Biofilm Control For Metal Mobility in ARD

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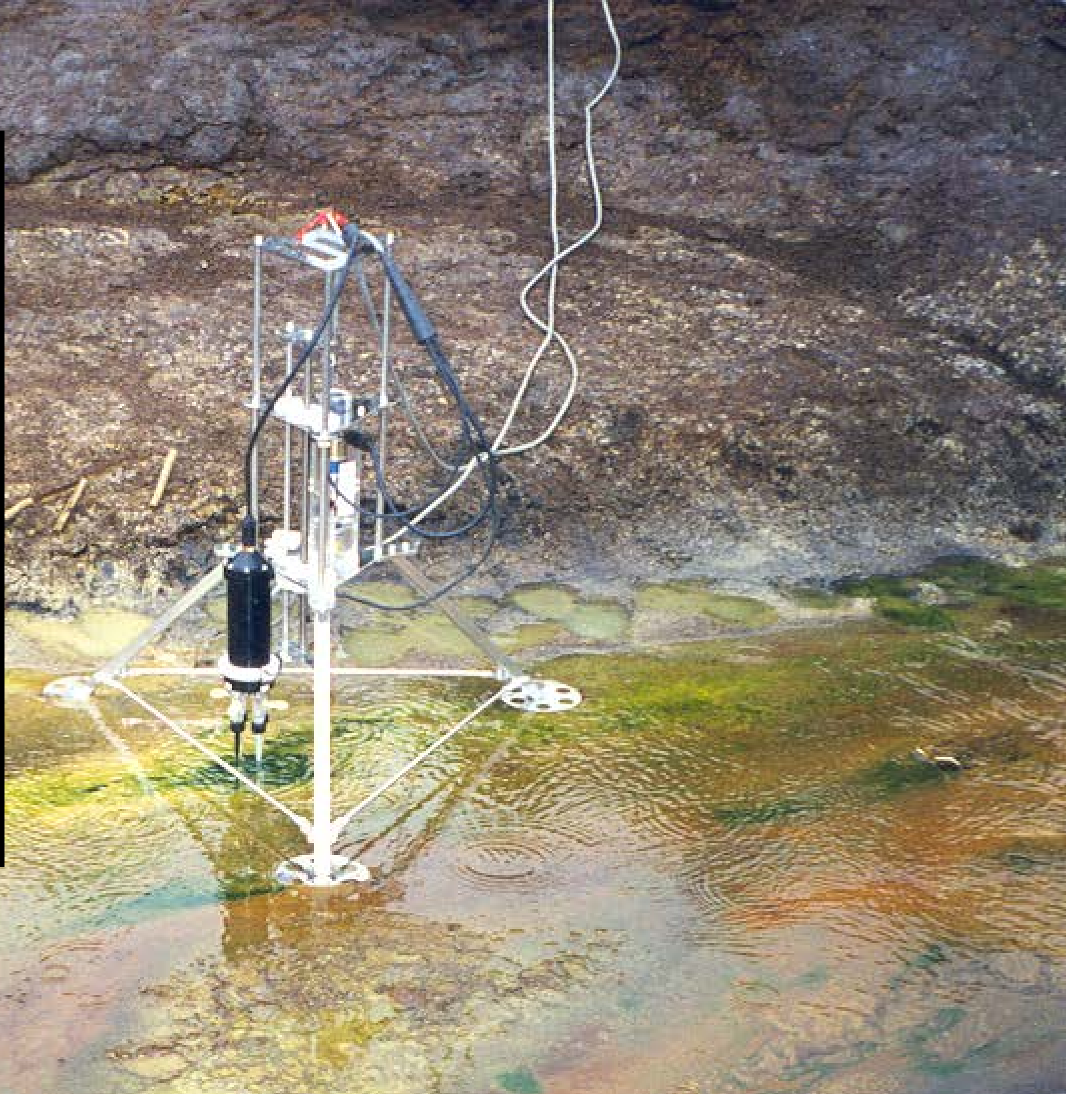
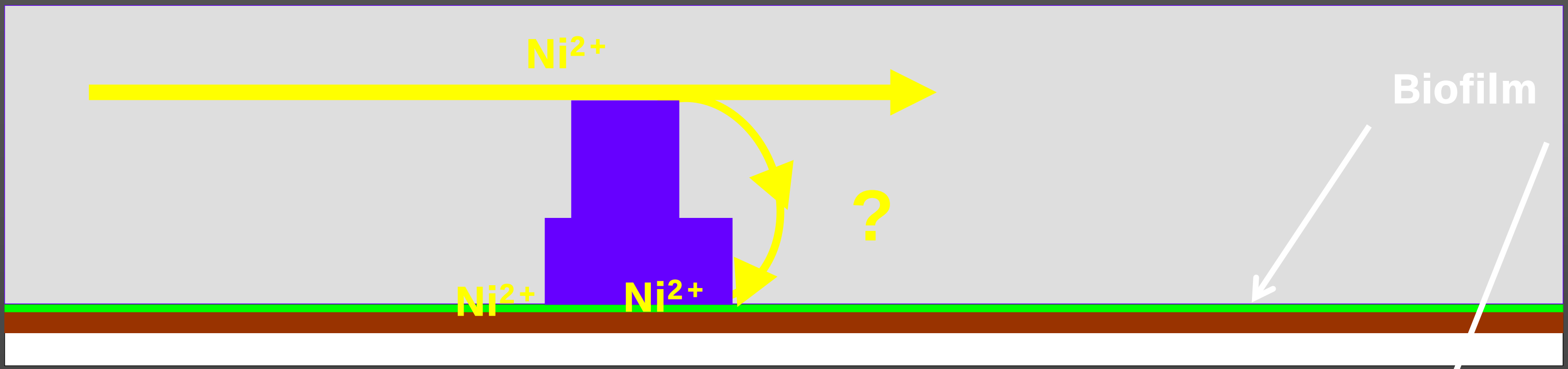
GOAL: Evaluate the potential of biofilm processes for development of bioremediation metal strategies for ARD



HOW and to **what extent** do biofilms affect reactive metal transport?

POTENTIAL OF BIOFILM MICROSCALE PROCESSES TO IMPACT BULK SYSTEM METAL TRANSPORT



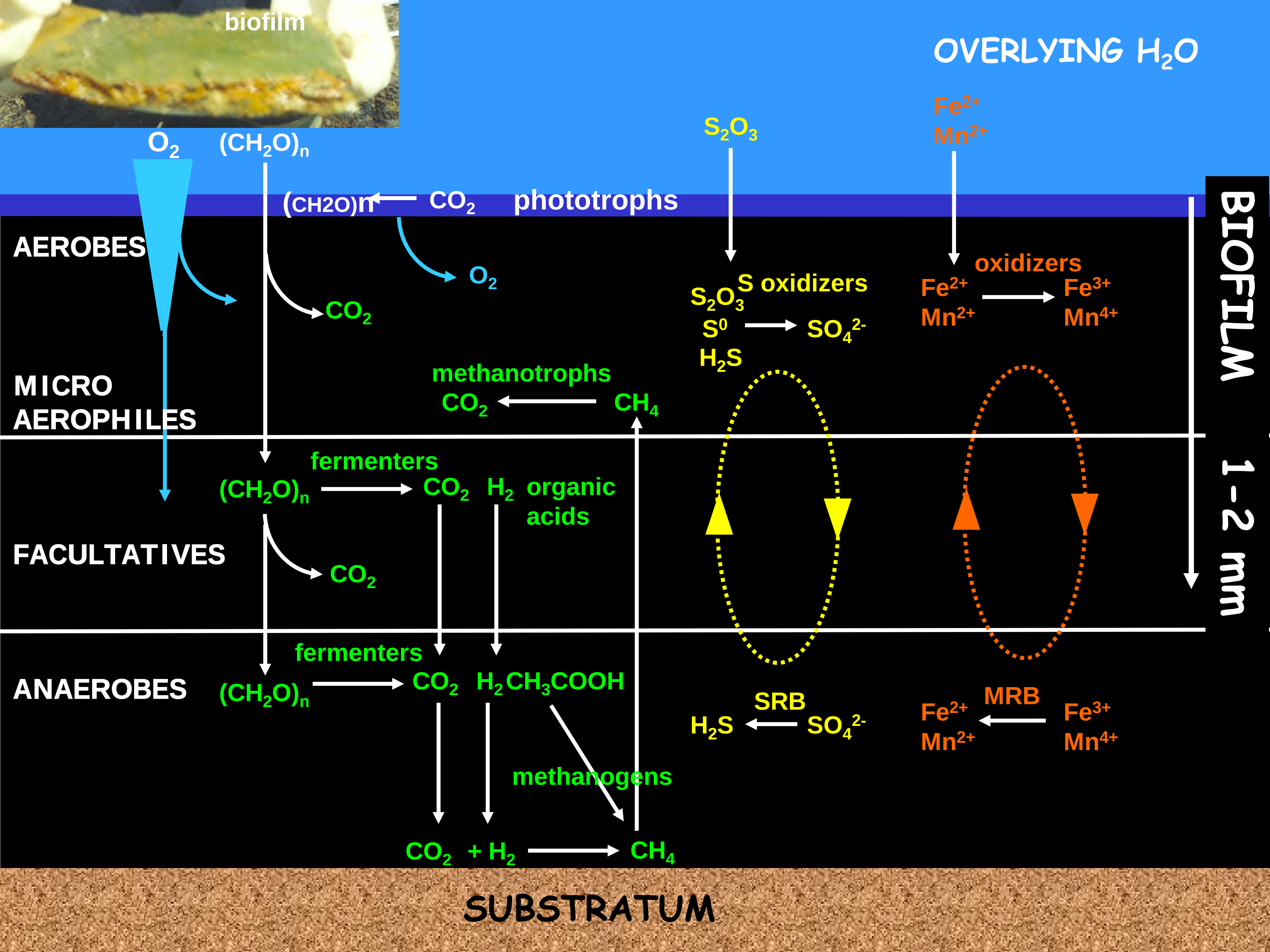
Biofilms:

biologically controlled solids: structure and zonation is metabolically ordered

highly concentrated stratified microbial populations: intense metabolic activity produces **strong geochemical gradients**

internal **geochemical microenvironments** differ substantially from bulk system conditions

biofilms may represent a significant, *in situ*, sequestration mechanism for metals with differing controlling processes from bulk H_2O


biofilm
OVERLYING H2O
O2
(CH2O)n
S2O3
Fe2+
Mn2+
(CH2O)n
CO2
phototrophs
BIOFILM
AEROBES
O2
CO2
S oxidizers
S2O3
S0
H2S
SO42-
oxidizers
Fe2+
Mn2+
Fe3+
Mn4+
methanotrophs
CO2
CH4
MICRO
AEROPHILES
fermenters
(CH2O)n
CO2
H2
organic
acids
1-2 mm
FACULTATIVES
CO2
fermenters
ANAEROBES
(CH2O)n
CO2
H2CH3COOH
SRB
H2S
SO42-
MRB
Fe2+
Mn2+
Fe3+
Mn4+
methanogens
CO2
+ H2
CH4
SUBSTRATUM

