with impact on**
- $\rightarrow$  water geochemistry**
  - $\rightarrow$  structural materials (corrosion)**
  - $\rightarrow$  dissolution chemistry and mobility of radionuclides**

- Oxygen
  - Most preferred E-acceptor for most microbes, can be consumed very fast
- Hydrogen from anoxic corrosion & radiolysis
  - very good 'fuel for microbes' (E-donor)
  - oxic (with  $O_2$  as E-acceptor)
  - anoxic (with nitrate or sulphate as E-acceptor)
- Carbon dioxide
  - As carbon source for autotrophic proliferation



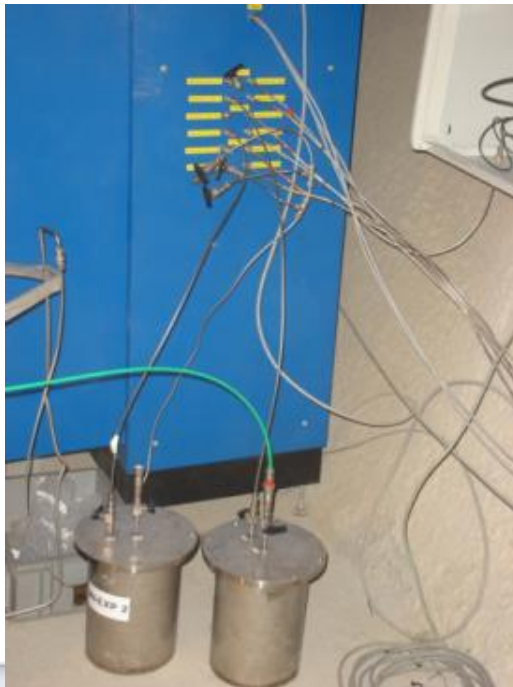
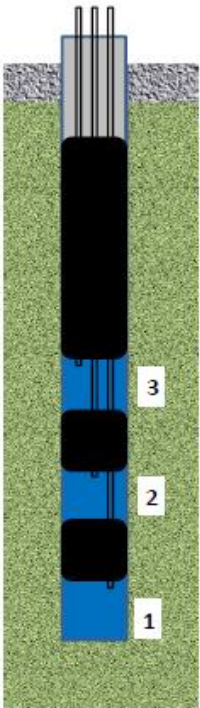
e.g. BN-Experiment, Mont Terri

