Bio Geo Sciences

PLANT SURVIVAL
National Centre of Competence in Research

Calcium carbonate biomineralization in ferralitic, tropical soils through the oxalate-carbonate pathway

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Institute of Geology ***Institute of Biology***

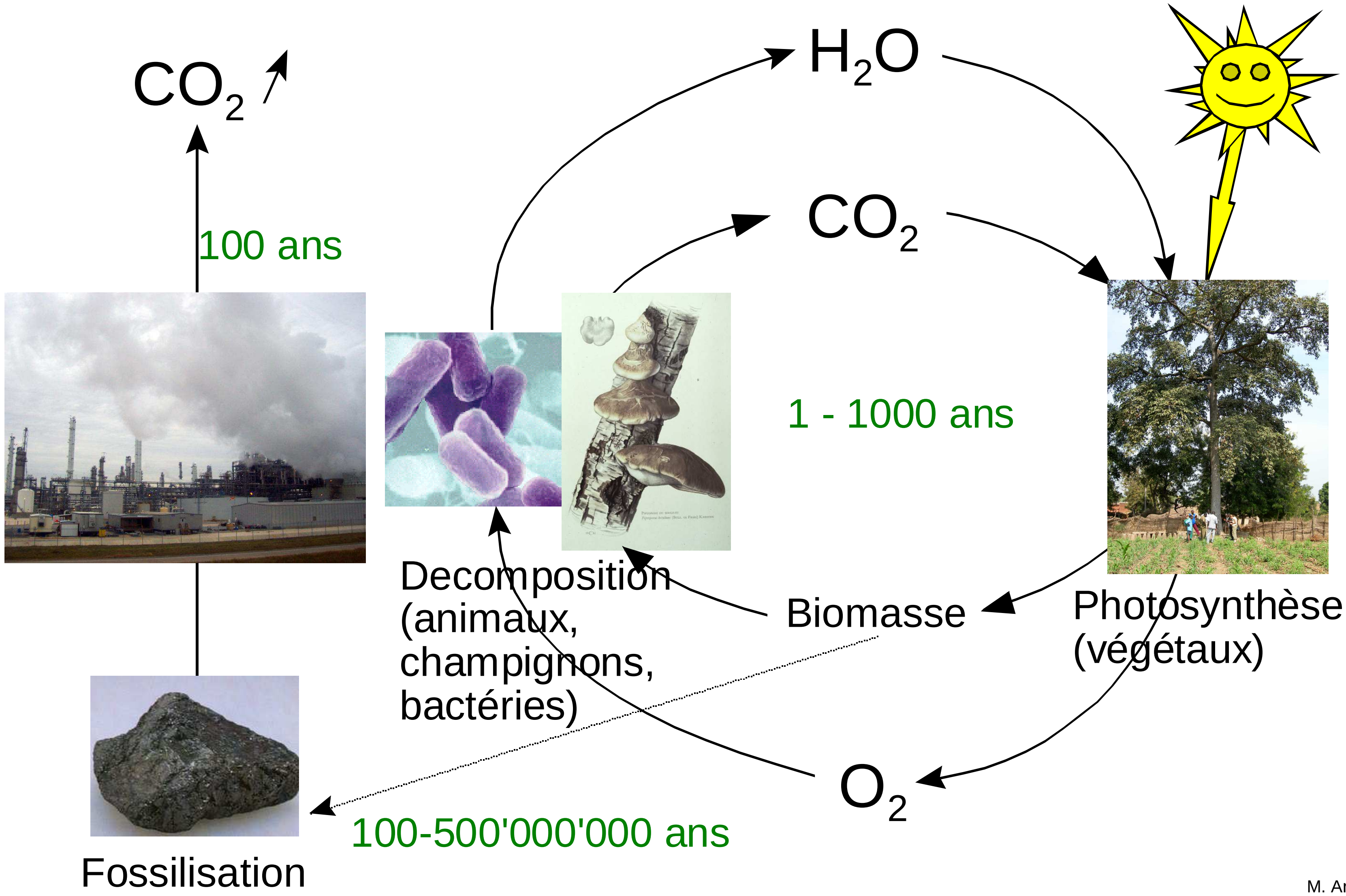
Master in Biogeosciences

Plan de l'exposé

1. Quelques idées sur le cycle du carbone
2. Quelques idées sur la biominéralisation du $CaCO_3$
3. Biominéralisation du $CaCO_3$ dans des sols tropicaux acides non carbonatés
4. La voie oxalate-carbonate
5. Importance des champignons
6. Performance de l'Iroko
7. Autres arbres biominéralisateurs
8. Perspectives et applications

1. Some ideas around the carbon cycle
2. Some ideas on $CaCO_3$ biomineralization
3. $CaCO_3$ biomineralization in tropical, non carbonateous acidic soils
4. The oxalate-carbonate pathway
5. Importance of fungi
6. Iroko performance
7. Other biomineralizing trees
8. Perspectives and applications

Carbon cycle



M. Aragno 9.2007

CO_2 sinks

CO_2

1-100 million years

1-1000 years

Limestone ($CaCO_3$)

Biomasse

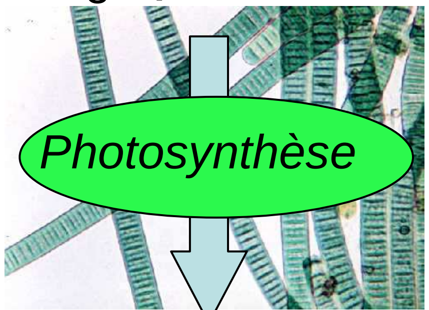
How to biomineralize limestone ?

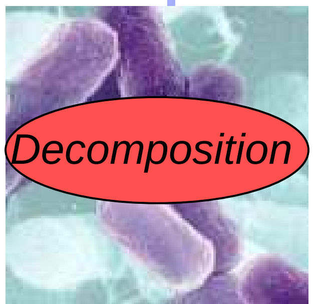
1. **From… limestone !**

$CaCO_3$ (limestone) + CO_2 + H_2O

Dissolution of limestone

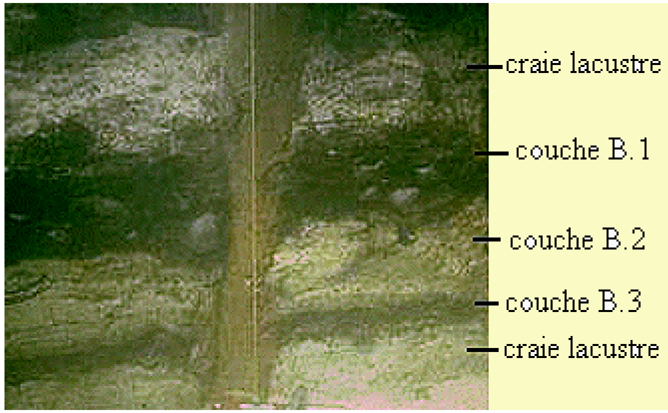
Ca^{+2} + $2\ HCO_3^-$ (bicarbonate)





Ca^{+2} + CO_3^{-2} + $C_{biomass}$

$CaCO_3$ (« lake chalk »)



Secundary limestone deposition: not a sink for CO_2 !

How to biomineralize limestone ?

2. From atmospheric CO_2 and calcium from non-carbonate minerals

Non carbonate minerals

↓

Alteration

↘

Transport

↘

Ex: feldspath

CO_2 (air)

Photosynthesis

$C_{biomass}$

Biological process ?

Ca^{+2} $CO_2 + 2\ OH^-$

$CaCO_3$

Primary limestone deposition: a sink for atmospheric CO_2 !

