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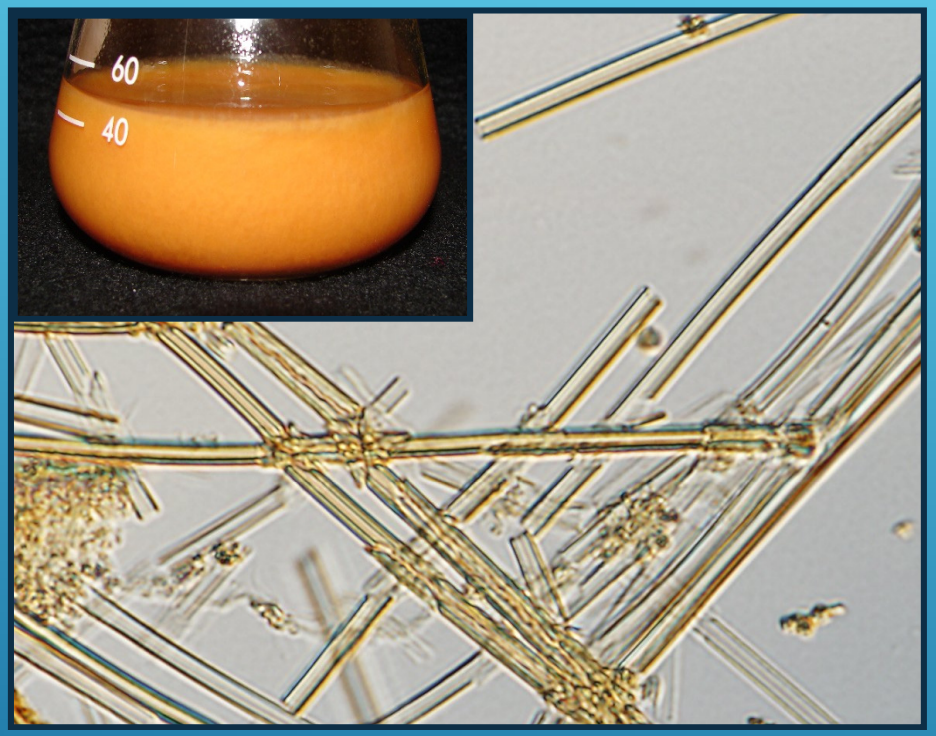
Unique organic/inorganic hybrid material produced by an iron-oxidizing bacterium, *Leptothrix* spp.

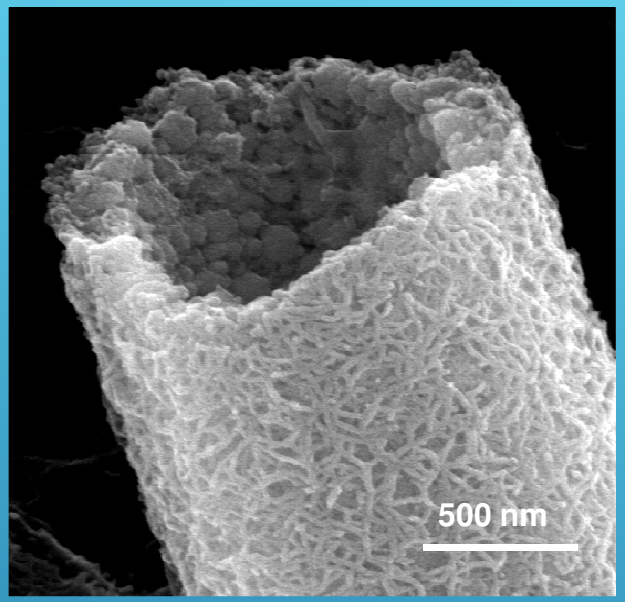
Biogenous **I**ron **Ox**ide
(BIOX)

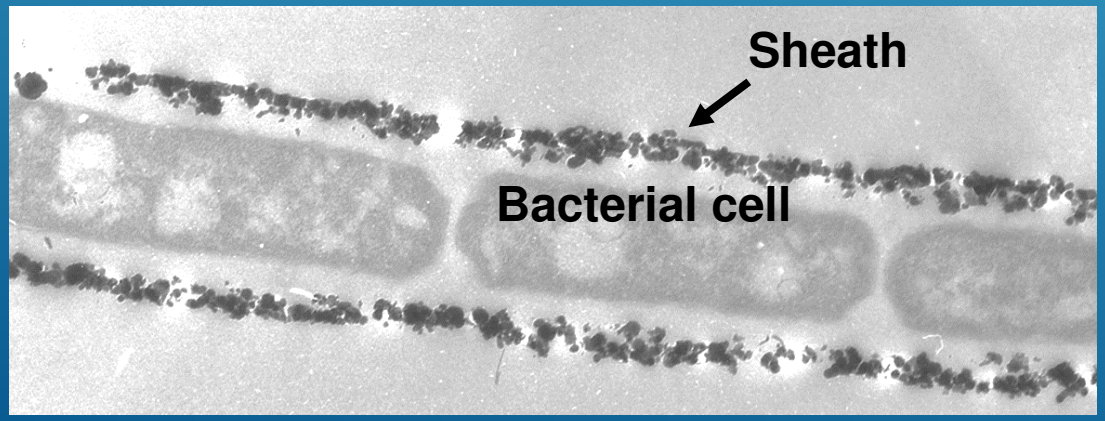
Hitoshi KUNOH
JST-CREST, Okayama University, Japan

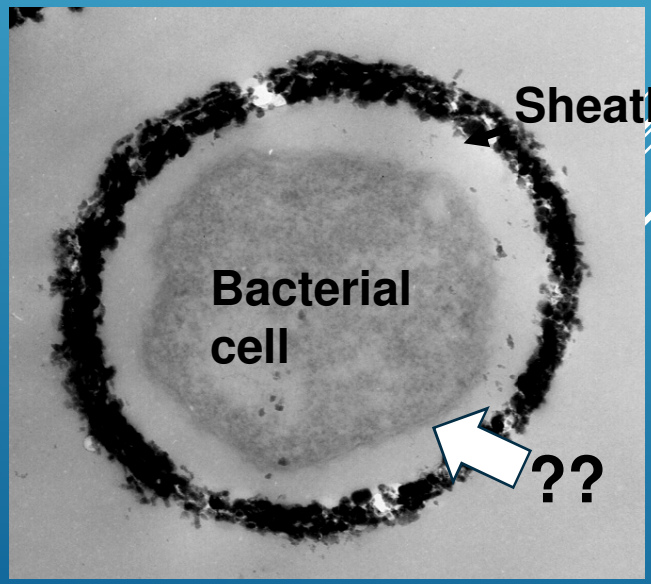
2014.08.04

Sheath-shaped iron oxide produced by *Leptothrix* spp. (L-BIOX)









Typical Sample Preparation for TEM

Chemical Fixation

Specimen → Chemically fixed with aldehyde and/or OsO_4 → Dehydrated through graded ethanol or acetone

→ Resin infiltration using acetone or propylene oxide → Resin embedding → Sectioning

Physical Fixation （No use of organic solvent）

Specimen → Rapid freezing (-200C) → Freeze fracture

→ Pt evaporation → Specimen removal with hypochlorite

→ Pt evaporation film (Cut surface mold)

Physical/chemical Fixation

Specimen → Rapid freezing (-200C)

→ Chemically fixed with acetone-mixed fixative at -200C

→ Gradually back to room temperature

→ Same as the chemical fixation

Journal of Bacteriology

Evaluation of freeze-substitution and conventional embedding protocols for routine electron microscopic processing of eubacteria.

