Terry Beveridge Memorial Lecture

Geochemical Reactivity of Bacterial Cell Surfaces

F.G. Ferris
Alumnus (Ph.D. 1985)
Department of Microbiology
University of Guelph
Supervised by TJB

“Nature Never Jests”

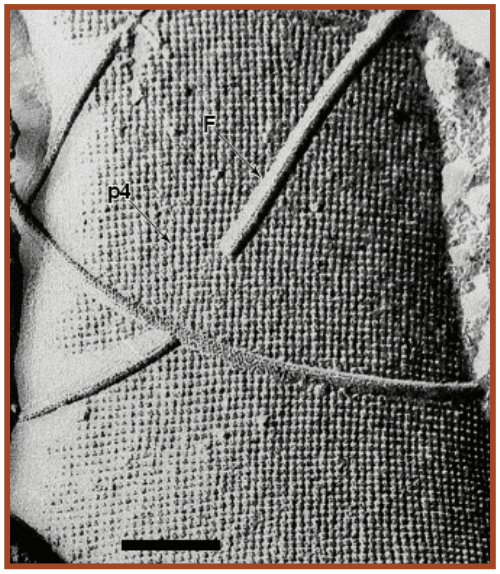
Victor Albrecht von Haller 1708–77 *Swiss physiologist and poet*
Réflexions sur le systême de la génération de M. de Buffon (1751)

The heritage and legacy of TJB

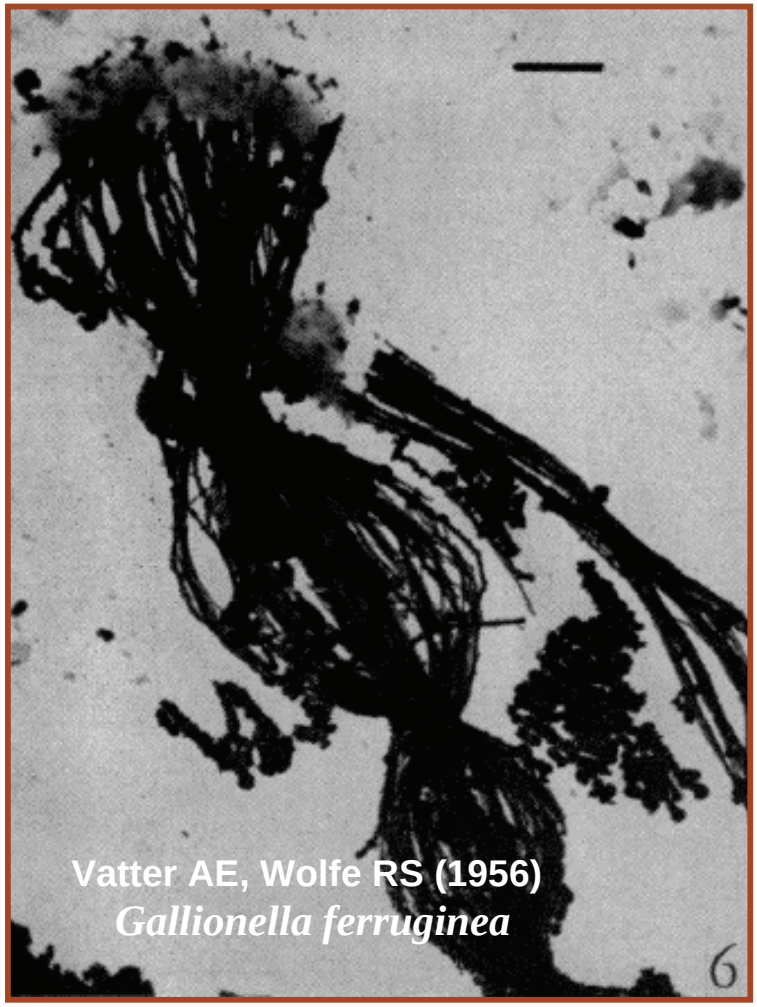
(amidst pioneering studies of bacterial ultrastructure)

Established that bacteria behave as Geochemically Reactive Solids – inspired a new field of research

- distinguished mentorship
- fundamentals
  - discovery of bacterial cell envelopes
- revelations
  - bacterial cell wall reactivity
- advances
  - sorption, precipitation, dissolution

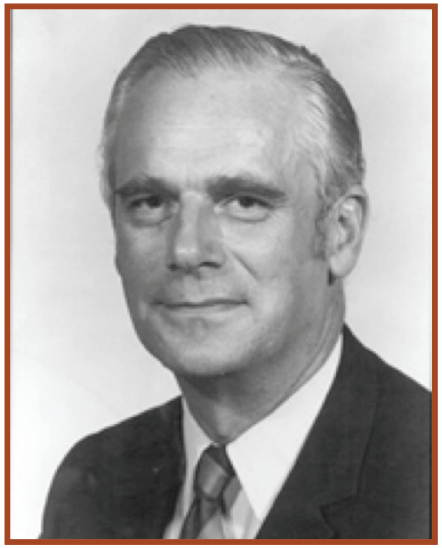


Caveat Emptor

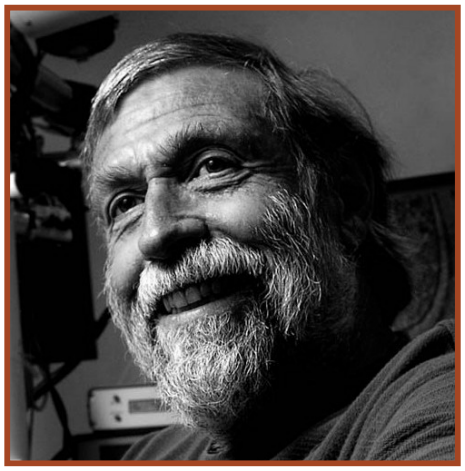


- Perspective based on my experiences
- Narrow in scope, especially considering the breadth of geomicrobiology and geochemistry
- A complete historical background of bacterial-mineral interactions is not provided, for example:
  - Gallionella first described by Ehrenberg in 1836 – proposed that iron bacteria contributed to formation of sedimentary iron ore deposits

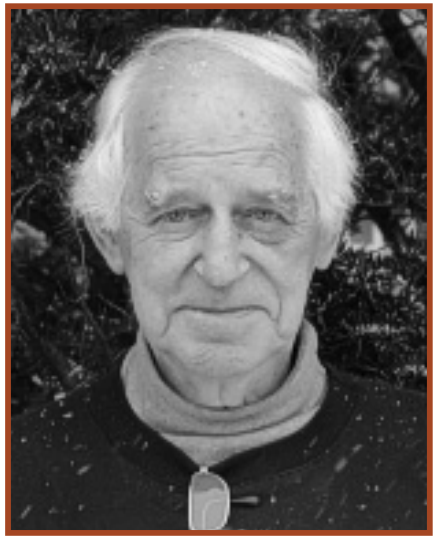
Inspiration from Extraordinary People



R.G.E. Murray
M.D.C.M., D.Sc., F.R.S.C.
Order of Canada



B.Sc. Thesis 1979 -1980
Lab Technician 1980 -1981
Ph.D. student 1981 - 1985



W.S. Fyfe
Ph.D., D.Sc., F.R.S.C.
Order of Canada

Breaking the "resolution barrier" of light microscopy

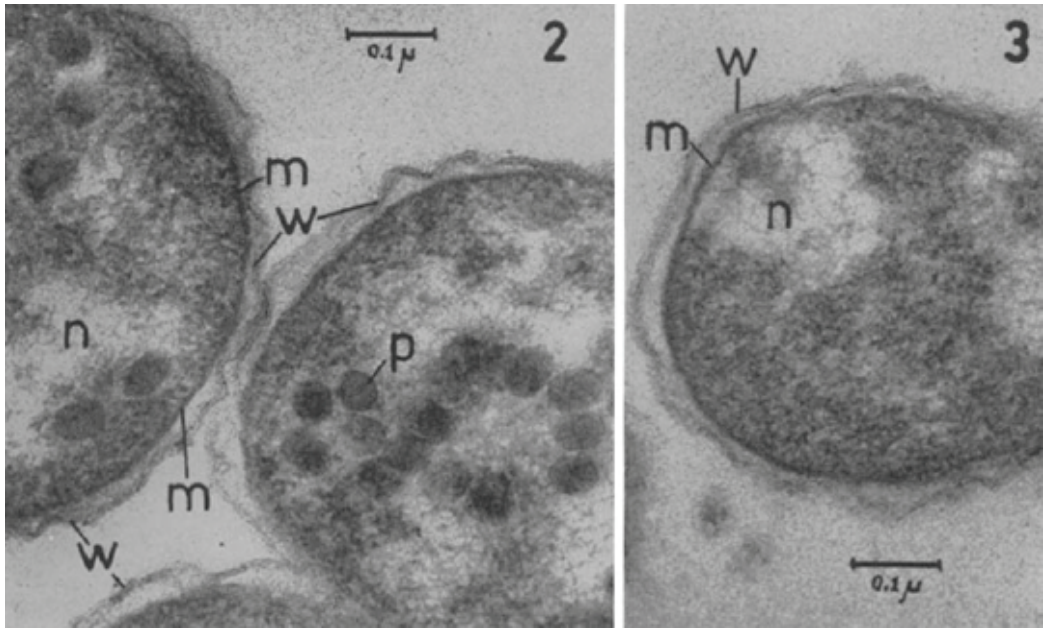
"what goes around comes around"

Electron micrograph of *Bacillus cereus* subjected to ether-induced cytolysis (done at Cambridge, UK).
Robinow CF, Murray RGE (1953)
***Exp. Cell. Res.* 4: 390-407**

- Wavelength of visible light limits resolution to ~ 0.2 μm; higher resolution is achieved using an electron beam with a far smaller wavelength in electron microscopes
- First practical electron microscope developed by E.F. Burton at the University of Toronto (Department of Physics) in late 1930's
  - studied colloids – "nanoparticles"
- First electron microscope acquired by RGE Murray at the University of Western Ontario in 1954 (Murray 1988. Ann.Rev. Microbiol. 42: 1-34)
  - Recognition of cell wall and cytoplasmic membrane
  - TJB - U of T alumnus / Ph.D. UWO
  - FGF - postdoc at UWO / prof U of T

“Cutting” to the Chase

Thin-sections and heavy metal staining



Kellenberger E, Ryter A. (1958)

Cell wall and cytoplasmic membrane of *Escherichia coli*

J. Biophys. Biochem. Cytol. 4: 323-326.

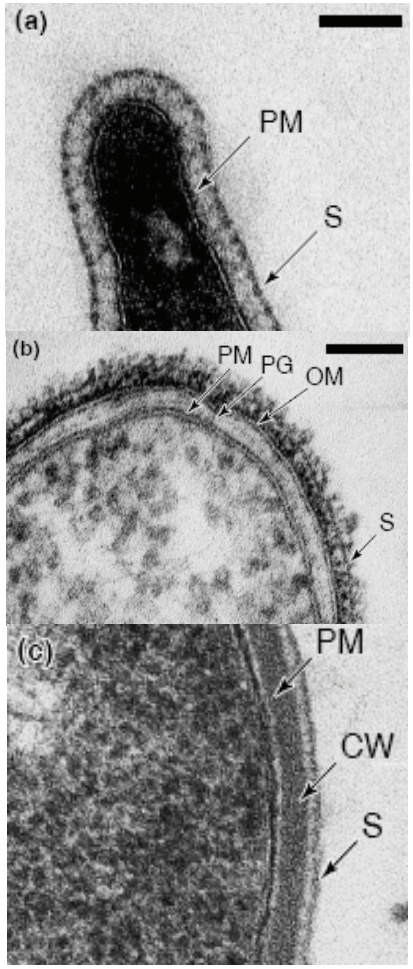
- Eduard Kellenberger at the Biozentrum, University of Basel, Switzerland
  - Developed classic method of osmium tetroxide fixation with uranyl acetate staining and thin-sectioning
- Revealed fine ultrastructural detail of bacterial cells for the first time
  - Improved electron contrast