Iroko (*Milicia excelsa, Moraceae)*, Ivory Coast

Photo E. Verrecchia

Soil profile (> 15 m from tree)

Photo M. Mota

Soil pH (> 15 m from tree)

Photo M. Mota

Soil profile (below the tree)

Photo M. Mota

Soil pH (below the tree)

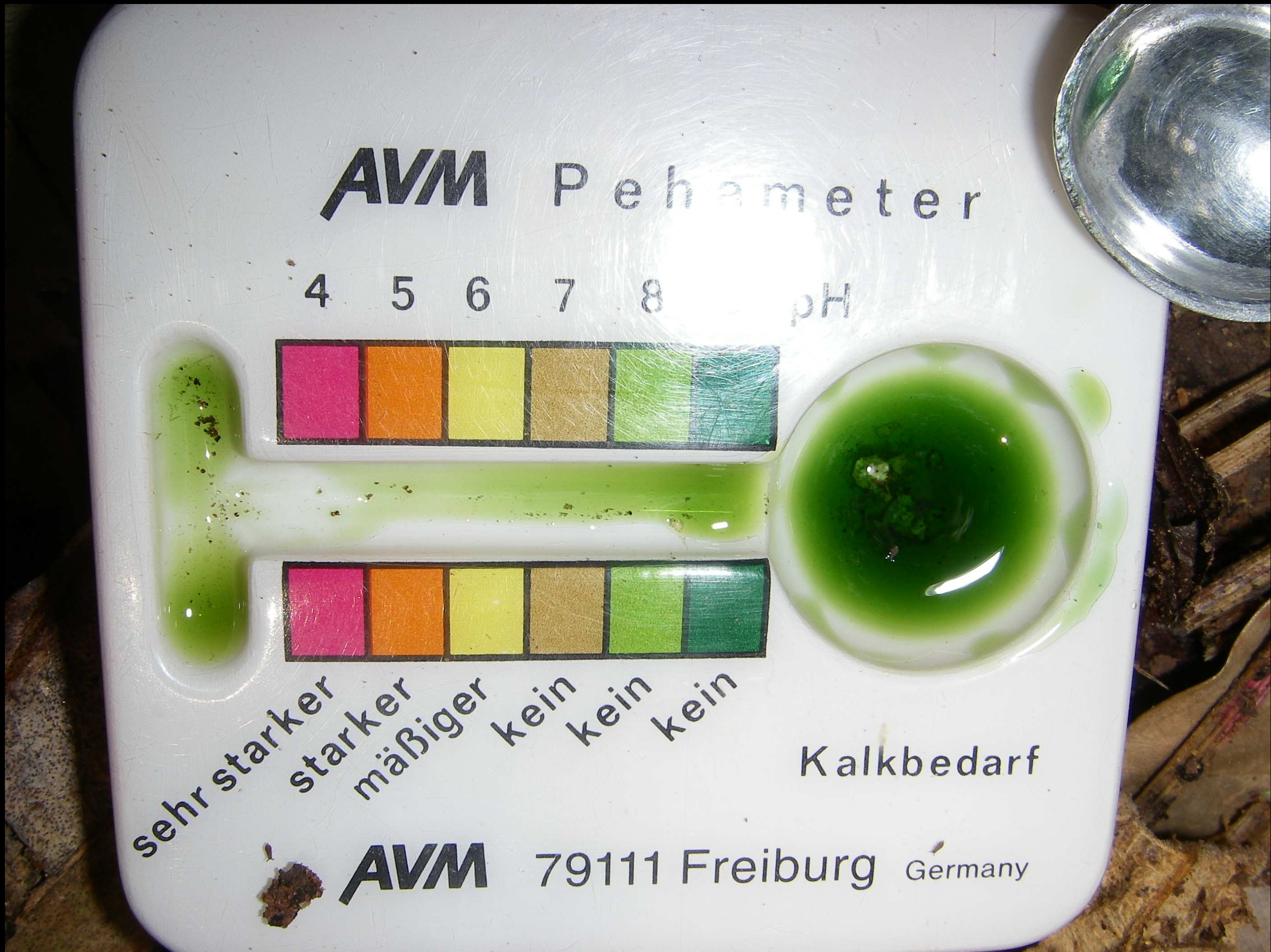


Photo M. Mota

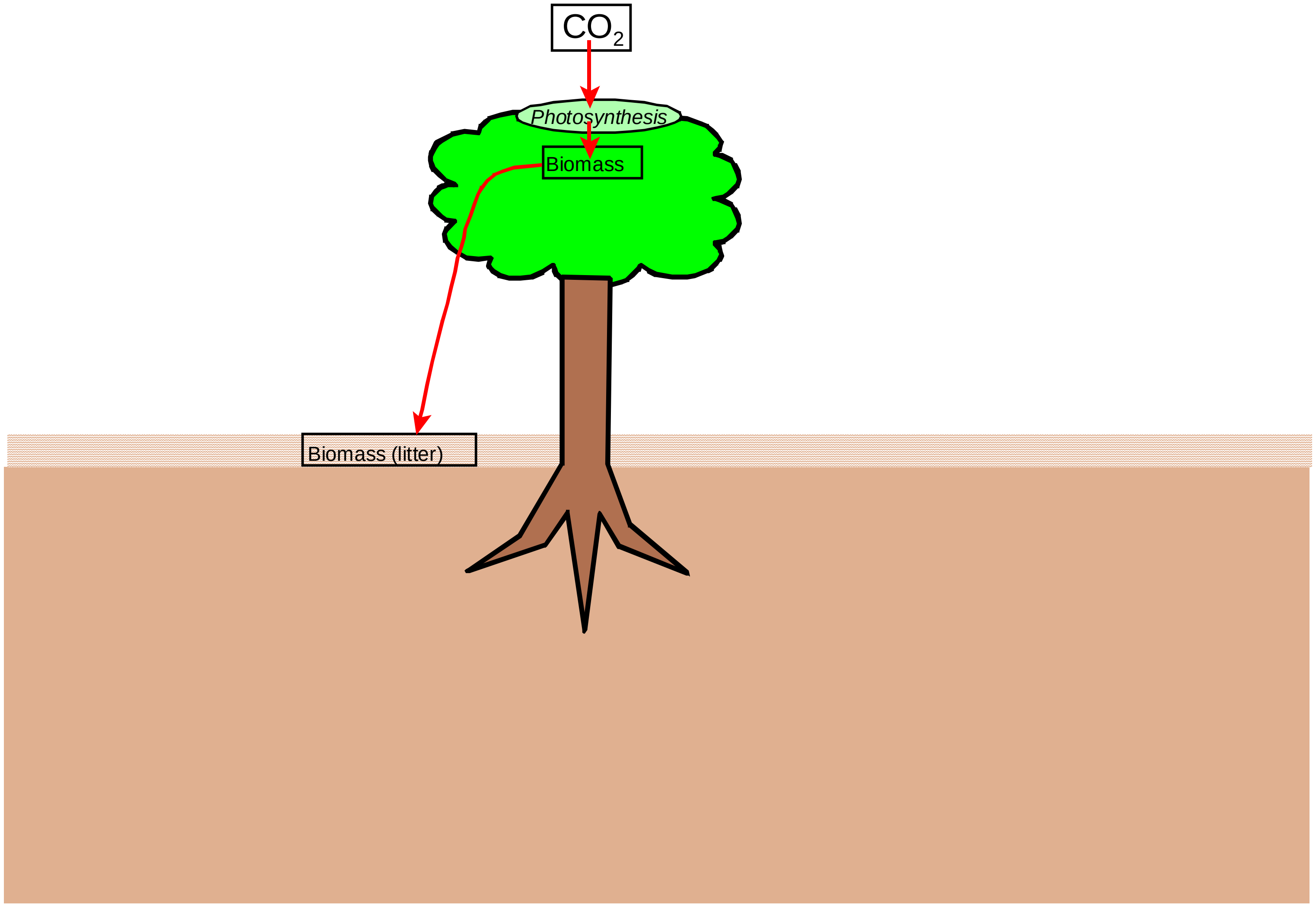
HCl test of the presence of carbonate in deadwood

Photo M. Mota

Wanted to form calcium carbonate:

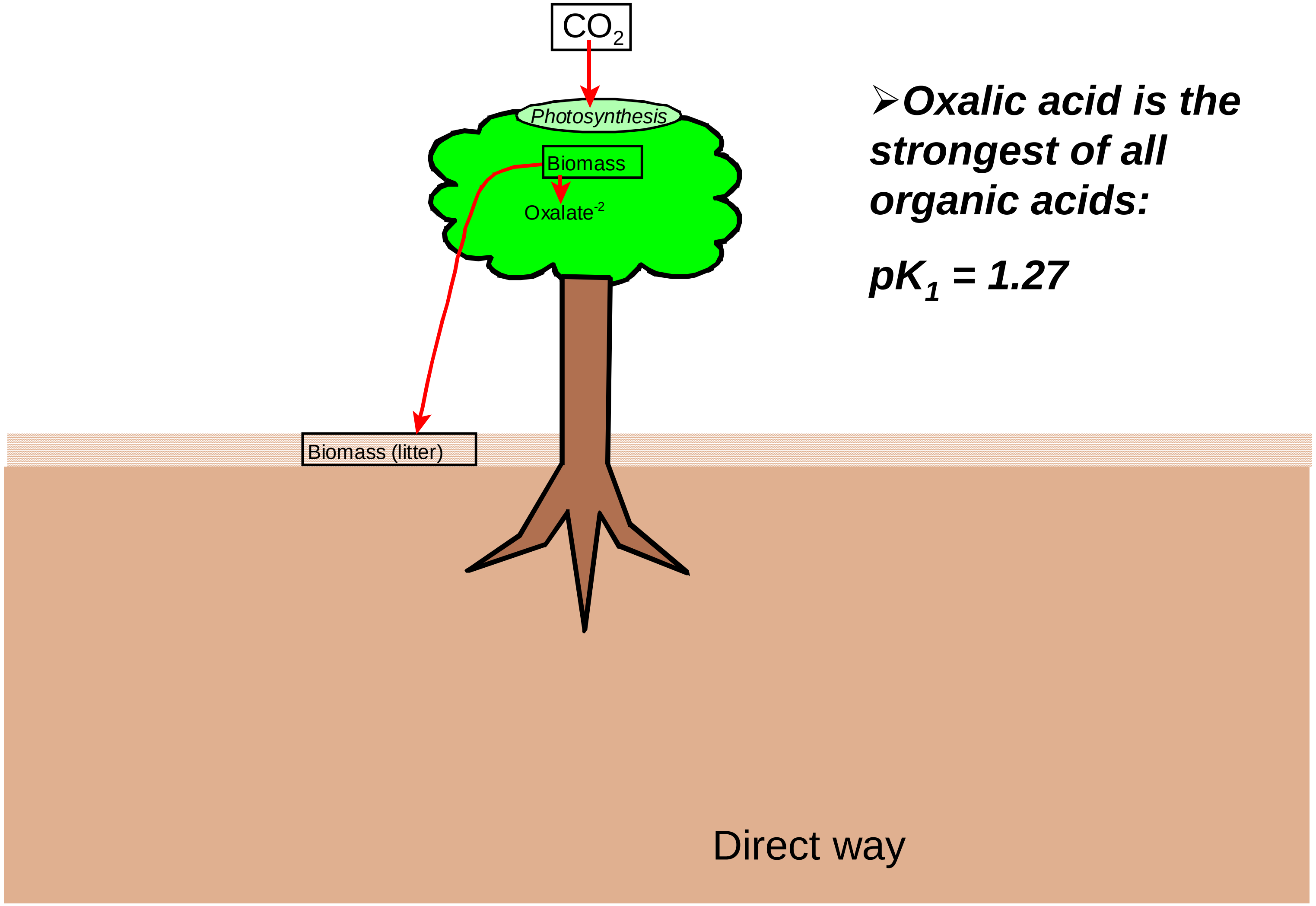
- CO_2
- Calcium
- Alkaline pH

Atmospheric CO_2 fixation in soil, through bio-mineralisation of calcite ($CaCO_3$) by the oxalate – carbonate pathway



M. Aragno 2.2006

Atmospheric CO_2 fixation in soil, through bio-mineralisation of calcite ($CaCO_3$) by the oxalate – carbonate pathway

