Enzyme-driven mechanisms in biocorrosion

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Contents

Aerobic corrosion

- Different enzyme-driven mechanisms and models
- Possible effect of catalase and porphyrin compounds
- but…

Anaerobic corrosion

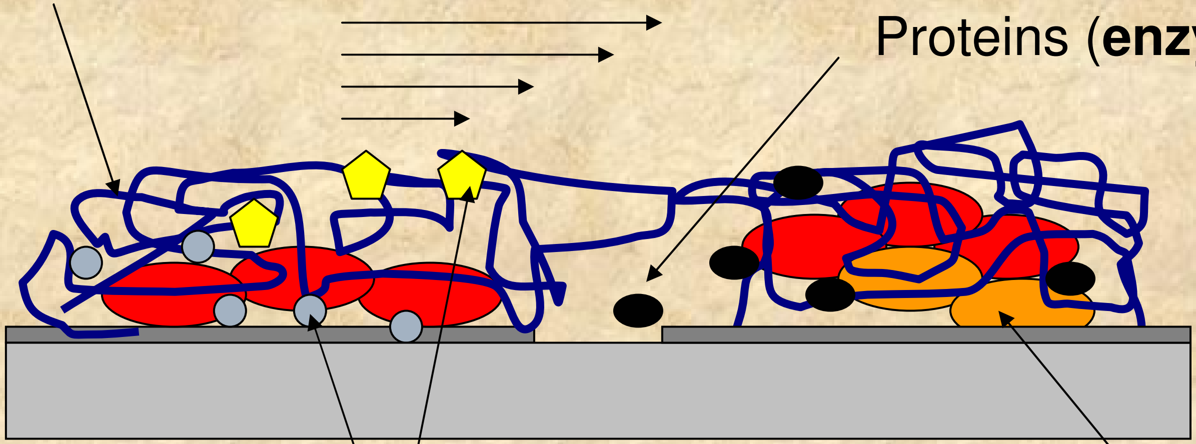
- Suphate reducing bacteria, FeS and hydrogenases
- New insights on the possible role of hydrogenases
- but…

Perspectives ?

What is a biofilm ?

Exopolysaccharides

Proteins (**enzymes**)



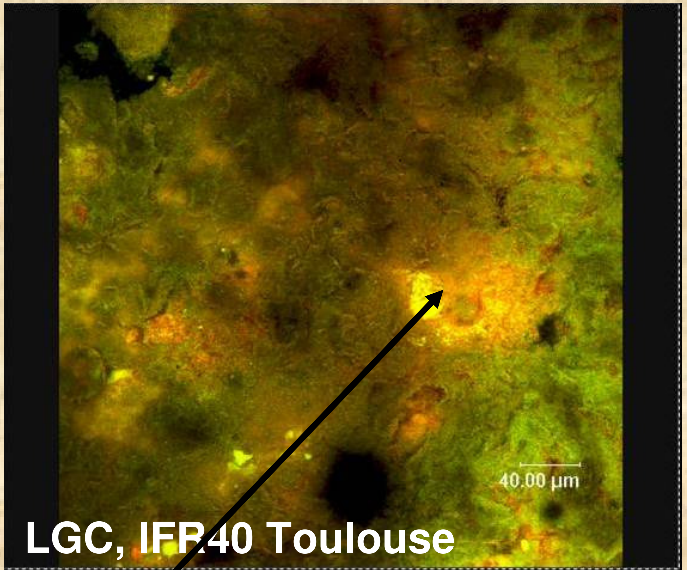
Accumulation of compounds
released from the surface
and from the environment

Microorganisms
different consortia

Being attached rather suspended makes a world of difference
(CBE, Montana)

What is a biofilm ?

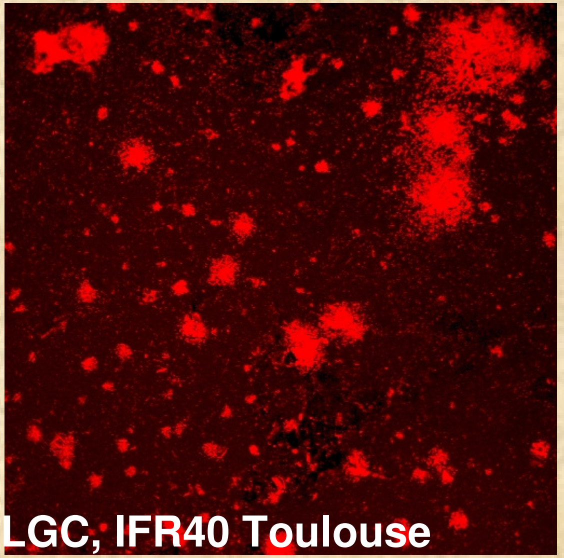
Epifluorescent microscopy



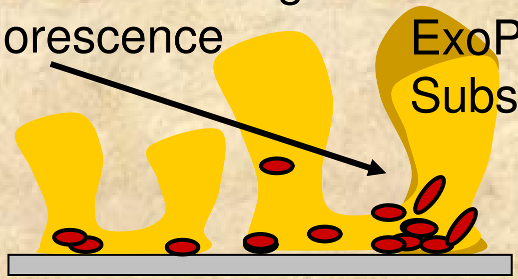
Pure culture of *G.sulfurreducens*

on carbon surface

on Ir/Ta oxides

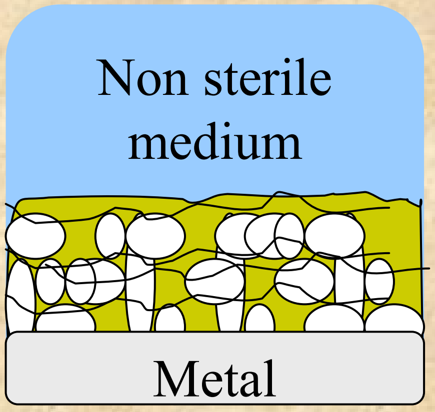


Bacteria with high fluorescence



ExoPolymeric Substance

What is biocorrosion ?



Modification of the local conditions : pH, $[O_2]$, **presence of enzymes**, exopolymers ...

Electrochemical reactions

Oxydation of the metal corrosion

$Fe \rightarrow Fe^{2+} + 2e^-$

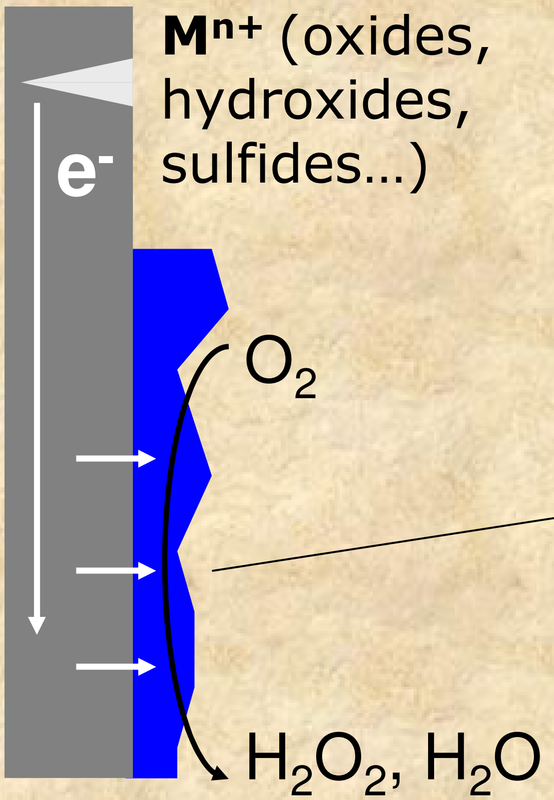
Reduction reactions

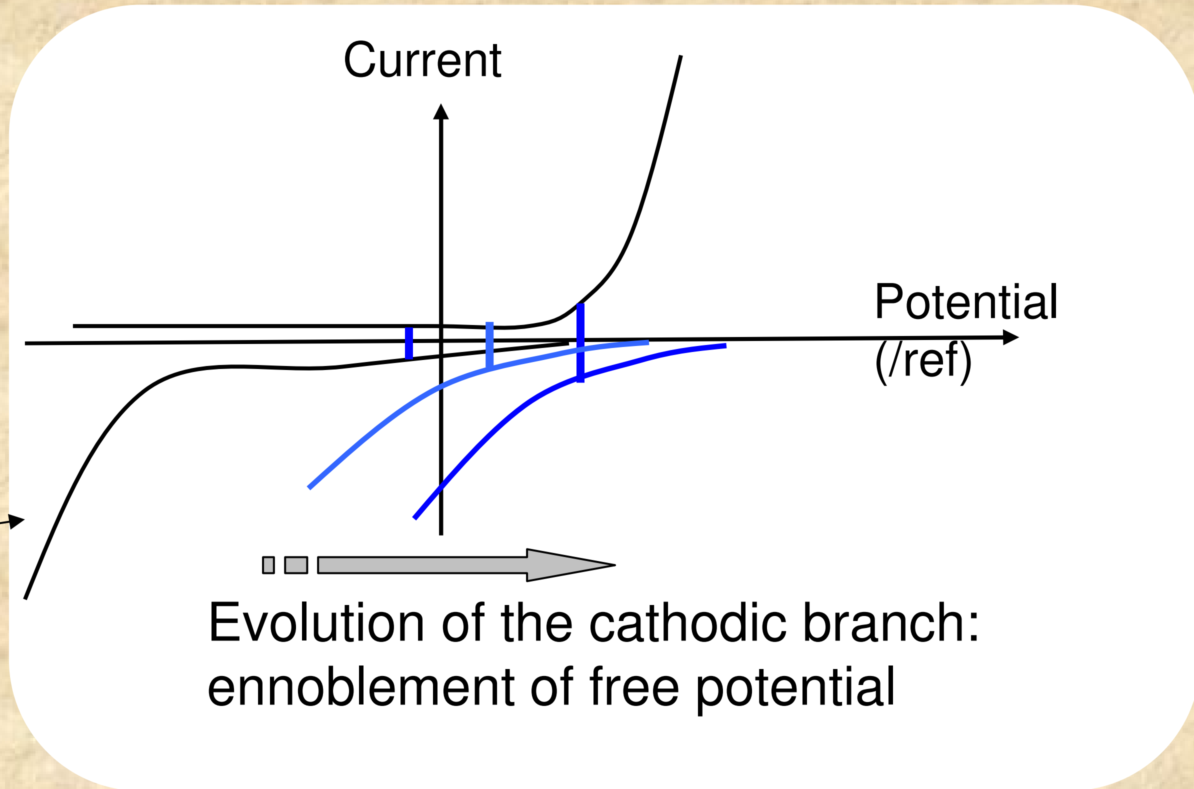
Aerobic medium
O_2

Anaerobic medium
H^+ ou H_2O

Biocorrosion = increase of electronic transfer in the presence of biofilm

Aerobic microbially influenced corrosion





Aerobic marine corrosion

• 1957 *Microbial factors of steel corrosion in seawater (Nikita N.S., Ulanovskii I.B.)*