Undressing Bacterial Cells

The haut couture of bacteriology

- Improved embedding resins, fixation (e.g., glutaraldehyde), and staining techniques (e.g., lead citrate for thin-sections, negative staining of whole mounts) were developed over the late 1950s and through the 1960s
- Biochemical analyses of cell envelope components continued apace
- Together established the composition and location of cell envelop components

Sleytr UB, Beveridge TJ (1999) Trends Microbiol. 258 (6)



Archeon

Sulfolobus acidocaldarius

Gram-negative

Aeromonas salmonicida

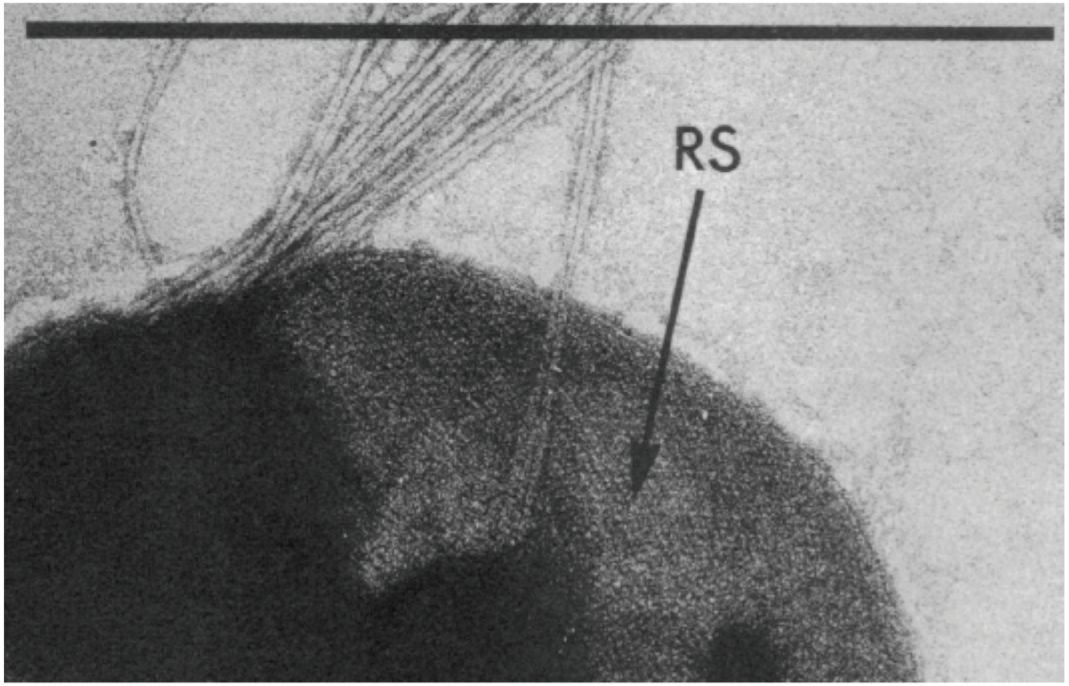
Gram-positve

Bacillus thuringiensis

bars = 50 nm

Order in Chaos

The intrigue of proteinaceous S-layers



Negative stain of *Aquaspirillum putridichonchylium*

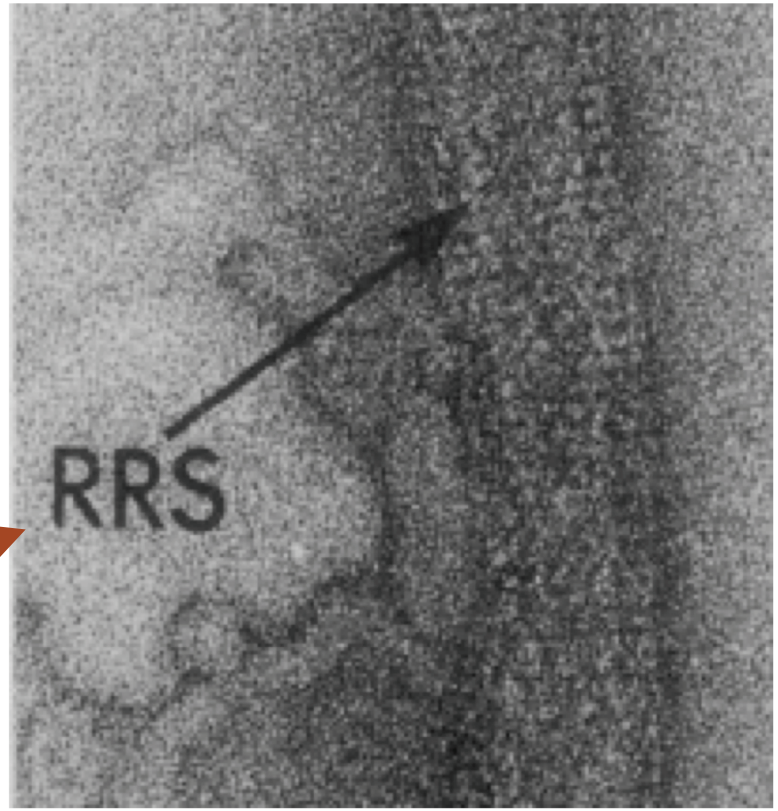
Beveridge TJ, Murray RGE (1974) J. Bacteriol. 119: 1019-1038

- Using negative staining, RGE Murray discovered structured surface-layers on a Deinococcous sp. (1958), and on a wide variety of other bacteria
- *"It was obvious that these arrays, or S-layers as they are now termed, were a common feature of bacteria in nature … They needed serious study …"*

Murray 1988. Ann.Rev. Microbiol. 42: 1-34

In Vitro Assembly of S-layers
The inquisition begins

- Murray's group isolates S-layer proteins from *A. putridichonchylium (TJB)* and *A. serpens* (Francis Buckmire)
  - discovered that they self-assembled on isolated outer membrane in the presence of Ca^{2+}, but stripped-off by Na^{+}
    - Beveridge TJ, Murray RGE (1976) J. Ultrastruct. Res. 55: 105-118
    - Buckmire F, Murray RGE (1976) J. Bacteriol. 125: 290-299



Lessons from Electron Microscopy and S-layers

One plus two equals four (curious math of TJB)

- *One* – enhanced electron contrast is provided by heavy metals

+

- *Two* – divalent metals like Ca^{2+} are essential for the maintenance of bacterial cell envelope structure

=

  - Metal ions bind to specific macromolecular constituents in bacterial cell envelopes
  - The amount of metal that is bound can be quantified
  - Sites of metal ion binding can be identified
  - Binding of different metal ions to cell envelopes can be distinguished by TEM (owing to atomic number *z*-contrast)

Reactivity of Bacterial Cell Envelopes

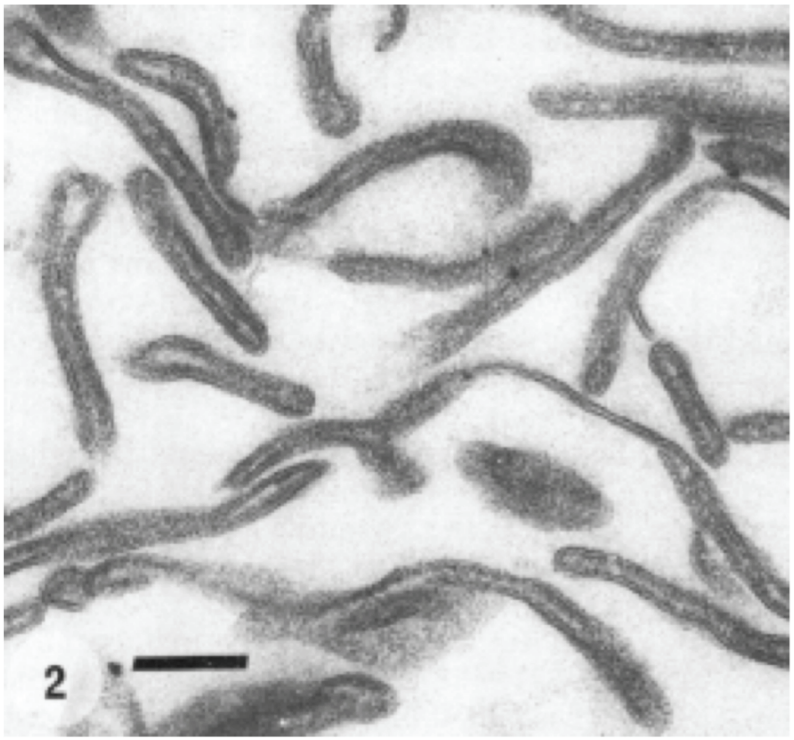
Pioneering developments

Beveridge TJ, Murray RGE (1976) Uptake and retention of metals by cell walls of Bacillus subtilis. J. Bacteriol. 127: 1502-1518.

Beveridge TJ, Murray RGE (1980) Sites of metal deposition in the cell wall of Bacillus subtilis. J. Bacteriol. 141: 876-887.

Beveridge TJ, Koval SF (1981) Binding of metals to cell envelopes of Escherichia coli K-12. *Appl. Environ. Microbiol.* 42: 325-335.

(opened a can of worms)



Connecting to Geochemistry

At the Faculty Club over some beers?

Murray comments (**1988. Ann.Rev. Microbiol. 42: 1-34)**

> "When T.J. Beveridge was working with me on his doctorate … we had many an occasion to discuss revealing substructure by staining with metal salts … But our geologist colleague, Professor W.S. Fyfe was more interested in why many ore bodies have a high percentage of organic residues and particular selections of metals."

The result:

> Beveridge TJ, Meloche JD, Fyfe WS, Murray RGE (1983) Diagenesis of metals chemically complexed to bacteria: laboratory formation of metal phosphate, sulfide, and organic condensates in artificial sediments. Applied Environ. Microbiol. 45: 1094-1108.