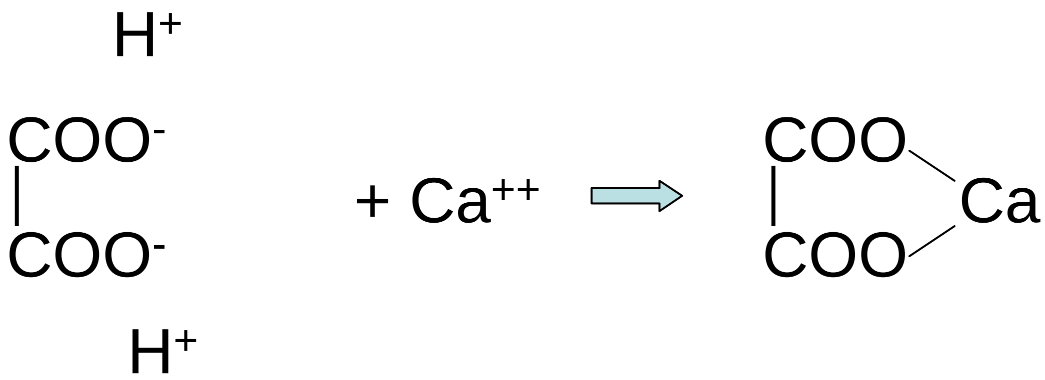


➢***Oxalic acid is the strongest of all organic acids:***

pK_1 = 1.27

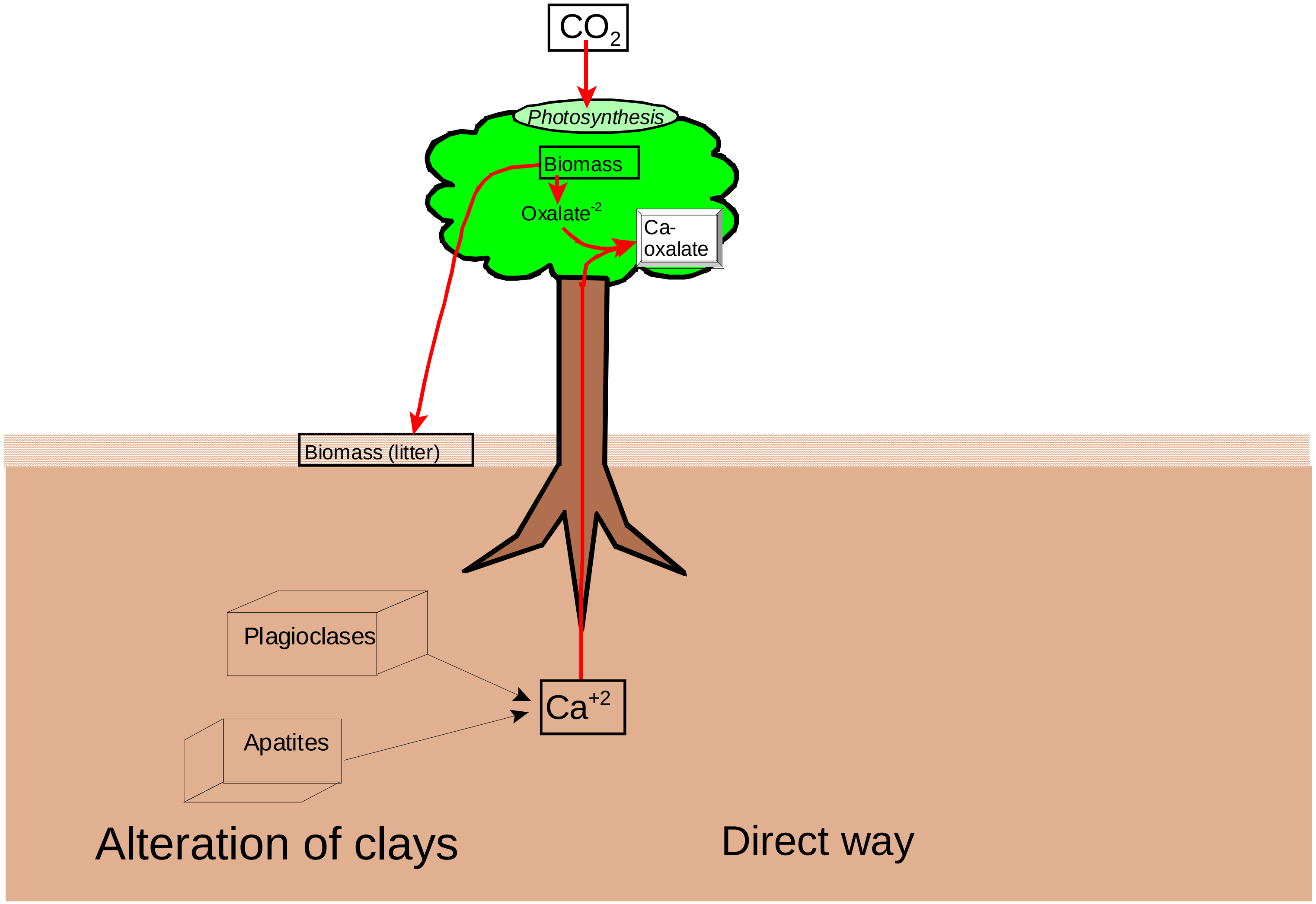
M. Aragno 2.2006

Ca-oxalate precipitation



Oxalate Calcium Calcium oxalate

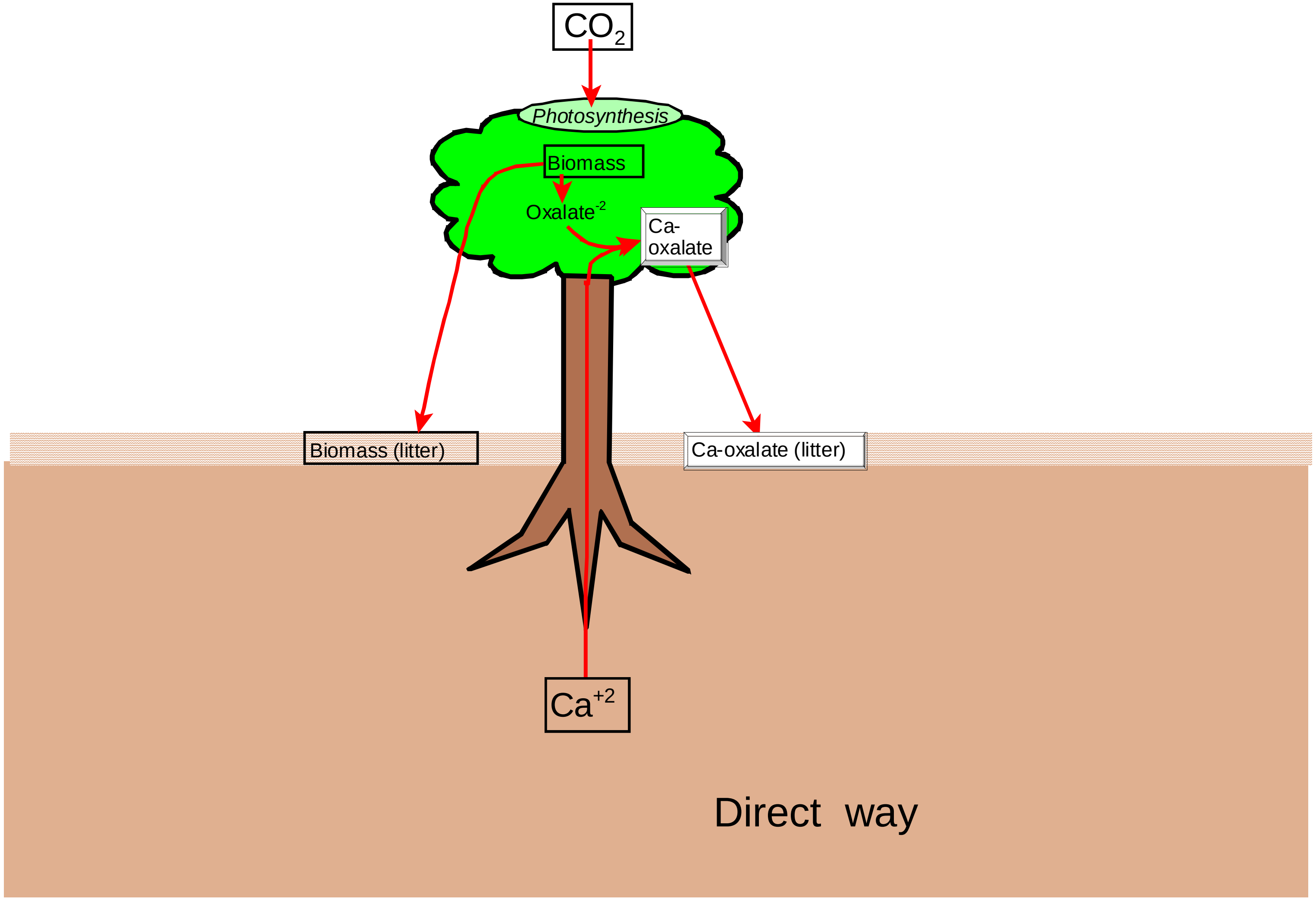
Atmospheric CO_2 fixation in soil, through bio-mineralisation of calcite ($CaCO_3$) by the oxalate – carbonate pathway



M. Aragno 2.2006

The tree co-accumulates calcium (taken up in the soil by alteration of calcium-containing minerals) and oxalate (synthesized from photosynthesized carbon) to form considerable amounts of calcium oxalate.

Atmospheric CO_2 fixation in soil, through bio-minéralisation of calcite ($CaCO_3$) by the oxalate – carbonate pathway



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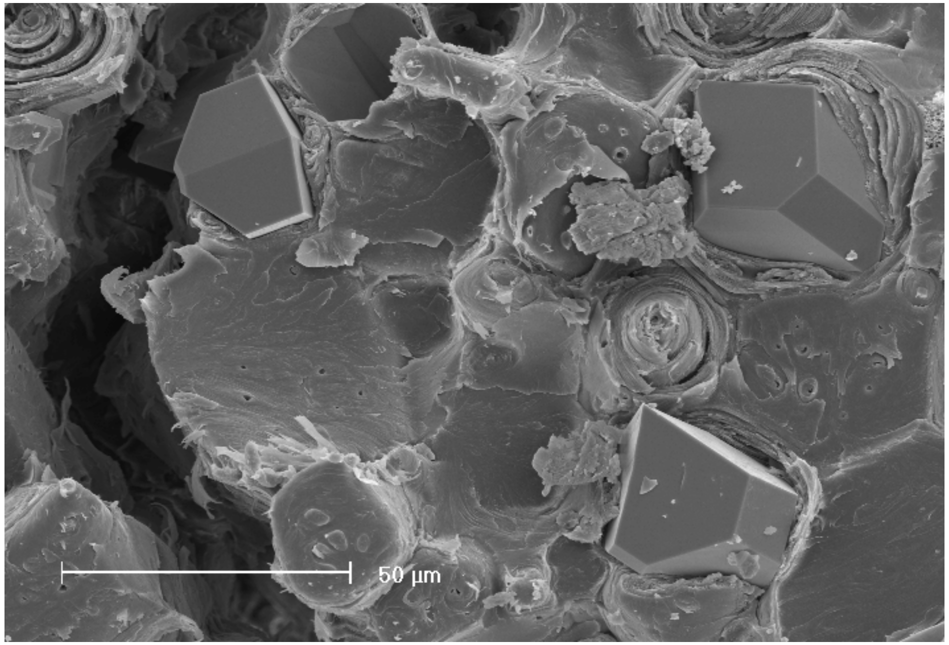
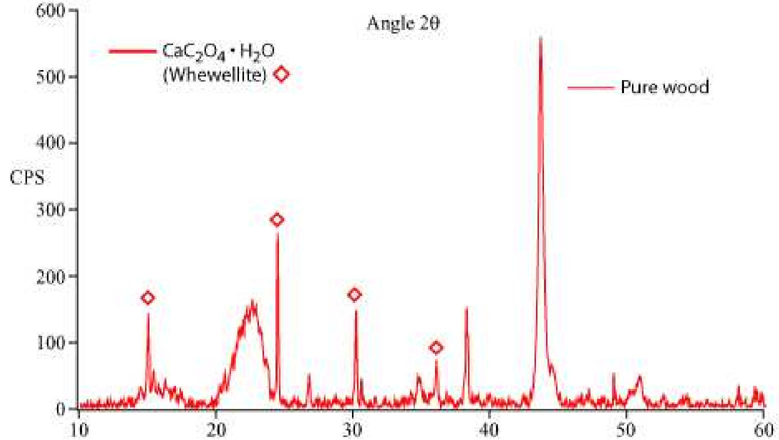
Whewellite (Ca oxalate monohydrate) crystals in Iroko's wood





Docs E. Verrecchia

Oxalate crystals released by lignolytic fungi in decaying wood

The iroko tree (*Milicia excelsa*)