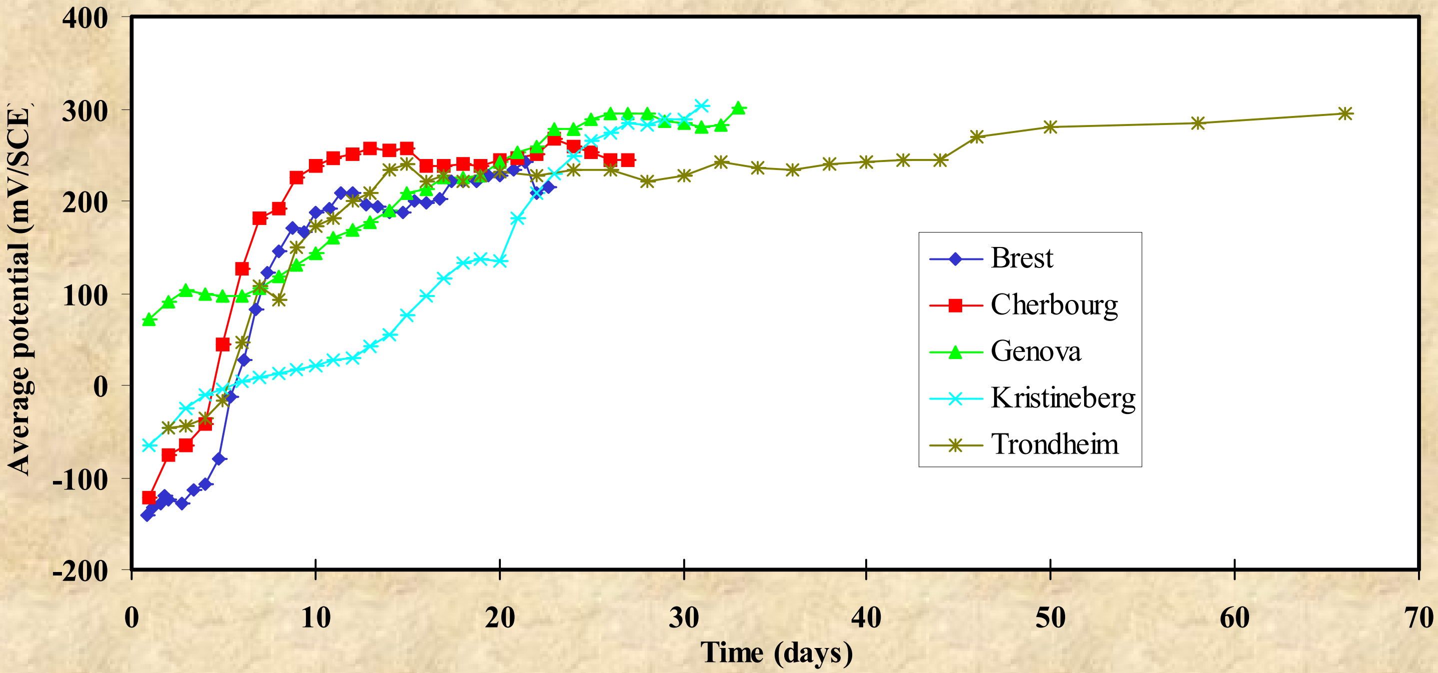
> Biofilm has an influence on marine corrosion

• 1976 *Corrélation entre la forme de la pellicule primaire et la modification de la cathodique sur des aciers inoxydables en eau de mer… (Mollica A., Trevis A.)*

> Seawater biofilms modify the cathodic reaction, without modification of the anodic branch

Aerobic marine corrosion

Potentiel ennoblement in Brest, Cherbourg, Genoa, Kristineberg, Trondheim



MAS programme, CNR-ICMM coordinator, from CEA-Saclay

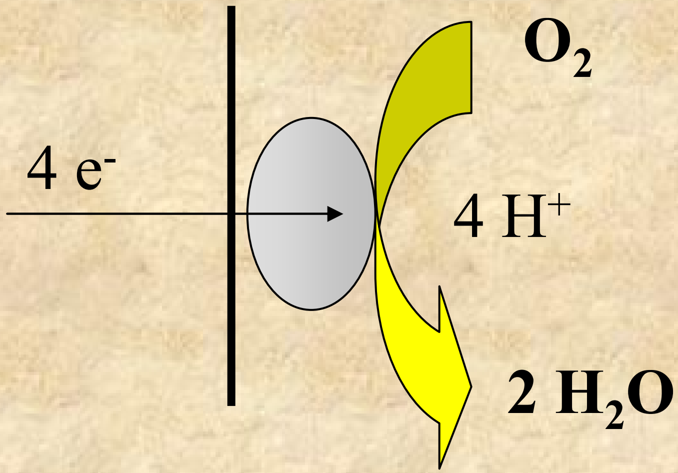
Mechanisms : complementary contributions

Modification of the cathodic branch due to reduction of the manganese oxides and hydroxides produced by Manganese Oxidizing Bacteria. *Shi, Avci, Lewandowski Corr. Sci. 2002, 44, 1027-1045*

Production of hydrogen peroxide in solution (GOD-catalysed) followed by reduction of hydrogen peroxide on the material surface
→ Easy-to-handle laboratory model (no micro-organism)
→ Able to produce same potential ennoblement as natural marine biofilms. *Dupont, Féron, Novel Int. Biodet. and Biodeg. 1998, 41, 13-18*

modification of the passive layer by the hydrogen peroxide produced (*phytoplanctons, bacteria, micro-algae*)
→ Easier to reduce O_2
LeBozec, Compère, L'Her, Laouenan, Costa, Marcus, Corr. Sci. 2001, 43, 765-786

Direct catalysis of oxygen reduction

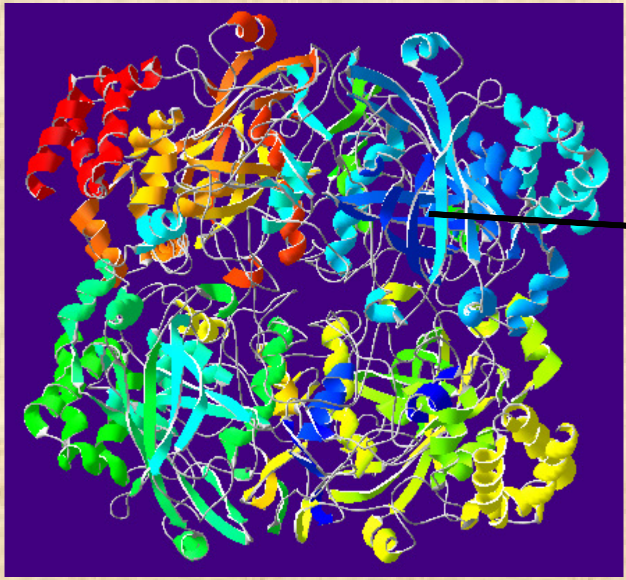


Laccase - active site = 4 Cu
- catalyses oxygen reduction with aromatic amines or phenol as electron donor

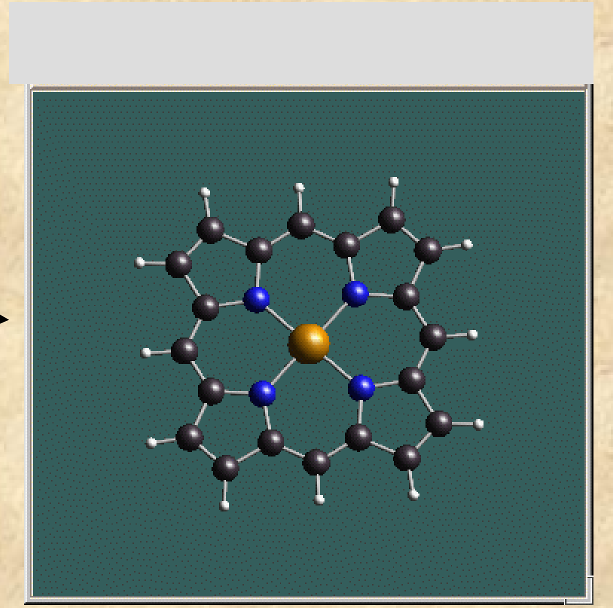
Catalase - Peroxidase - Superoxide dismutase ?