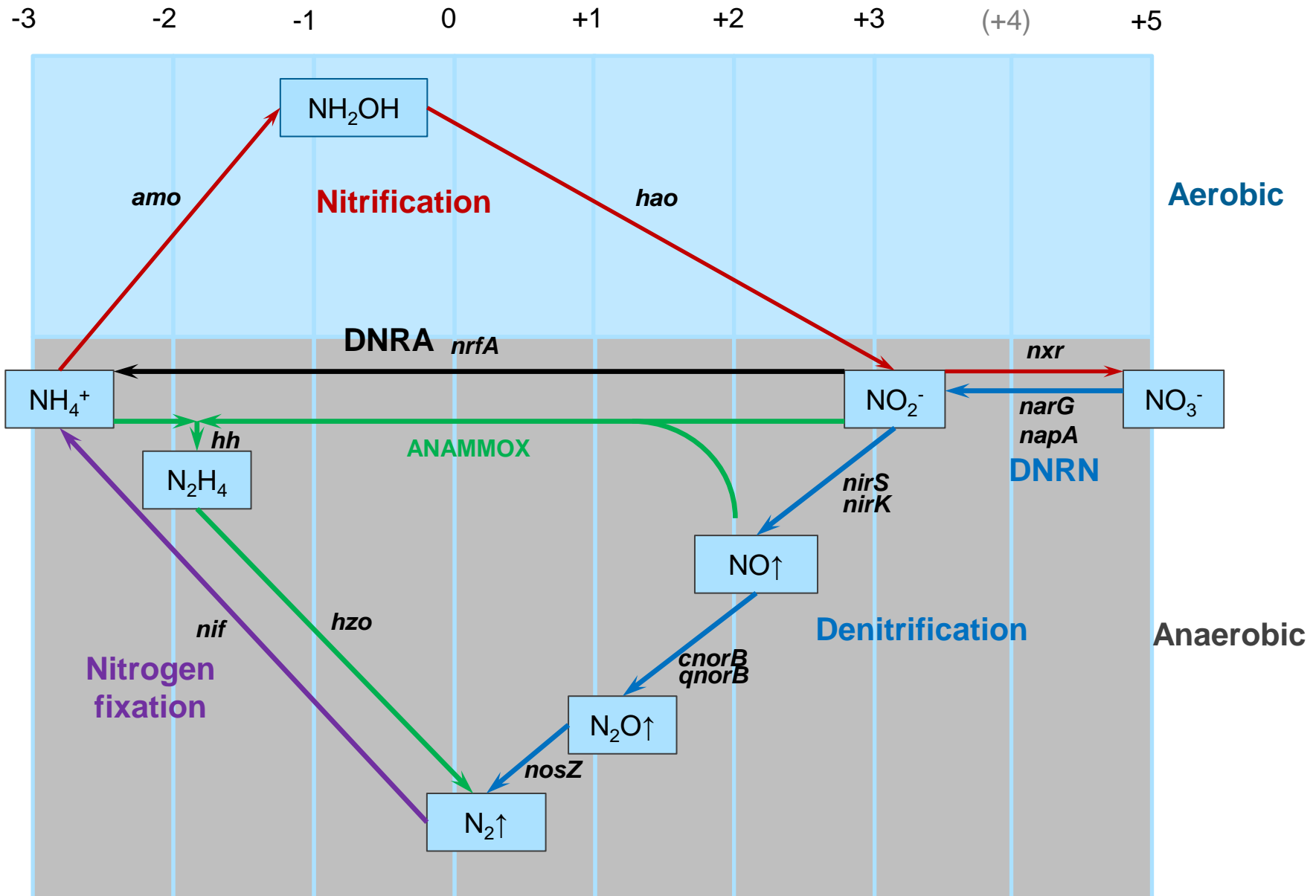
Interaction clay – bitumen waste

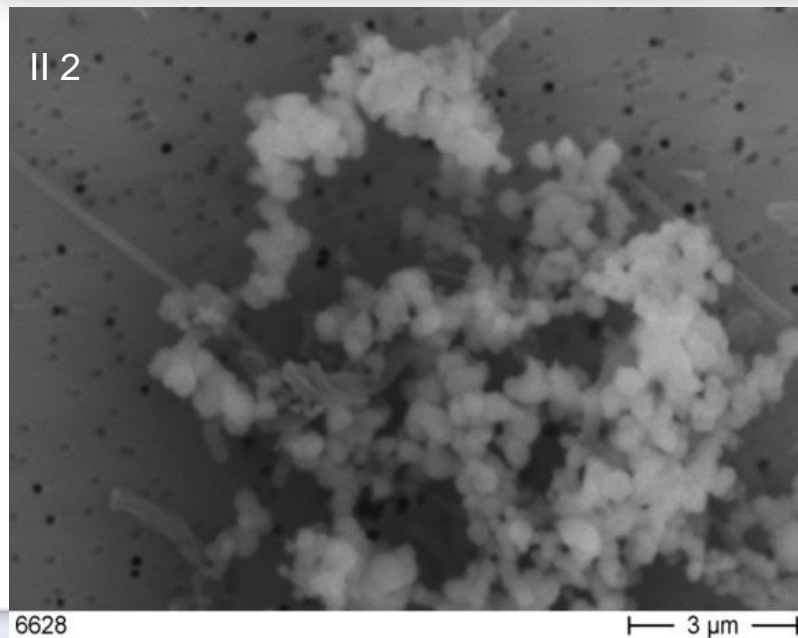
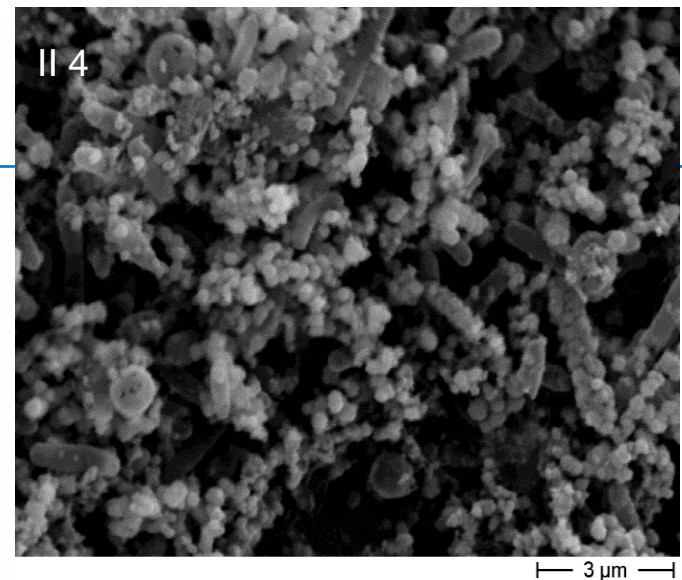
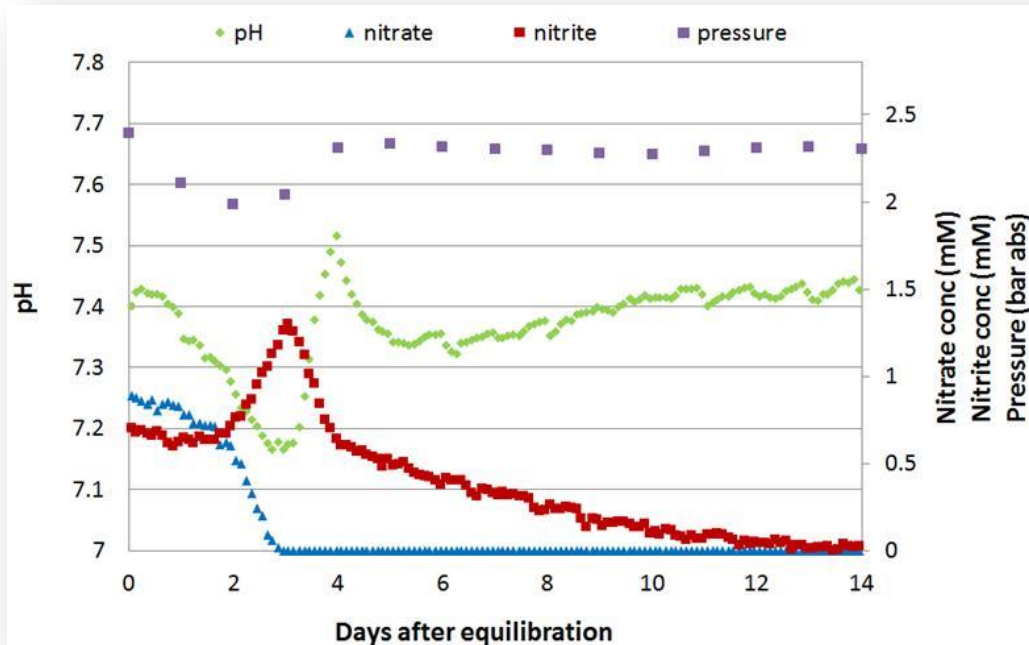
**Nitrate & Acetate injection** as products