Photo E. Verrecchia

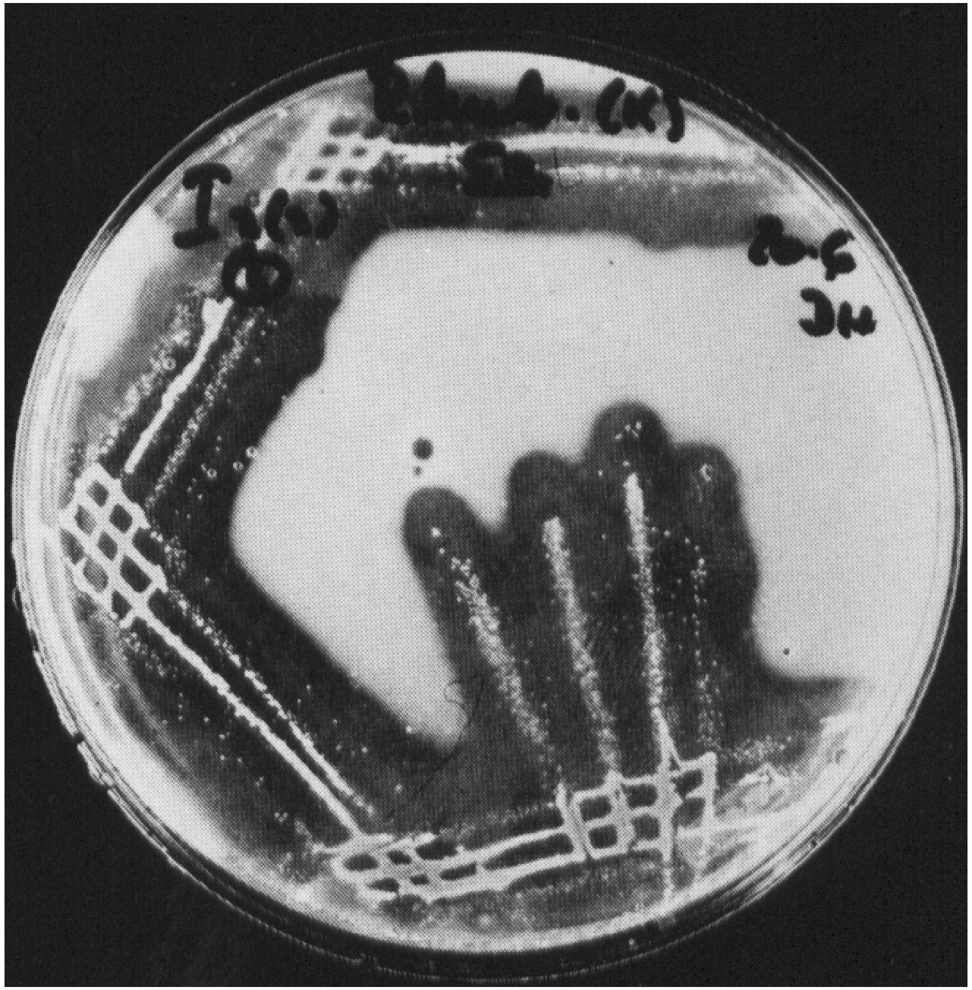
However:

- Calcium oxalate solubility is very low ($K_{ps} = 4*10^{-9}\ M^2$)
- Calcium oxalate does not oxidize spontaneously in air or in water
- Calcium oxalate accumulation was never observed in geological records

Question of the geologist (Verrecchia) to the microbiologist (Aragno) in october 2000:

Do bacteria exist which are able to feed on oxalate and to oxidize it to CO_2 ?

Answer:



Solubilization of Ca-oxalate by *Ralstonia eutropha.*

From Tamer and Aragno, ***1980***

Oxalate catabolism
Ca-oxalate dissolution

$$\underline{Ca(COO)_2} \longleftrightarrow Ca^{+2} + (COO)_2^{2-}$$

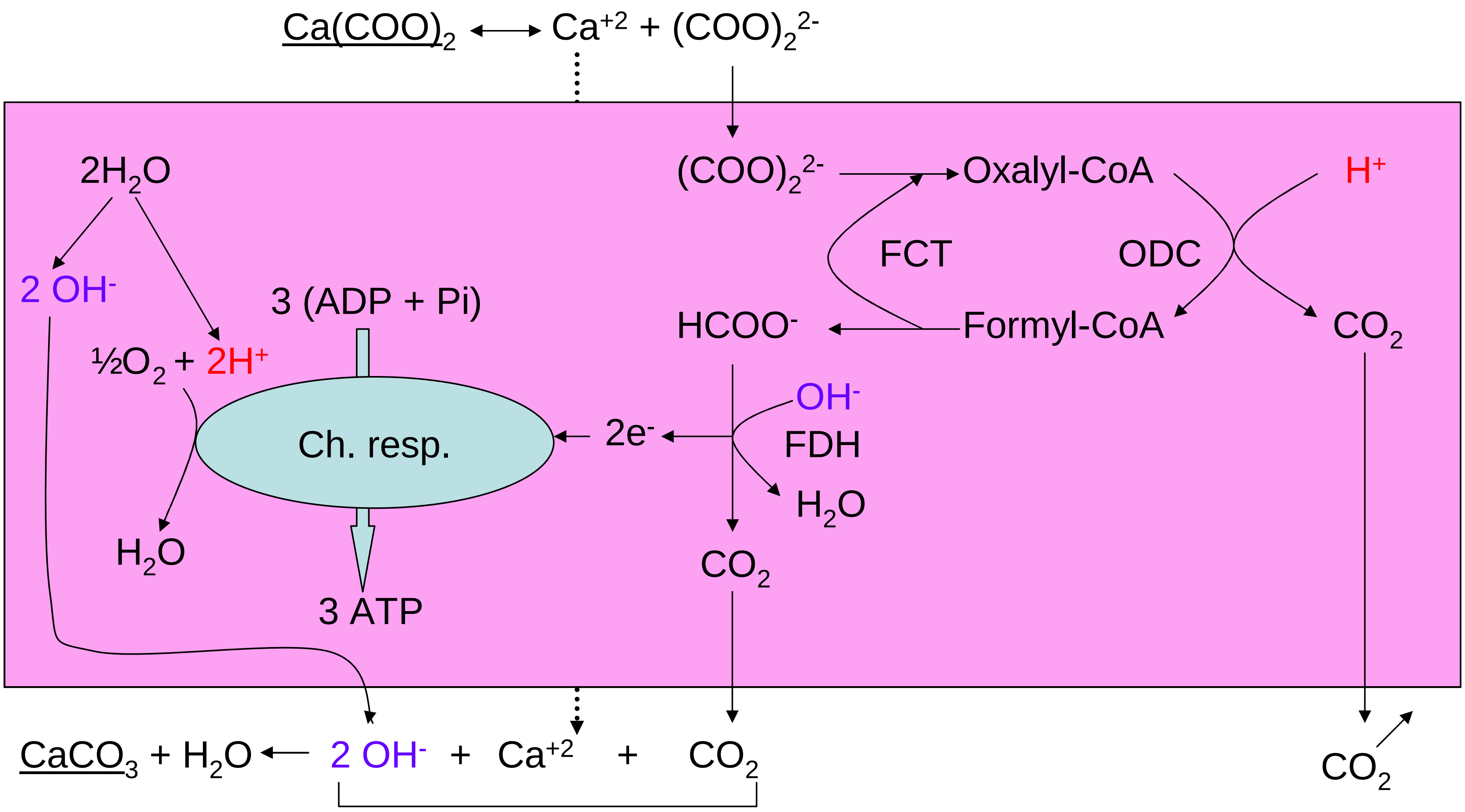
Kps (oxalate) = $4 \cdot 10^{-9}$ M^2

$\{(COO)_2^{2-}\}$ = 63 µM

K_s(oxalate) ≈ 1µM (order of magnitude)

(K_s: affinity constant of bacterial growth, according to Monod's model)