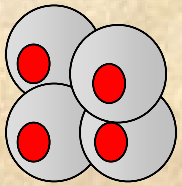
Catalase

$$H_2O_2 + H_2O_2 \rightarrow O_2 + 2\ H_2O$$



Active site
porphyrin ring



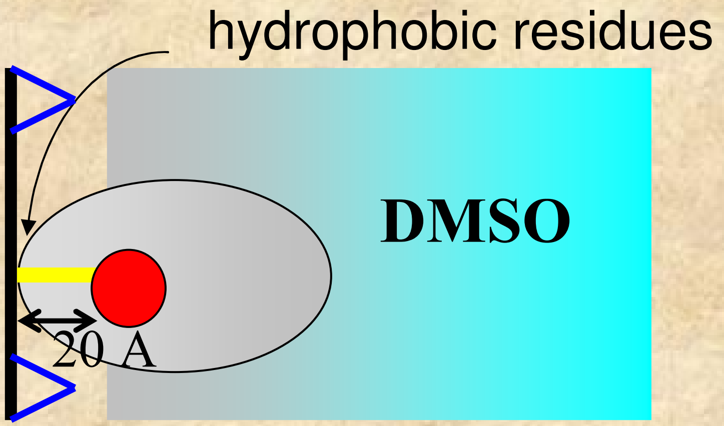


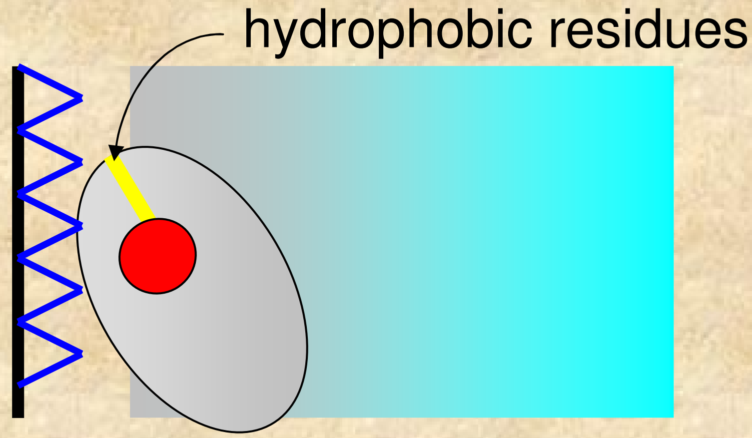
4 equal subunits each 57000 Da

One molecule of catalase can convert 5 million molecules of hydrogen peroxide to water and oxygen each minute

Direct catalysis of oxygen reduction?

Glassy carbon electrode





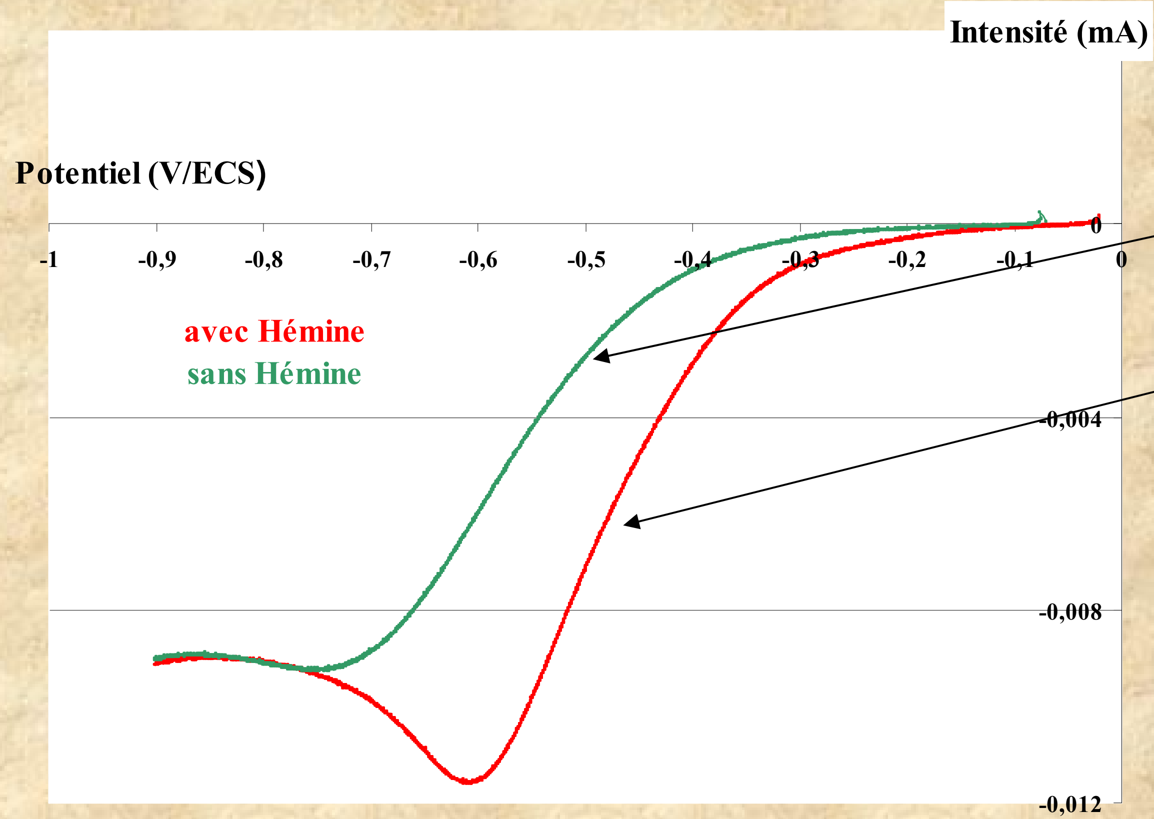
No preliminary treatment
=> hydrophobic groups on the surface (only partial coverage with water)

With preliminary treatment
=> hydrophilic surface (fully wetted)
=> carboxyl groups which react with amines of the polypeptide chain

CNRS-LGC, M.E.Lai, JEAC 2000

Direct catalysis of oxygen reduction?

Iron porphyrin adsorbed on stainless steel URB66



Voltammetry 10mV/s

Clean SS

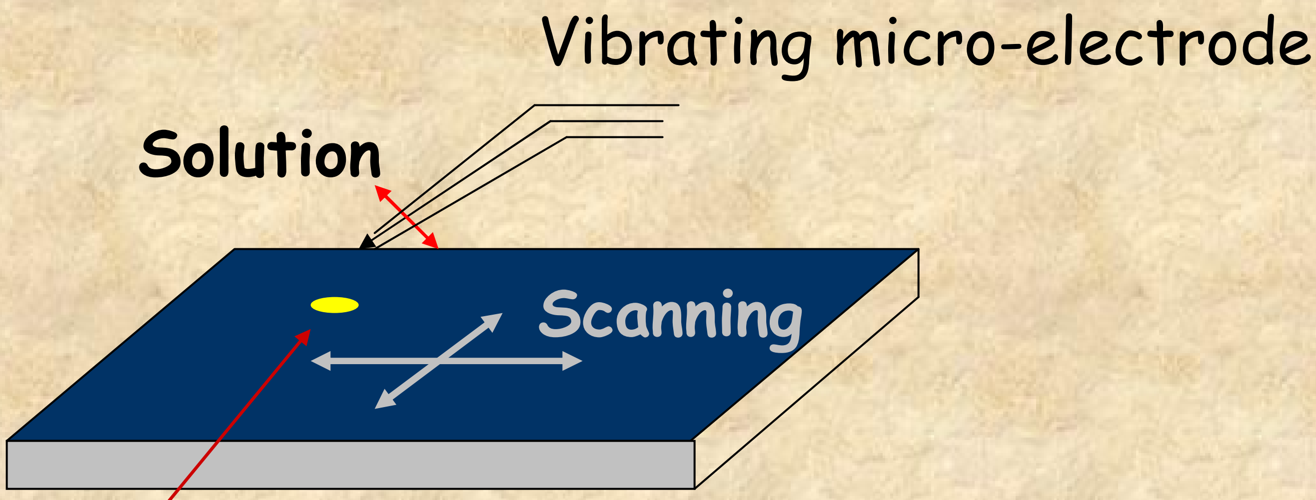
Porphyrin adsorbed on SS

NaCl 24.5 g/l

URB66 = Cr 23.92 – Ni 21.90 – Mo 5.44 – Mn 2.99 – Cu 1.54 – W 1.86 - N 0.47

EFC 45 chap.5 R.Basséguy et al.

SVET: local electrochemical analysis



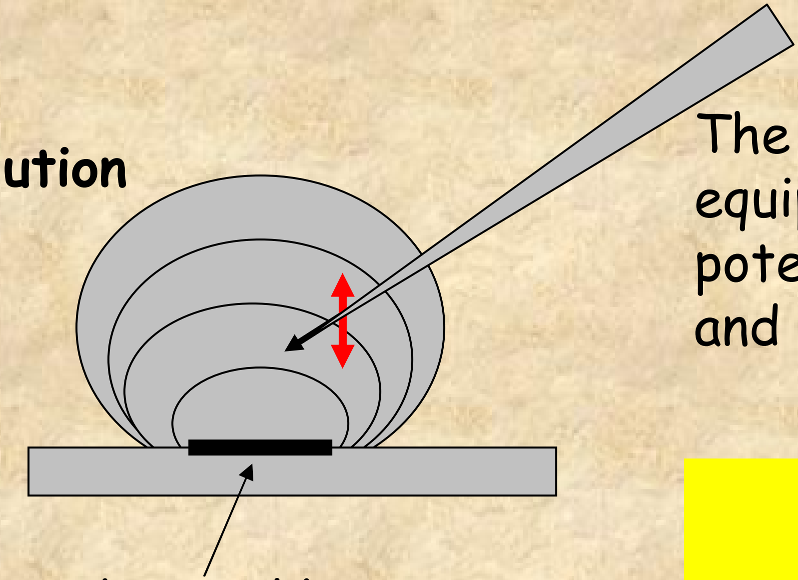
Electrochemical heterogeneity =

- pitting (anode) on a metal
- disruption of a protective coating over a metallic material
- local electrochemical reaction (micro-sensor)