- 3 packed-off intervals
- Sintered stainless steel filter screen surrounding a central tube
- Water lines connecting intervals with recirculation cabinet in gallery

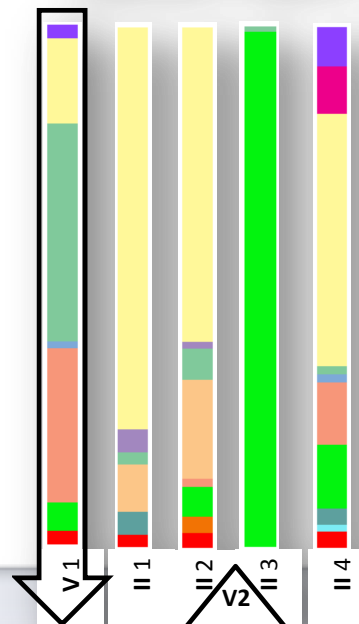
→ Providing 'space' and 'water' & 'rock contact'







### Interval II N/C=1



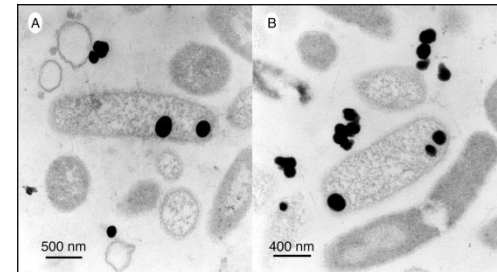
- Actinobacteria;Actinobacteria;Actinomycetales
- Bacteroidetes;Bacteroidia;Bacteroidales
- Bacteroidetes;Sphingobacteria;Sphingobacteriales
- Chloroflexi;Anaerolineae;Anaerolineales
- Chloroflexi;Thermomicrobia;Sphaerobacterales
- Cyanobacteria\_Chloroplast;Chloroplast;Chloroplast\_order\_incertae\_sedis
- Firmicutes;Bacilli;Bacillales
- Firmicutes;Bacilli;Lactobacillales
- Firmicutes;Clostridia;Clostridiales
- Firmicutes;Clostridia;unclassified
- Firmicutes;Erysipelotrichia;Erysipelotrichales
- Proteobacteria;Alphaproteobacteria;Caulobacterales
- Proteobacteria;Alphaproteobacteria;Rhizobiales
- Proteobacteria;Alphaproteobacteria;Rhodobacterales
- Proteobacteria;Alphaproteobacteria;Rhodospirillales
- Proteobacteria;Alphaproteobacteria;Sphingomonadales
- Proteobacteria;Alphaproteobacteria;unclassified
- Proteobacteria;Betaproteobacteria;Burkholderiales
- Proteobacteria;Betaproteobacteria;Nitrosomonadales
- Proteobacteria;Betaproteobacteria;Rhodocyclales
- Proteobacteria;Deltaproteobacteria;Desulfobacterales
- Proteobacteria;Gammaproteobacteria;Alteromonadales
- Proteobacteria;Gammaproteobacteria;Enterobacterales
- Proteobacteria;Gammaproteobacteria;Pseudomonadales
- Proteobacteria;unclassified;unclassified
- unclassified;unclassified;unclassified



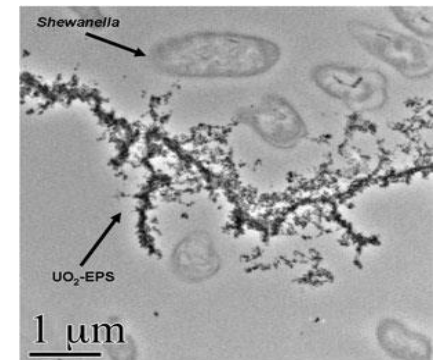
- RN : selenium (Se), uranium (U), neptunium (Np), plutonium (Pu), curium (Cm)
- Microbial impact on RN chemical form (speciation)
  - Microbial RN reduction (RN as *E*-acceptor, anoxic)  
→ e.g. metallic nanoparticles
- Microbial impact on RN ligands
  - Degradation of organic ligands (as *C*-source)
  - Production of complexation compounds
    - biomineralisation with phosphate, carbonate, carboxylate ...
    - Extracellular polymeric substances (EPS)
    - metallophores (e.g. organic acids, peptides, ...)
- Microbial impact on RN sorption
  - RN sorption on microbial biofilm on wetted surfaces
  - RN sorption on microbial colloids

alkalinity (pH),  
redox potential (Eh),

*Selenihalanaerobacter shriftii*  
& selenate (A) and selenite (B)  
→ Black = solid elemental Se.