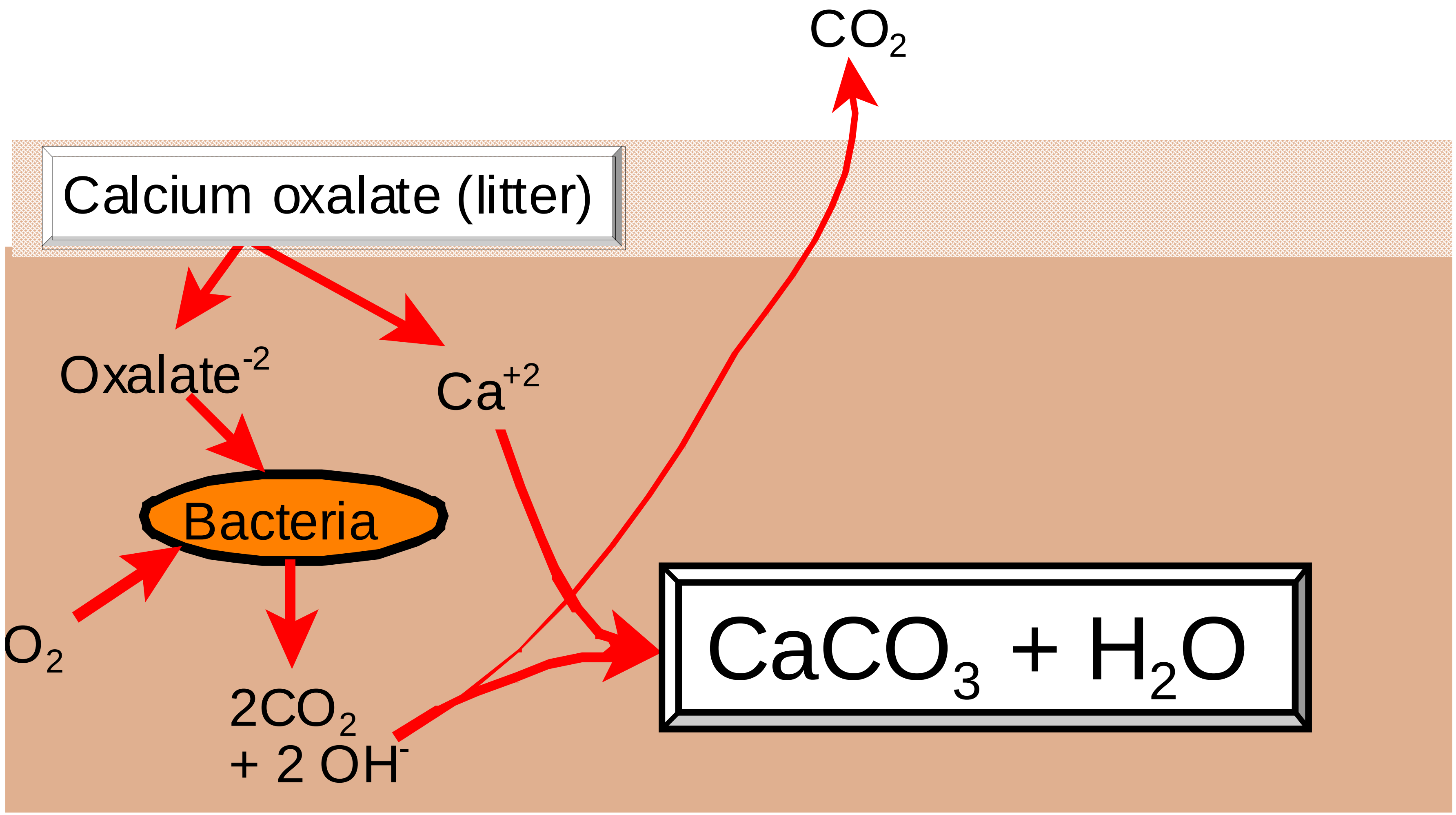
Aerobic oxalate catabolism (ex: Ralstonia eutropha)



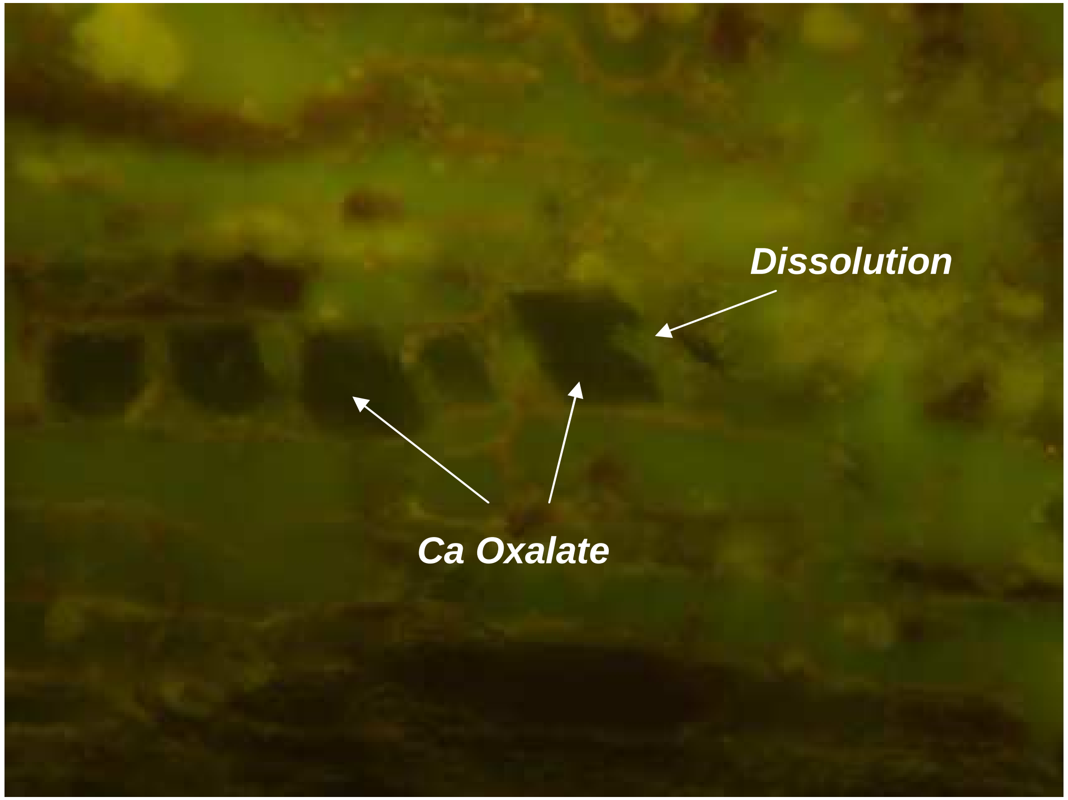
FCT: formyl-CoA-transferase ODC: oxalyl-CoA-decarboxylase FDH: formiate-DH

MA 11.2006

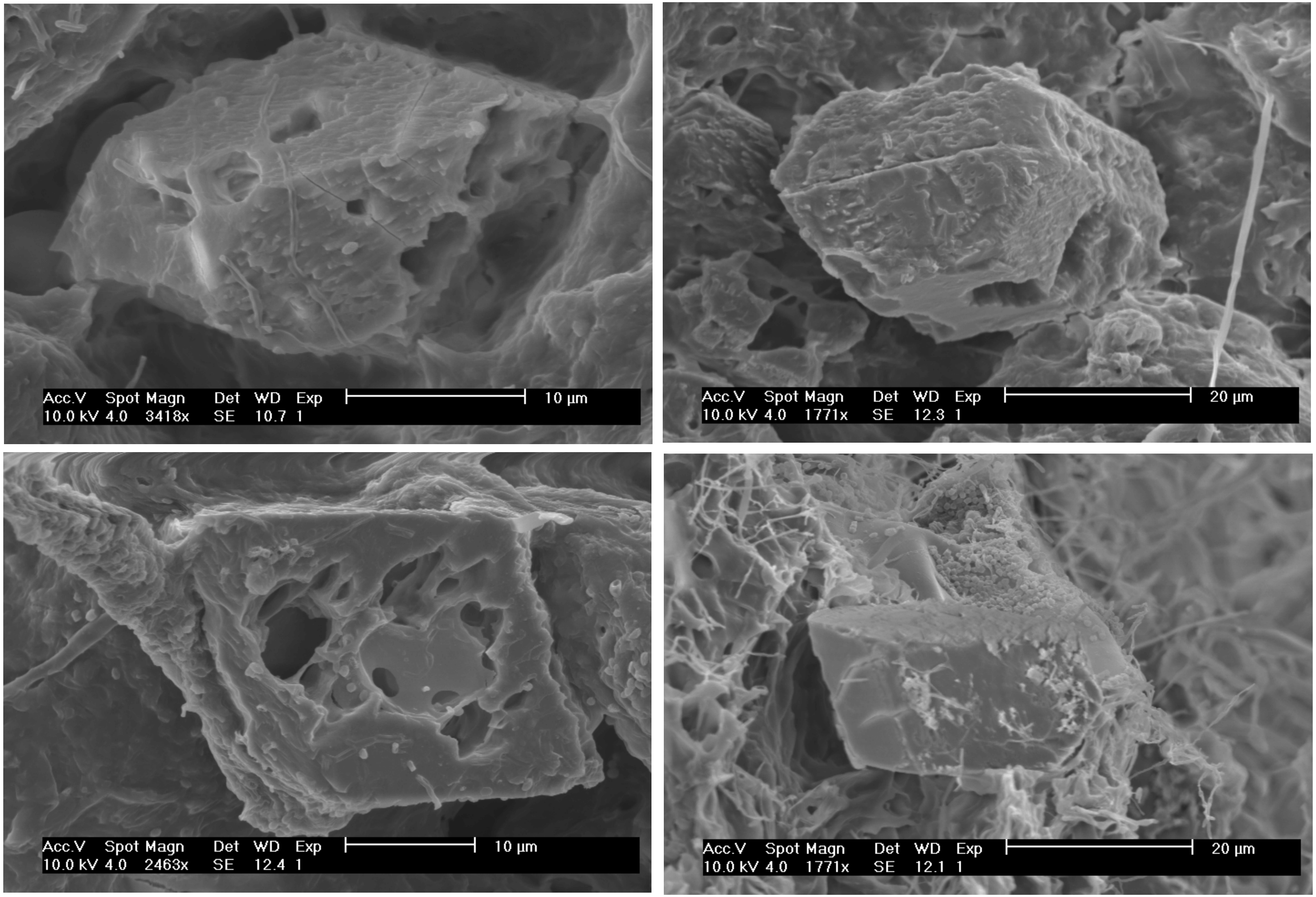
Transformation of calcium oxalate into calcium carbonate (biomineralization) by bacteria