C.Jacques

SVET

Solution



Electrochemical heterogeneity induces local currents and potential differences

The electrode vibrates across the equipotential shells and measures the potential drop ΔU between the top level and the bottom level

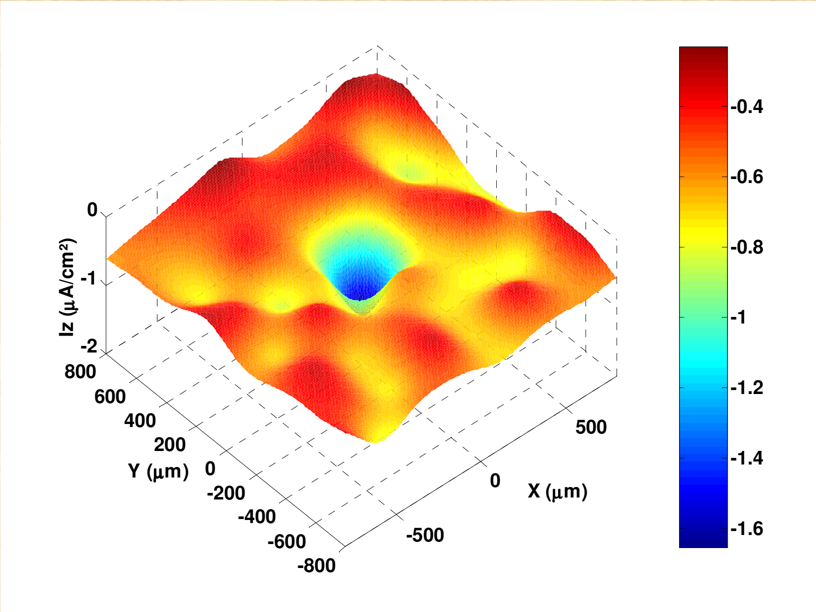
$$\Delta U = R\ I$$

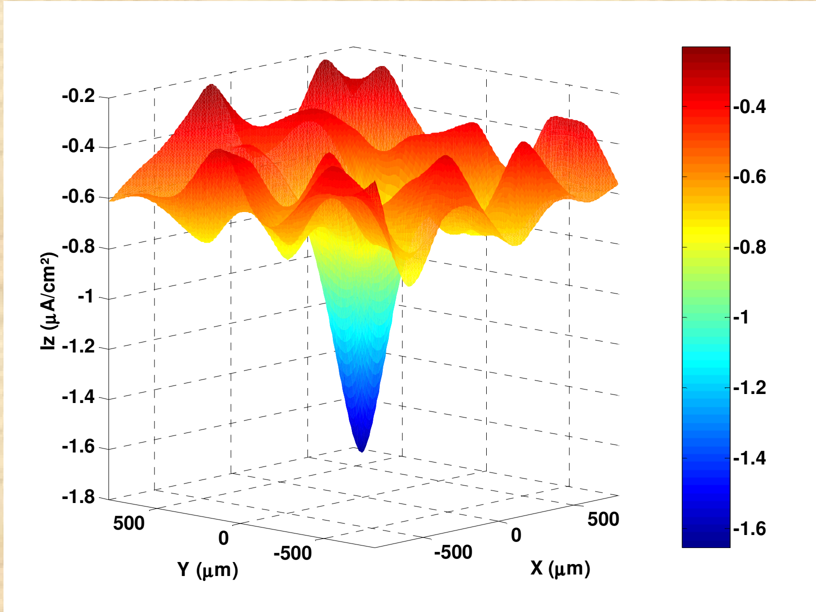
Potential drop — Resistivity of the solution — Local current

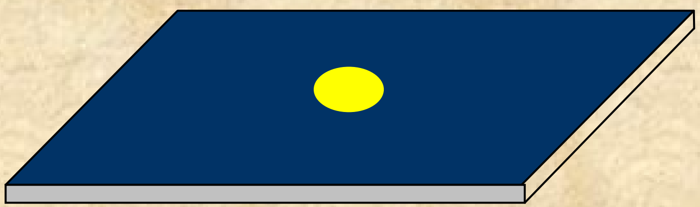
Result = 2D mapping of the local current

Hemin on GC electrode

SVET on glassy carbon electrode





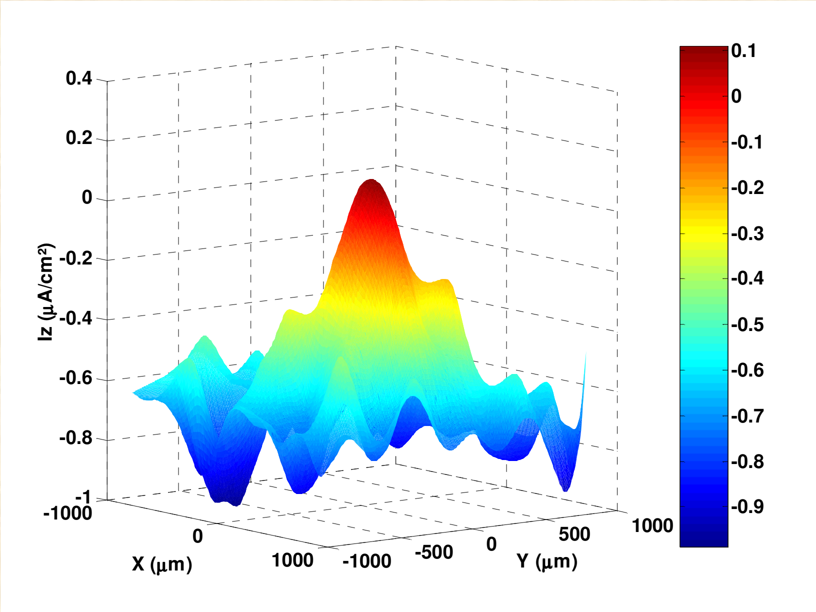


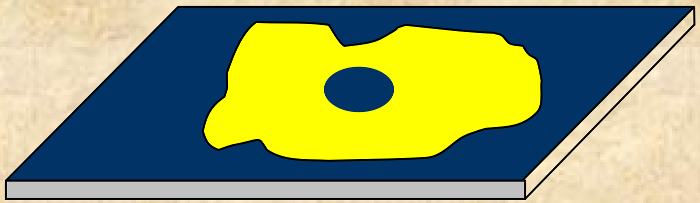
Porphyrin 1 central plot
-0.32 V/SCE

CNRS-LGC, H.Iken, unpublished

Hemin on GC electrode

SVET on glassy carbon electrode



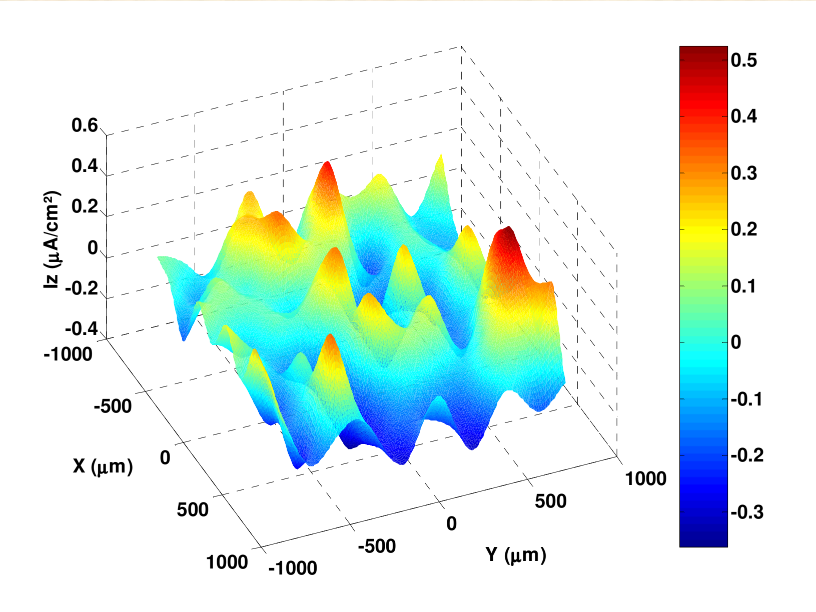


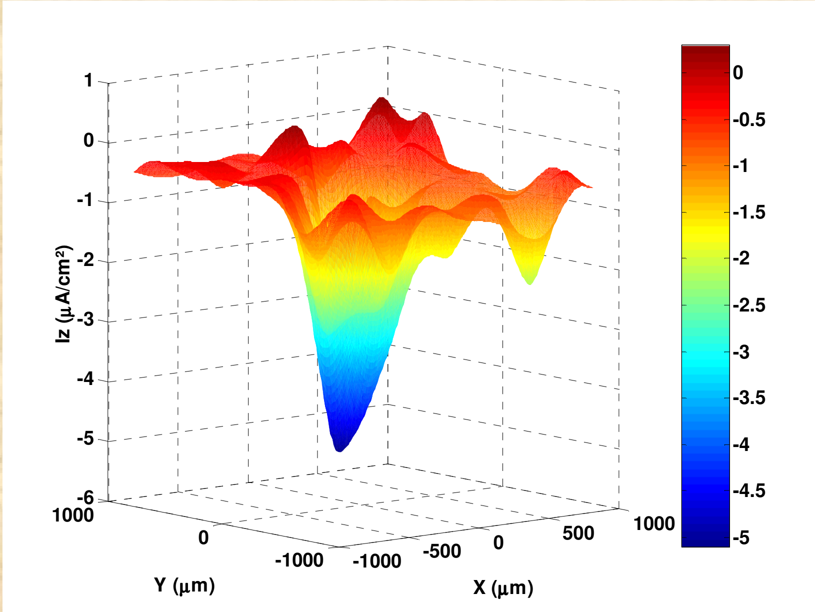
Porphyrin surrounding a clean central plot
-0.32 V/SCE

CNRS-LGC, H.Iken, unpublished

Hemin on stainless steel electrode

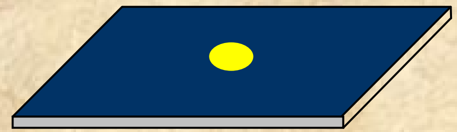
SVET on stainless steel URB66





without hemin, scale X 7

Porphyrin 1 central plot
-0.50 V/SCE



CNRS-LGC, H.Iken, unpublished

Aerobic corrosion: is the truth in the models?