L L Graham and T J Beveridge

J. Bacteriol. 1990, 172(4):2141.

TABLE 1. Total percent ^3H and ^{14}C cpm detected as soluble material in processing fluids during conventional embedding of *E. coli* SFK11 and W7 and *B. subtilis* 168 and W23 with acetone and ethanol as dehydrating agents

Dehydrating agent	% of added cpm[a]									
	E. coli						*B. subtilis*			
	SFK11		SFK11		W7		168		W23	
	[^{3}H]DPM	[^{14}C]Ura	[^{3}H]Thy	[^{14}C]Gal	[^{3}H]DPM	[^{14}C]Ura	[^{3}H]GlN	[^{14}C]Ura	[^{3}H]GlN	[^{14}C]Ura
Acetone	4.85	3.93	3.82	2.52	2.3	2.65	6.47	2.64	9.56	1.94
Ethanol	4.86	4.74	5.03	3.88	2.58	3.36	6.05	2.69	7.14	2.07

[a] DPM, Diaminopimelic acid; Ura, uracil; Thy, thymidine; Gal, galactose; GlN, *N*-acetylglucosamine.

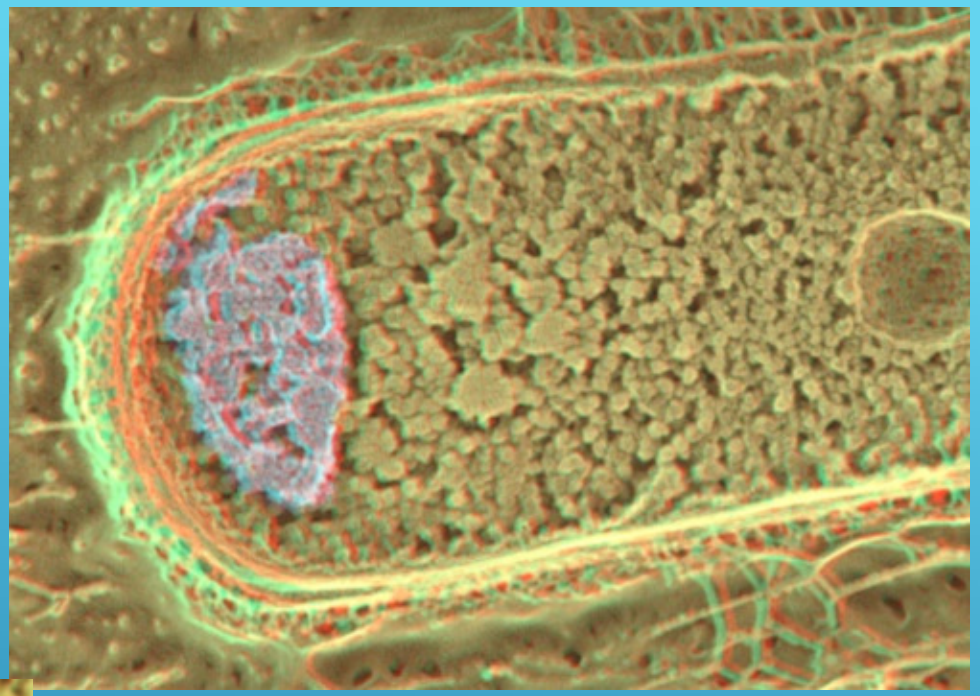
TABLE 2. Percent ^3H and ^{14}C cpm detected as soluble material in processing fluids during freeze-substitution of *E. coli* SFK11 and W7 and *B. subtilis* 168 and W23

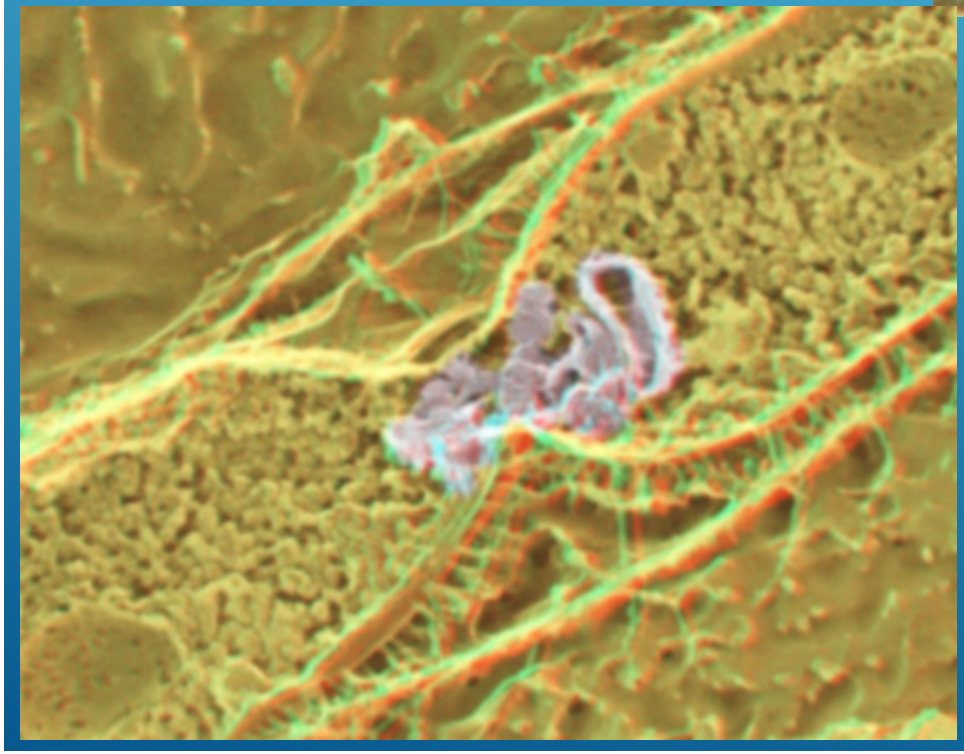
Processing fluid	% of added cpm[a]									
	E. coli						*B. subtilis*			
	SFK11		SFK11		W7		168		W23	
	[^{3}H]DPM	[^{14}C]Ura	[^{3}H]Thy	[^{14}C]Gal	[^{3}H]DPM	[^{14}C]Ura	[^{3}H]GlN	[^{14}C]Ura	[^{3}H]GlN	[^{14}C]Ura
Substitution medium	<0.1	<0.1	<0.10	<0.1	3.09	0.42	2.25	0.37	0.83	0.06
Acetone washes	33.5	31.92	44.14	42.17	4.93	26.53	8.59	23.24	8.91	28.81
Infiltration resin	1.6	1.46	3.64	3.7	2.67	16.55	34.73	6.41	27.65	10.28
Total	35.1	33.38	47.77	45.87	10.69	43.5	45.57	30.02	37.39	39.15

[a] DPM, Diaminopimelic acid; Ura, uracil; Thy, thymidine; Gal, galactose; GlN, *N*-acetylglucosamine.