BACTERIA metabolic activity METAL BEHAVIOUR

system chemical status

agents of geochemical change (pH,redox)

concentrations & types of minerals

passive / active biomineralization

metal solid-solution partitioning

bacterial surfaces are geochemically reactive

OBJECTIVES

Linking **BACTERIA**

- **evaluate** metal retention by ARD biofilms
- **identify** key biofilm solids for metal sequestration
- **characterize** microbial – geochemical linkages involved in metal retention

REACTIVE METAL TRANSPORT

MECHANISTIC APPROACH:

Characterize microbial functional zonation

microbial community structure using molecular RNA probes

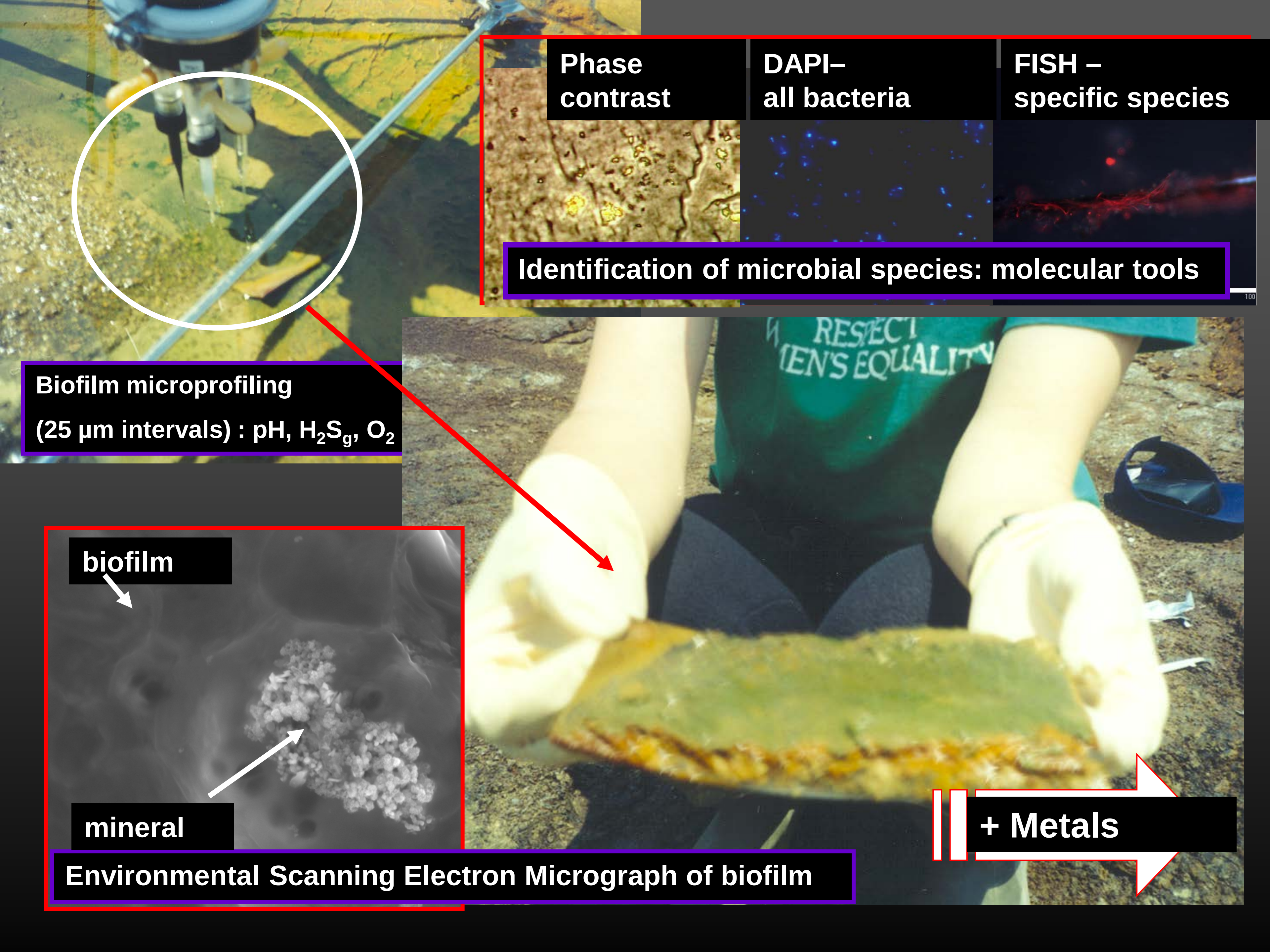
Link to geochemical gradients

microprofiling of internal geochemical gradients

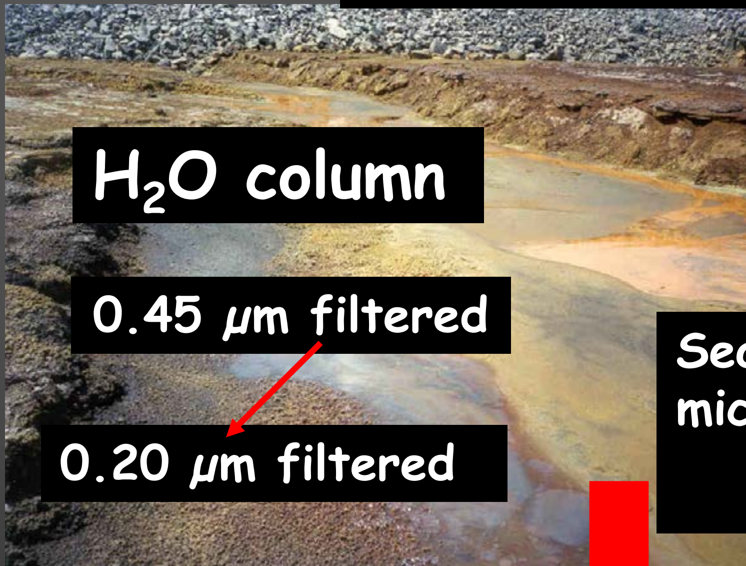
and metal partitioning

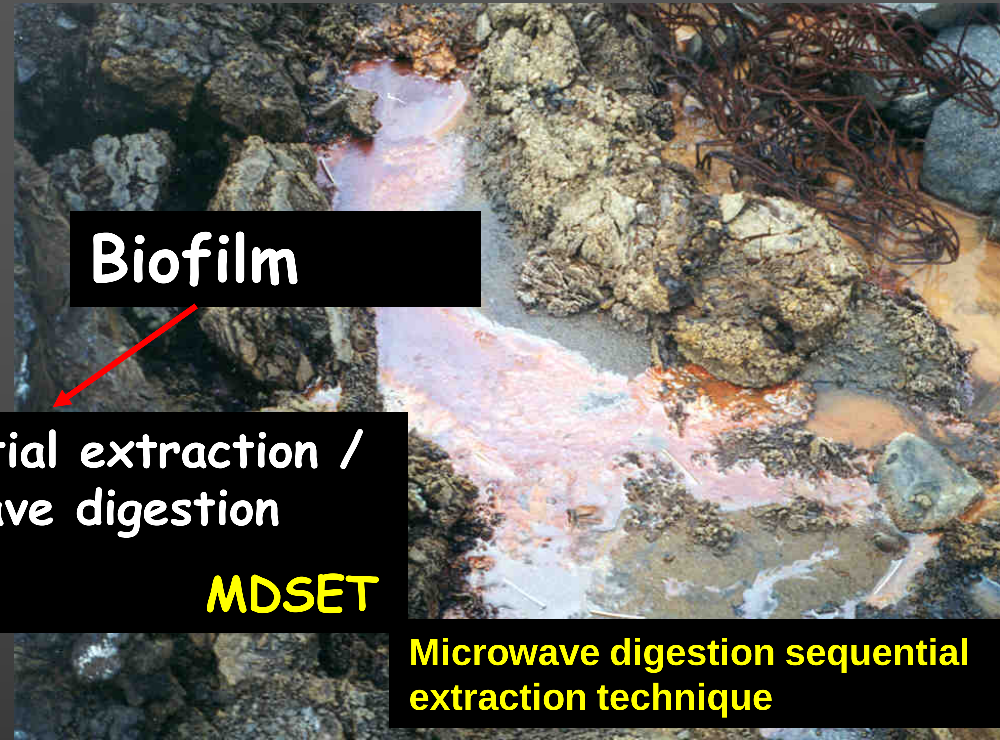
identification of key solids (MDSET); microscopy - ESEM/SEM/TEM + EDS; mineralogy XRD

IDENTIFY key microbial processes controlling metal sequestration


Phase contrast
DAPI– all bacteria
FISH – specific species
Identification of microbial species: molecular tools
Biofilm microprofiling
(25 µm intervals) : pH, H_2S_g, O_2
biofilm
mineral
Environmental Scanning Electron Micrograph of biofilm
+ Metals