✓ Powerful models able to reproduce biocorrosion in the lab (MOB, GOD, hopefully porphyrin…)

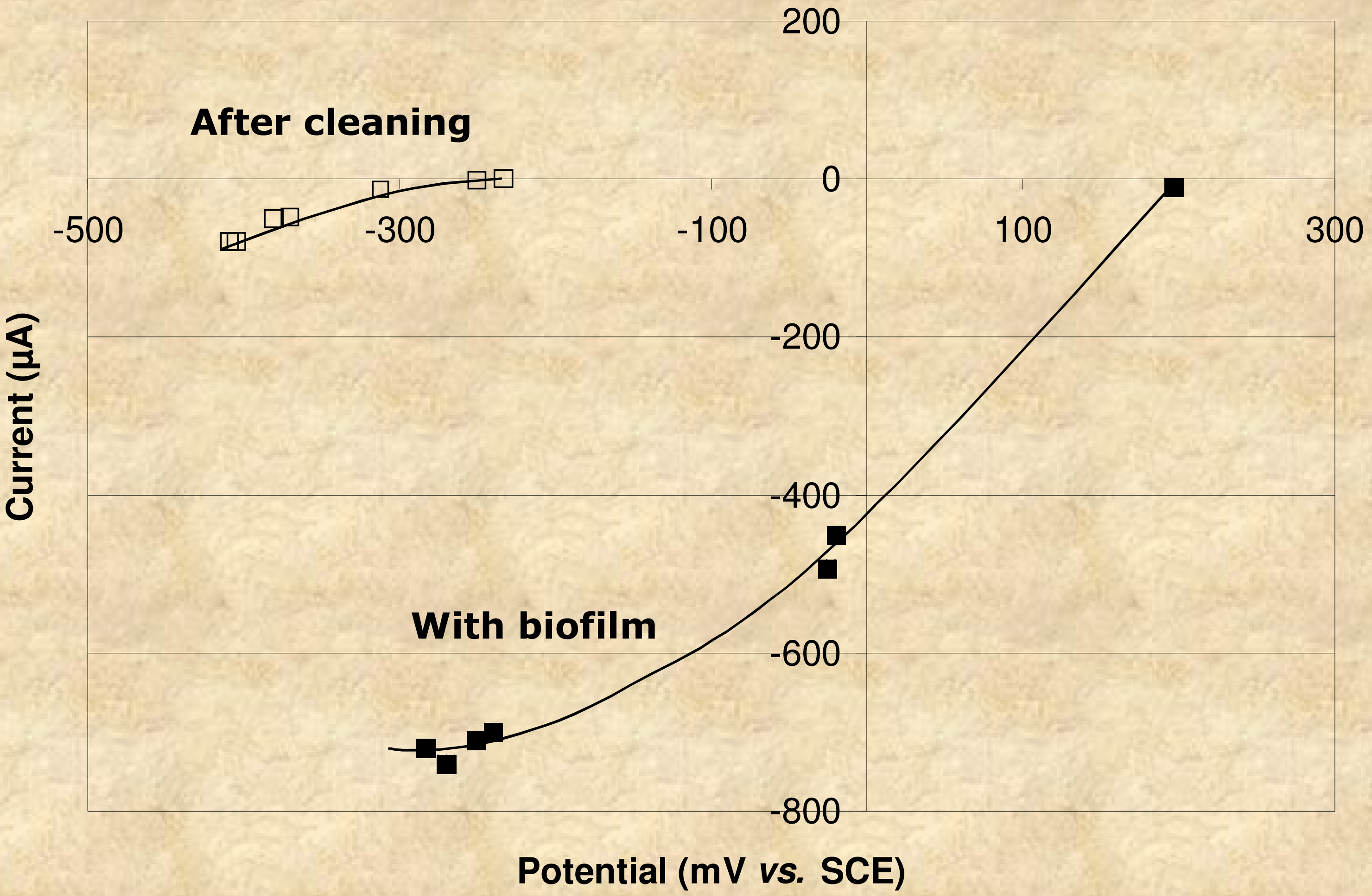
✓ Each model corresponds to particular natural conditions (high concentration of Mn ions, high concentration of substrate…)

✓ Each has a part of the "code"

➢ Even more inter-penetration of the thematic areas is still required…

➢ And more…

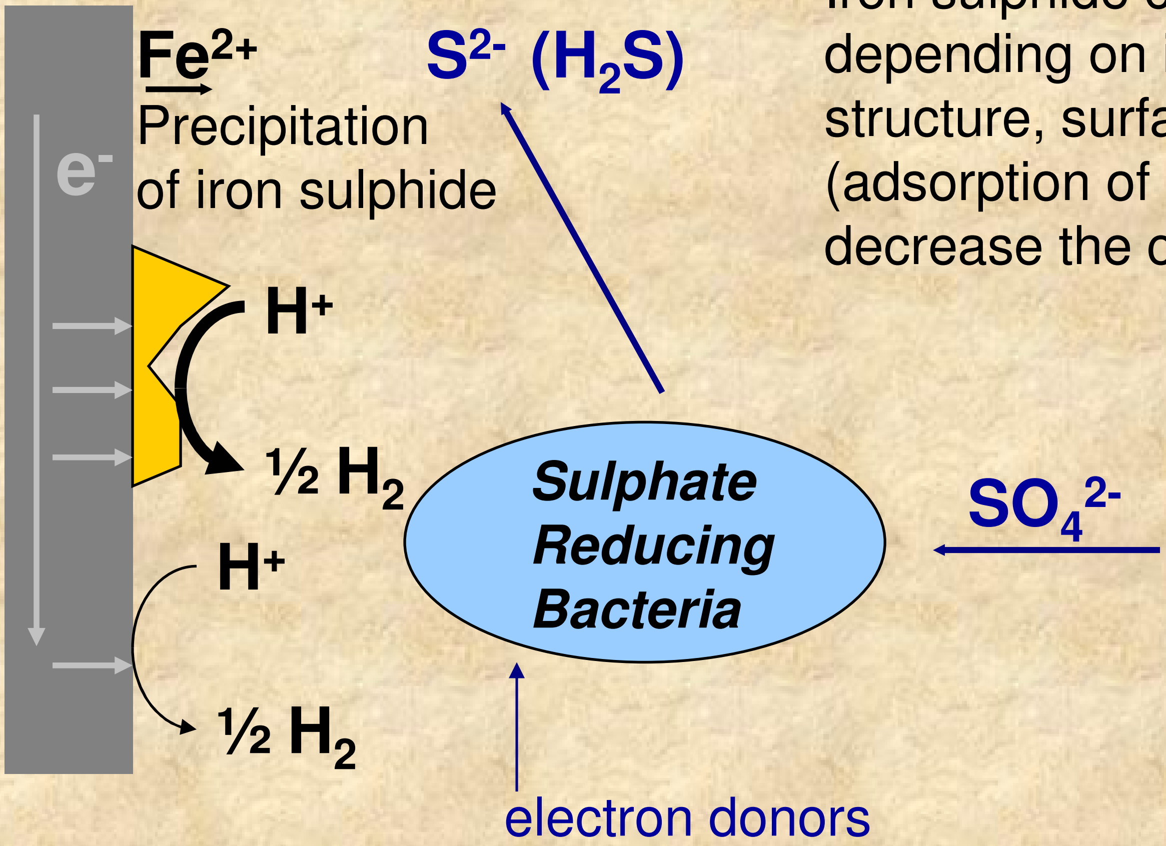
Whole marine biofilm on stainless steel



Stainless steel
URB66
Current density
up to 1.3 A/m^2

A.Bergel, D.Féron, A.Mollica, Electrochem.Comm. 2005

Anaerobic biocorrosion: basics



Iron sulphide catalyses proton reduction, depending on its composition, crystal structure, surface defects, age… (adsorption of atomic hydrogen may decrease the catalytic effect…)

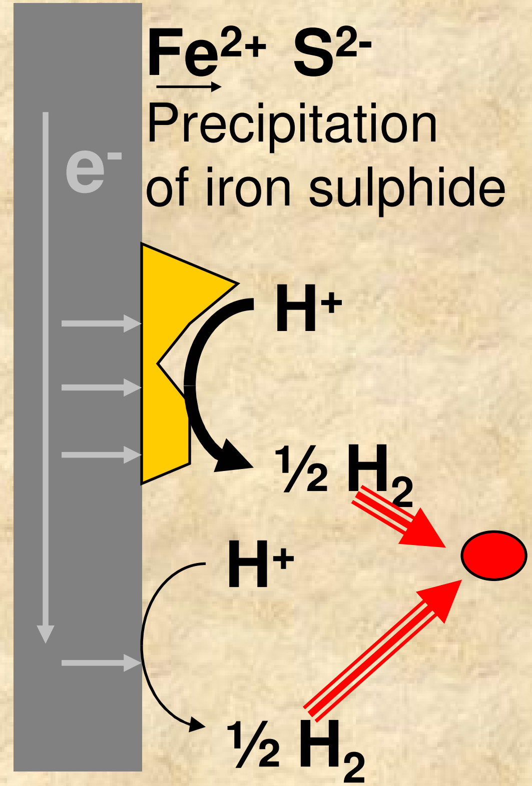
Anaerobic biocorrosion: basics

What may be the role of hydrogenase ? (if one?)