*Shewanella* & uranyl  
→ Black = solid uraninite (UO<sub>2</sub>).



e.g. Characterising and 'engineering' bacteria that bioaccumulate or bioprecipitate metals & RN

## Our model bacterium: *Cupriavidus metallidurans* CH34

Isolated in 1976 from polluted sludge in metallurgical plant (Prayon-Liege, Belgium), by Dr. C. Houba (Ulg) & Dr. M. Mergeay (SCK•CEN), > 30 years of research in SCK, studied > 30 labs all over the world

**Metals for which the interaction with bacterium *C. metallidurans* CH34 was studied**

- C** Solid
- Hg** Liquid
- H** Gas
- Rf** Unknown

- Metals**
  - Alkali metals
  - Alkaline earth metals
  - Lanthanoids
  - Actinoids
  - Transition metals
  - Poor metals
- Nonmetals**
  - Other nonmetals
  - Noble gases

1 <b>H</b> Hydrogen 1.00794	2 <b>He</b> Helium 4.002602																	3 <b>Li</b> Lithium 6.941	4 <b>Be</b> Beryllium 9.012182	5 <b>B</b> Boron 10.811	6 <b>C</b> Carbon 12.0107	7 <b>N</b> Nitrogen 14.0067	8 <b>O</b> Oxygen 15.9994	9 <b>F</b> Fluorine 18.9984032	10 <b>Ne</b> Neon 20.1797										
11 <b>Na</b> Sodium 22.98976928	12 <b>Mg</b> Magnesium 24.3050	13 <b>Al</b> Aluminum 26.9815386	14 <b>Si</b> Silicon 28.0855	15 <b>P</b> Phosphorus 30.973762	16 <b>S</b> Sulfur 32.06	17 <b>Cl</b> Chlorine 35.453	18 <b>Ar</b> Argon 39.948	19 <b>K</b> Potassium 39.0983	20 <b>Ca</b> Calcium 40.078	21 <b>Sc</b> Scandium 44.955912	22 <b>Ti</b> Titanium 47.867	23 <b>V</b> Vanadium 50.9415	24 <b>Cr</b> Chromium 51.9961	25 <b>Mn</b> Manganese 54.938045	26 <b>Fe</b> Iron 55.845	27 <b>Co</b> Cobalt 58.933195	28 <b>Ni</b> Nickel 58.6934	29 <b>Cu</b> Copper 63.546	30 <b>Zn</b> Zinc 65.38	31 <b>Ga</b> Gallium 69.723	32 <b>Ge</b> Germanium 72.64	33 <b>As</b> Arsenic 74.92160	34 <b>Se</b> Selenium 78.96	35 <b>Br</b> Bromine 79.904	36 <b>Kr</b> Krypton 83.798										
37 <b>Rb</b> Rubidium 85.468	38 <b>Sr</b> Strontium 87.62	39 <b>Y</b> Yttrium 88.90585	40 <b>Zr</b> Zirconium 91.224	41 <b>Nb</b> Niobium 92.90638	42 <b>Mo</b> Molybdenum 95.96	43 <b>Tc</b> Technetium (97.9072)	44 <b>Ru</b> Ruthenium 101.07	45 <b>Rh</b> Rhodium 102.90550	46 <b>Pd</b> Palladium 106.42	47 <b>Ag</b> Silver 107.8682	48 <b>Cd</b> Cadmium 112.411	49 <b>In</b> Indium 114.818	50 <b>Sn</b> Tin 118.710	51 <b>Sb</b> Antimony 121.760	52 <b>Te</b> Tellurium 127.60	53 <b>I</b> Iodine 126.90447	54 <b>Xe</b> Xenon 131.293	55 <b>Cs</b> Caesium 132.9054519	56 <b>Ba</b> Barium 137.327	57-71	72 <b>Hf</b> Hafnium 178.49	73 <b>Ta</b> Tantalum 180.94788	74 <b>W</b> Tungsten 183.84	75 <b>Re</b> Rhenium 186.207	76 <b>Os</b> Osmium 190.23	77 <b>Ir</b> Iridium 192.217	78 <b>Pt</b> Platinum 195.084	79 <b>Au</b> Gold 196.966569	80 <b>Hg</b> Mercury 200.59	81 <b>Tl</b> Thallium 204.3833	82 <b>Pb</b> Lead 207.2	83 <b>Bi</b> Bismuth 208.98040	84 <b>Po</b> Polonium (209.9824)	85 <b>At</b> Astatine (209.9871)	86 <b>Rn</b> Radon (222.0176)
87 <b>Fr</b> Francium (223)	88 <b>Ra</b> Radium (226)	89-103	104 <b>Rf</b> Rutherfordium (261)	105 <b>Db</b> Dubnium (262)	106 <b>Sg</b> Seaborgium (266)	107 <b>Bh</b> Bohrium (264)	108 <b>Hs</b> Hassium (277)	109 <b>Mt</b> Meitnerium (268)	110 <b>Ds</b> Darmstadtium (271)	111 <b>Rg</b> Roentgenium (272)	112 <b>Uub</b> Ununbium (285)	113 <b>Uut</b> Ununtrium (284)	114 <b>Uuq</b> Ununquadium (289)	115 <b>Uup</b> Ununpentium (288)	116 <b>Uuh</b> Ununhexium (292)	117 <b>Uus</b> Ununseptium (294)	118 <b>Uuo</b> Ununoctium (294)																		