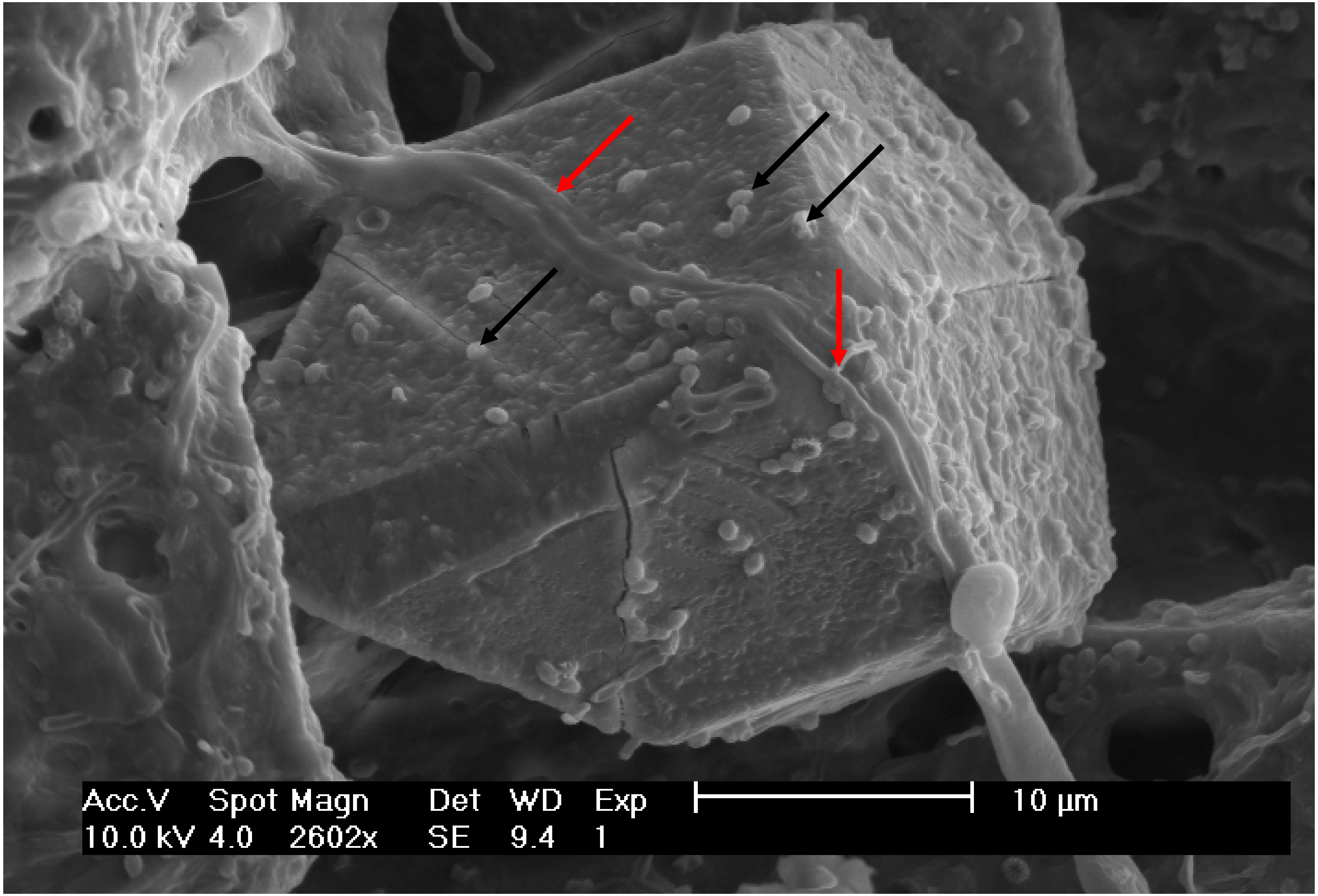
Ca oxalate's dissolution in dead wood's webs



Corroded oxalate crystals

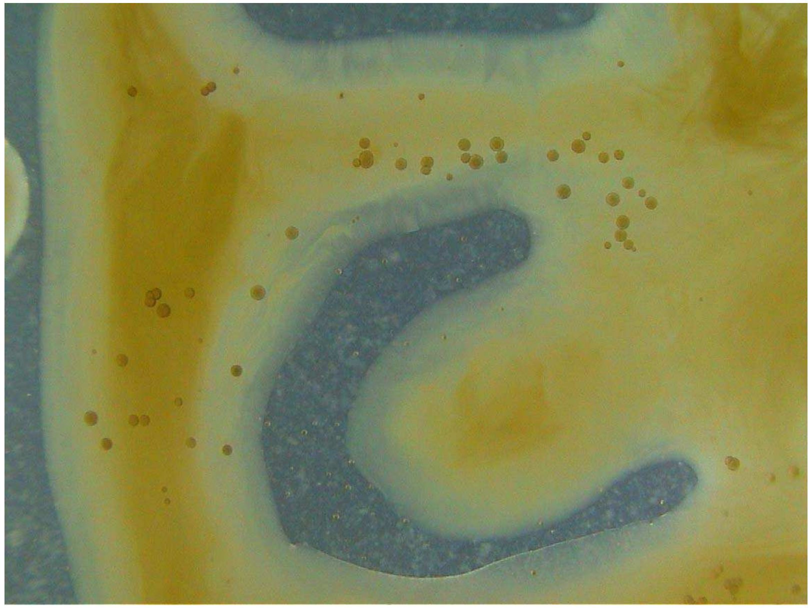


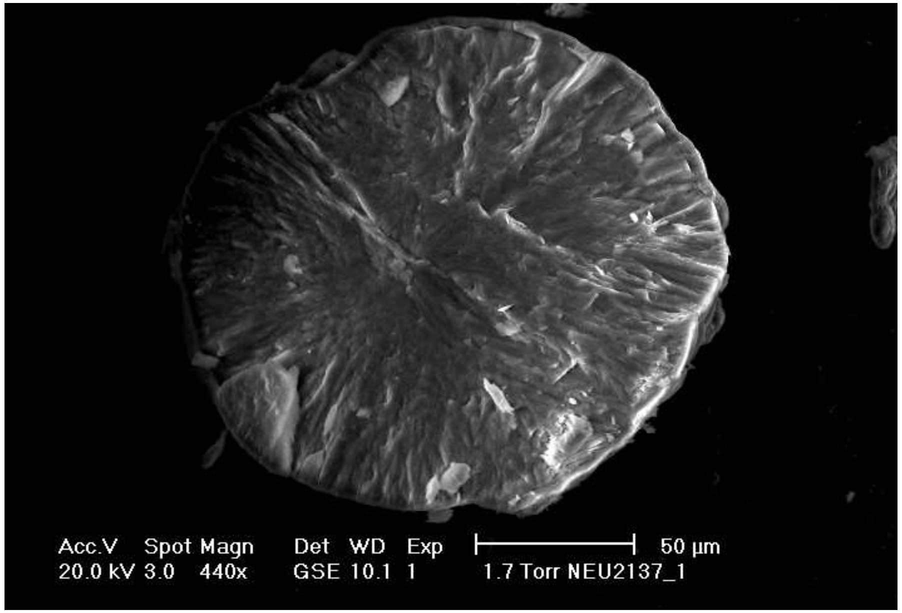
Hypha (actinobacterium ?) and bacterial cells attached to an oxalate crystal