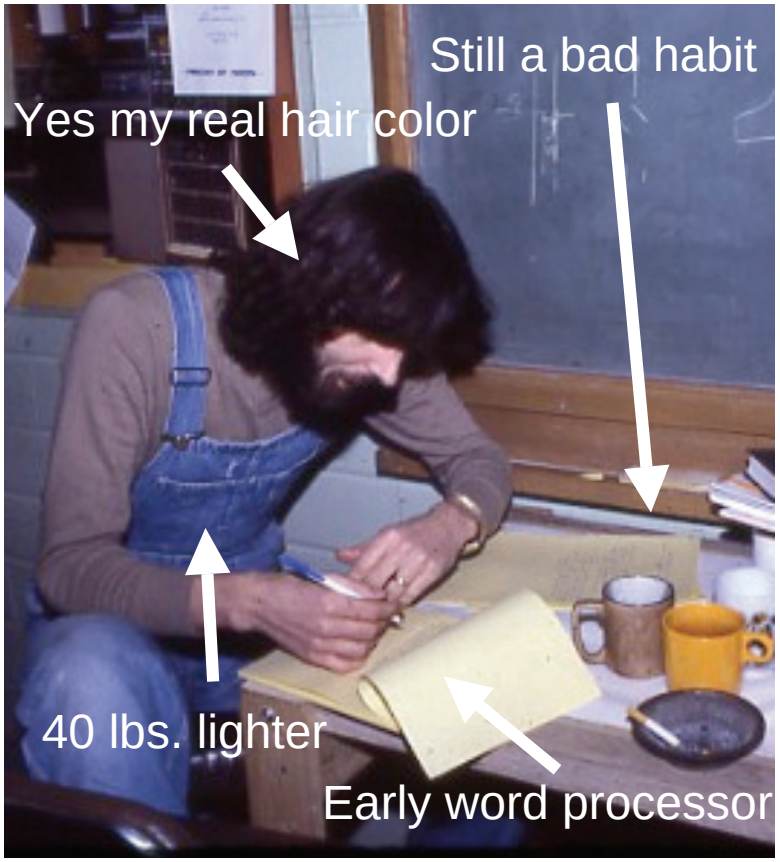
The Beveridge Laboratory – Early Days

Meanwhile in the depths of the Chemistry & Microbiology Building, University of Guelph (now destroyed)

- Into this fertile mix arrives a wide-eyed, naive, freshly-minted B.Sc. in microbiology (1980, that would be me)
- Joined Brian Hoyle (TJB's first grad student) and Magda (our technician) in a "lab" comprised of a sink with a small island bench surrounded by six walk-in incubators (and overhead pipes that tended to leak) – Philips EM 300 (workhorse)
- Started to work along with Brian on Terry's can of worms … trying to figure out sites of metal deposition in the cell wall of (Gram-negative) *E. coli* K-12 (strain AB264)
  - Brian (now a freelance science and technology writer) – quantification of metal ion binding to the outer membrane and petidoglycan (in comparison to the envelopes of Beveridge and Koval, PM/PG/OM)
  - Grant – identification of metal binding sites in the outer membrane (a new challenge in the form of physical membrane biochemistry)

The Gram-negative Story

A new wave of discovery inspired by TJB



- Peptidoglycan sacculus and outer membrane (of *E. coli*) implicated in the binding of metallic ions (Brian)
- Phosphoryl groups of outer membrane lipopolysaccahride (LPS) implicated as sites of metal sorption from ^{31}P-NMR studies (Grant)
- Established the importance of Ca^{2+} (and to a lesser extent Mg^{2+}) for the structural integrity of the outer membrane from EDTA extraction of LPS (Grant)

Diagenesis ? (had to look it up)

TJB introduces WSF to FGF – Postdoc gone wild

- To paraphrase Terry's mentor R.G.E. Murray

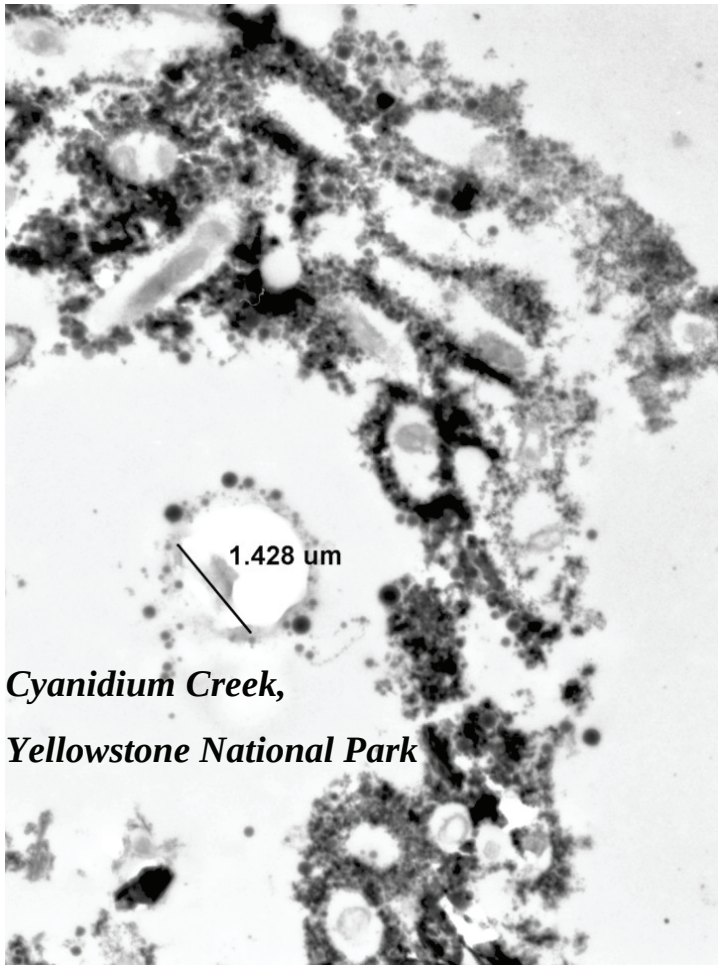
  "When I was working with Terry on my doctorate we had many an occasion to discuss bacterial interactions with metallic ions … but a geologist colleague of Terry and R.G.E. Murray, Professor W.S. Fyfe was more interested in bacterial mineral formation."

- Postdoctoral studies turned into an avalanche of different revelations
  - NASA Planetary Biology Internship with Ken Nealson at Scripps (thermodynamics is not just a good idea … it's the law)
  - NSERC PDF with W.S. Fyfe
  - Henry Ehrlich (RPI) and Bill Ghiorse (Cornell) (Iron and manganese oxidizing bacteria, Joint-Chief Editors, Geomicrobiology Journal)
  - Rolf Hallberg (Professor, University of Stockholm, sulfate-reducing bacteria), Jacques Berthelin (Professor, CNRS Nancy, France, soil microbiology), Yuri Gorby (grad student, magnetotactic bacteria), Joel Thompson (grad student, microbial carbonate precipitation)

Sorption to Mineral Precipitation

A controversial hypothesis even to this day

- Breaking the rules … thin-sectioning of natural sediments with a (second-hand) diamond knife
  - Ferris FG, Beveridge TJ, Fyfe WS, (1986) Iron-silica crystallite nucleation by bacteria in a geothermal sediment. Nature 320: 609-611.
  - Ferris FG, Fyfe WS, Beveridge TJ, (1987) Bacteria as nucleation sites for authigenic minerals in a metal contaminated lake sediment. Chem. Geol. 63: 225-232.

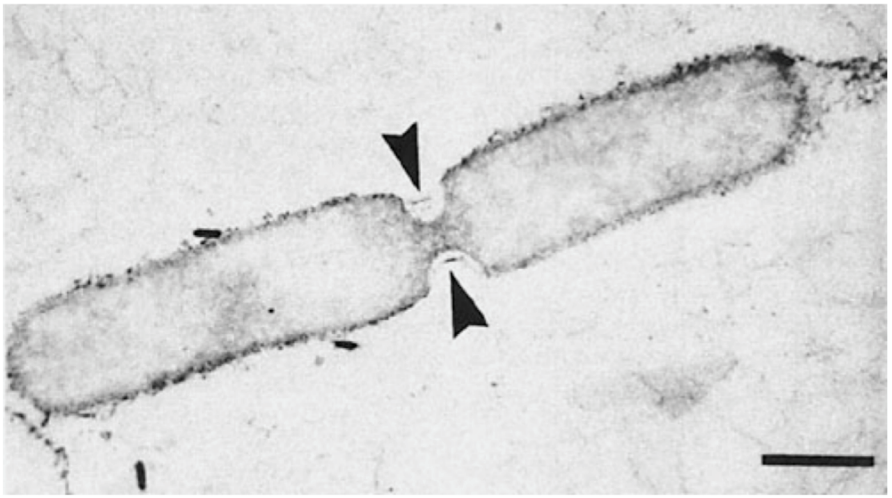


Cyanidium Creek,

Yellowstone National Park

Silicification of Bacteria

- Experimental studies using *B. subtilis*
  - rapid (days at *SI* ~ 10)
  - presence of Fe^{3+} inhibited cell autolysis; ensured preservation of structurally intact cells
  - Good model for siliceous sinters and well preserved bacterial microfossils in ancient cherts

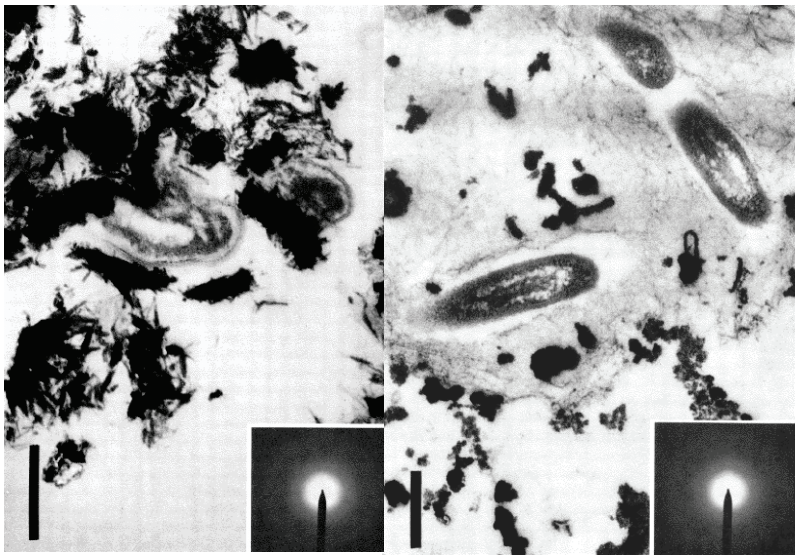


Ferris FG, Fyfe WS, Beveridge TJ (1988) Metallic ion binding by *Bacillus subtilis*: Implications for the fossilization of microorganisms. Geology 16: 149-152.

***B. subtilis* at 90 days bar = 500 nm**

Metallic Ion Sorption by Bacteria in Nature

Getting wet feet … still an uncommon undertaking



- Ferris FG, Schultze (Douglas) S, Witten TC, Fyfe WS, Beveridge TJ, (1989) Metal interactions with microbial biofilms in acidic and neutral pH environments. Applied Environ. Microbiol. 55: 1249-1257.
  - Improvised biofilm substratum using coat hangers, epoxy empregnated filter paper, and 35 mm slide mounts
  - Suspended in natural and acid mine drainage-contaminated lakes (Sudbury, Ontario, Canada)
  - Observed Fe, Mn, Ni, Cu, Co accumulation in excess of controls (0.22 μm membrane filter covered substratum)
  - Fe accumulation = mineral precipitation (neutral pH, 2-line ferrihydrite; acidic pH, jarosite or Schwertmannite) – probably metals too!