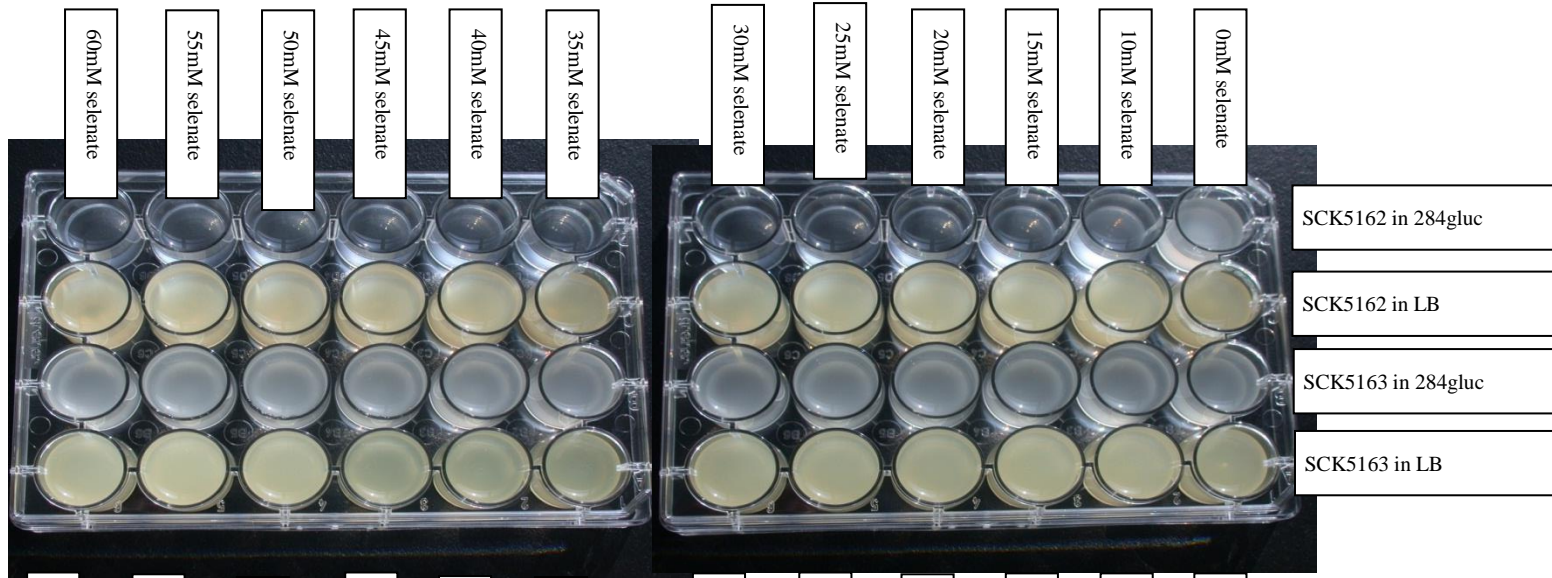
For elements with no stable isotopes, the mass number of the isotope with the longest half-life is in parentheses.