METALS





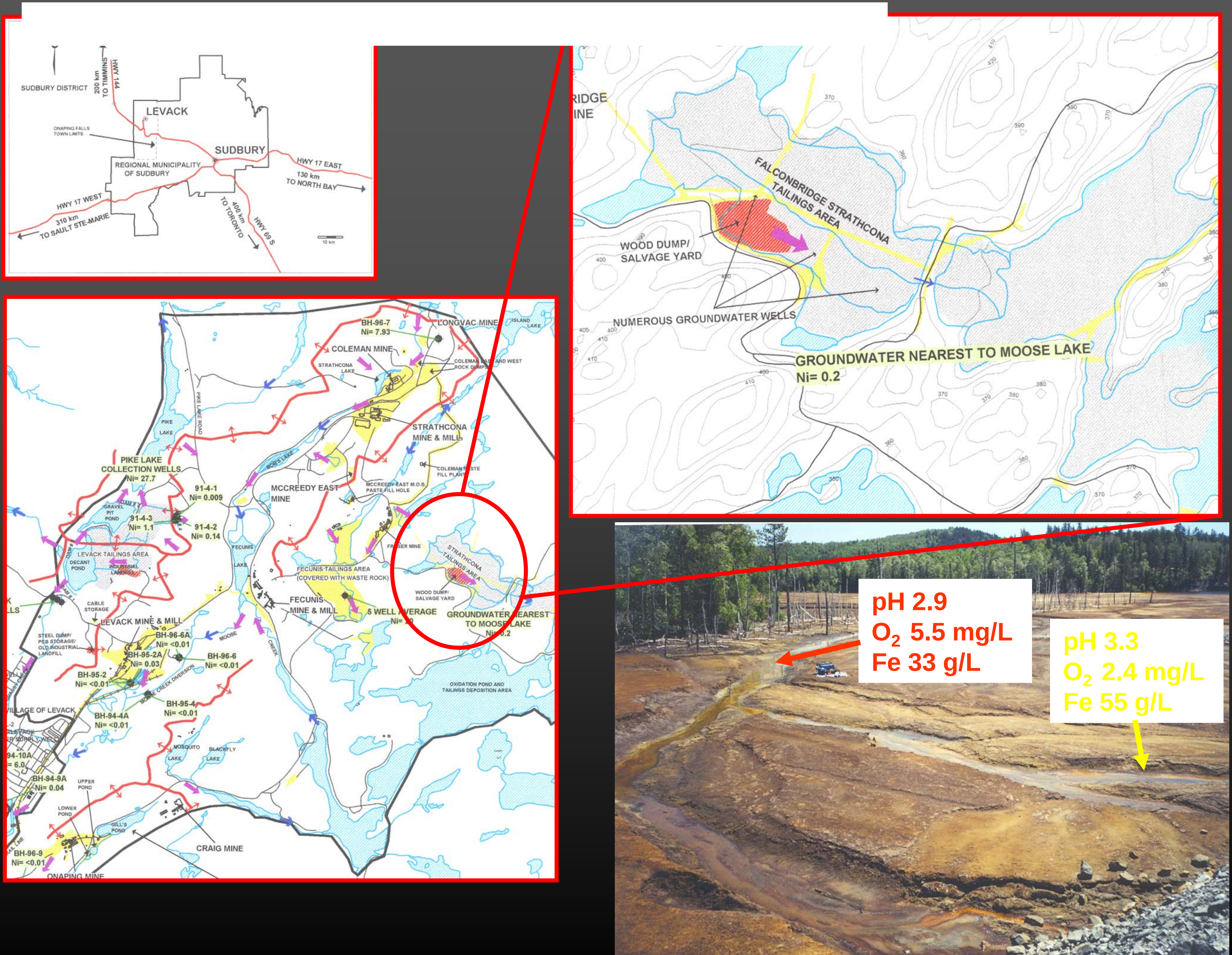
Analyses -> ICP-MS

TAILINGS-BIOFILM MINERALOGY (XRD)
FALCONBRIDGE TECHNOLOGY CENTRE

Biofilm Sample	Main	Minor	Trace (>5%)
31-05, 15:00	Jarosite, quartz, albite	Clinochlore, biotite, actinolite	Gypsum
21-06, 15:00	Quartz, albite, gypsum	Jarosite, clinochlorite, biotite	Actinolite

1. **Exchangeable**
2. **Carbonates**
3. **Amorphous Oxyhydroxides**
4. **Crystalline Oxyhydroxides**
5. **Organics/Sulfides**
6. **Residual**

Haack & Warren, *Env. Sci. Technol* submitted



SUDBURY DISTRICT
200 km TO TIMMINS
HWY 144
LEVACK
ONAPING FALLS TOWN LIMITS
SUDBURY
REGIONAL MUNICIPALITY OF SUDBURY
HWY 17 EAST
130 km TO NORTH BAY
HWY 17 WEST
310 km TO SAULT STE-MARIE
400 km TO TORONTO
HWY 69 S
10 km
BH-96-7
Ni= 7.93
LONGVAC MINE
ISLAND LAKE
COLEMAN MINE
STRATHCONA LAKE
COLEMAN EAST AND WEST ROCK DUMPS
PIKE LAKE
PIKE LAKE ROAD
STRATHCONA MINE & MILL
PIKE LAKE COLLECTION WELLS
Ni= 27.7
BOB'S LAKE
COLEMAN PASTE FILL PLANT
MCCREEDY EAST MINE
MCCREEDY EAST M.O.B. PASTE FILL HOLE
91-4-1
Ni= 0.009
GRAVEL PIT POND
91-4-3
Ni= 1.1
91-4-2
Ni= 0.14
FECUNIS LAKE
FRASER MINE
STRATHCONA TAILINGS AREA
LEVACK TAILINGS AREA
DECANT POND
INDUSTRIAL LANDFILL
FECUNIS TAILINGS AREA (COVERED WITH WASTE ROCK)
WOOD DUMP/ SALVAGE YARD
FECUNIS MINE & MILL
5 WELL AVERAGE
GROUNDWATER NEAREST TO MOOSE LAKE
Ni= 0.2
CABLE STORAGE
LEVACK MINE & MILL
STEEL DUMP/ PCB STORAGE/ OLD INDUSTRIAL LANDFILL
BH-96-6A
Ni= <0.01
BH-95-2A
Ni= 0.03
BH-96-6
Ni= <0.01
MOOSE CREEK
BH-95-2
Ni= <0.01
MOOSE CREEK DIVERSION
OXIDATION POND AND TAILINGS DEPOSITION AREA
VILLAGE OF LEVACK
BH-94-4A
Ni= <0.01
BH-95-4
Ni= <0.01
MOSQUITO LAKE
BLACKFLY LAKE
BH-94-9A
Ni= 0.04
UPPER POND
LOWER POND
GILL'S POND
CRAIG MINE
BH-96-9
Ni= <0.01
ONAPING MINE
FALCONBRIDGE STRATHCONA TAILINGS AREA
WOOD DUMP/ SALVAGE YARD
NUMEROUS GROUNDWATER WELLS
GROUNDWATER NEAREST TO MOOSE LAKE
Ni= 0.2
pH 2.9
O2 5.5 mg/L
Fe 33 g/L
pH 3.3
O2 2.4 mg/L
Fe 55 g/L

METAL TRANSPORT VS. RETENTION

H_2O Metal (0.2 µm)

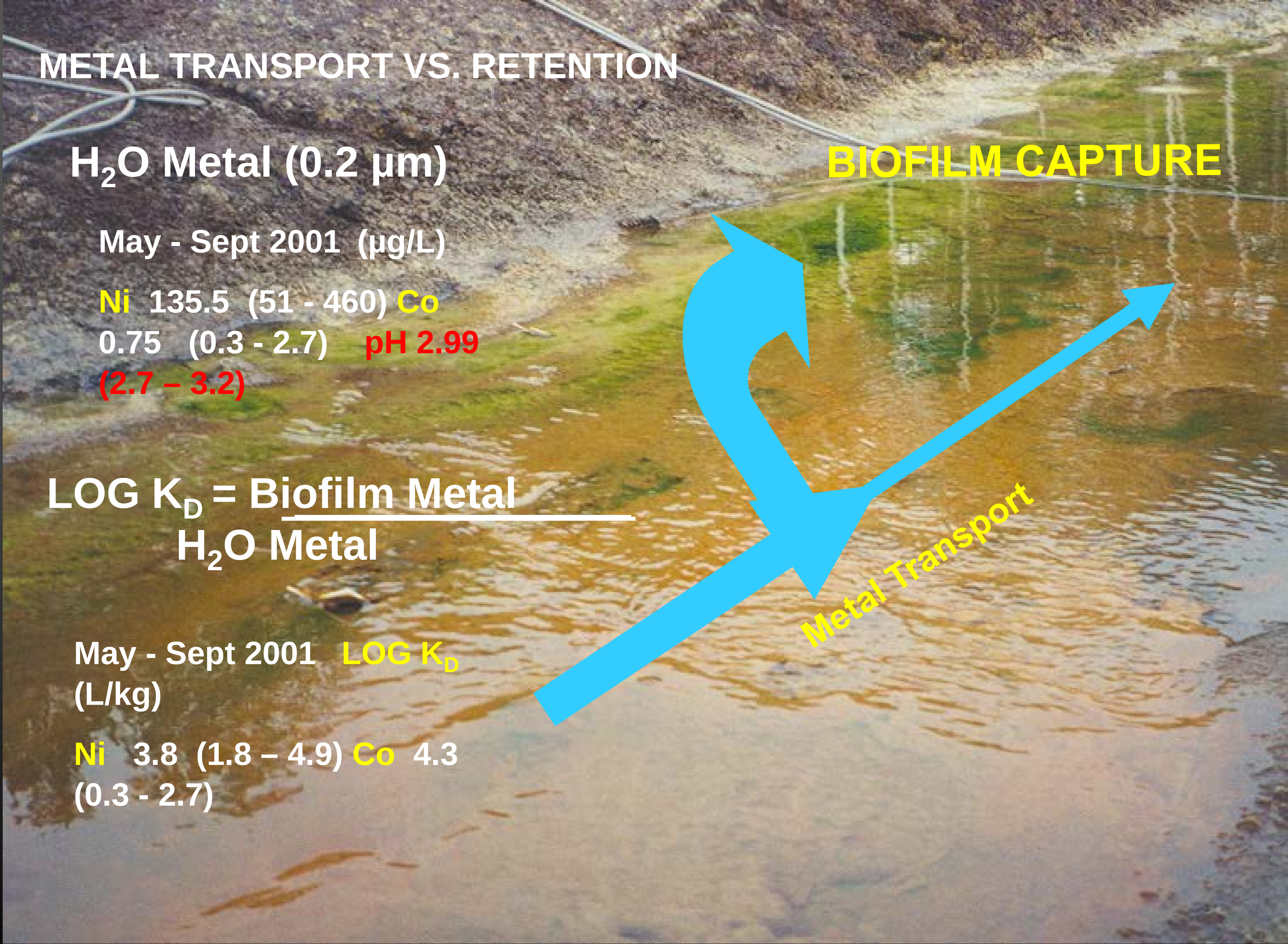
May - Sept 2001 (µg/L)

Ni 135.5 (51 - 460) Co 0.75 (0.3 - 2.7) pH 2.99 (2.7 – 3.2)

