SIGNIFICANCE, MECHANISMS AND ENVIRONMENTAL IMPLICATIONS OF MICROBIAL BIOMINERALIZATION PROCESSES
Acid Mine drainage: Carnoules (France)

Pb-Zn abandoned mine
Water pH~3

20-60% As removed after 30 m of downflow

Casiot et al., Water Res. 2003
Casiot et al., Sci Total Environ 2005

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As is trapped by the formation of diverse As-Fe-rich minerals (up to 22% As)

<table>
<thead>
<tr>
<th>Mineral</th>
<th>As(III)/Fe</th>
<th>As(V)/Fe</th>
<th>As(III,V)/Fe</th>
</tr>
</thead>
<tbody>
<tr>
<td>tooeleite</td>
<td>0.60</td>
<td>0.72</td>
<td>0.16</td>
</tr>
<tr>
<td>amorphous + to</td>
<td>0.42</td>
<td></td>
<td>0.30</td>
</tr>
<tr>
<td>schwertmannites</td>
<td>0.20</td>
<td>0.16</td>
<td>0.07</td>
</tr>
<tr>
<td>2L-ferrihydrite</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Morin et al., ES&T 2003

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**Microbes indirectly impact the formation of minerals by redox transformations**

**Fe oxidizers**

\[ \text{Fe}^{2+} + \text{As}^{3+} + \text{SO}_4^{2-} + \text{H}_2\text{O} + \text{O}_2 \rightarrow \text{Fe(III)}-\text{As(III)} \text{ phase (Tooeleite)} \]

**As- and Fe-oxidizers**

\[ \text{Fe}^{2+} + \text{As}^{3+} + \text{SO}_4^{2-} + \text{H}_2\text{O} + \text{O}_2 + \text{CO}_2 \rightarrow \text{Fe(III)}-\text{As(V)} \text{ amorphous phase} \]

**Metabolic activity catalyzes the formation of minerals**

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Diversity of minerals results from the diversity of bacteria

As(III) tooeleite

Amorphous As(V)-Fe(III)

Schwertmannite

★ X-Ray Diffraction and XAS:
(e.g. Morin et al. ES&T 2003; Morin et al. Am Min 2007)

Spatial and temporal variation of the mineralogy of sediments

Microbial diversity:
→ Low diversity
→ Species able to oxidize Fe/ As
→ Spatial and temporal variations of these communities

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Use of microscopy to look at microbial precipitates

Need to characterize both organics and minerals at the submicrometer scale

Synchrotron-based X-ray Spectromicroscopy
STXM

Transmission Electron Microscopy

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Scanning Transmission X-ray Microscope

He, air or vacuum

Objective = Fresnel lens

Sample

Aperture

monochromatic X-ray beam

Synchrotron= source of high brightness X-ray radiations

Microscope chamber

Images with 20 nm resolution

500 nm

500 nm

High spectral resolution XAS
By tuning incident energy: study of C, Fe, As speciation

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Mapping As redox in Carnoules sediments at the 50-nm scale

Difficult to evidence a correlation between As redox and specific microorganisms

Benzerara et al. GCA 2008
Bacteria cell walls provide nucleation sites for the precipitation of Fe-As minerals

1. Diverse patterns of biomineralization
2. Fossilization of microorganisms

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However, most of the precipitates are extracellular…

STXM at the C K-edge shows the presence of pervasive organic carbon polymers

![Organic C map](image)

- 284.4 eV: 1s-π* quinone
- 285 eV: 1s-π* aromatics
- 287.6 eV: 1s-π* ketone
- 288.5 eV: 1s-π* carboxylic
- 290.7 eV: 1s-π* Fe-carboxyls

Benzerara et al. GCA 2008

★ Likely significant impact on sedimentation properties, maybe surface reactivity?

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Abundance of mineralized vesicles in Carnoules: An overlooked biomineralization process?
What do we learn?

Microbes and mineral precipitation:

★ indirect role via metabolic activity
★ nucleation surfaces: cell wall, extracellular polymers, vesicles

What needs to be further studied?

Why are there different biomineralization patterns?

What are the molecular mechanisms of these biomineralization processes?

How bacteria deal with mineral precipitation?

→ Is it lethal?
→ Beneficial?
→ Are there adaptative responses to mineral precipitation?
Precipitation of minerals and Bacteria

**Lethal:**
- Disruption of membranes
- Limitation of nutrient transfers

**Advantageous:**
- Production of $\text{H}^+$ used for energy generation
- Protection against antibiotics/radiation/dessication/toxic metals...
- Magnetic sensing (e.g., magnetotactic bacteria)

Some microbes develop strategies to limit precipitation within the cell.

*From Jennyfer Miot (IMPMC)*

*From Xavier Chatellier (CAREN, Rennes)*

*From Nicolas Menguy (IMPMC)*

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EXPERIMENTAL APPROACH
Experimental Fe biomineralization by neutrophilic anaerobic bacteria

Dilemma: source of energy but production of a highly insoluble compound

\[ 4 \text{Fe}^{2+} + 11 \text{H}_2\text{O} + \text{CO}_2 \rightarrow 4 \text{Fe(OH)}_3 + 8 \text{H}^+ + \text{CH}_2\text{O} \]

Encrusts

\[ 2 \text{Fe}^{2+} + 5 \text{H}_2\text{O} + \text{NO}_3^- \rightarrow 2 \text{Fe(OH)}_3 + 4 \text{H}^+ + \text{NO}_2^- \]

BoFeN1

SW2

Does not encrust

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Fe biomineralization by BoFeN1 (encrusting cells)

- Where is Fe(II) oxidized?
- Where does Fe(III) precipitate?
Extracellular and periplasmic Fe-precipitates

40nm-thick layer of iron minerals

Globules at the cell surface

40nm-thick layer = Periplasmic encrustation

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Temporal evolution of iron redox state (STXM)

Both bacteria AND extracellular precipitates are eventually oxidized!
Iron gets oxidized on/in the cells

Miot et al., Geobiology (2009)
Cell encrustation vs. Time: Cryo-TEM on cryo-sections

Precipitation starts on the plasmic membrane
Then extends and fills the entire periplasm
Fe-oxidation likely occurs within the periplasm
Proteins are preserved in the encrusted periplasm

Interesting for the one looking for fossils in old rocks
Biomineralization of Fe by neutrophilic Fe-oxidizing bacteria

• Where is Fe(II) oxidized?
→ probably within the periplasm. Sometimes outside the cells but…

• Where does Fe(III) precipitate?
→ clearly within the periplasm then at the surface of the cells
Is encrustation of BoFen1 cells lethal?

Biomass vs. time

% of encrusted cells vs. time

Number of viable cells vs. time

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SW2 does not encrust
Fe precipitates on extracellular filaments

Miot et al. AEM 2009
Fe-redox gradients along the filaments

Fe(II) and Fe(III) distributions in the cell and medium.

Graph showing the Fe(III)/Fe total ratio against distance in the cell medium.

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Fe-bio mineralization by neutrophilic Fe-oxidizing bacteria

Different patterns of biomineralization. Is there a genetic basis?

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Bacterial biomineralization is not limited to Fe-minerals, AMD or modern Earth...

Banded Iron Formation
Precambrian (up to 3.8 Ga)

Stromatolites
modern and fossil (up tp 3.5 Ga)

Pathogenic aortic calcification

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Conclusions

What is the quantitative importance of bacterial biomineralization?

What do biominerals tell us about past environments?

Are there adaptations for/against biomineralization?

→ Need for coupling mineralogy/geochemistry and (molecular) biology tools
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