Freeze Fracture Replica

(Acetone- or ethanol-dehydration is not necessary)

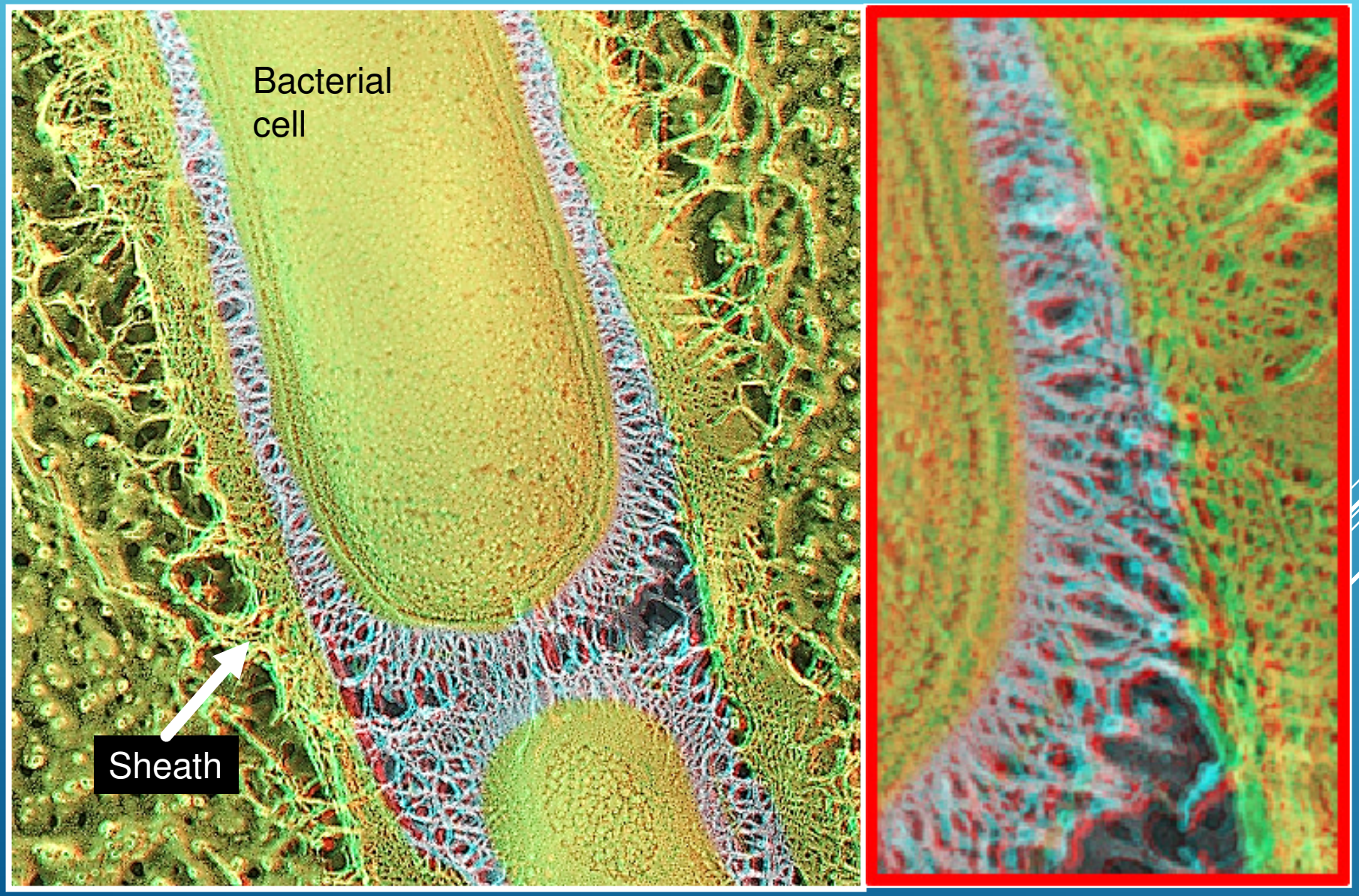




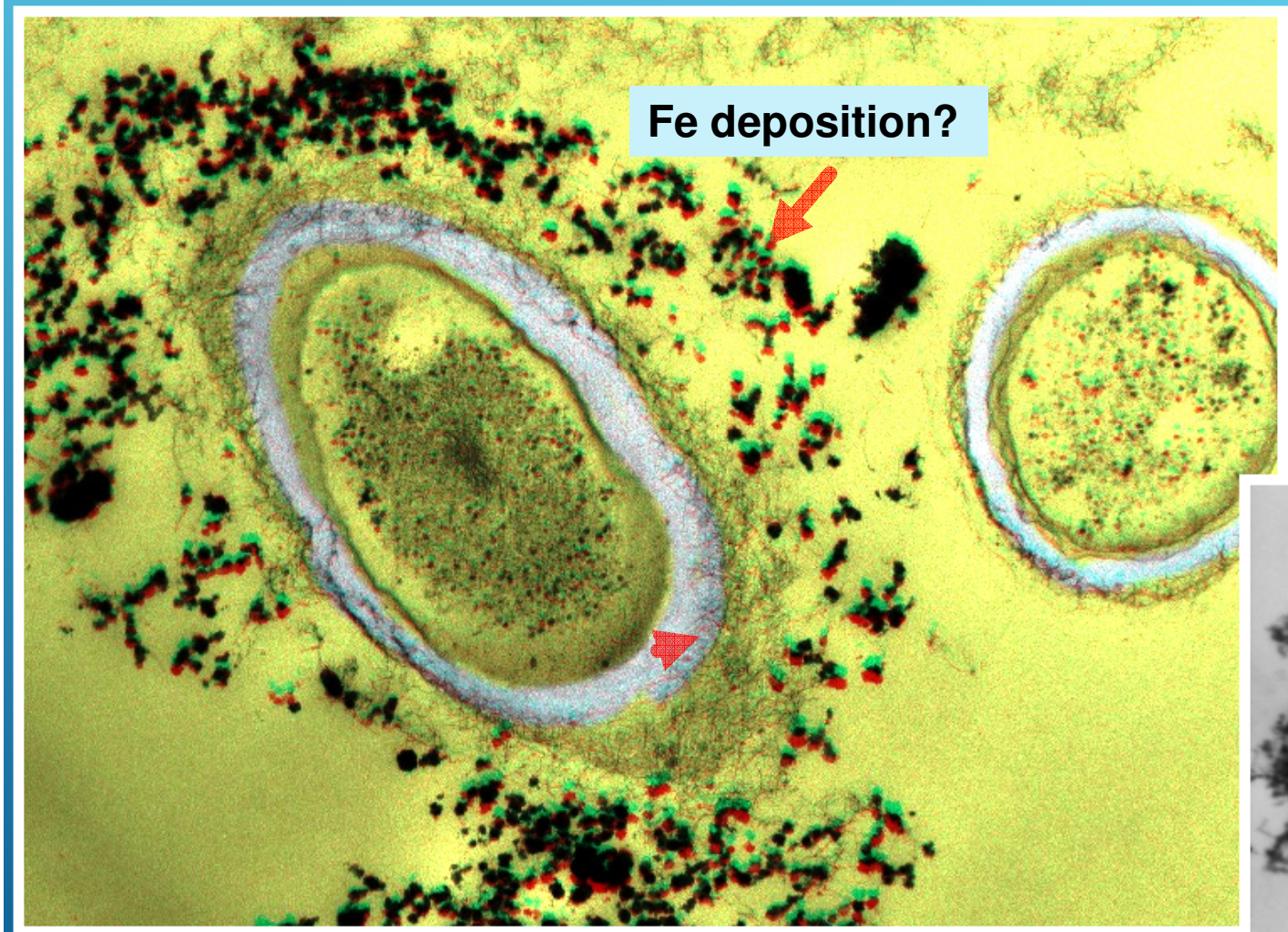
Numerous fibrils arising from outer membrane of cell