$$H_2 \Longleftrightarrow 2\,H^+ + 2\,e^-$$

- It has been claimed that the presence of HYDROGENASE may have a key role
- It has also been claimed that the presence or not of hydrogenase has no effect
- … but kits are commercialized to measure hydrogenase activity in order to assess corrosion risks

Hydrogenase: the wrong answer



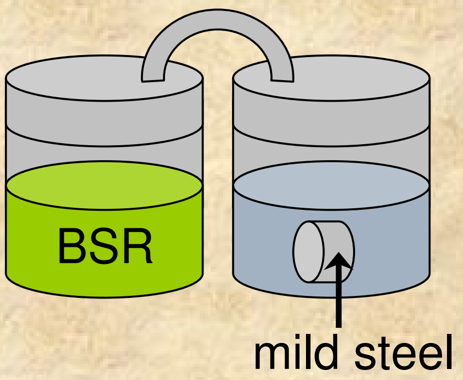
~~Consumption of H_2 shifts the equilibrium towards proton reduction~~

H_2 evolution is a non-reversible process on carbon steel

H_2 removal cannot be rate-limiting

But however experiments said

Rajagopal and Le Grall (1989)
Belay and Daniels (1990)



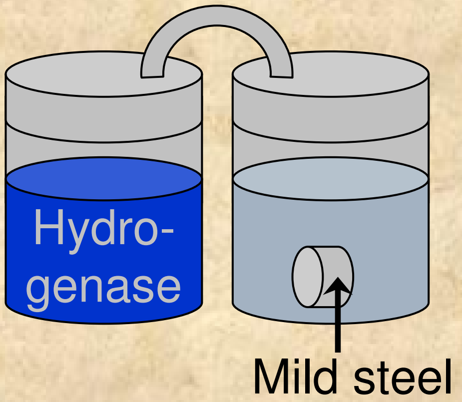
Corrosion increase

~~Intervention of FeS~~

Consumption of H_2

Displacement of the equilibrium of reduction de H^+ or H_2O?

Bryant et Laishley (1990)

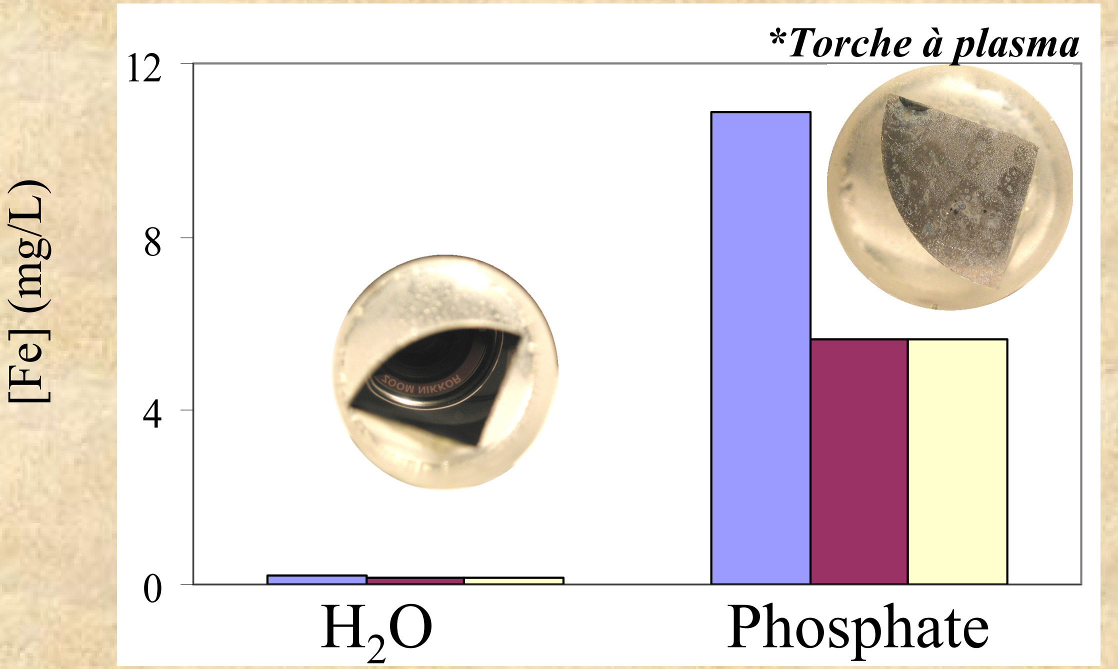


Corrosion increase + **Confirmation of consumption of H_2**

Only in the presence of phosphate

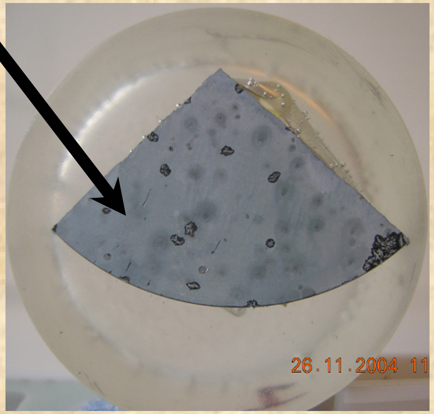
Phosphate role

[Iron] in solution



0,5 mM, 6 h of immersion

Vivianite



phosphate 150 mM
3h d'immersion

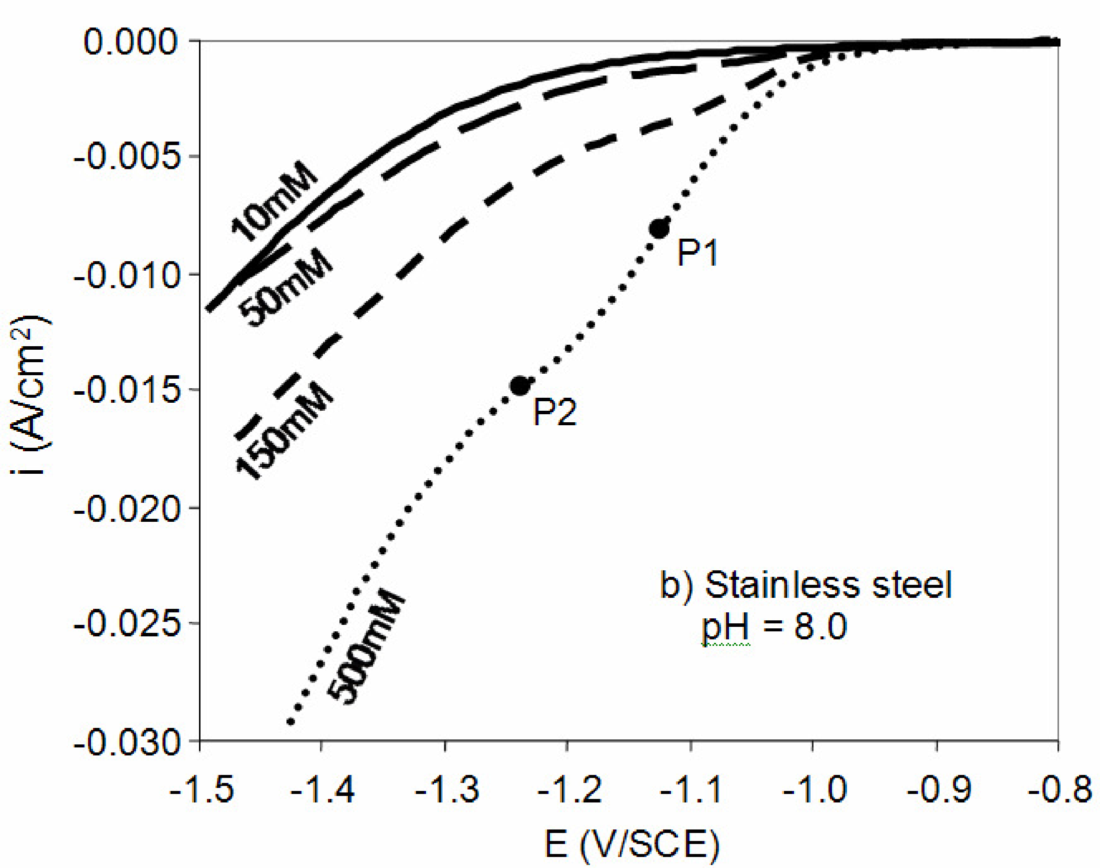
Mild steel corrosion in the presence of phosphate

*L.DeSilva-Munoz, **A.Bergel, R.Basseguy***

Corrosion Science 2007

Electro-deprotonation of phosphate

K-phosphate 10, 50, 150, 500 mM, pH 8.0 - RDE 2mm SS316L electrode

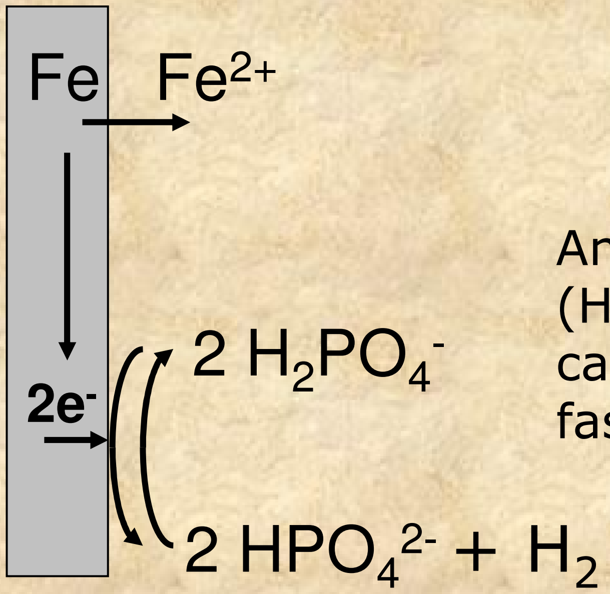


0.1M KCl, rotating speed 1000 rds/min, potential scan rate 20 mV s^{-1}. Preliminary 5-minute electrolysis at -0.50V/SCE before each scan.