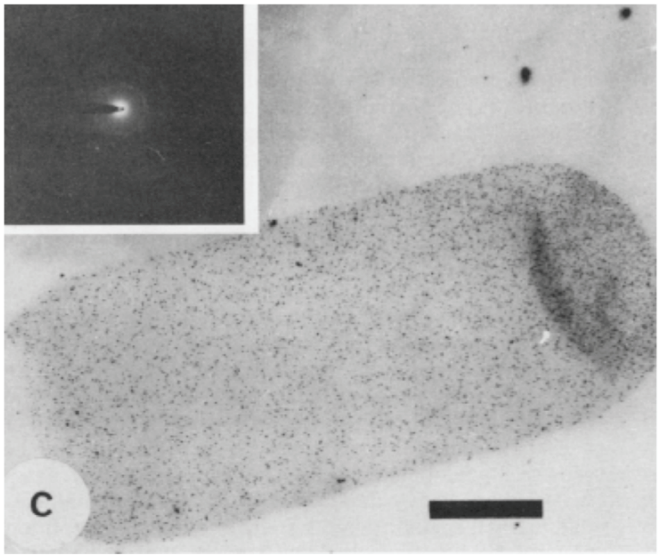
The Beveridge Laboratory – Takes Flight

FGF off to the oil patch (1988)

- Metal/mineral interactions with bacterial walls (other than *E.coli* and *B. subtilis*)
- S-layers (always!)
- Interaction of antibiotics with bacteria
- Freeze-substitution as a new way to study ultrastructure

Walker SG, Flemming CA, Ferris FG, Beveridge TJ , Bailey GW (1989) Appl. Environ. Microbiol. 55: 2976-2984

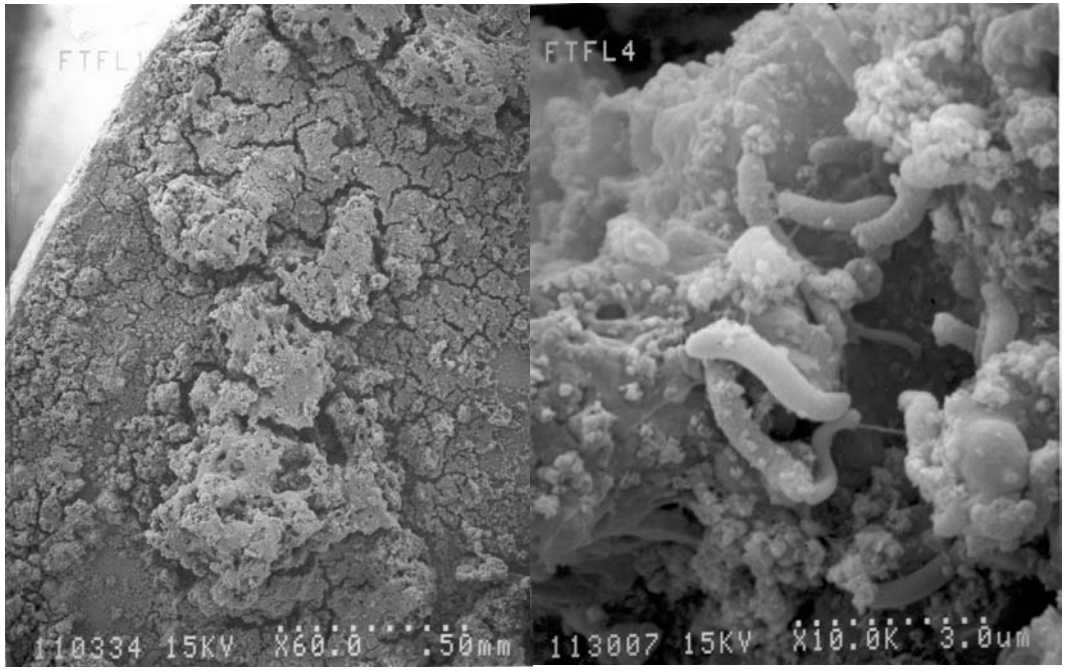
Precipitation of elemental Ag on isolated B. subtilis walls in a solution of AgCl … still a very curious phenomenon as source of reducing equivalents has never been identified (happens with Au too)



Anaerobic Corrosion of Steel

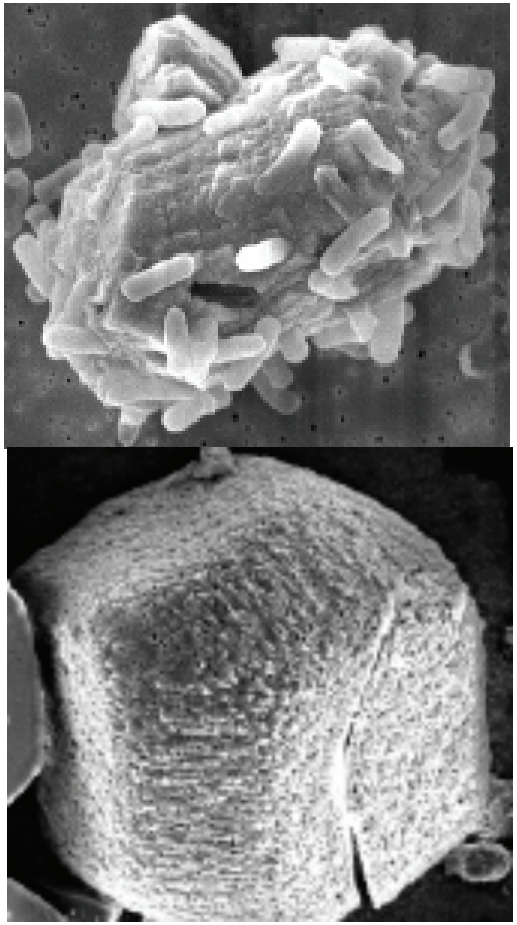
Major liability in oil and gas production

- Biofilms of sulfate-reducing bacteria in FeS corrosion product
- Intact bacteria only on outside (from thin-sections cut perpendicular to steel surface)
- Cathodic depolarization theory of corrosion
  - requires that FeS behaves as semiconductor
  - bacteria utilize cathodic hydrogen that forms in outer region of the FeS corrosion product



Microbially Enhanced Oil Recovery

An alternative to biomass plugging

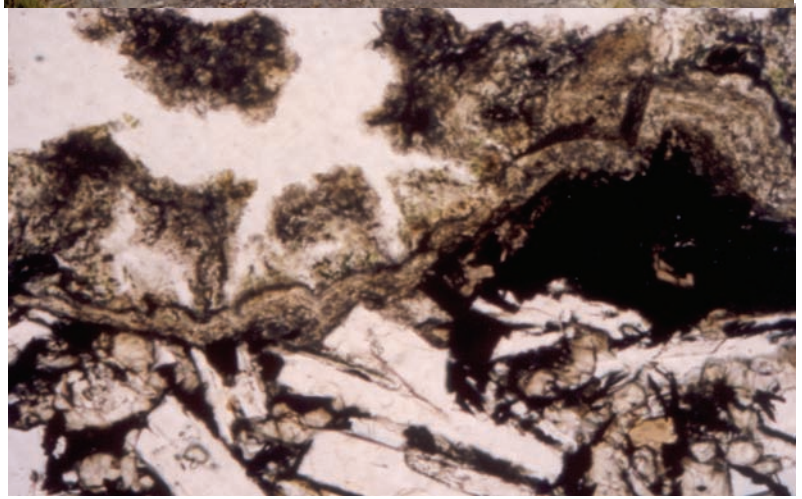


- Concept – use bacteria to precipitate calcium carbonate as a cementing/plugging agent to improve sweep efficiency of water flooding
- Used ureolytic bacteria to increase pH and alkalinity, thereby inducing carbonate precipitation
- Ferris FG, Stehmeier LG (1992) Bacteriogenic mineral plugging. U.S. Patent No. 5,143,155

Carbonates and Silica

Join U of T (1991) – working again with TJB / WSF

- Silicification of bacteria in Iceland hot springs
- Cyanobacterial formation of carbonate crusts on weathering basalt
- Accretion of cyanobacterial carbonate microbialites



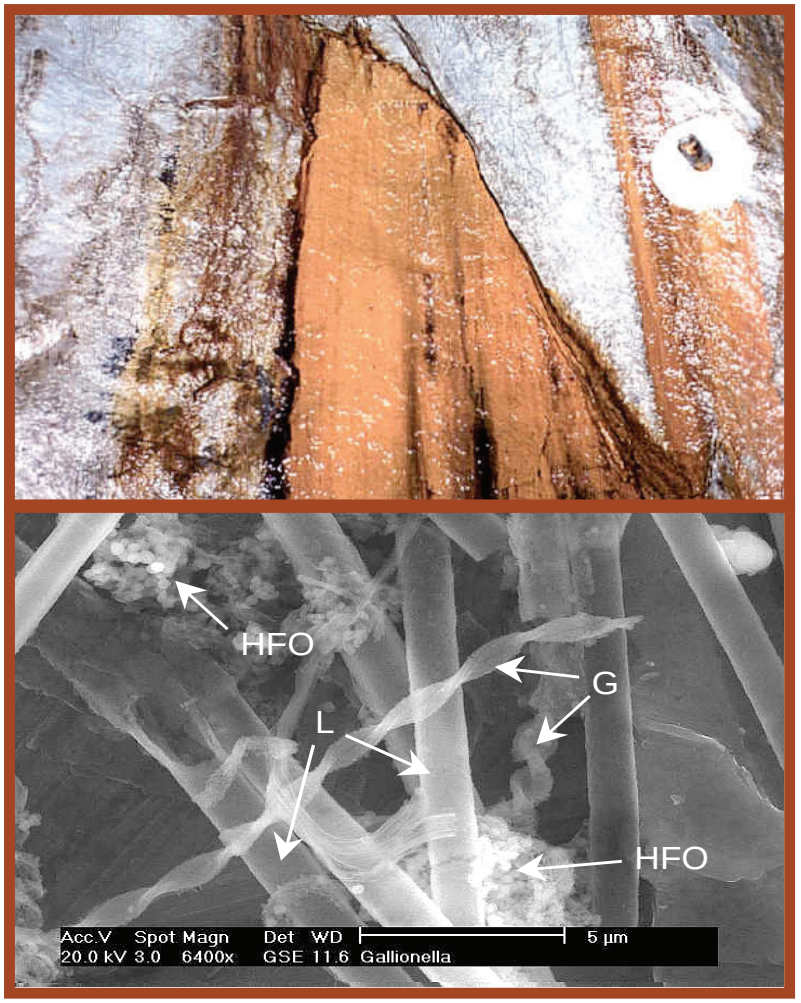
Origin of the Strontium Carbonate Concept for Bioremediation

Ferris FG, Fratton CM, Gerits JP, Schultze-Lam S, Sherwood Lollar B (1995) Microbial precipitation of a strontium carbonate phase at a groundwater discharge zone near Rock Creek, British Columbia, Canada. Geomicrobiol. J. 13: 57-67

- Discovered a strontium rich carbonate crust that was formed by cyanobacteria growing at a groundwater discharge zone
- Curiosity at the time (still focused on microbialites)
  - Suggested that Sr^{2+} could be captured as a result of solid-solution formation
  - Inspired later work for DOE (EMSP) with Eric Roden (then at Alabama) and Bob Smith (then at INEEL)

Bacterial Cell Surface Reactivity and Iron Oxides Revisted

- Introduced to Karsten Pedersen by WSF
  - first visit to the Aspo Hard Rock Laboratory in Sweden (operated by the Swedish Nuclear Waste Management Company)
- Introduced to John Zachara at a Subsurface Science program meeting
  - discussed potential of investigating surface chemical interactions between bacteria and iron oxides



Bacteriogenic Iron Oxides (BIOS): Surface Reactivity

- Structural polymers in cell walls and external sheaths of bacteria contain abundant acidic functional groups (*carboxyl, phorphoryl, phosphodiester, amino*)

- Iron oxide surfaces are characterized by presence of amphoteric functional groups:

$$\equiv S\text{-}OH_2^+ \leftrightarrow \equiv S\text{-}OH + H^+ \leftrightarrow \equiv S\text{-}O^- + 2H^+$$

- Proton exchange reactions involving these functional groups impact surface charge development, and mediate chemical interactions with dissolved ions and solids

- ***BIOS composites thus exhibit a high degree of surface chemical heterogeneity***

Dissolved and solid phase metal concentrations in Aspo HRL BIOS

Metal	Concentration (ppm)[a]				log $K_{d\ BIOS}$
	Aqueous phase[b]	BIOS	Oxide (%)	Bacterial (%)	
Al	0.975	6708	2048 (30.5)	4659 (69.5)	3.8
Cr	0.003	42.56	25.4 (59.8)	17.1 (40.2)	4.2
Cu	0.032	67.34	14.3 (21.3)	53.0 (78.7)	3.3
K	20.2	4797	1058 (22.1)	3738 (77.9)	2.4
Mg	113	5573	820 (14.7)	4752 (85.3)	1.7
Mn	1.42	15745	15722 (99.8)	22.5 (0.2)	4.0
Na	1600	2796	1087 (38.9)	1708 (61.1)	0.2
Sr	15	1153	915 (79.4)	237 (20.6)	1.9
Zn	0.047	141.2	31.2 (22.1)	109 (77.9)	3.5