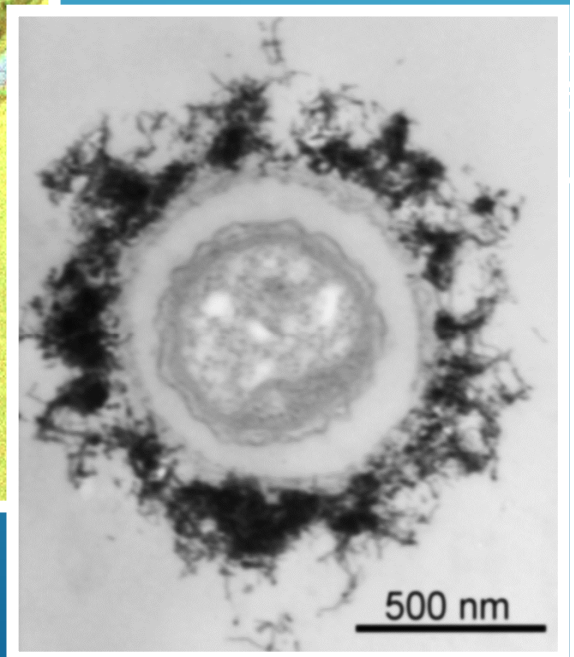
(1 dpi)

Basic skeleton of sheaths is composed of numerous fibrils released from outer membrane of bacterial cell (3dpi)



OUMS1 cell and associated sheath prepared by freeze substitution





Assumption

Exopolymers immediately after released could be sensitive to organic solvents even after fixed but those linked with aquatic ions could be tolerant to the solvents

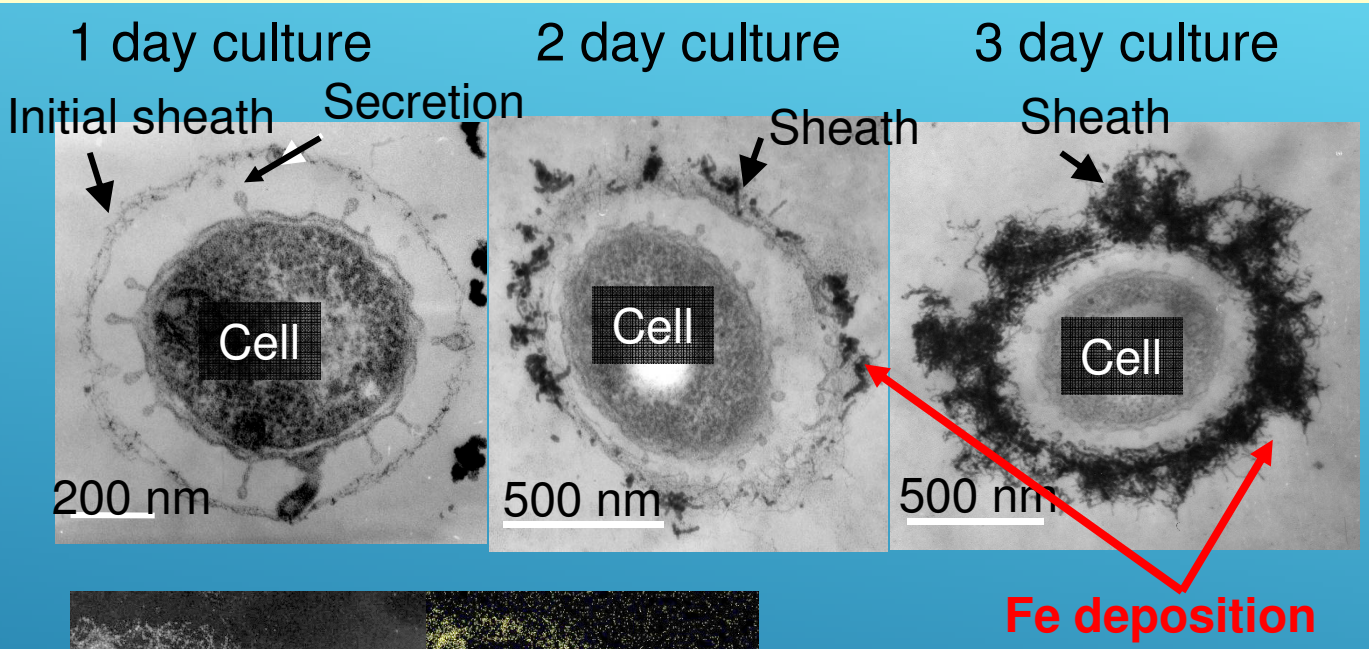
Only sheath is detected but released fibrils not by conventional fixation and freeze substitution in which organic solvents are used for specimen preparation

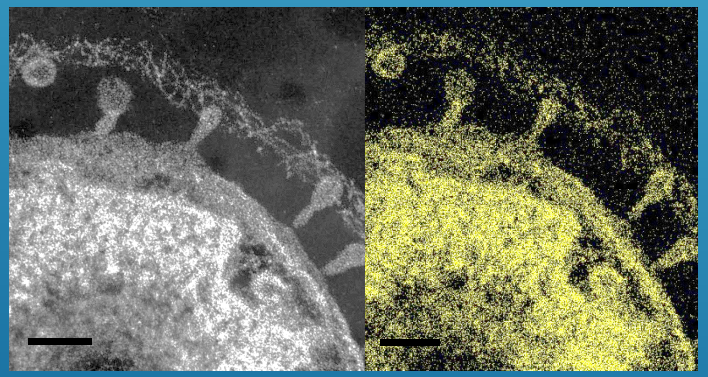
Freeze Fracture/Replica proved

1. Exopolymer fibrils are released from outer membrane of bacterial cell
2. Basic structure of sheath is composed of the released fibrils
3. No intervening space between bacterial cell and sheath

¥

Sheath Formation Steps (OUMS1)