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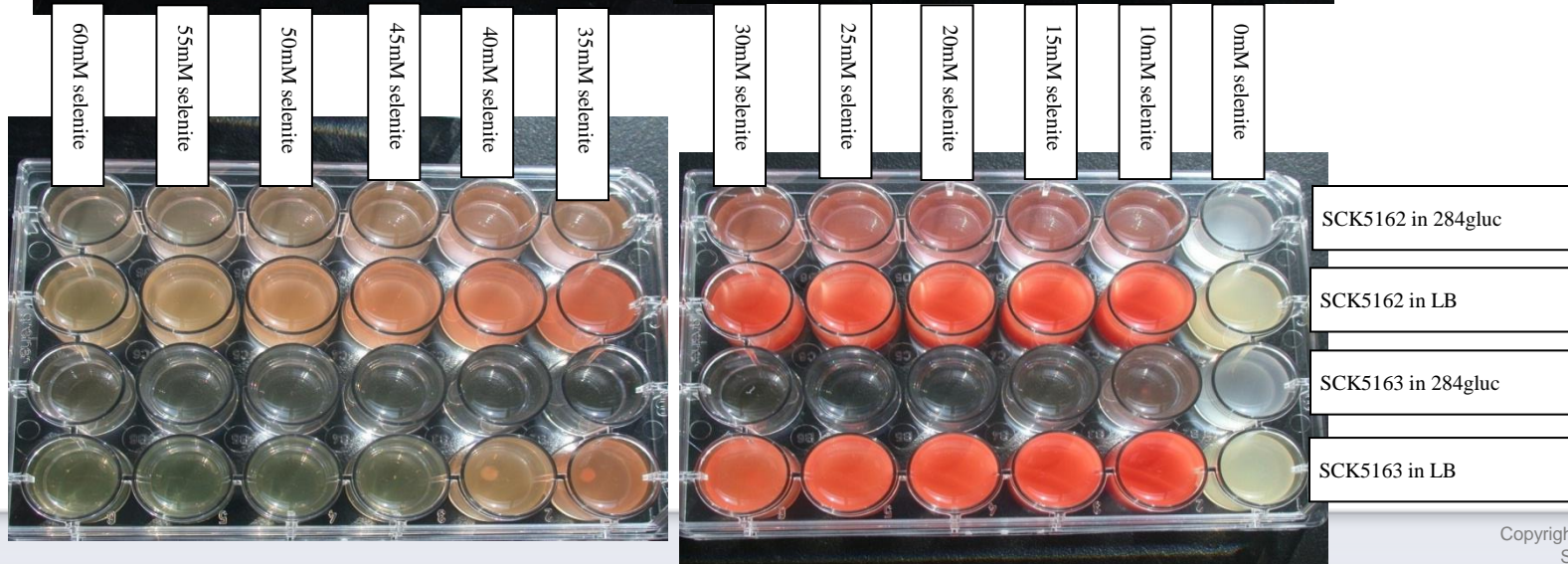
57 <b>La</b> Lanthanum 138.90547	58 <b>Ce</b> Cerium 140.116	59 <b>Pr</b> Praseodymium 140.90765	60 <b>Nd</b> Neodymium 144.242	61 <b>Pm</b> Promethium (145)	62 <b>Sm</b> Samarium 150.36	63 <b>Eu</b> Europium 151.964	64 <b>Gd</b> Gadolinium 157.25	65 <b>Tb</b> Terbium 158.92534	66 <b>Dy</b> Dysprosium 162.500	67 <b>Ho</b> Holmium 164.93032	68 <b>Er</b> Erbium 167.259	69 <b>Tm</b> Thulium 168.93421	70 <b>Yb</b> Ytterbium 173.054	71 <b>Lu</b> Lutetium 174.9668
89 <b>Ac</b> Actinium (227)	90 <b>Th</b> Thorium 232.03806	91 <b>Pa</b> Protactinium 231.03688	92 <b>U</b> Uranium 238.02891	93 <b>Np</b> Neptunium (237)	94 <b>Pu</b> Plutonium (244)	95 <b>Am</b> Americium (243)	96 <b>Cm</b> Curium (247)	97 <b>Bk</b> Berkelium (247)	98 <b>Cf</b> Californium (251)	99 <b>Es</b> Einsteinium (252)	100 <b>Fm</b> Fermium (257)	101 <b>Md</b> Mendelevium (258)	102 <b>No</b> Nobelium (259)	103 <b>Lr</b> Lawrencium (262)