Sorption and Precipitation of Fe^{3+} on Bacterial Cells

Sorption of Fe^{3+}

$$BH + Fe^{3+} + 2H_2O = BFe(OH)_2 + 3H^+$$

$$K_{sorb} = [BFe(OH)_2]\,[H+]^3 / [Fe^{3+}]\,[BH]$$

Solubility of hydrous ferric oxide (HFO)

$$Fe(OH)_3 + 3H^+ = Fe^{3+} + 3H_2O; \quad K_{so} = [Fe^{3+}] / [H+]^3$$

Sorption Equilibria: Mass Action and Mass Balance

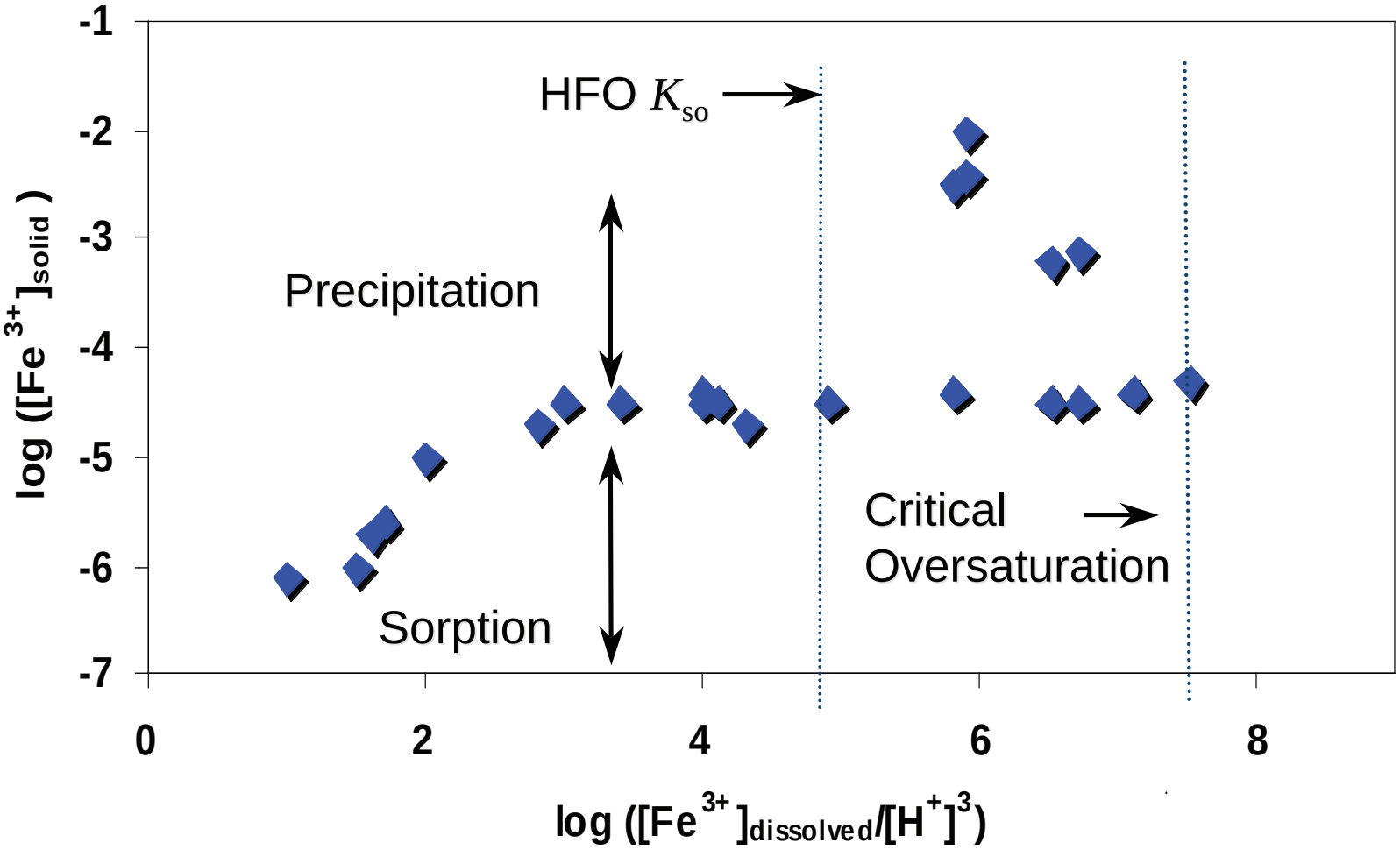
- mass balance

$$B_{Total} = BH + BMe(OH)_n^{z-(n+1)}$$

- Langmuir sorption isotherm (single site)

$$BMe(OH)_n^{z-(n+1)} = B_{Total}\, Ks\, (Me^{z+}/H^{+(n+1)}) \,/\, 1 + Ks\, (Me^{z+}/H^{+(n+1)})$$

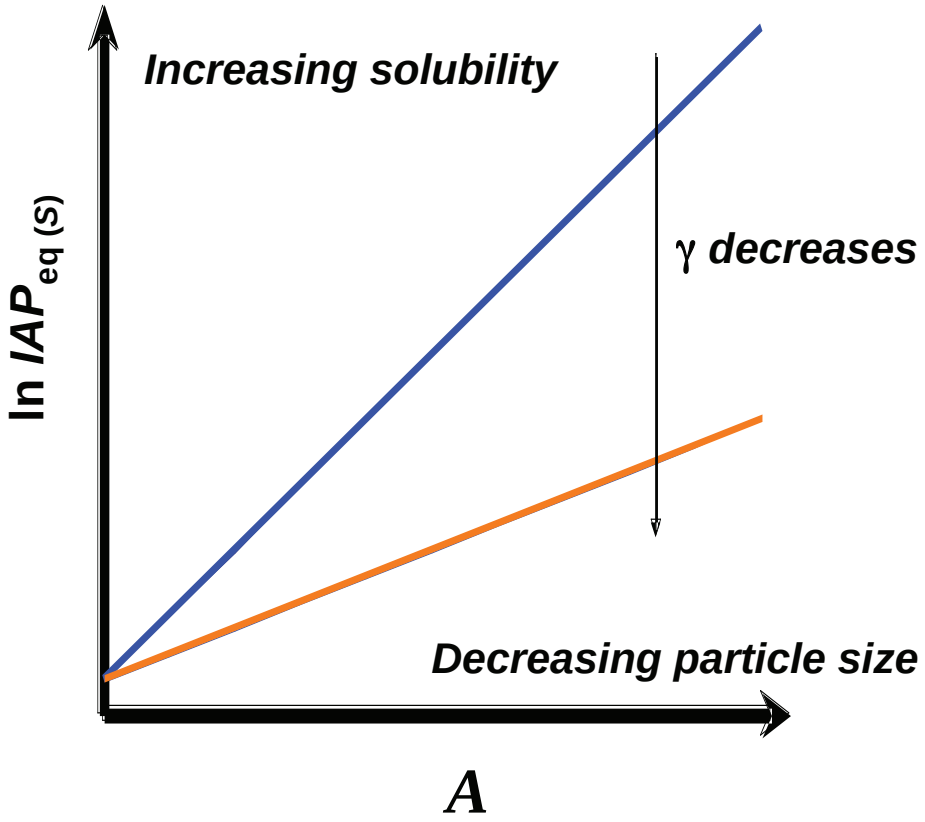
Solid Phase Partioning of Fe^{3+} in the Presence of *Bacillus subtilis*



Warren LA, Ferris FG (1998) Continuum between sorption and precipitation of Fe(III) on bacterial cell surfaces. Environmental Science and Technology 32: 2331-2337.

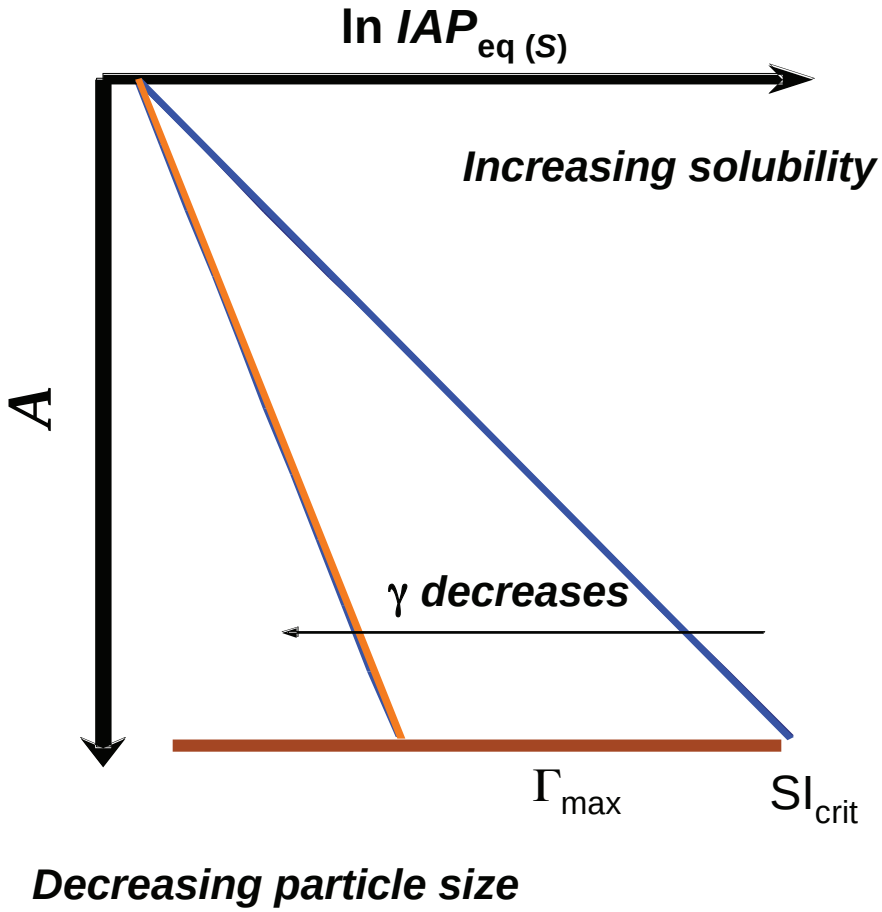
Influence of Surface Area and Surface Tension on Mineral Solubility