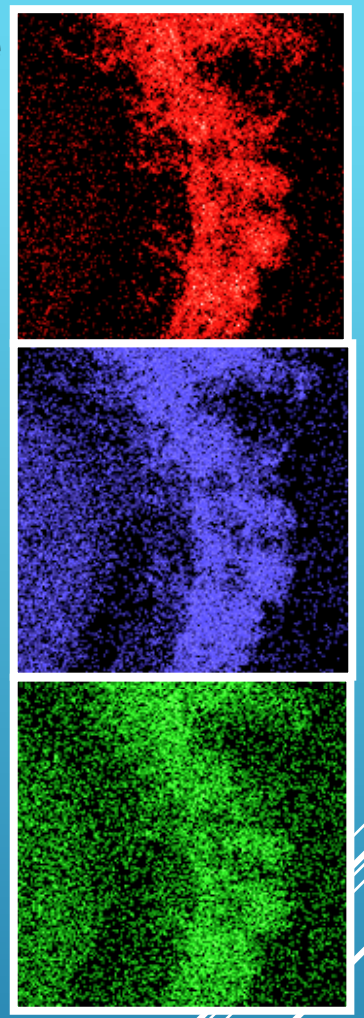


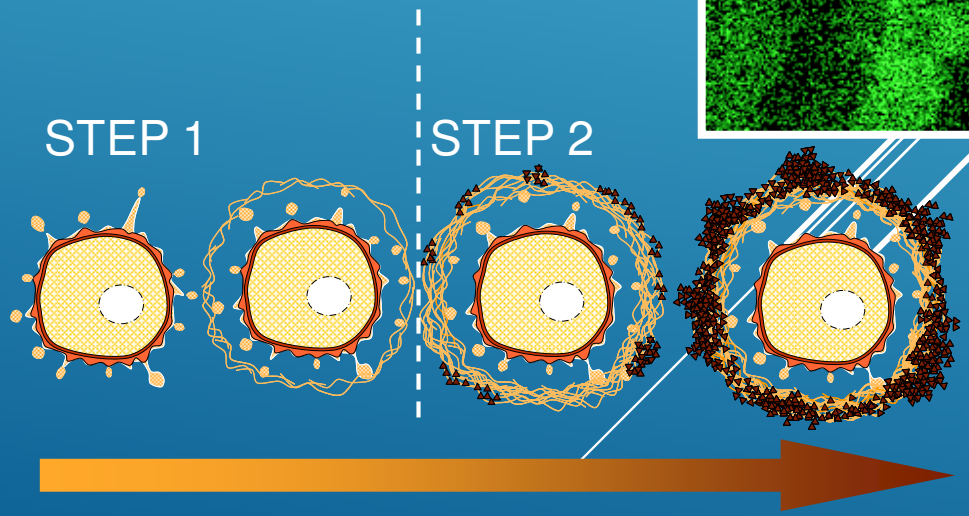


HAADF-STEM Bi-stain

BIOX contains saccharic materials

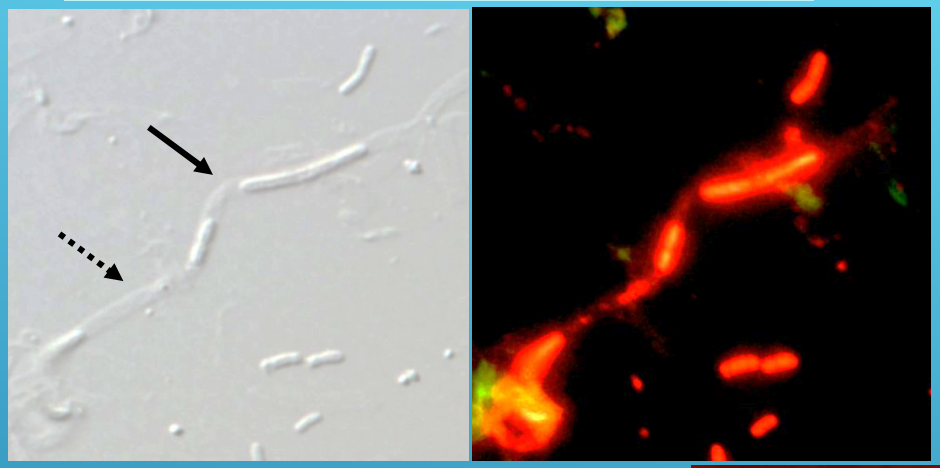
C content : ~1 (wt)%



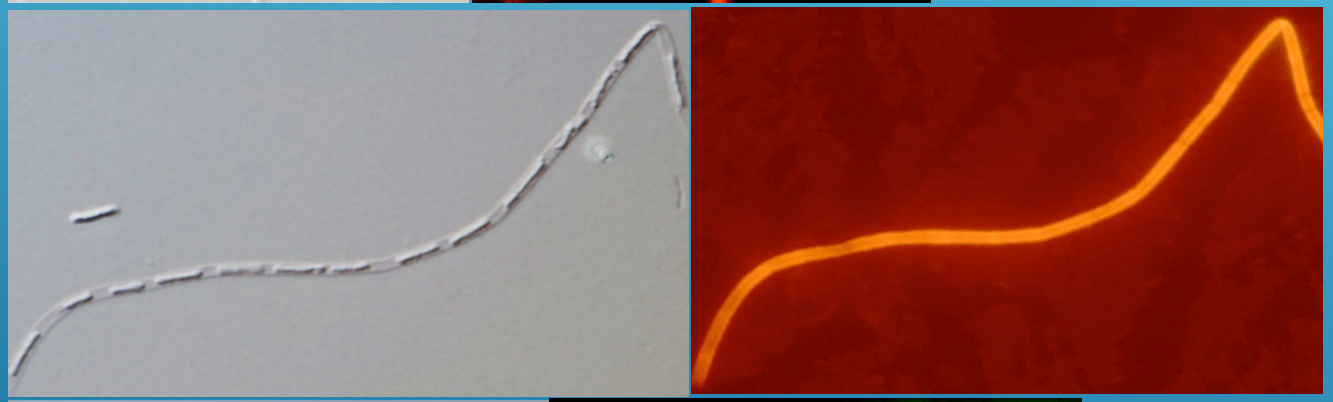


BIOX contains protein?

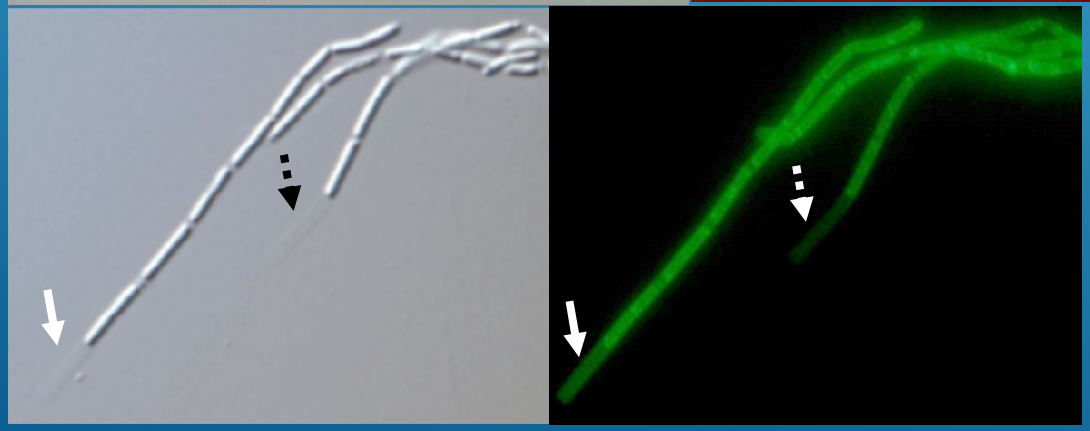
OUMS1 2-3 dpi



Detection of amino acids
（Ruby)



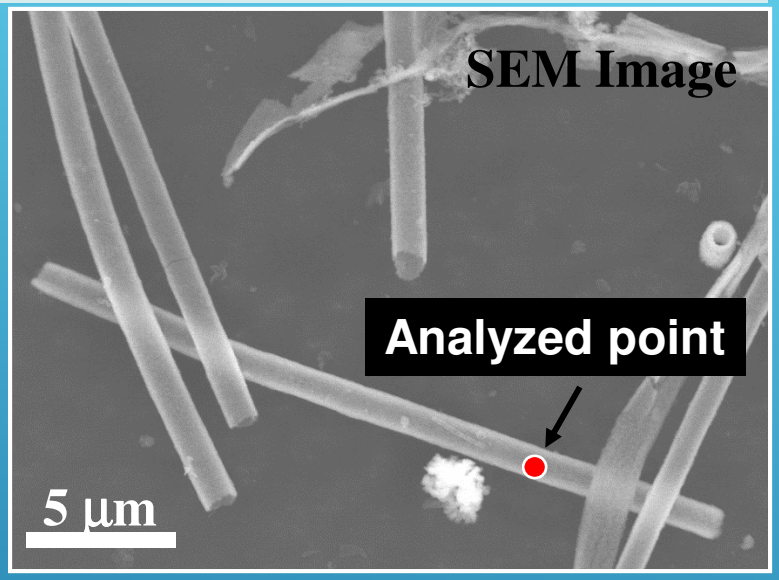
-SH Detection
(R-phycoerythrin
labeled antibody）