L.DeSilva-Munoz, A.Bergel, R.Basseguy Corrosion Science 2007

Electro-deprotonation of phosphate

Cathodic deprotonation of phosphate species



Any cause of consumption of dihydrogen (Hydrogenase, SRB, others) can enhance the cathodic branch, as phosphate deprotonation is a fast reversible reaction.

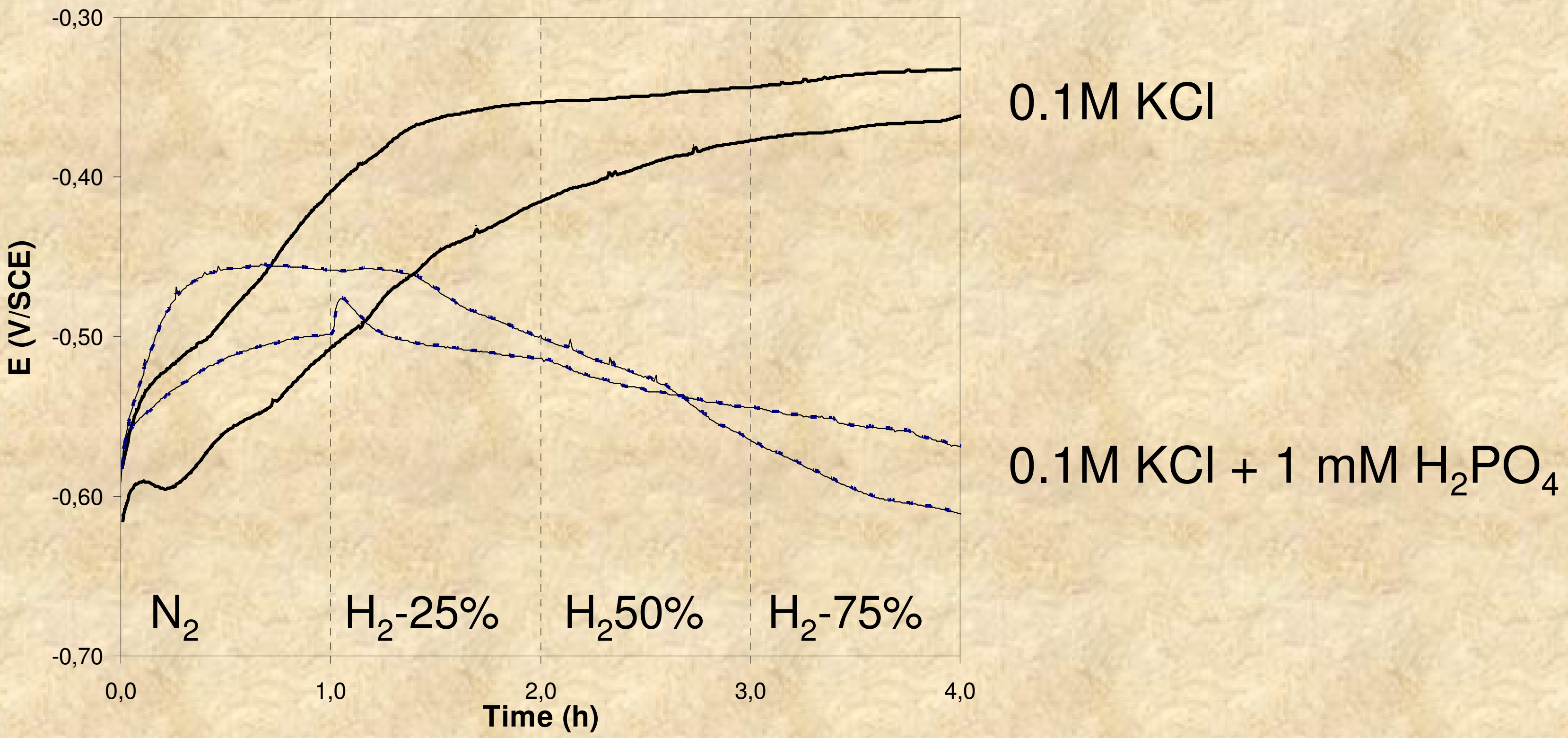
- ✓ Sole explanation of the Belays and Daniels 'two-bottles' experiment (90)
- ✓ Already suggested by Bryant and Laishley (90, 93)

L.DeSilva-Munoz, A.Bergel, R.Basseguy
Corrosion Science 2007

Electro-deprotonation of phosphate

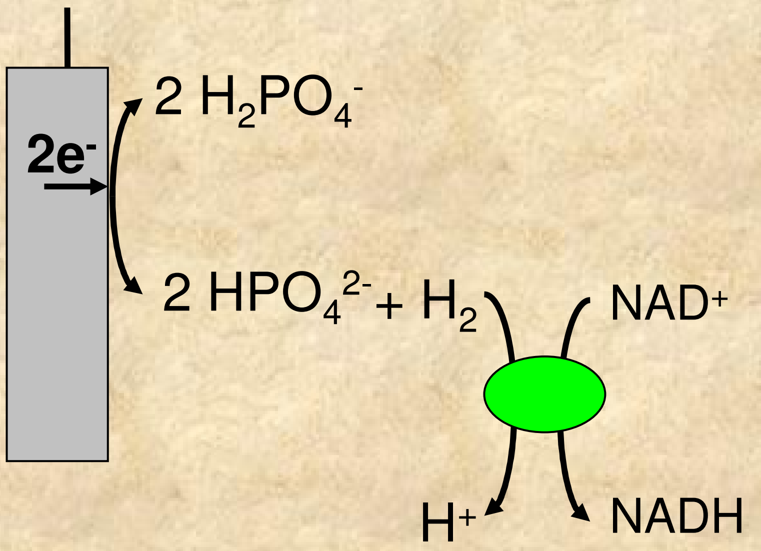
Looking for reversibility ?

SS 316L

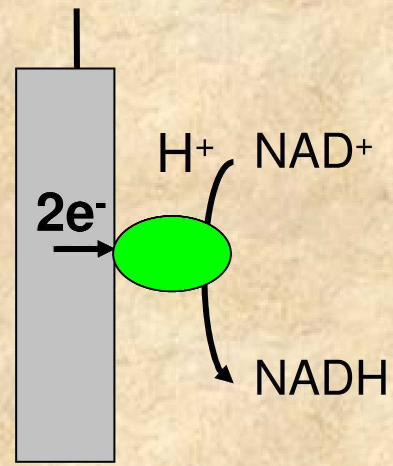


Carbon steel / phosphate / hydrogenase

What could be the cathodic reactions that enhance corrosion rate?



Electrochemical deprotonation of phosphate species



Direct electron transfer

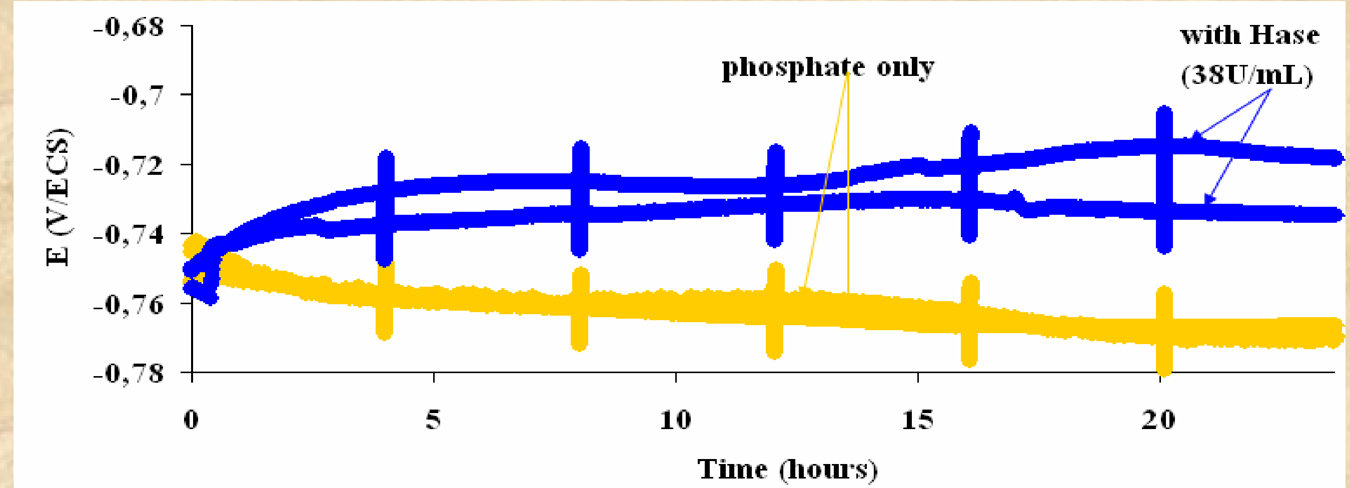
demonstrated on stainless steel
Recently direct oxidation of metallic powders with H_2 production (Zadvorny et al. 2006)