Change in equilibrium solubility with respect to molar surface area A

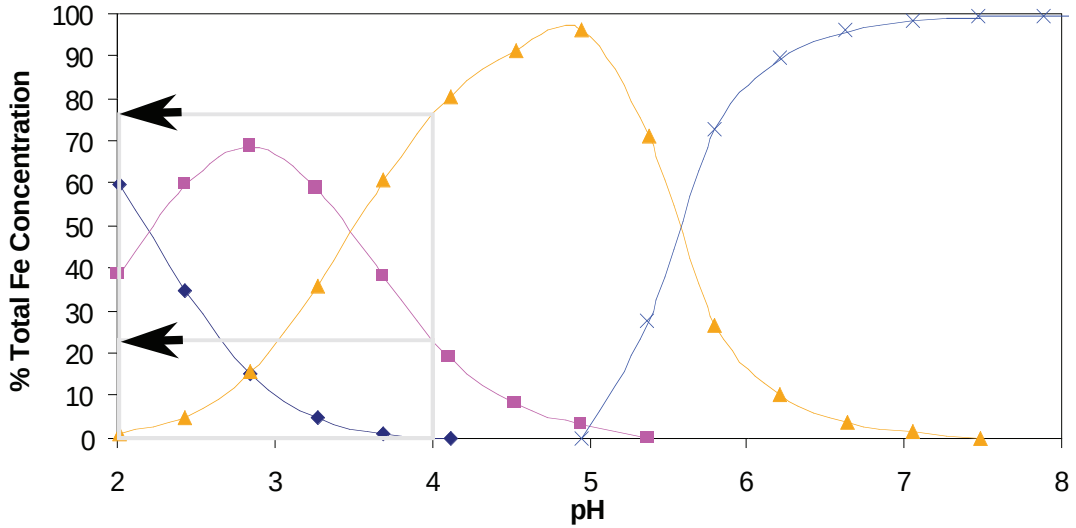


Influence of Surface Area and Surface Tension on Mineral Solubility

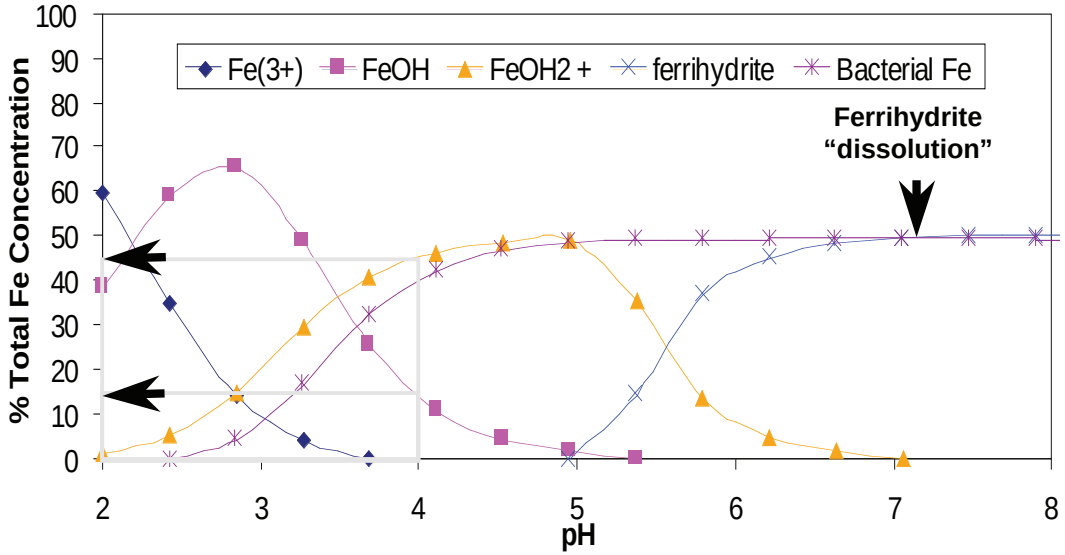
Change in equilibrium solubility with respect to molar surface area A



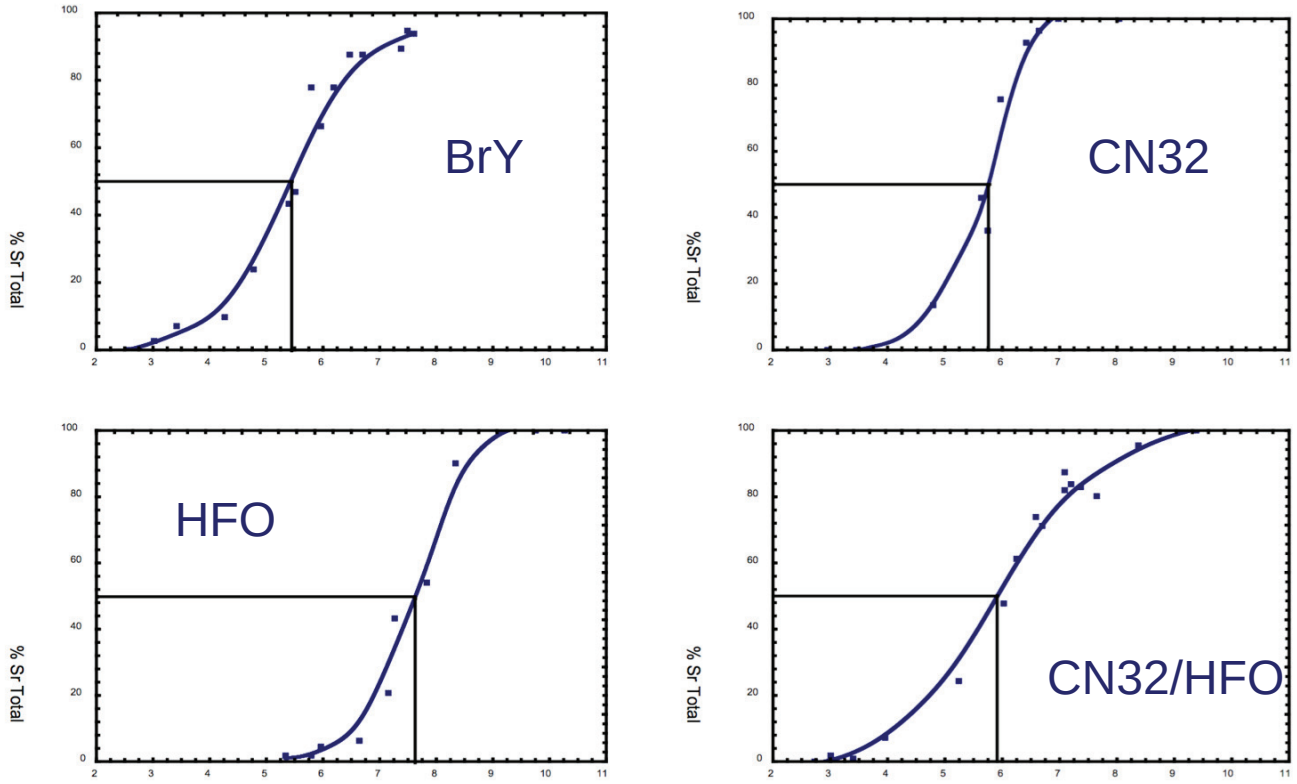
Microbial Influence on Equilibrium Fe^{3+} Speciation



- Total Fe^{3+} = 2 x 10^{-6} M
- Bacteria
  - Total sorption sites = 1 x 10^{-6} M
  - Cells = 1 x 10^{-5} / mL
  - log *K* = -3.0

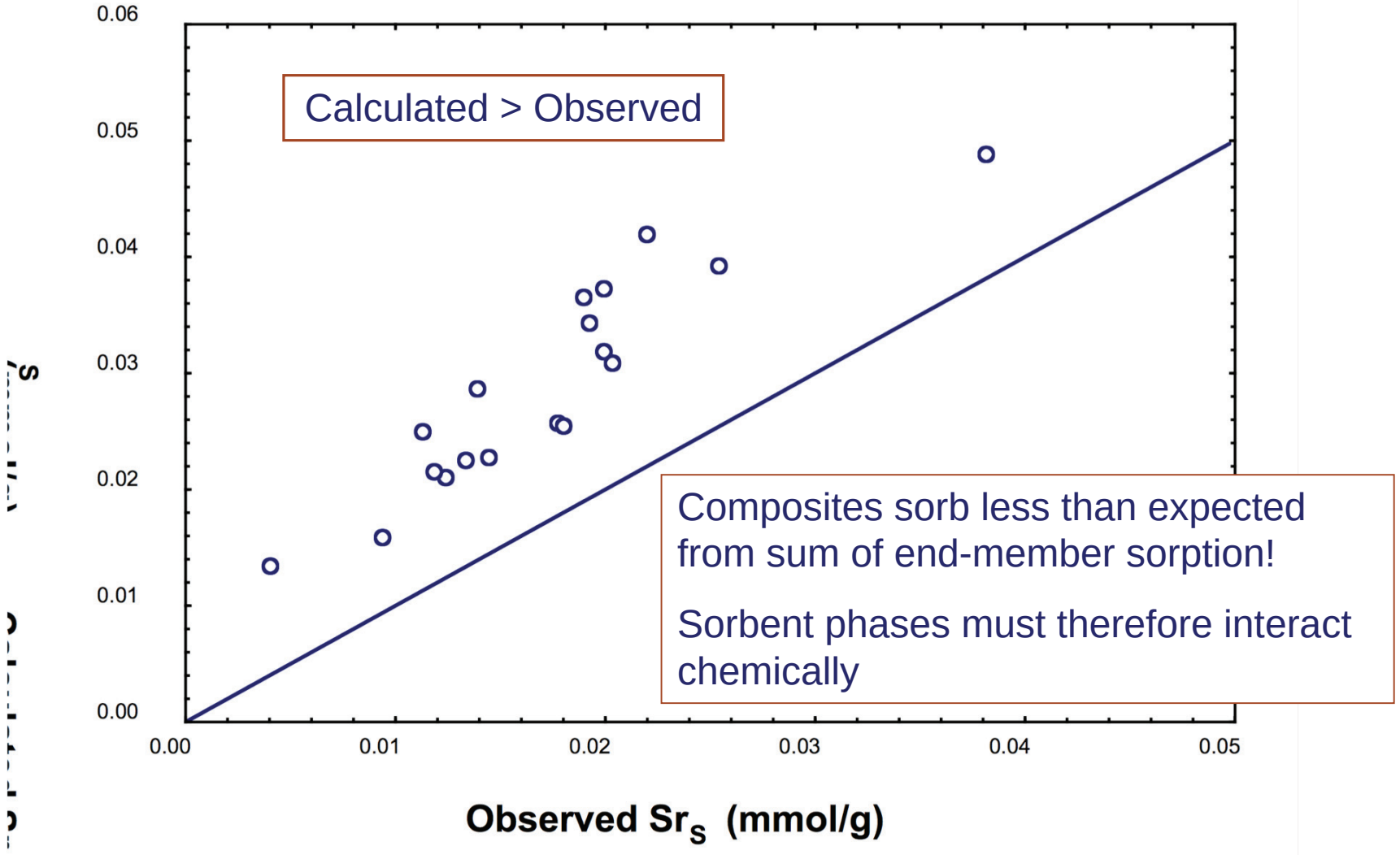


Sorption of Strontium to Bacteria and Bacteria – Iron Oxide Composites



Small TD, Warren LA, Roden EE, Ferris FG (1999) Sorption of strontium by bacteria, Fe(III) oxide and bacteria-Fe(III) oxide composites. Environmental Science and Technology 33: 4465-4470.