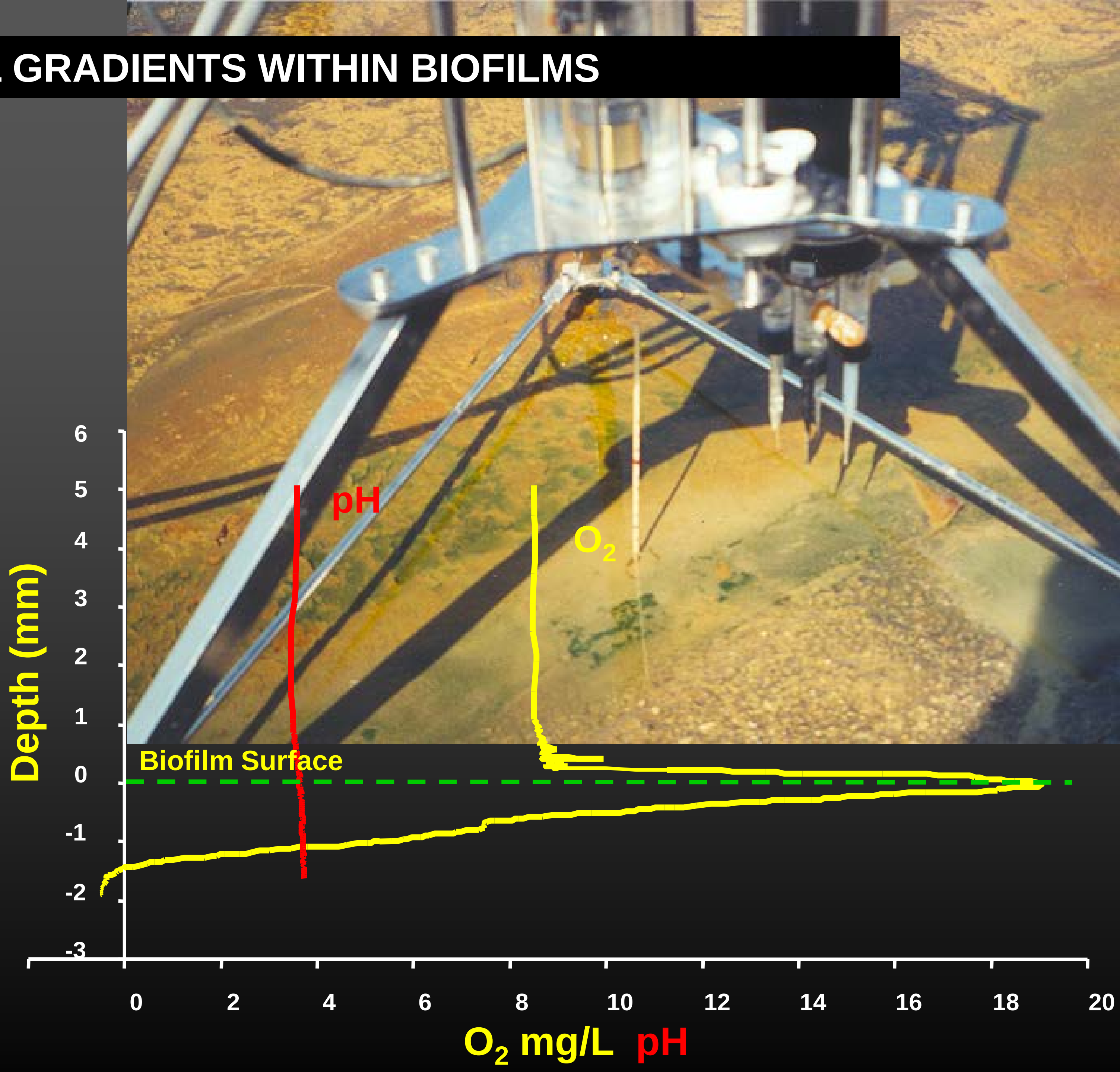
$$\text{LOG } K_D = \frac{\text{Biofilm Metal}}{H_2O \text{ Metal}}$$

May - Sept 2001 LOG K_D (L/kg)

Ni 3.8 (1.8 – 4.9) Co 4.3 (0.3 - 2.7)



GEOCHEMICAL GRADIENTS WITHIN BIOFILMS

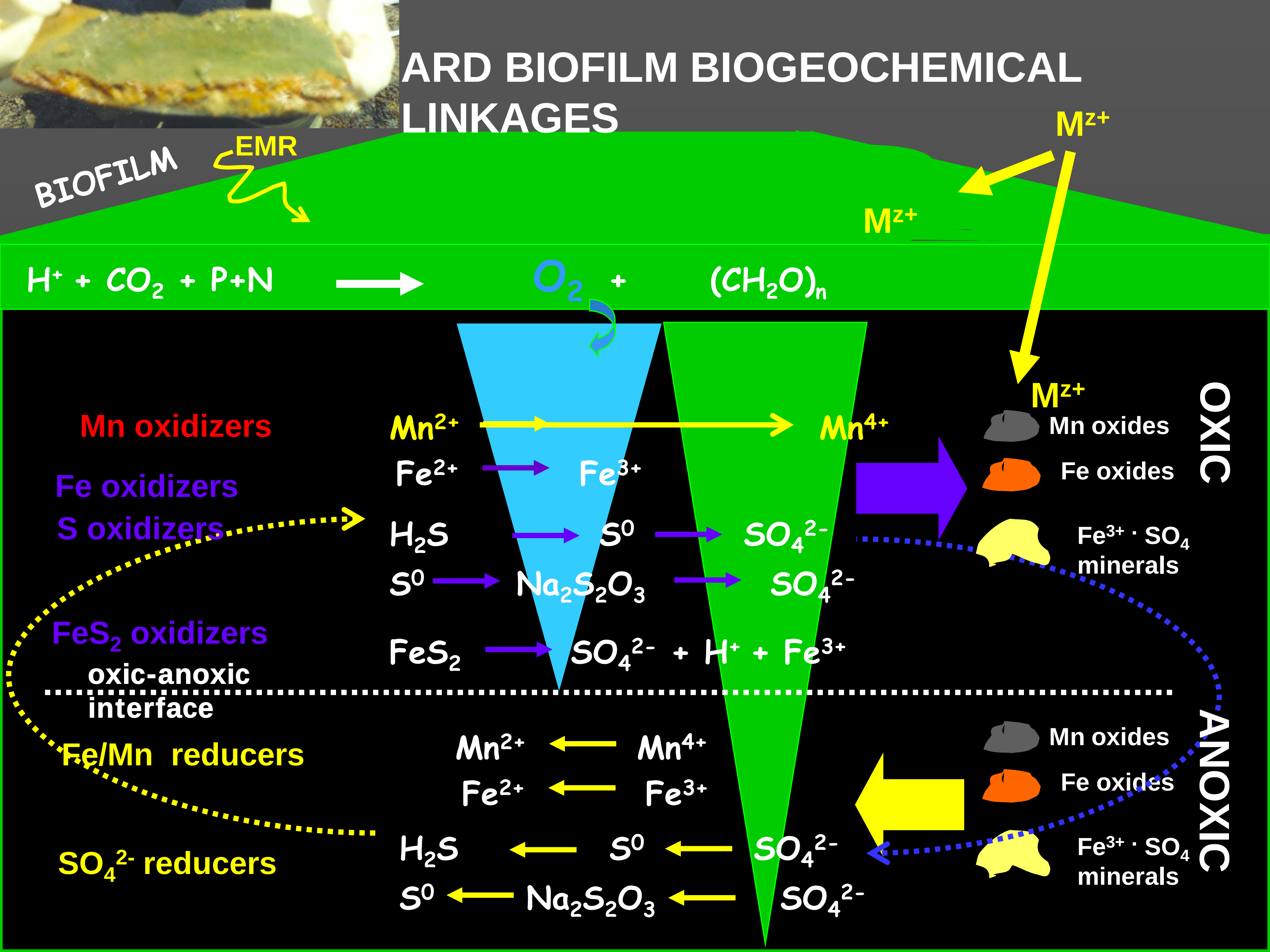


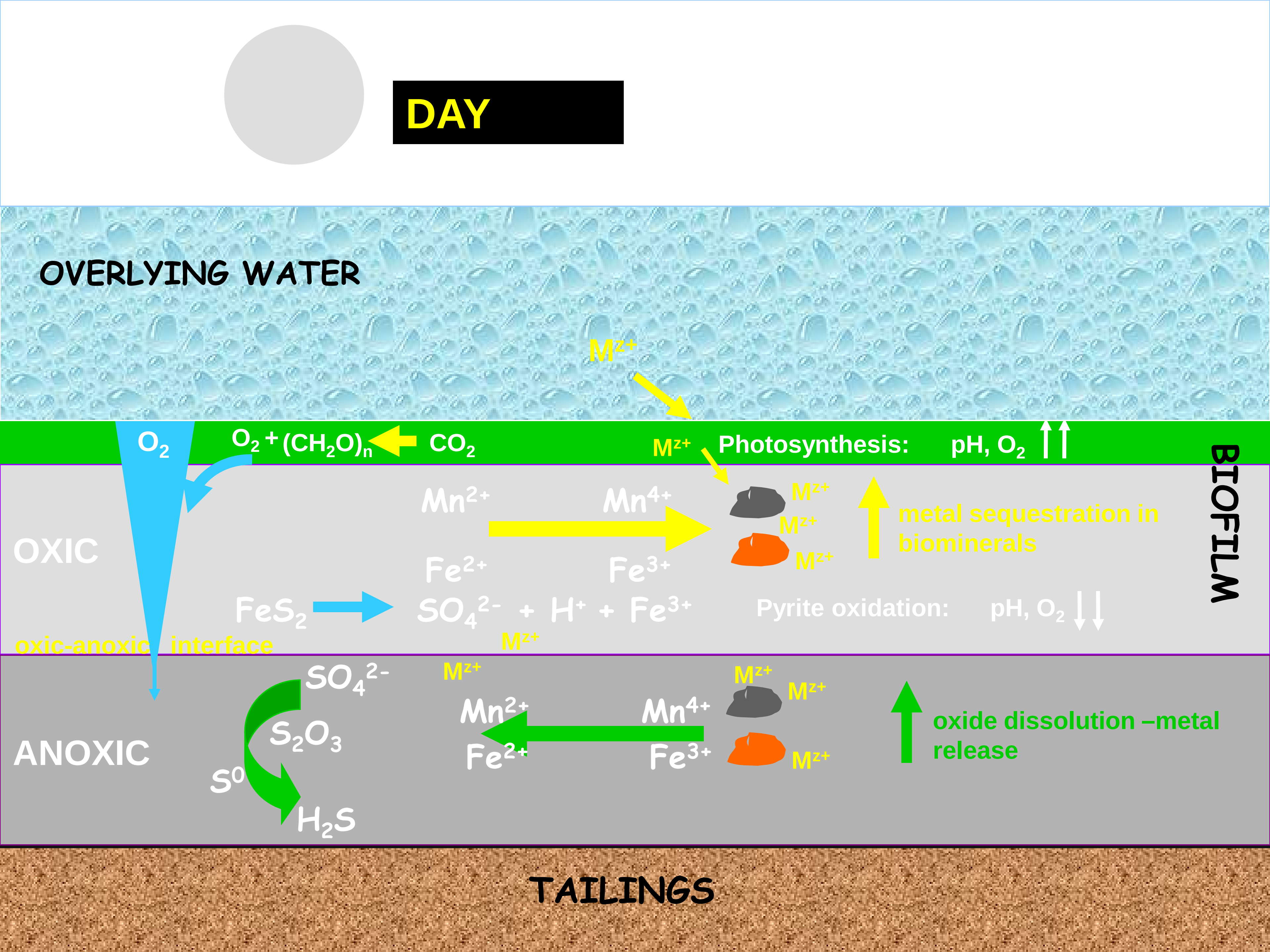
METAL PARTITIONING AMONG BIOFILM SOLIDS

TOTAL [M^{z+}] (µmol/g)	Fe (100)	Mn (8.66)	Ni (200)	Co (1.03)	Cu (1800)
% Exchangeable % Carbonates			<10%		
% Amorphous Oxide *Mn*	78-93	44-48	8-37	7-35	35-73
% Crystalline Oxide	2.5-3.5				
% Organic / Sulfide			41-88	58-88	7-54
% Residual	3.5-4.5	27-47			