## e.g. Toxicity and Reduction testing

### Selenate

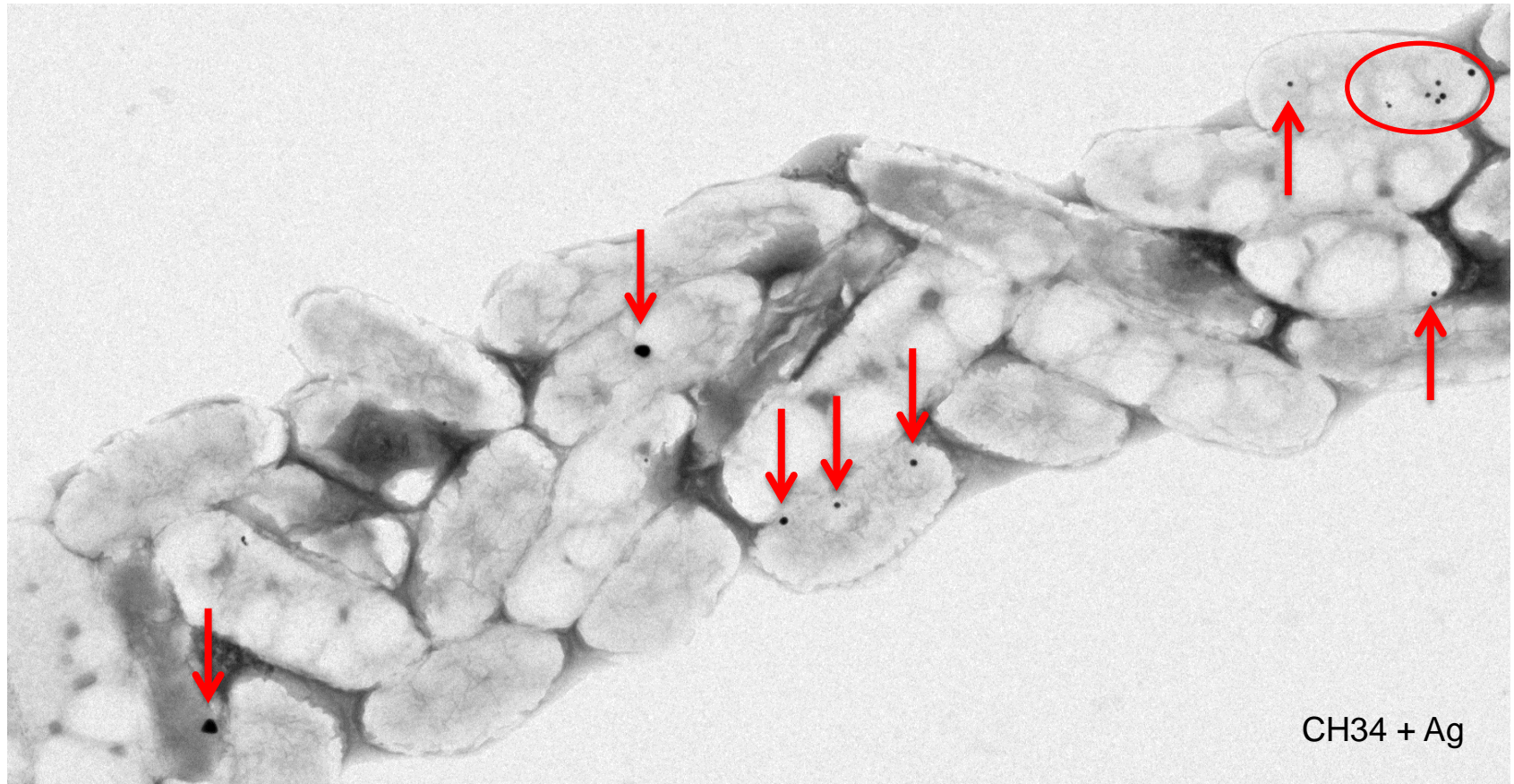


### Selenite





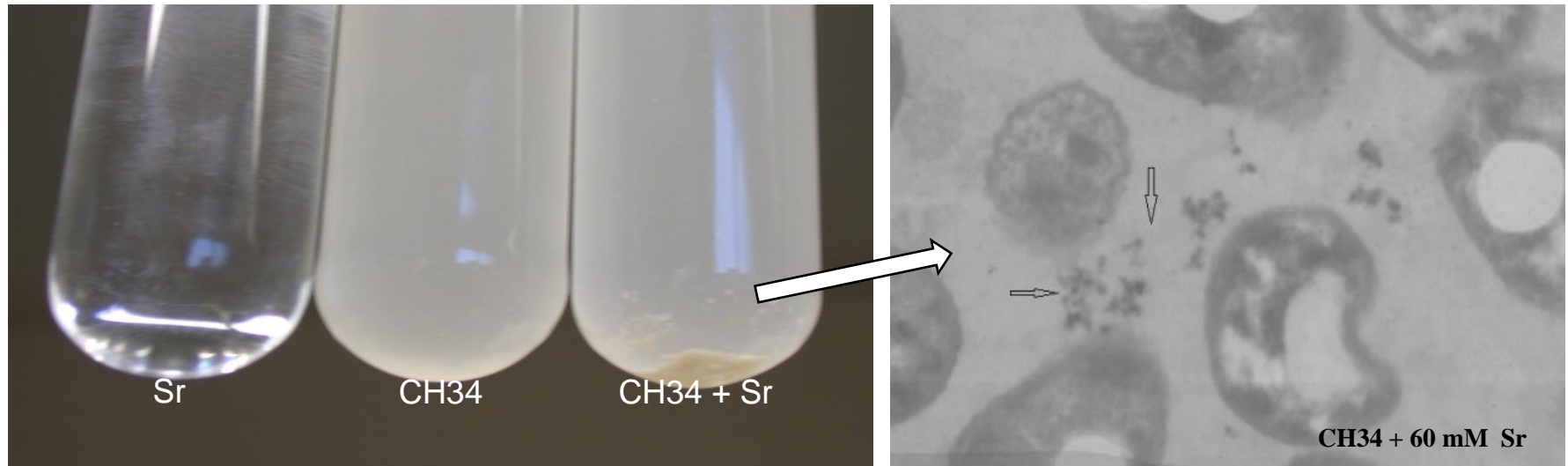
## e.g. Biogenic metal nanoparticles



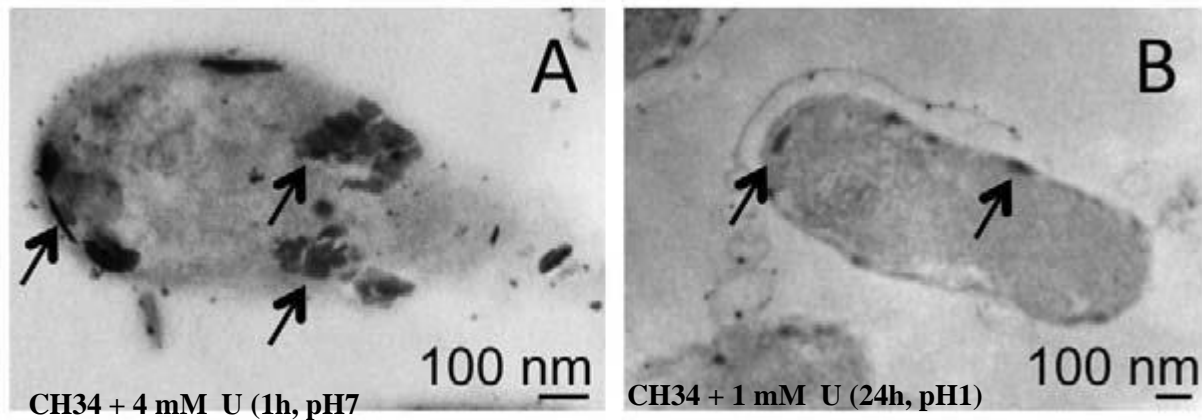
→ silver, paladium, gold, ....