The "Additive" Problem of Sr^{2+} sorption to Bacteria-iron oxide composites



Interrogation of Bacterial Cell Surface Chemical heterogeneity

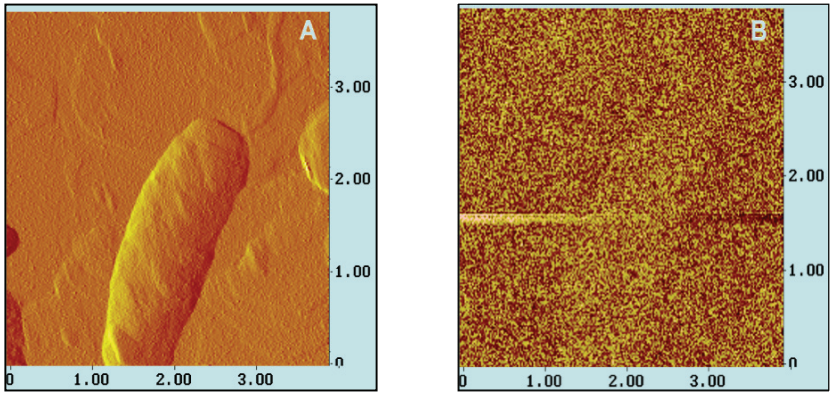
- Electrostatic force microscopy
- acid-base titration
  - fit experimental data to combined mass action and charge balance expression
    - nonlinear least-squares (FITEQL)
    - pK_a spectrum (linear programming, FOCUS)

Cox JS, Smith DS, Warren LA, Ferris FG (1999) Characterizing heterogeneous bacterial surface functional groups using discrete affinity spectra for proton binding. Environmental Science and Technology 33: 4514-4521

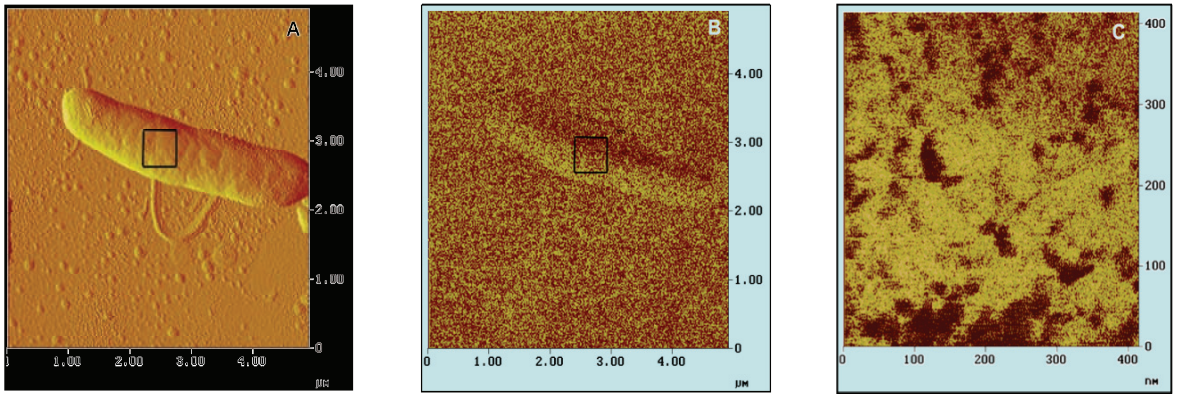
Sokolov I, Smith DS, Henderson GS, Gorby YA, Ferris FG (2001) Cell surface electrochemical heterogeneity of the Fe(III)-reducing bacteria *Shewanella putrefaciens*. Environmental Science and Technology 35: 341-347.

Heterogeneity of Surface Charge on *Shewanella putrefaciens* CN32

pH 4.0



pH 7.0



Paramter summary from FOCUS analysis[#] of *B. subtilis* and *E. coli* Titration Data

*(Matinez et al 2002 J. Colloid Interface Sci. **253**:130)*

Bacteria	pK_a^{int} *	Individual SD[b] (µmoles/mg)	Total SD[b] (µmoles/mg)	Functional Group Assignment
B. Subtilis	3.59 ± 0.38	0.10	0.43	carboxyl/phosphoryl
pzc	4.33 ± 0.57	0.11		carboxyl
6.63 ± 0.21	5.94 ± 0.66	0.08		phosphoryl
	8.64 ± 0.57	0.14		amine
E. Coli	3.72 ± 0.44	0.06	0.35	carboxyl/phosphoryl
pzc	4.85 ± 0.71	0.15		carboxyl
5.73 ± 0.23	6.56 ± 0.64	0.05		phosphoryl
	8.79 ± 0.62	0.09		amine

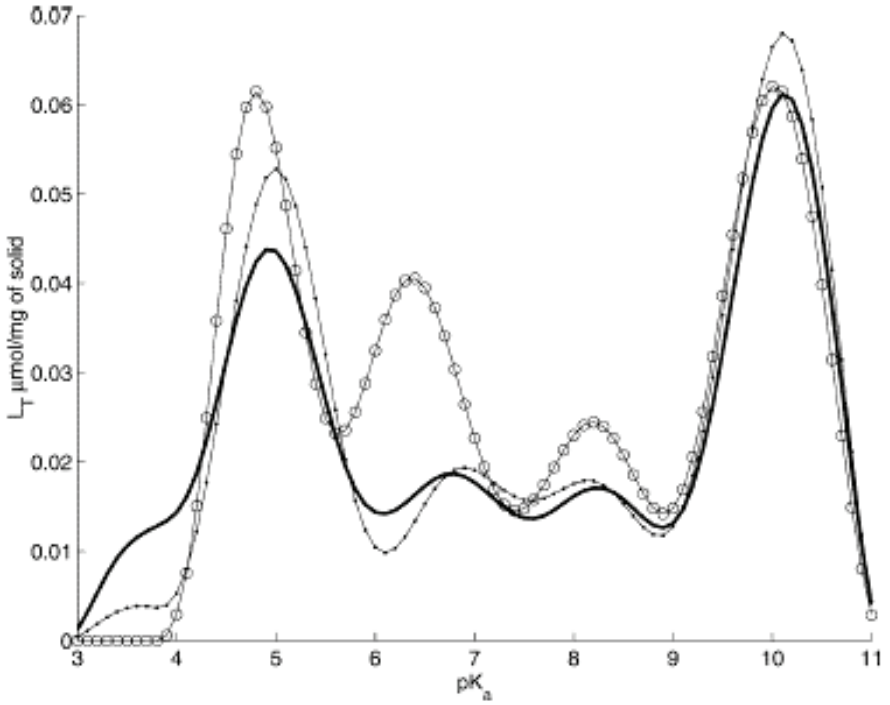
[#] after removal of electrostatic effects using a Donnan potential model

*** pK_a^{int} = -log K_a^{int}, where K_a^{int} is the intrinsic proton dissociation constant.**

b SD refers to the binding site density on the cell surface.

pK_a Spectra of CN32 coated and mixed with HFO compared to calculated spectrum

- Low pKa sites lost in spectra of cells coated and mixed with HFO
- Site densities of coated cells higher than those of cells mixed with HFO
  - Attributed to a surface area effect; smaller particles on coated yields an increased number of sites



Smith DS, Ferris FG (2003) Specific surface chemical interactions between hydrous ferric oxide and iron reducing bacteria determined using pK spectra. Journal of Colloid and Interface Science 266, 60-67.

Profile of Bacterial Cell Surface Reactivity

My last publication with TJB

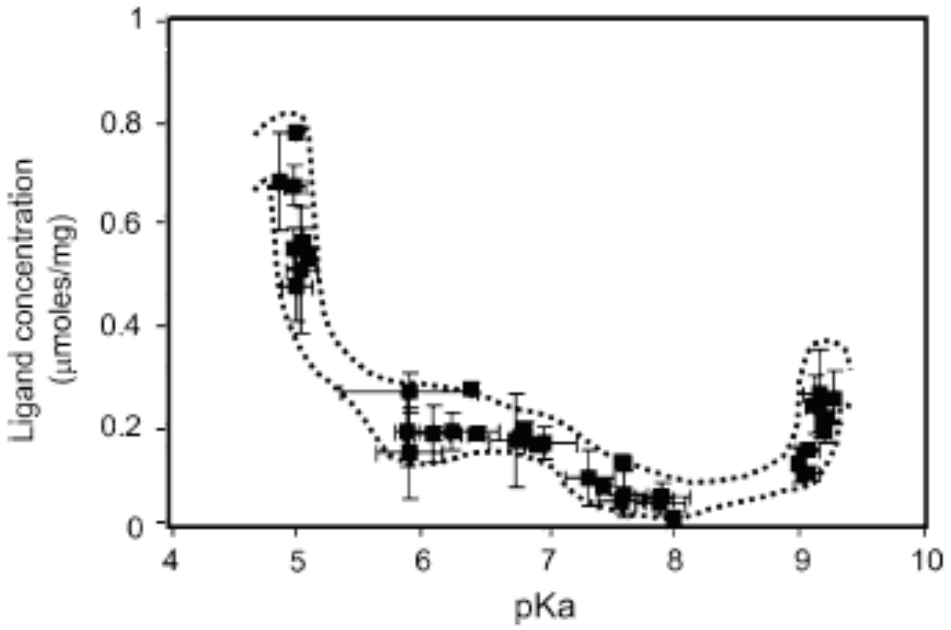


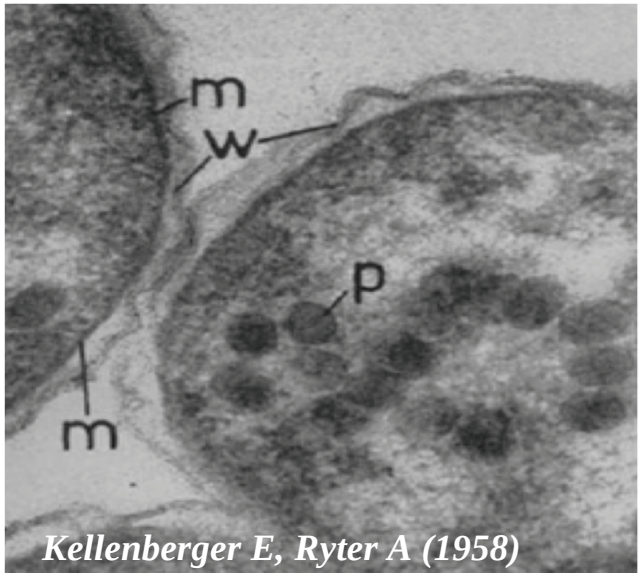
Fig. 3. Composite plot of pK_a spectra from all eight *Shewanella* strains. Error bars are standard deviations ($n = 3$; $\sigma = 1$). The combined data creates a common trend, outlined by the dashed line.

- A measure of universality in structure and function?
  - Something like antlers?
  - moose vs. deer or caribou?

Phoenix VR, Korenevsky AA, Ferris FG, Gorby YA, Beveridge TJ (2007) Influence of lipopolysaccharide on the Surface proton binding behavior of Shewanella spp. Current Microbiology 55: 152-157