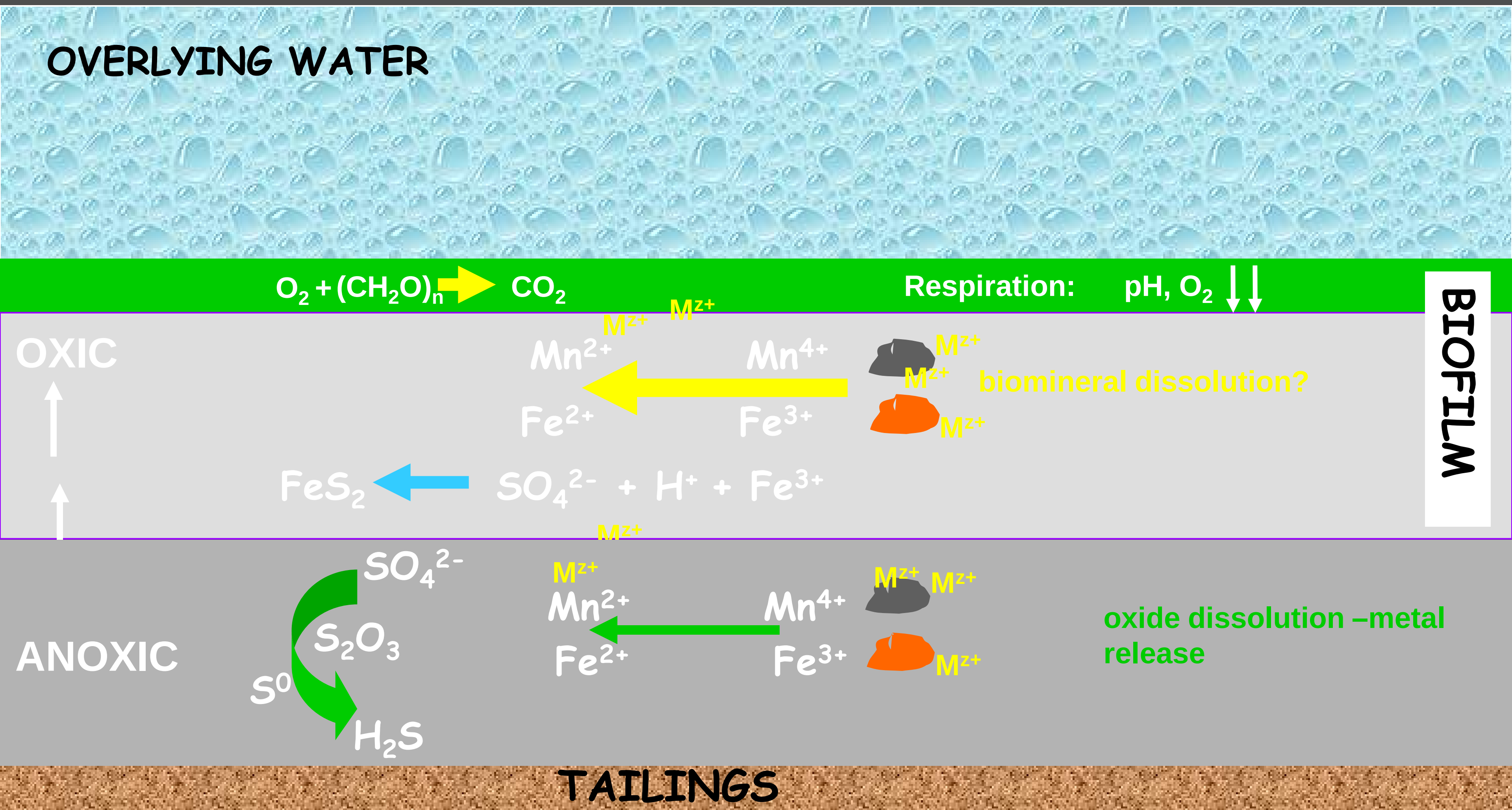
biofilms ARE significant metal sequestrators 1. Biominerals of Mn oxyhydroxides

2. organic matrix of biofilm itself

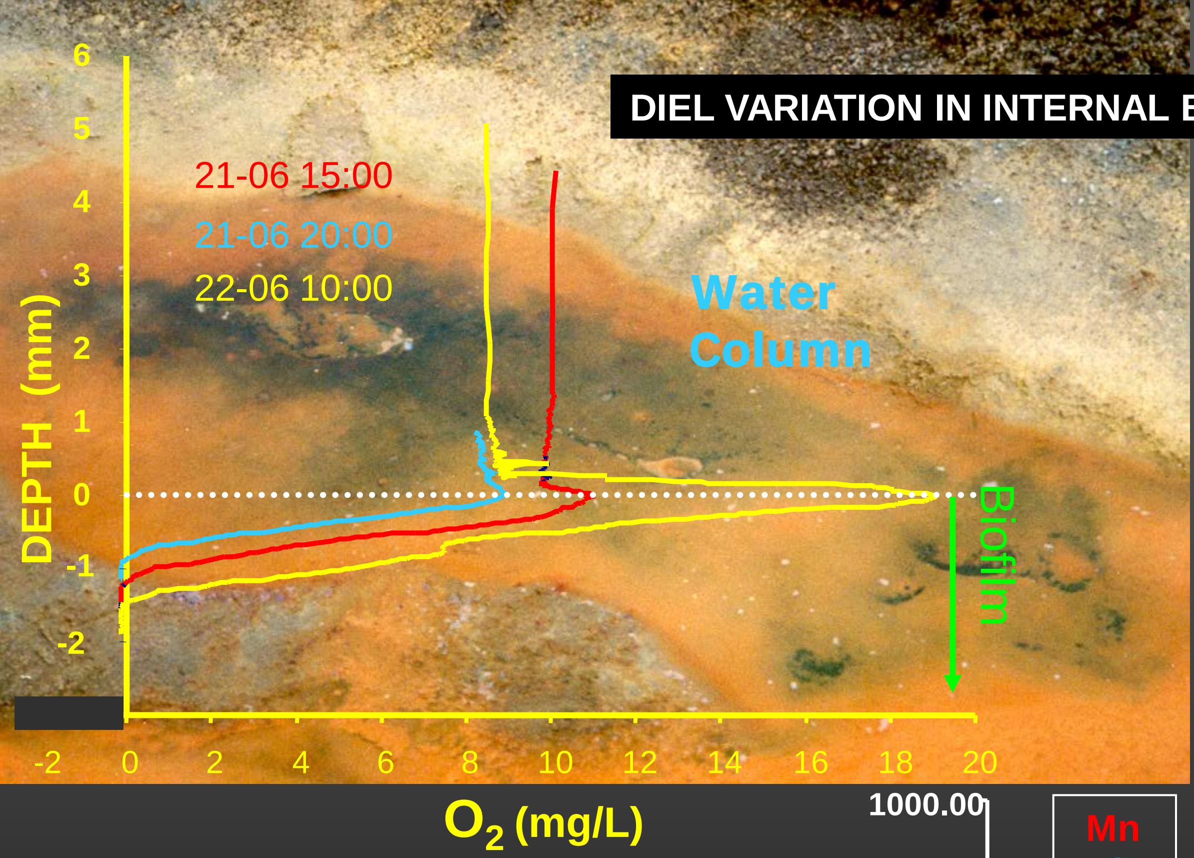

ARD BIOFILM BIOGEOCHEMICAL LINKAGES
BIOFILM
EMR
M^{z+}
M^{z+}
$H^+ + CO_2 + P+N \rightarrow O_2 + (CH_2O)_n$
M^{z+}
Mn oxidizers
Fe oxidizers
S oxidizers
FeS_2 oxidizers
$Mn^{2+} \rightarrow Mn^{4+}$
$Fe^{2+} \rightarrow Fe^{3+}$
$H_2S \rightarrow S^0 \rightarrow SO_4^{2-}$
$S^0 \rightarrow Na_2S_2O_3 \rightarrow SO_4^{2-}$
$FeS_2 \rightarrow SO_4^{2-} + H^+ + Fe^{3+}$
Mn oxides
Fe oxides
$Fe^{3+} \cdot SO_4$ minerals
OXIC
oxic-anoxic interface
Fe/Mn reducers
SO_4^{2-} reducers
$Mn^{2+} \leftarrow Mn^{4+}$
$Fe^{2+} \leftarrow Fe^{3+}$
$H_2S \leftarrow S^0 \leftarrow SO_4^{2-}$
$S^0 \leftarrow Na_2S_2O_3 \leftarrow SO_4^{2-}$
Mn oxides
Fe oxides
$Fe^{3+} \cdot SO_4$ minerals
ANOXIC


DAY
OVERLYING WATER
M^{z+}
O_2
$O_2 + (CH_2O)_n$
CO_2
M^{z+}
Photosynthesis: pH, O_2
BIOFILM
OXIC
Mn^{2+}
Mn^{4+}
M^{z+}
M^{z+}
M^{z+}
metal sequestration in biominerals
Fe^{2+}
Fe^{3+}
FeS_2
$SO_4^{2-} + H^+ + Fe^{3+}$
Pyrite oxidation: pH, O_2
M^{z+}
oxic-anoxic interface
SO_4^{2-}
M^{z+}
M^{z+}
M^{z+}
S_2O_3
Mn^{2+}
Mn^{4+}
ANOXIC
Fe^{2+}
Fe^{3+}
M^{z+}
oxide dissolution –metal release
S^0
H_2S
TAILINGS