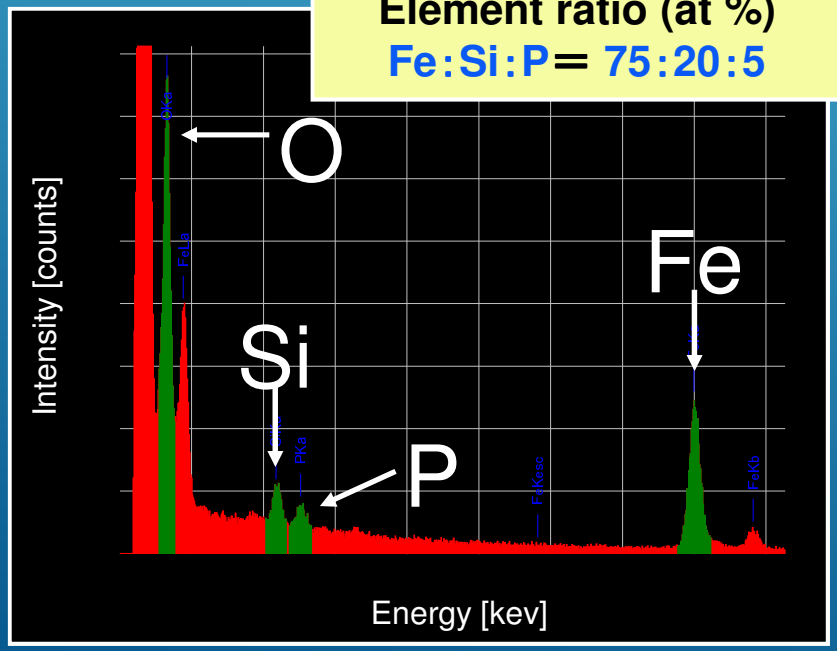
-NH_2 Detection
(Fluorescein-labeled NH_2 kit）

BIOX contains saccharic and proteinacious materials of bacterial origin

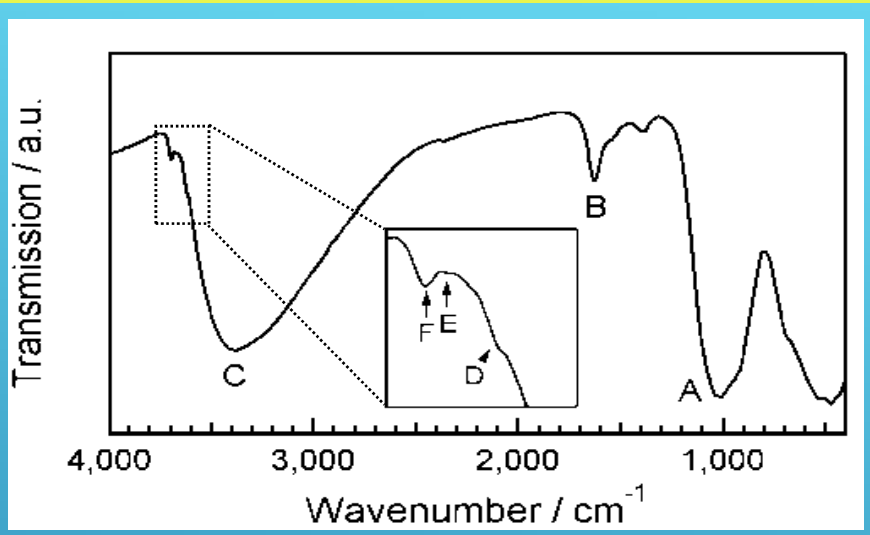
ELEMENTAL ANALYSIS OF L-BIOX (EDX)