E. Verrecchia

Calcite biomineralization by *Xanthobacter autotrophicus*



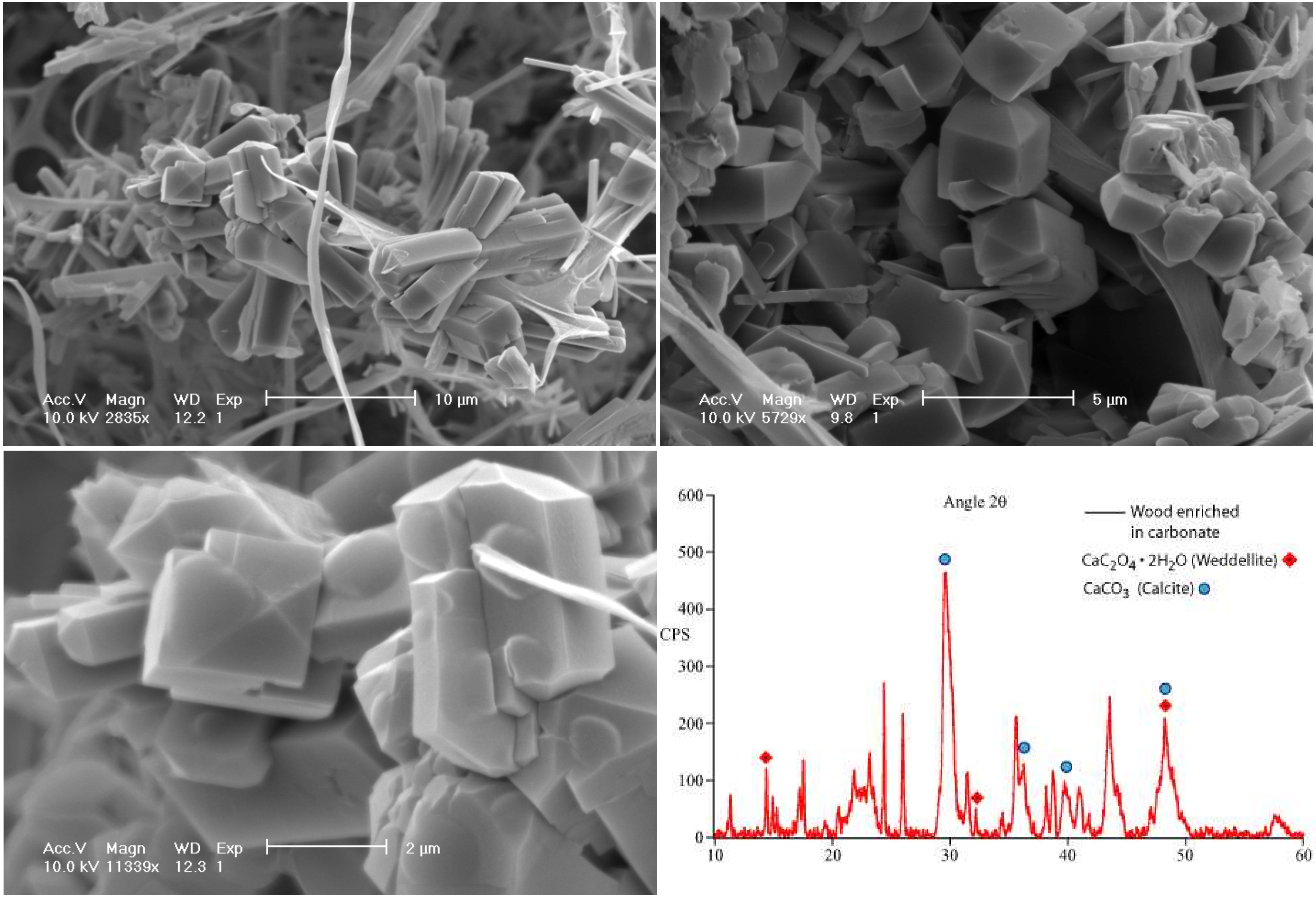


Calcite sphaerolithes

Calcium oxalate formation: the fungal pathway

Certain xylophageous fungi secrete calcium oxalate dihydrate (weddellite), whose crystals adhere to the surface of hyphae

Ca-oxalate crystals (dihydrate, weddellite) on fungal hyphae



Docs E. Verrecchia

- One Iroko tree fixes 5.6 kg C /yr as $CaCO_3$
- With the present rate of atmospheric CO_2 increase (2 ppm/yr, that is, 1 mg/m^3.yr C), one such tree would stabilize CO_2 concentration in 5’600’000 m^3 air, that is, in the air column above a ca. 1000 m^2 surface
- Calcite has a residence time up to 10^6 years, organic carbon 3-4 orders below
- Most present research on C-biosequestration deals with organic carbon accumulation…

Some economically important tree species which induce a strong calcite biomineralization

Africa (Ivory Coast, Burkina Faso):

- Iroko (*Milicia excelsa*, Moraceae)
- Kapok tree *(Bombax costatum*, Bombacaceae)
- Lingué *(Afzelia africana*, Cesalpinaceae)

Lingué (*Afzelia africana*, Cesalpinaceae)

Foto K. Ferro

The Sapecho region (Alto Beni, Bolivian Amazona)

« J'ai trouvé (…) des specimens de ces espèces dont le sol réagit à HCl et dont le pH atteint par endroit 8 ou même 9 (les sols ici dépassent rarement 6 et notre profil-témoin est à 4 au fond...) »

Translation: « I found specimens of these species whose soil reacts to HCl and whose pH reaches values of 8 or even 9 (the soils here rarely exceed pH 6 and our control profile is at 4 in the bottom…) »

Matteo Mota, Sapecho (Bolivie), 26.9.06 (by e-mail)

Some economically important tree species which induce a strong calcite biomineralization

Africa (Côte d'Ivoire, Burkina Faso):
Iroko (*Milicia excelsa*, Moraceae)
Kapok *(Bombax costatum*, Bombacaceae)
Lingué *(Afzelia africana*, Cesalpinaceae)

Bolivia (Alto Benin, Amazonia):
Ajipa *(Pentaplaris davidsmithii,* Tiliaceae)
Flor de Mayo *(Ceiba speciosa,* Bombacaceae)
Verdolago colorado *(Terminalia amazonica,* Combretaceae)
Quina Quina (*Myroxolon balsamum,* Leguminosae-Caesalpinaceae)

The « Flor de Majo »
(*Ceiba speciosa, Bombacaceae)*

Photo M. Mota

Perspectives for application

- ***Sustainable forestry (« gardening forestry »)***
- ***Efficient, long term sink for atmospheric*** CO_2 ***through calcite biomineralization***
- ***Soil fertilization through neutralization and calcite accumulation***
- ***Opportunity for agro-forestry by cultivation at three levels: herbaceous crops (e.g. legumes), fruit trees (e.g. cocoa trees, bananas) and biomineralizing big trees (wood production)***

Cultivation of banana trees in Sapecho (Bolivia)

Photo R Romero

Literature

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Khammar, N., Martin, G., Ferro, K, Job, D., Aragno, M., Verrecchia, EP. 2008. Use of the *frc* gene as a molecular marker to characterize oxalate-oxidizing bacterial abundance and diversity structure in soil. J. Microbiol. Methods (in press)

Recent developments and future research

- Molecular tools to recognize, characterize, identify and quantify the oxalotrophic community: *frc*-universal PCR primers, DGGE profiles, Q-PCR quantification

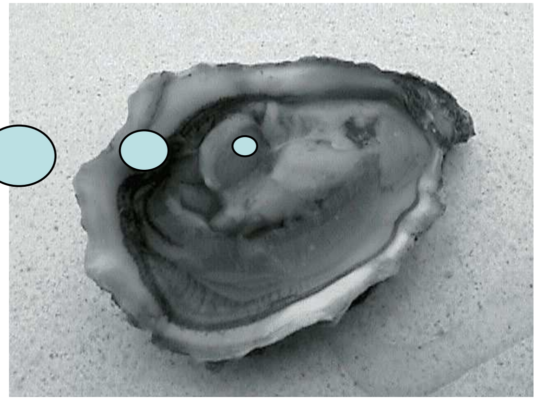
- The fungal way: oxalogenic isolates, alteration of calcium containing minerals, calcium translocation by the mycelium, syntrophic association between oxalogenic fungi and oxalotrophic bacteria

- Research at bench level (Petri dishes, microcosms)

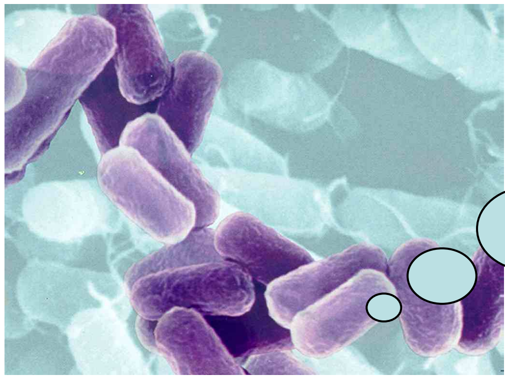
- Back to the field: Africa, Bolivia… and Australia ?

I'm the best, too !

I'm making limestone, too !



Thank you for your attention !



OK, but who is making all the job ? Once more, it's the poor proletarian bacterium !