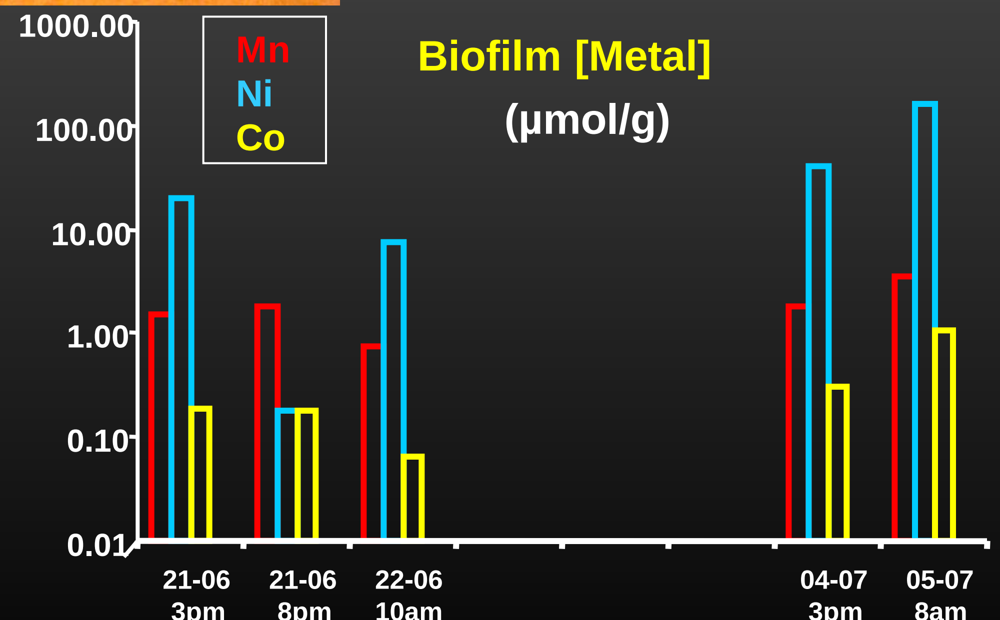
NIGHT


OVERLYING WATER
$O_2 + (CH_2O)_n$ → CO_2
Respiration: pH, O_2 ↓↓
BIOFILM
OXIC
M^{z+}
Mn^{2+} Mn^{4+}
Fe^{2+} Fe^{3+}
biomineral dissolution?
FeS_2 ← SO_4^{2-} + H^+ + Fe^{3+}
ANOXIC
SO_4^{2-}
S_2O_3
S^0
H_2S
Mn^{2+} Mn^{4+}
Fe^{2+} Fe^{3+}
oxide dissolution –metal release
TAILINGS

DIEL VARIATION IN INTERNAL BIOFILM GRADIENTS



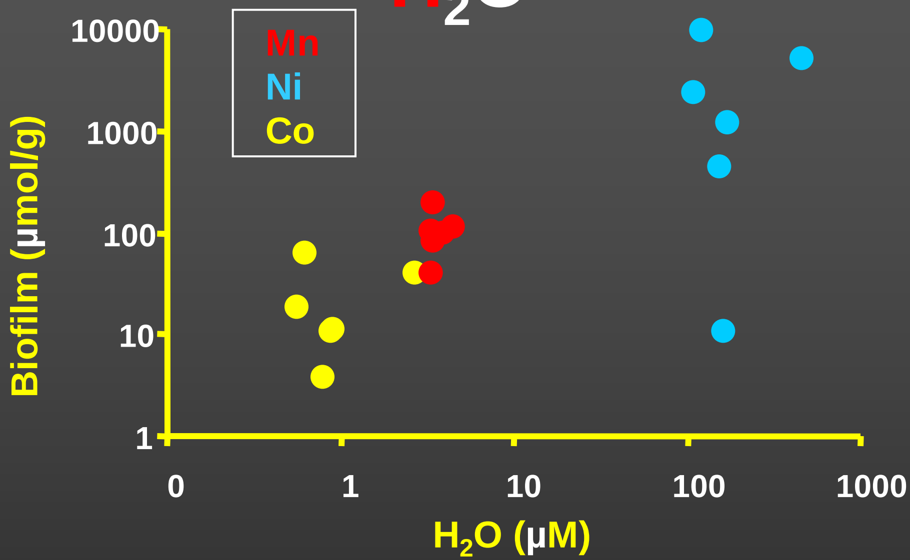
DIEL VARIATIONS IN METAL CONCENTRATIONS

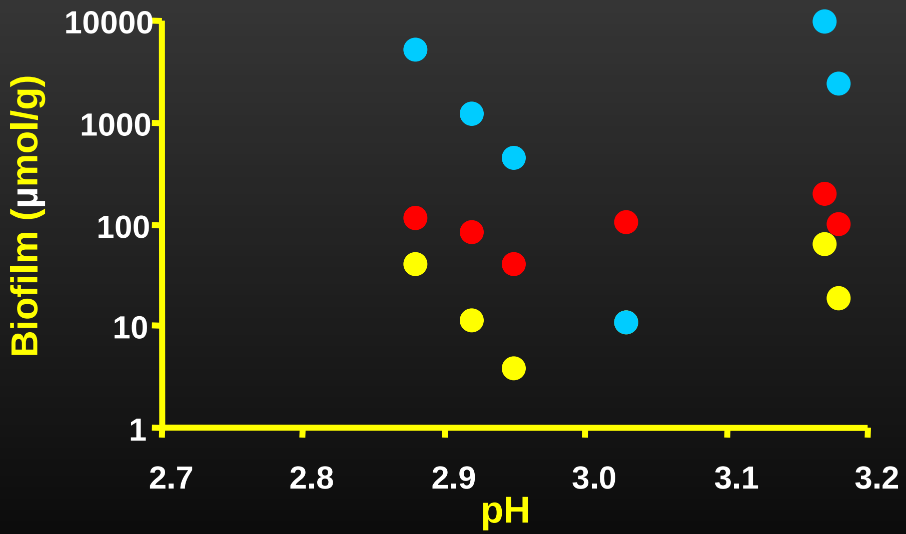


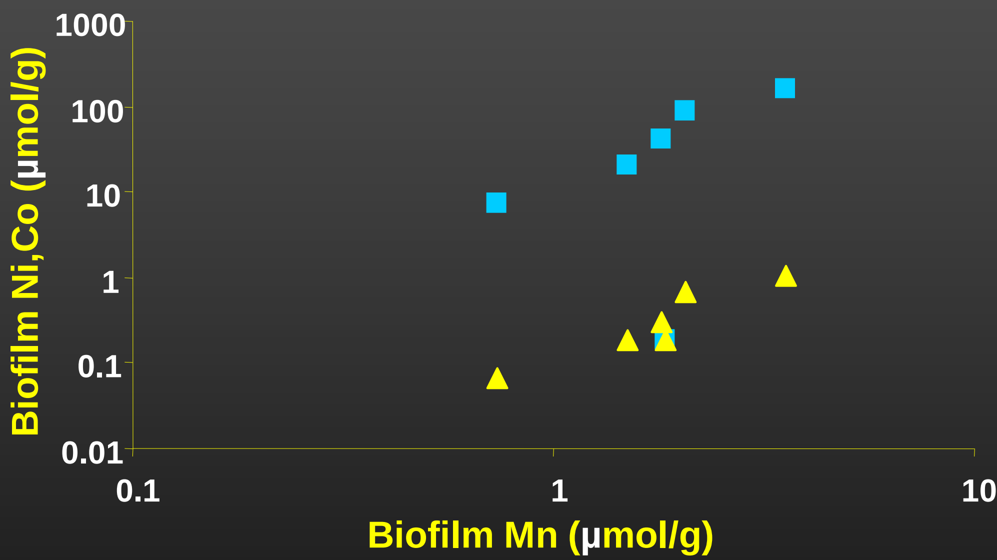
Biofilm vs. control ?