Element ratio (at %)
Fe:Si:P= 75:20:5



Chemical bonds detected by FTIR Spectroscopy



A: Si–O–Fe, P–O–Fe
B: O–H, C: O–H
D: P–O–H, E: Fe–O–H, F: Si–O–H

Si and P linked with Fe via O

Inorganic elements are mutually linked through chemical connections

Elemental composition of groundwater

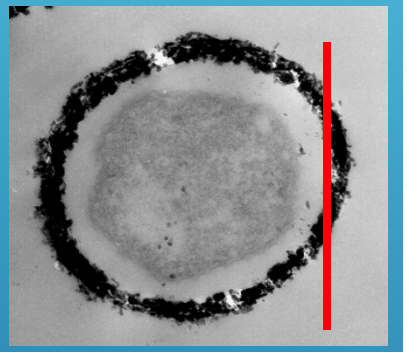
Fe:Si:P = 13:14:1 （wt. %）

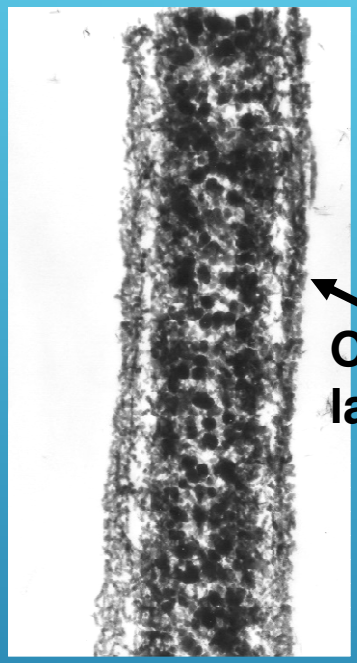
Ca, Na, K : minor elements

Distribution of Fe, C, and O in L-BIOX Texture （EELS ）

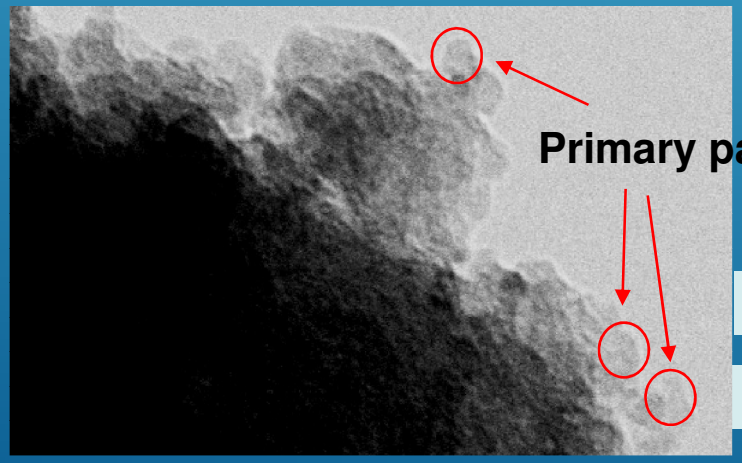
Longitudinal section

Cross section

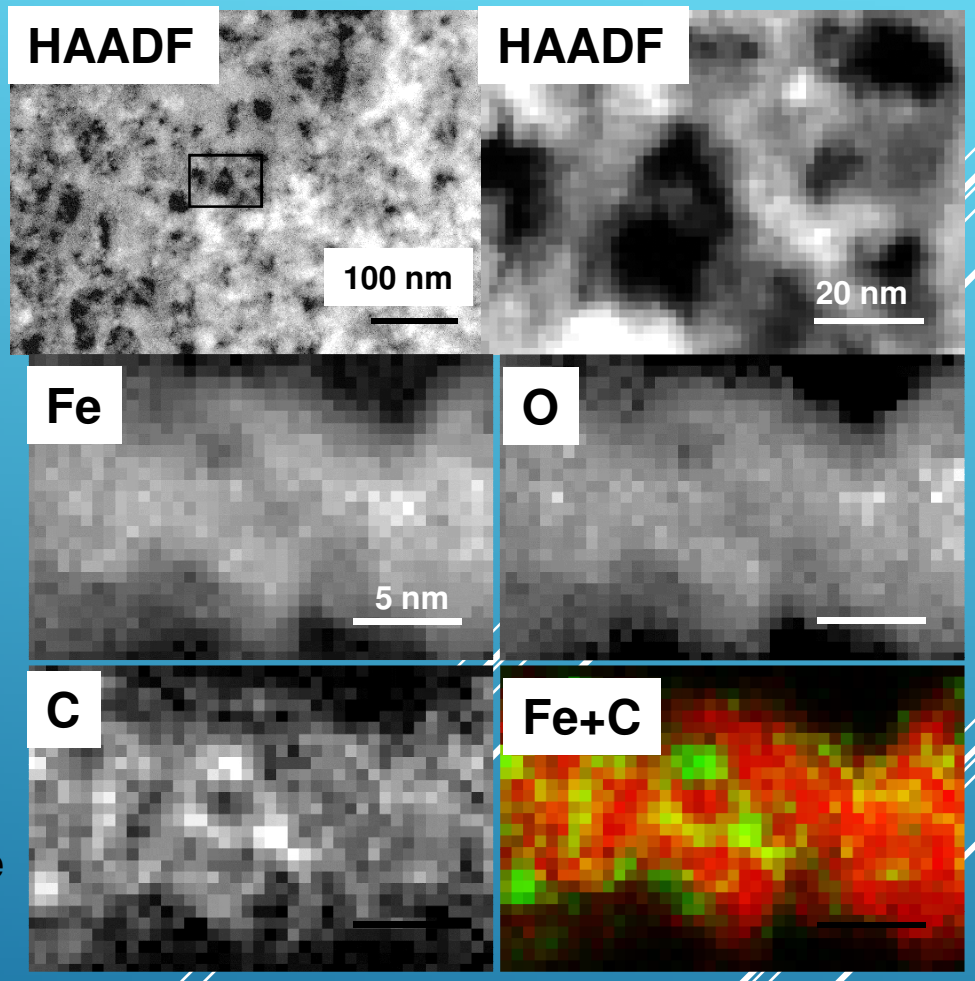




Outer layer



Primary particle



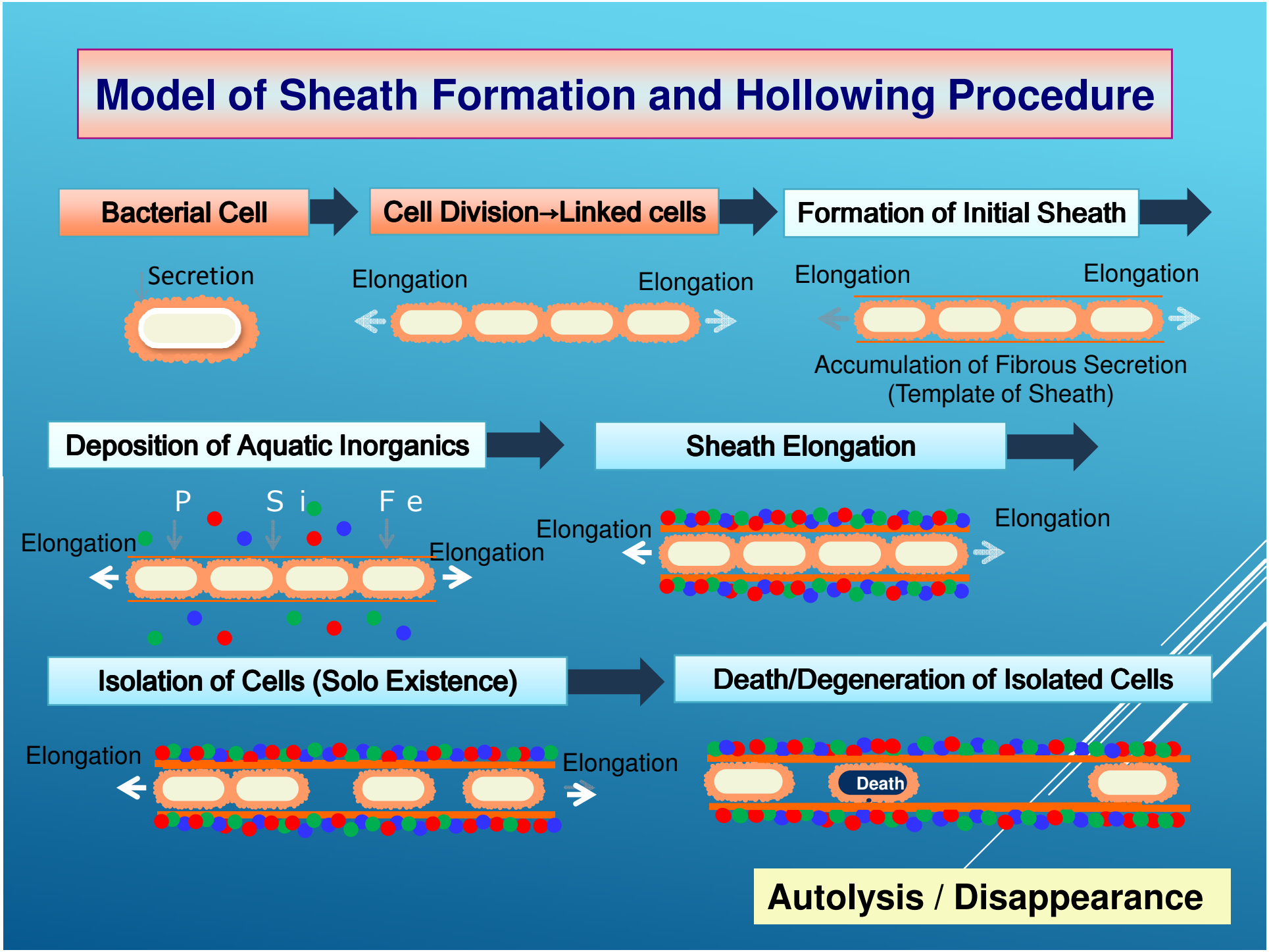
Identical localization of Fe and O → Exist as Iron oxides?

Inconsistent localization of Fe and C → C and Fe irregularly connected?