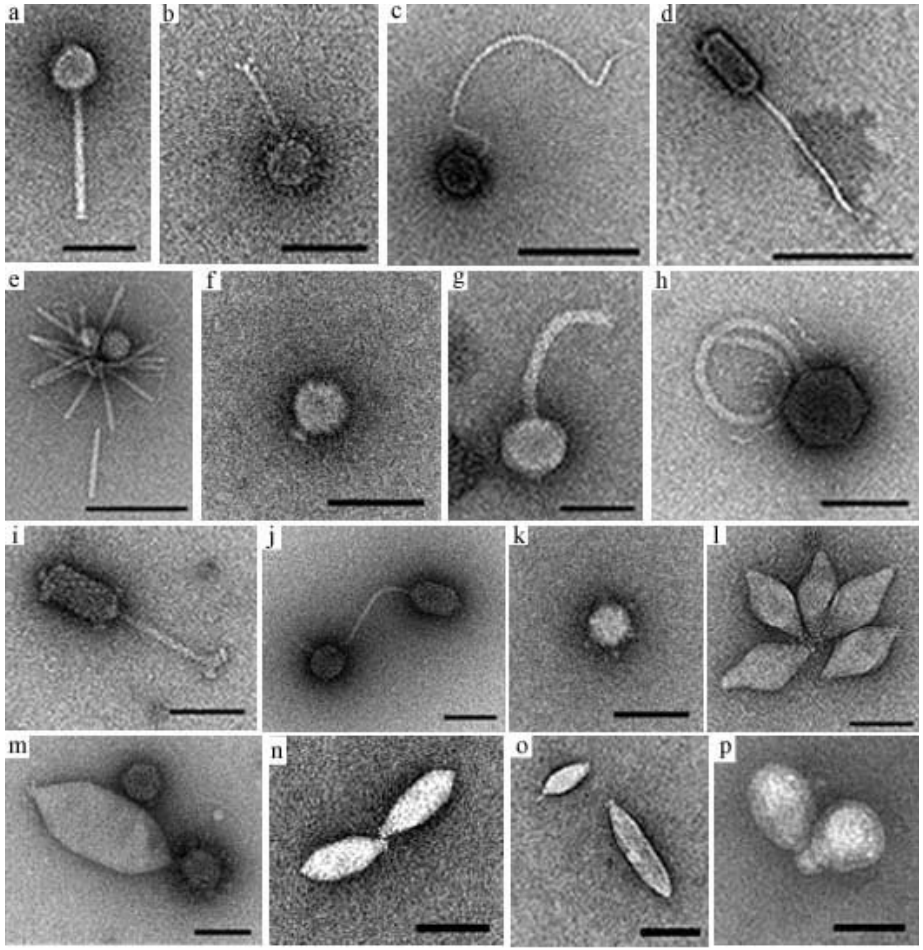
Bacteriophage – there and back again

Last trip to TJB's Lab



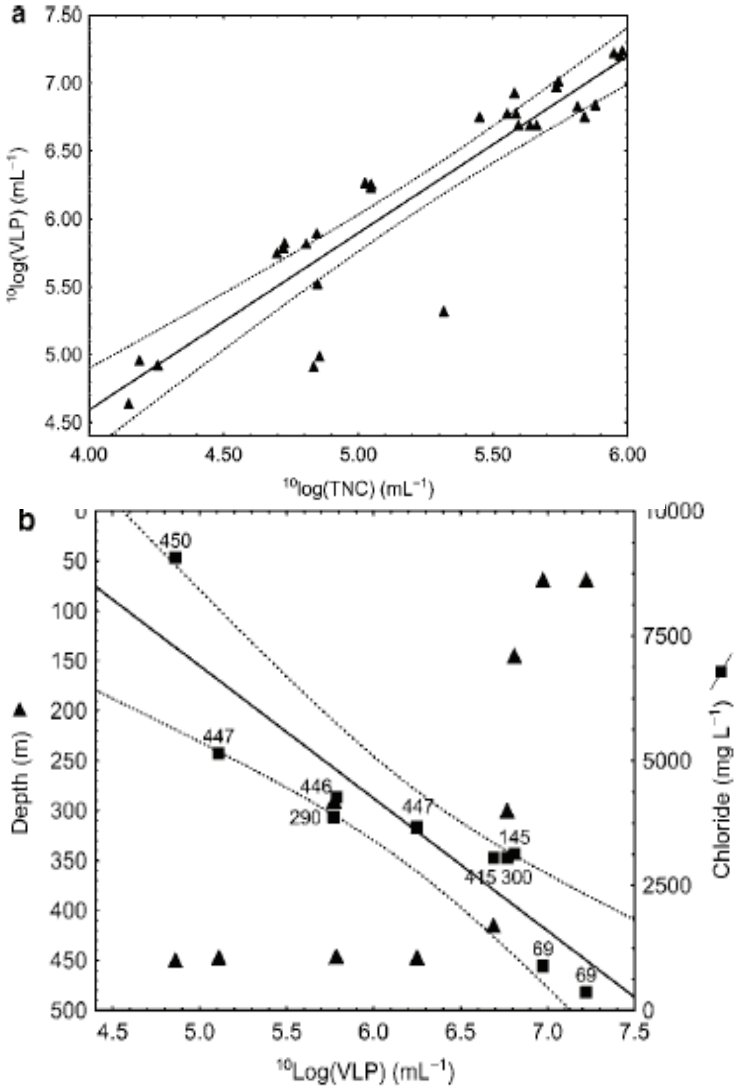
Kellenberger E, Ryter A (1958)

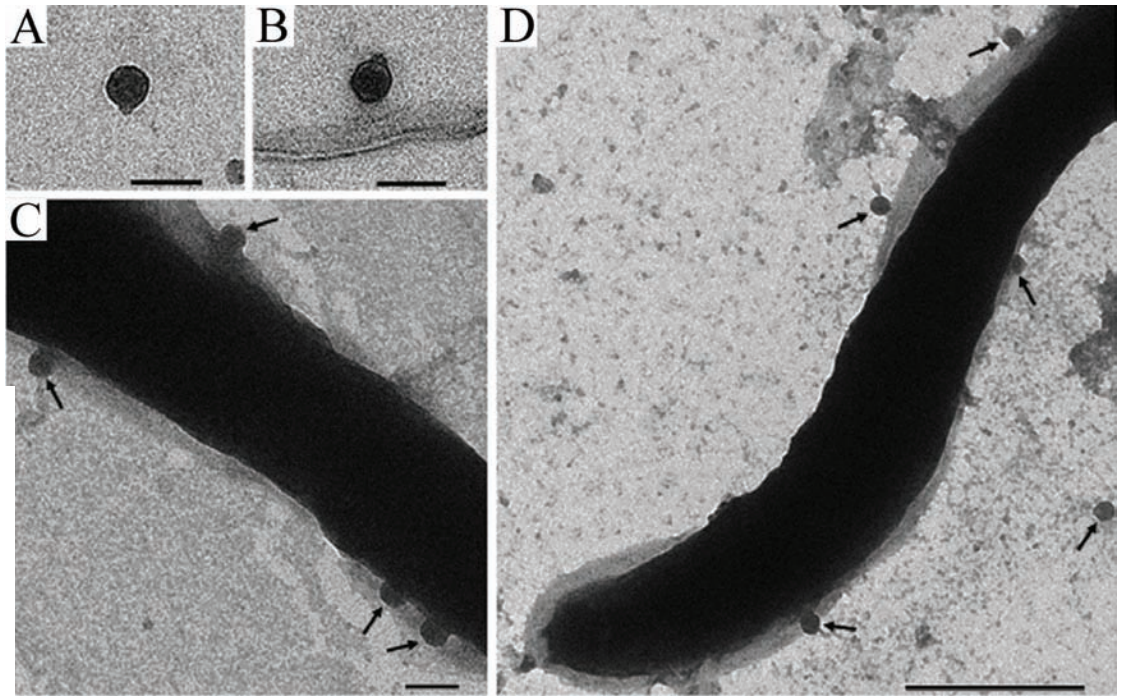
Aspo HRL, Sweden 60 to 450 m depth



Kyle et al (2008) ISME Journal doi: 10.1038/ismej.2008.18

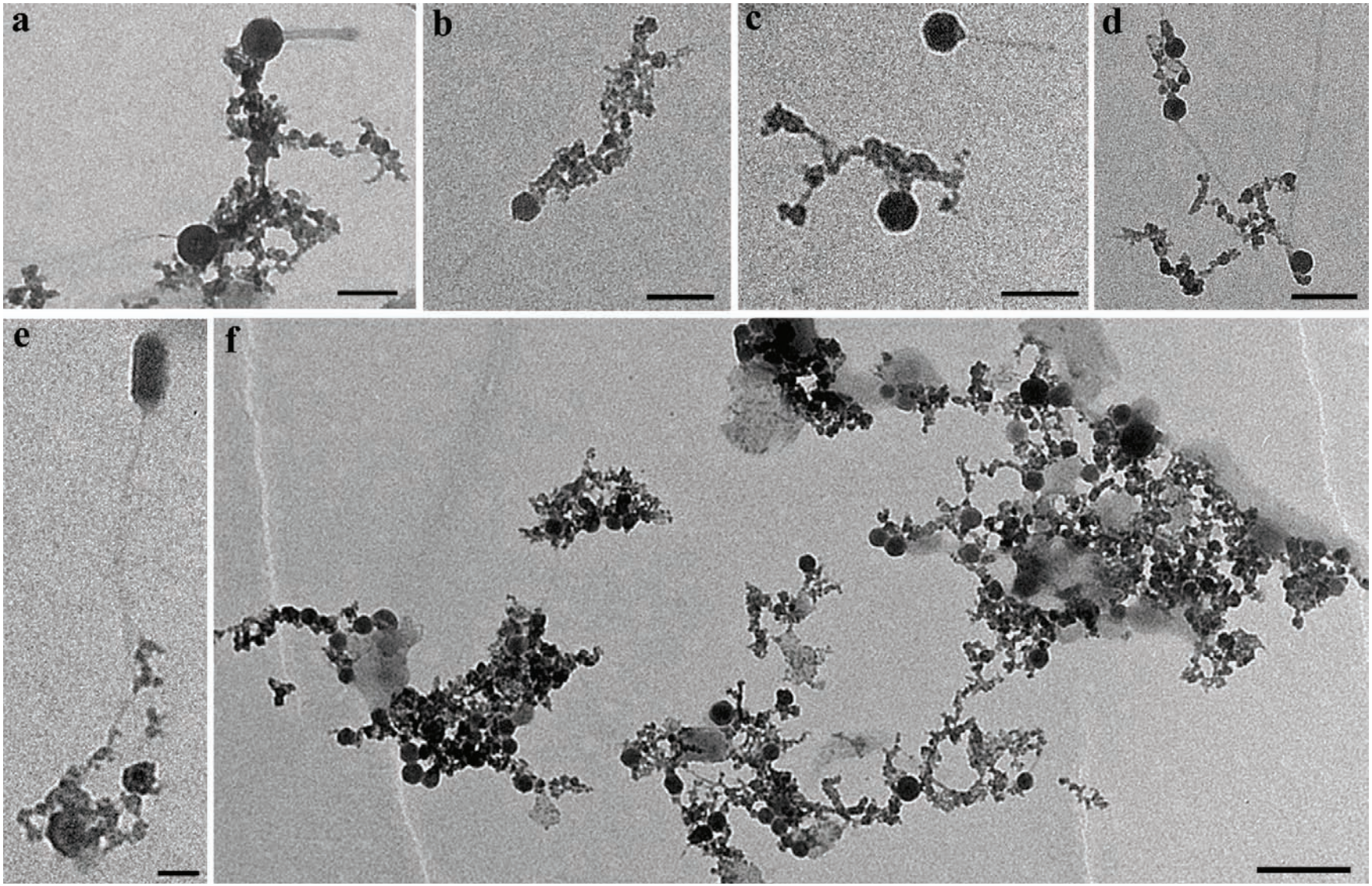
Aspo HRL Bacteriophage





Phage isolates of *Desulfovibrio aespoeensis*

Mineral-Bacteriophage Interactions pH 2.5, Rio Tinto, Spain



Bacterial Ultrastructure and Function
Some parting comments.

- Geochemical reactivity of bacterial cell surfaces – some additional aspects always emphasized by TJB
  - Bacteria (and phage) are subject to diffusion and viscous shear rather than advection in turbulent eddies (i.e., Kolmogorov scale larger than bacteria/phage size range, even at high rates of fluid shear)
    - stresses kinetic importance of diffusion-limited reactions
    - bacterial design strategies – maximize surface area, minimize diffusion distances, steepen diffusion gradients
  - Bacteria grow from the inside out and shed older wall fragments (and sometimes phage) into their surroundings
    - away go the sorbed metals and precipitated minerals … ?
  - Economy and efficiency of design
    - energy is expended to synthesize and assemble cell wall components; given the antiquity of bacteria, one must conclude that cell wall reactivity is representative of an early and highly conserved evolutionary adaptation

Nature Never Jests!

Dedicated to my
Teacher Supervisor Mentor Colleague ***Friend***

The Microbe is so very small
you cannot make him out at all.

But many sanguine people hope
to see him down a microscope.

Of lovely pink and purple spots
composed of forty separate… “dots”.

His eyebrows of a tender green
all these have never yet been seen.

But Scientists, who ought to know
assure us they must be so.

Oh! let us never, never doubt
what nobody is sure about!

Hilaire Belloc
***More Beasts for Worse Children** (1897)*