## e.g. Biogenic metal carbonates and phosphates

e.g. strontium carbonate



e.g. uranium phosphate

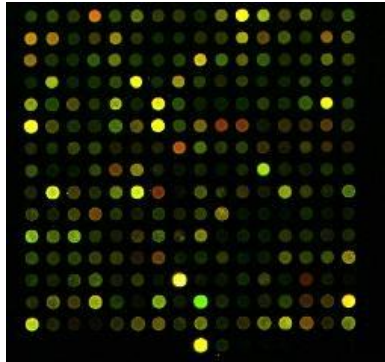


*Llorens et al. 2012*

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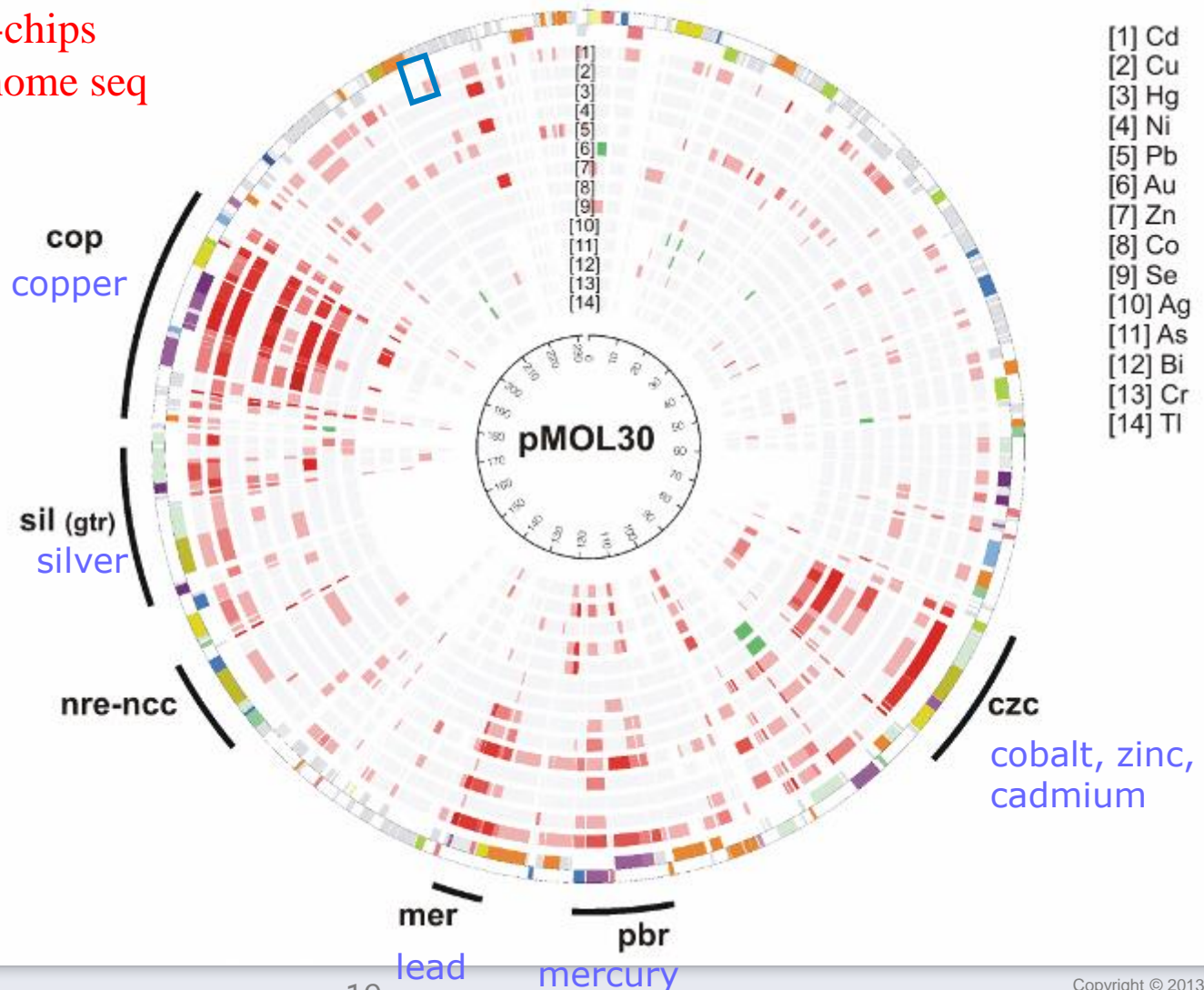


# e.g. Characterising genes and proteins in bacteria involved in the metal processing



DNA-chips  
& genome seq

- Novel **genes & proteins involved** : mechanisms - efflux pumps, reduction, metallophores ...
- **Regulation** : transcription, regulatory RNA, ...
- **Mobility** : MGE - genomic islands, transposons plasmids, IS, ...
- **Bioreporters** : makers, specificity, sensitivity, ...



excavation & installation

exploitation

storage



*contribute to geology & geochemistry & material investigations, to address microbiology aspects in those*

*ex situ laboratory batch and in situ field tests, specific for geomicrobiology*

- *Exploitation of existing In situ facilities & their 'diversity' (e.g. investigate same bioprocess but in different environment)*
- *Multidisciplinary (materials, molecular bio, biochemistry, modeling, ...)*
- *Involvement of young researchers (e.g. PHDs, mobility, training)*
  - *Data comparison*

...

**In Situ Environmental Conditions :  
Restrictions & Opportunities**



We are ready to jump on the new scientific challenges ....