Primary Particle: 3 nm diameter

Organic/inorganic Hybrid

Model of Sheath Formation and Hollowing Procedure



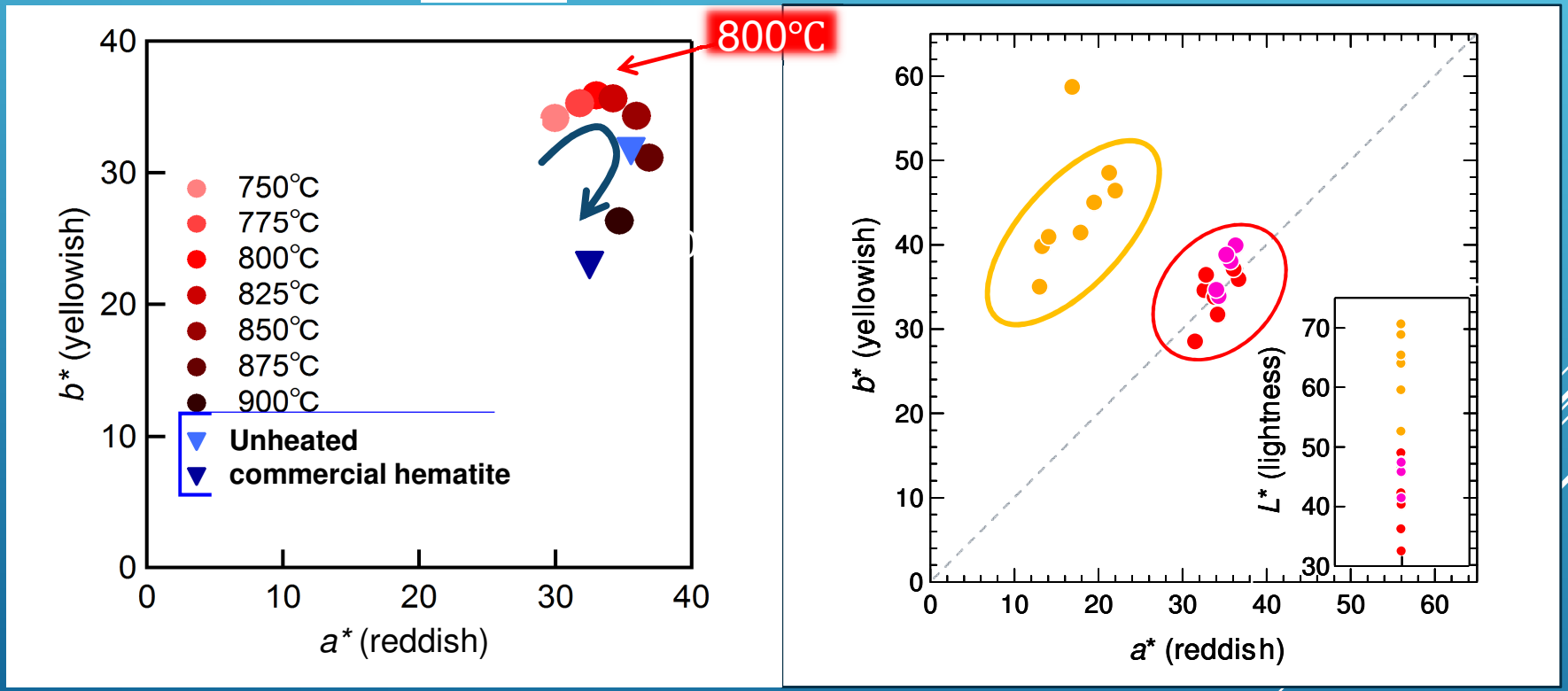
Practical Use of BIOX in Diverse Fields

1. Pigment (glaze) for pottery
2. Enhancer of catalytic activity
3. Electrode of Lithium battery
4. Cell culture (cell affinity)
5. Plant protectant

Ceramic artists always seek bright color pigments (glazes)

COLOR CHANGE OF HEATED OR CULTURED BIOX

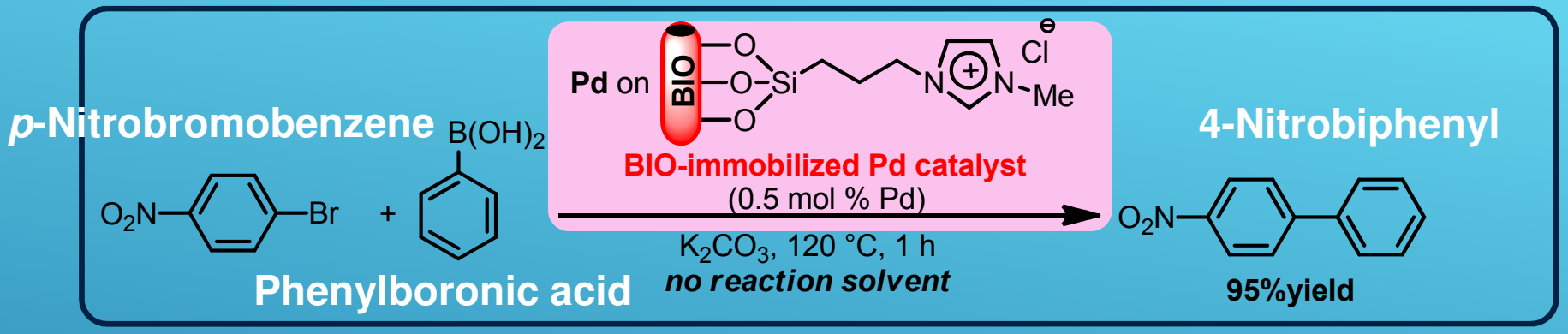
Culture at low pH and with CO_2



Hashimoto H. et al. **(2012)**. *Dyes and Pigments*, **95**, 639-643,

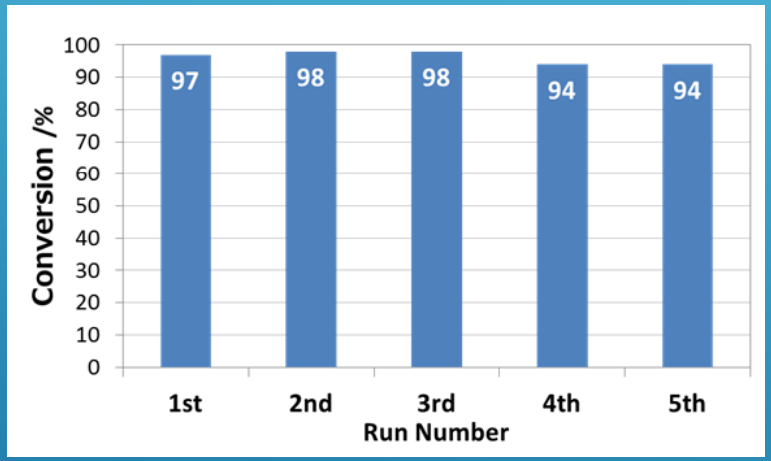
BIOX heated at 800 C gives an ideal reddish glaze for ceramic works

BIOX-immobilized Pd catalyst for solvent-free Suzuki- Miyaura cross coupling reaction



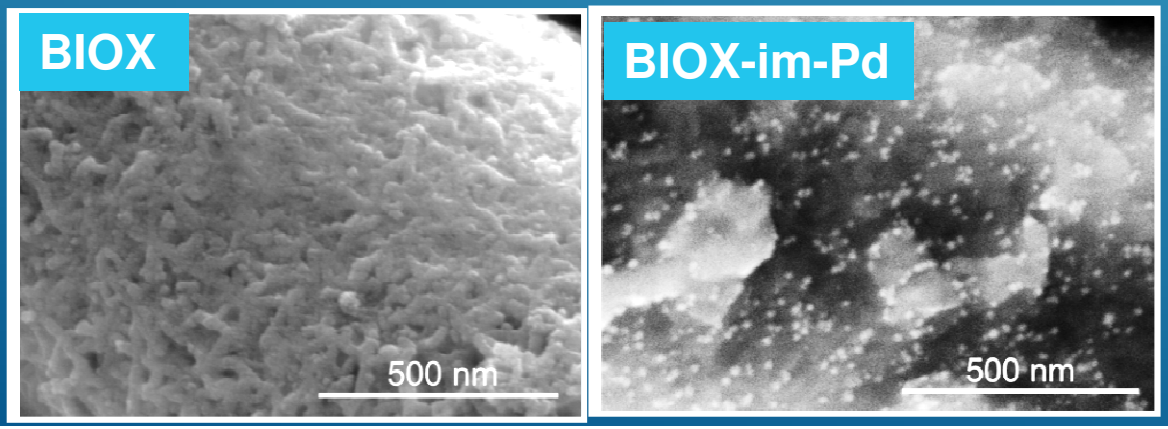
Pd: palladium
Me: methyl

Recyclability



Recyclable four times without significant loss of catalytic activity

SEM



Pd nanoparticles on BIOX-im-Pd facilitates the reaction

Tetrahedron Lett. 2012, 53(3), 329-332.

Acknowledgement

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Dr. Hiromichi Ishihara (Molecular biology/microbiology)

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Professor Kazuhiro Toyoda (Plant pathology)

<Kyoto University>
Professor John Heuser (Bacteriology/microscopy)
Dr. Nobuhiro Morone (Bacteriology/microscopy)

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