Hydrogenase from *R.eutropha*

CNRS-LGC, S.DaSilva JEAC 04, EA 04

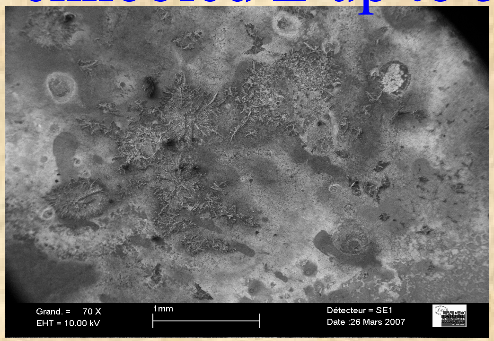
Another Hydrogenase /phosphate
from *Clostridium acetobutylicum*

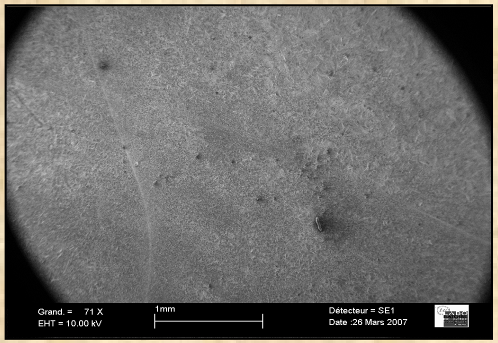
E = f(t) Phosphate 100mM, pH 7.2 with or without Hase



Mild steel
XC45

Hase ennobled E up to 50 mV



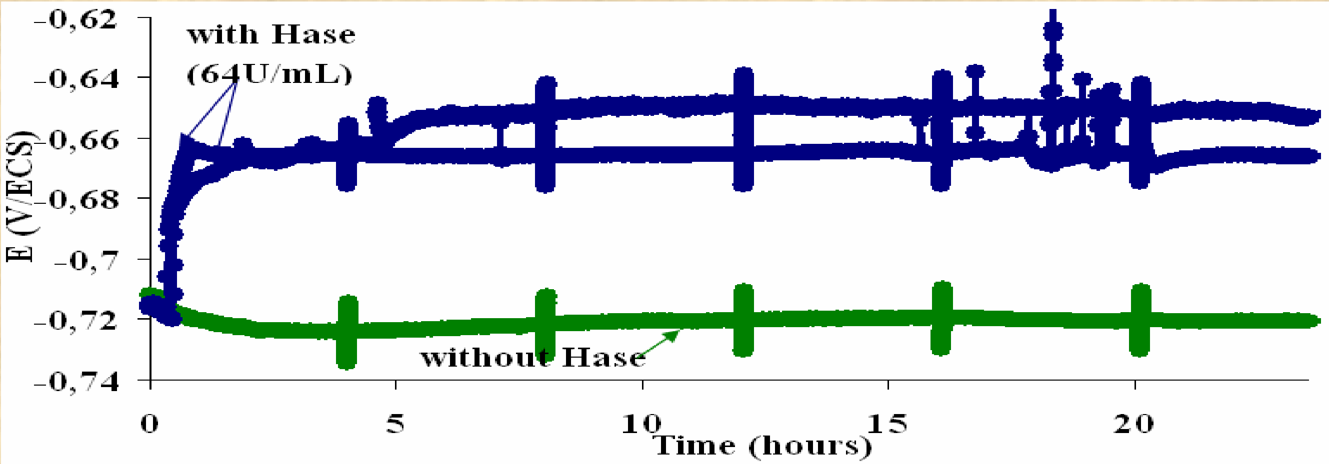


SEM **with (38U/mL) Hase** **without Hase**

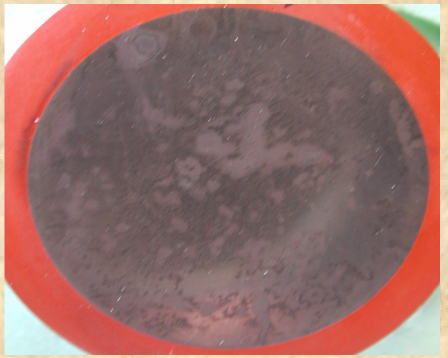
CNRS-LGC, M.Mehanna, unpublished

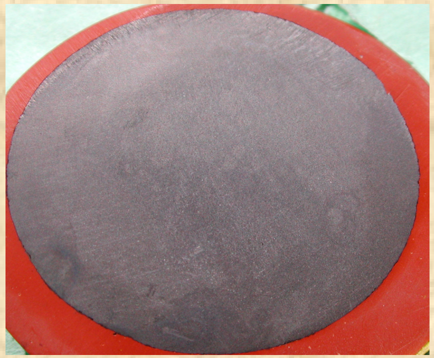
The same enzyme without phosphate

E = f(t) Tris-HCl 50mM, pH 6.3 with or without Hase



Hase ennobled E up to 70 mV



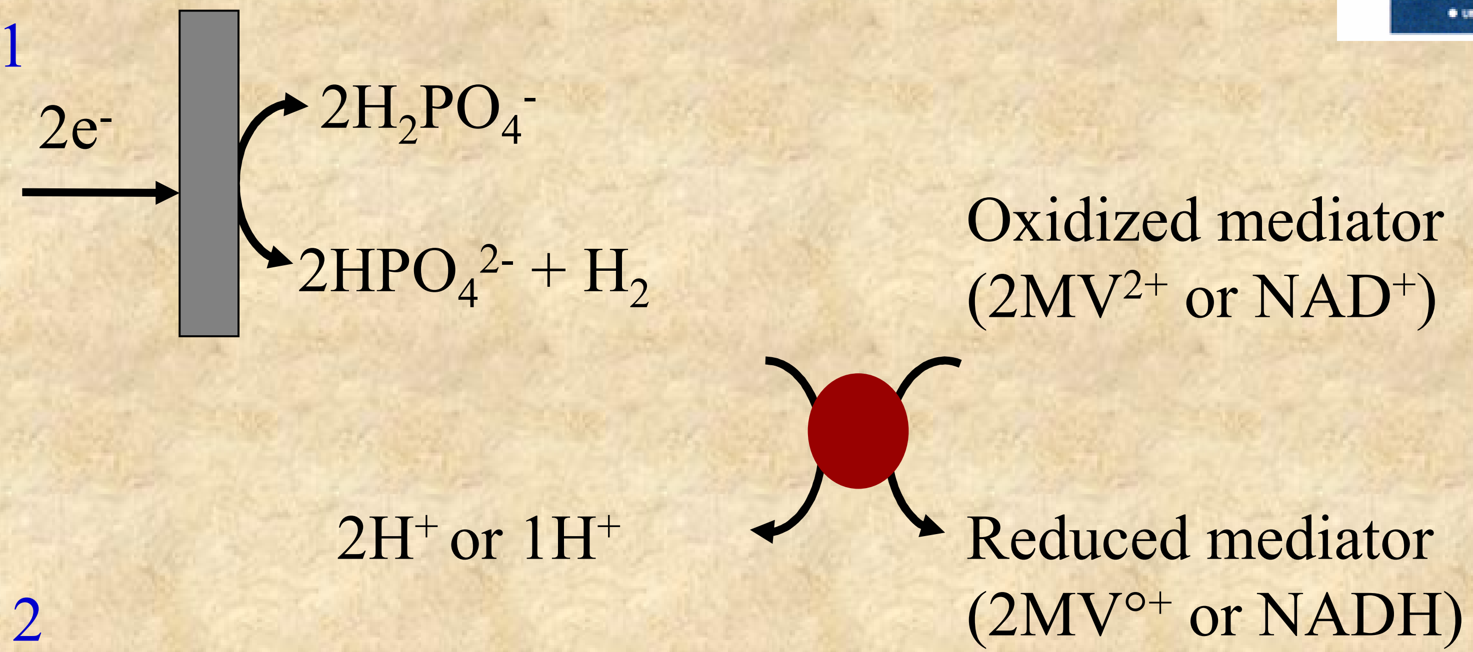


SEM **with (64U/mL) Hase** **without Hase**

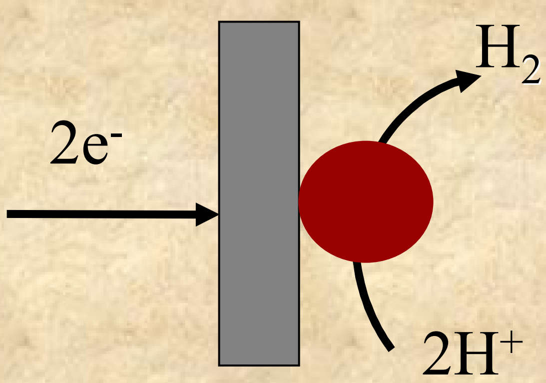
CNRS-LGC, M.Mehanna, unpublished

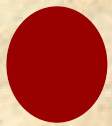
Possible direct catalysis of cathode reactions by Hase

Mechanism 1



Mechanism 2





Hydrogenase from
***C.** acetobutylicum*

Trends in anaerobic biocorrosion

? Revisiting the previous lab studies taking into account possible involvement of phosphate species (common buffer)

? Towards the end of the domination of the FeS hypothesis?

YES
- SRB corrosive activity has been detected before significant sulfide production (*D.capillatus*, from oil field separator Mexico, Miranda et al. Corr. Sci. 2005)
- Direct electron transfer from iron to *Desulfobacterium*-like (Dinh et al. Nature 04)
- Direct electron transfer of different free hydrogenases
- Electro-Deprotonation of weak acids present in biofilm (LGC-CNRS De Silva under preparation)

NO
- Switching SRB from sulfate metabolism to nitrate metabolism stopped corrosion

Thank you for your attention

Nothing had been possible without

Late Dr.Vittoria SCOTTO and Dr.Alfonso MOLLICA,
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CEA-Saclay, France

My colleagues

Alain Bergel, Marie-Line DELIA, Luc ETCHEVERRY

Previous students	Dr.Maria Elena LAI
	Dr.Serge DA SILVA
	Dr.Jonathan IDRAC
PhD students	Leonardo DE SILVA
	Maha MEHANNA