METAL BEHAVIOUR

H_2O

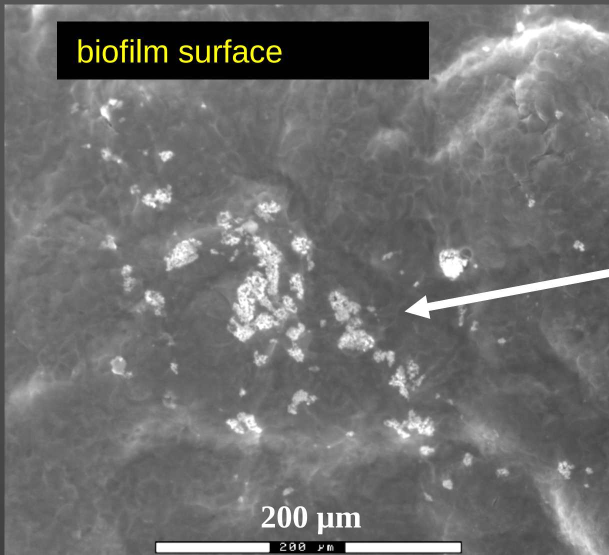






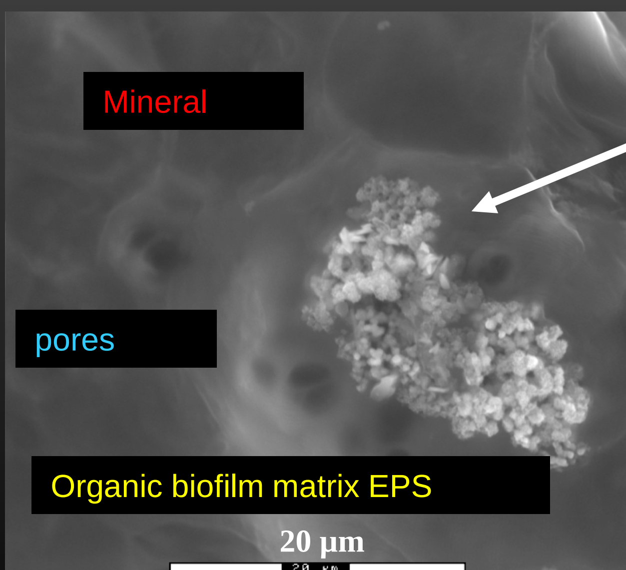
ESEM-EDS

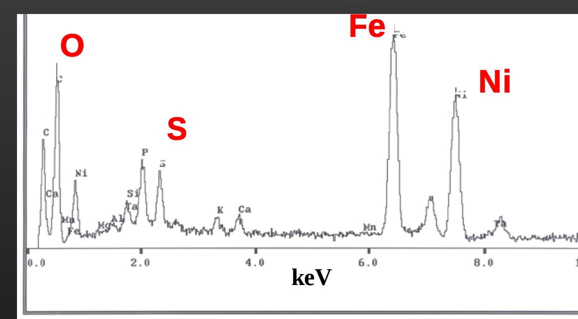
BIOGEOCHEMICAL BIOFILM ARCHITECTURE



Microtopography of biofilm surface indicates distinct zones of mineralization

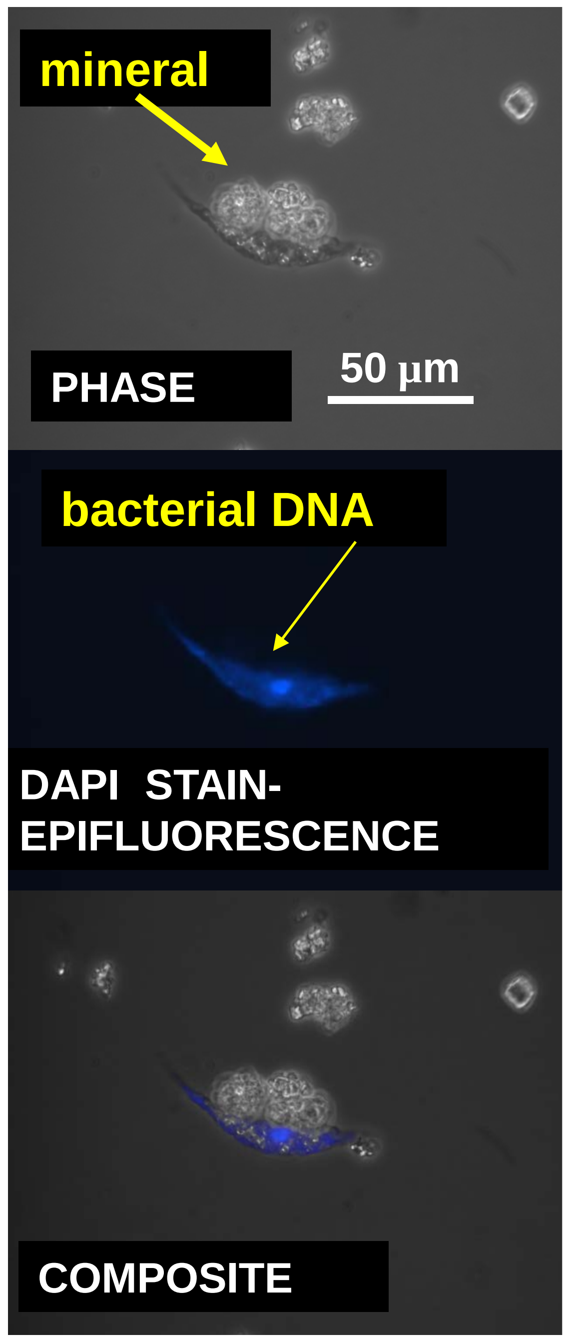
Amorphous finegrained minerals evident at biofilm surface (Ni, S, Fe –EDS)

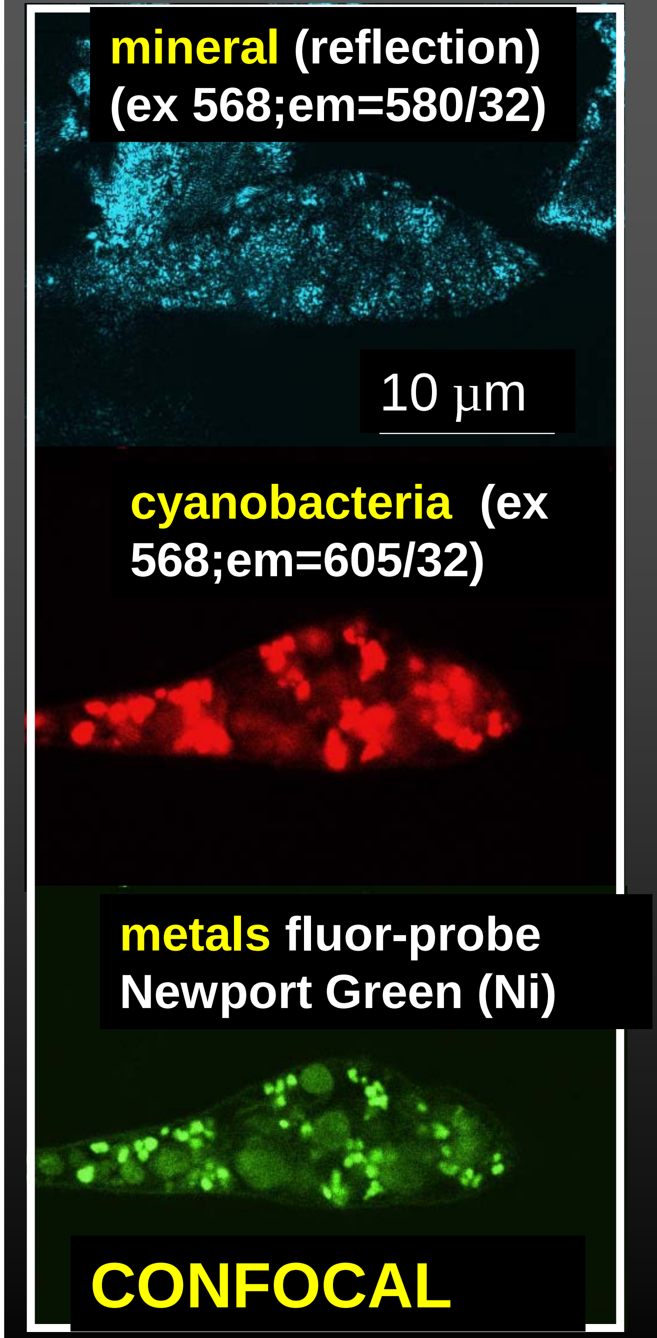




High Ni associations found in pores and in bacterial cells

MICROSCOPY: BACTERIAL – MINERAL – METAL ASSOCIATIONS





Bacterial – mineral assemblages occur within biofilm structure: bacterial pods

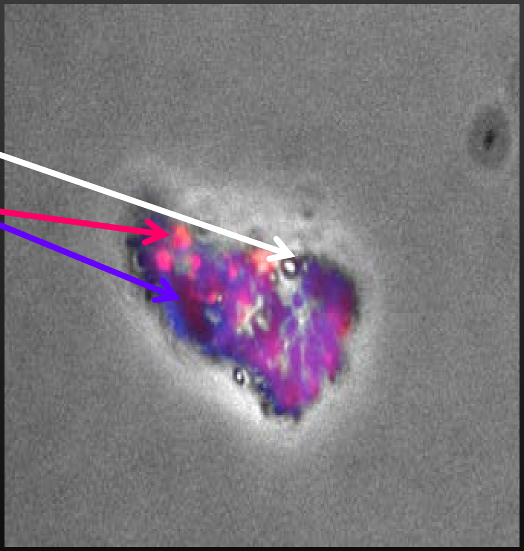
Extremely fine grained minerals within bacterial pods occur

Metals are associated specifically with bacterial cells and fine grained minerals

Molecular probes to identify bacterial groups (i.e. likely function) within biofilm pods: Fluorescence In Situ Hybridization FISH - using 16S RNA oligonucleotide probes

DAPI: all bacterial DNA
gamma proteobacteria RNA

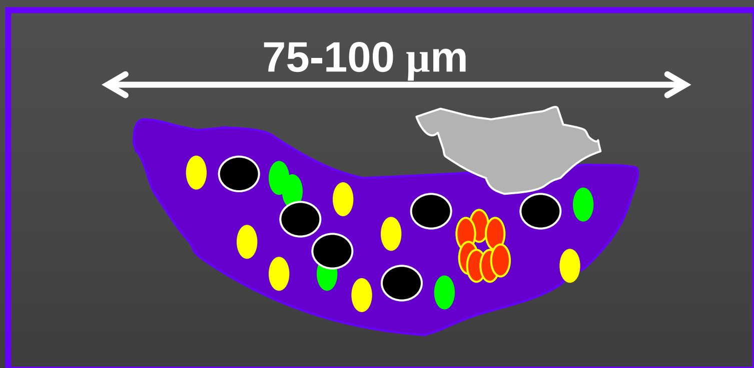
Composite: minerals
DAPI (all bacterial DNA)
gamma proteobacteria



Microbial geochemical architecture of biofilms: relevance to metal behaviour

Bacterial Pods

microbial consortia within a sheath structure



phototrophic cyanobacteria

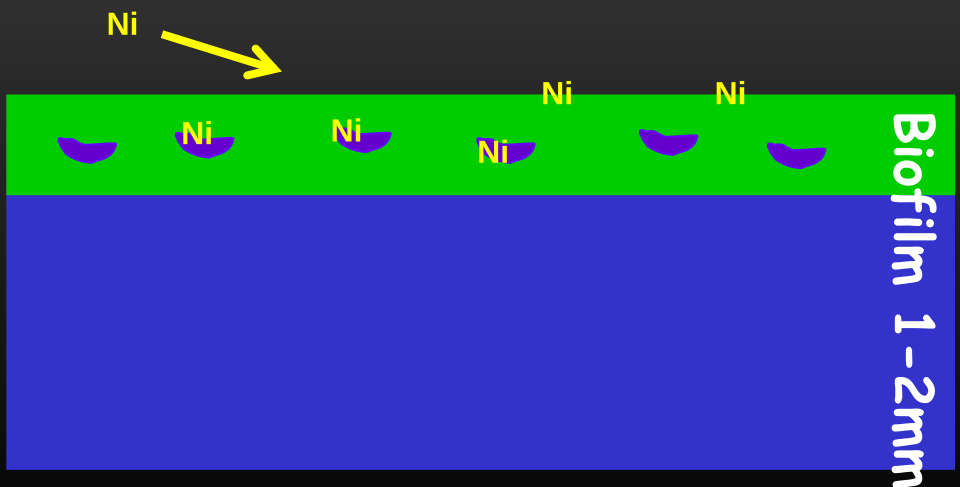
S oxidizers (gamma proteo-)

Fe/Mn oxidizers (beta proteo-)

associated interior biominerals and exterior minerals

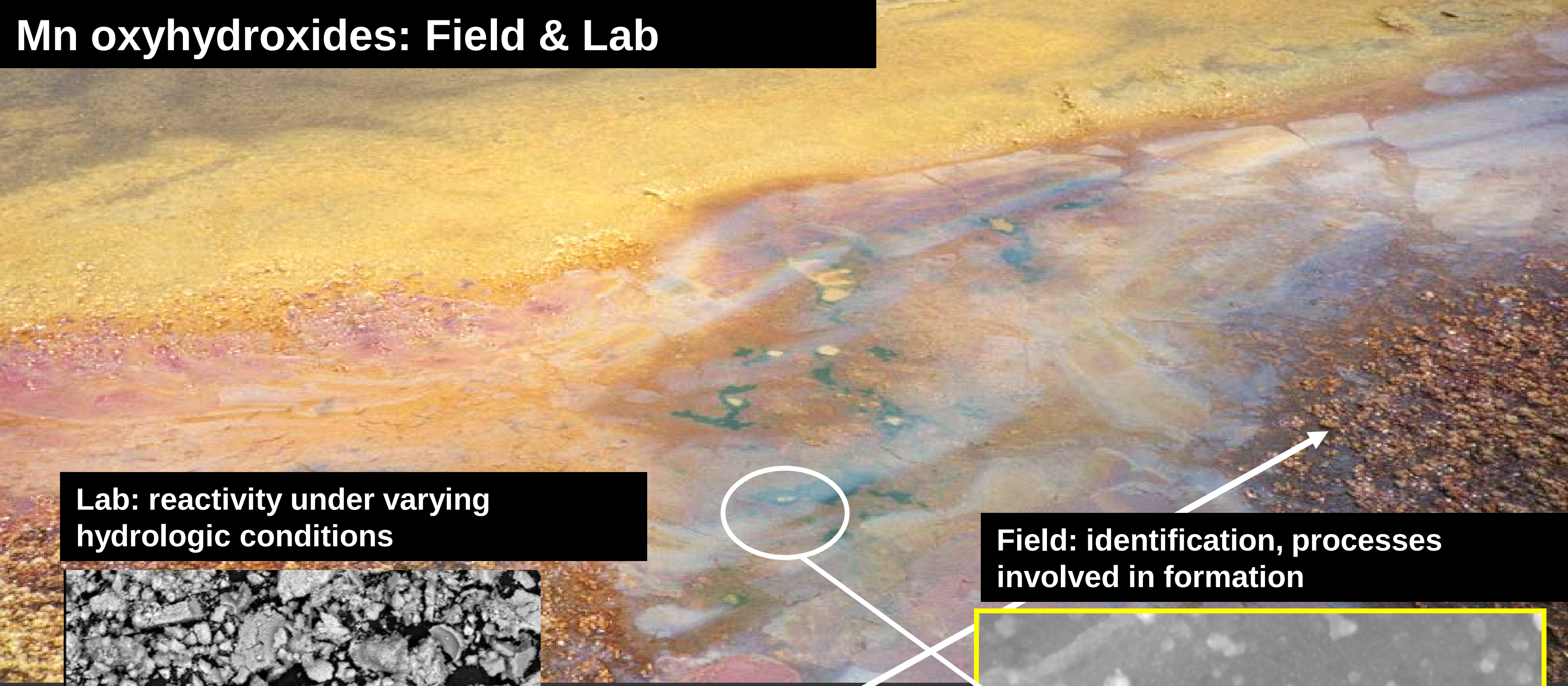
Mn oxyhydroxides?

???????

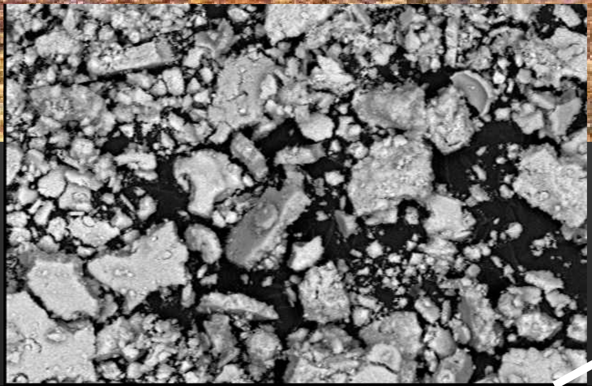


METAL SEQUESTRATION SITES

Mn oxyhydroxides: Field & Lab



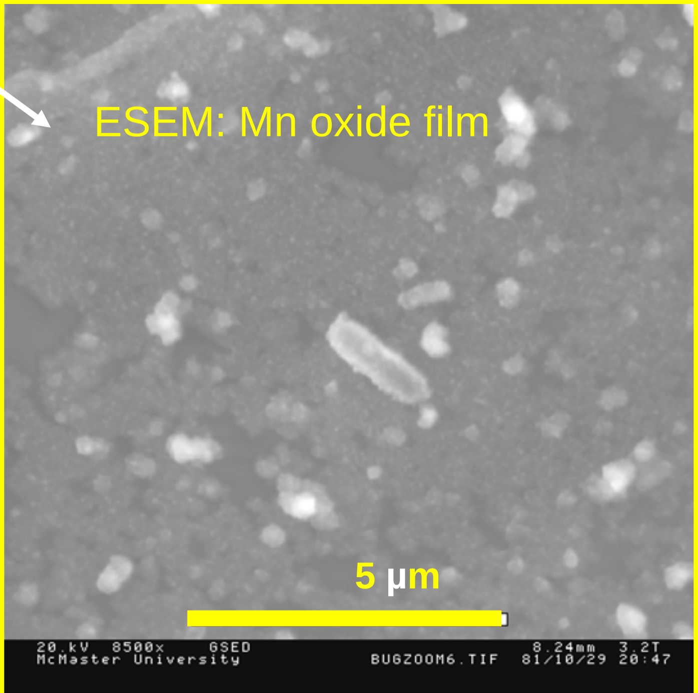
Lab: reactivity under varying hydrologic conditions



Repeated wetting and drying results in changes in surface reactivity and an increased sorptive capacity for metals.

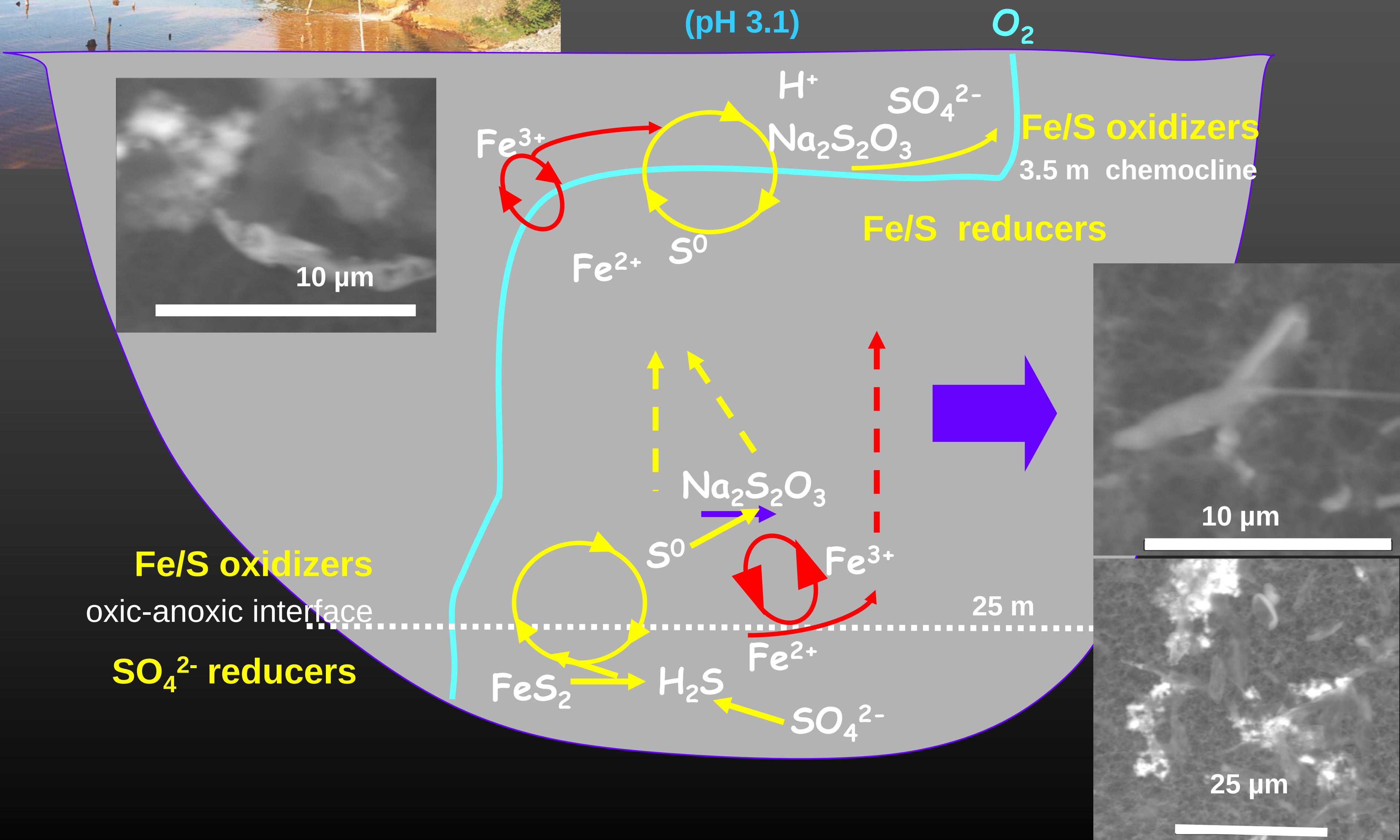
Kennedy, Smith and Warren, *Env. Sci. & Technol*. submitted

Field: identification, processes involved in formation



MOOSE LAKE: OXIDATION POND (thiosalt, H^{+}, Ni^{2+})

Fe – S cycling



CONCLUSIONS

OBJECTIVES

Linking

BACTERIA

evaluate metal retention by ARD biofilms

STRONG METAL CONCENTRATION BY BIOFILMS : 10^3 – 10^5 fold over H_2O

identify key biofilm solids for metal sequestration

Mn oxyhydroxides (biomineral) and organic components: i.e. biofilm specific solids generated by microbial function

characterize microbial – geochemical linkages involved in metal retention

Bacterial “pods”: microbial – mineral assemblages micro-hotspots for metals

REACTIVE METAL TRANSPORT

→ **MICROBIAL ECOLOGY**

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