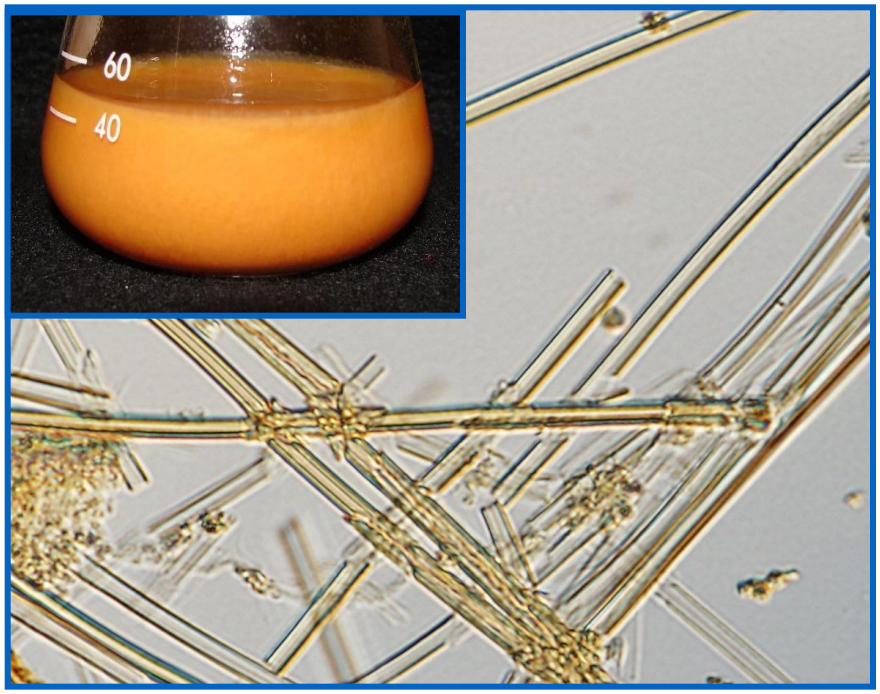
Novel functional materials created by mimicking Fe-oxidizing bacterium-associated iron oxides complex

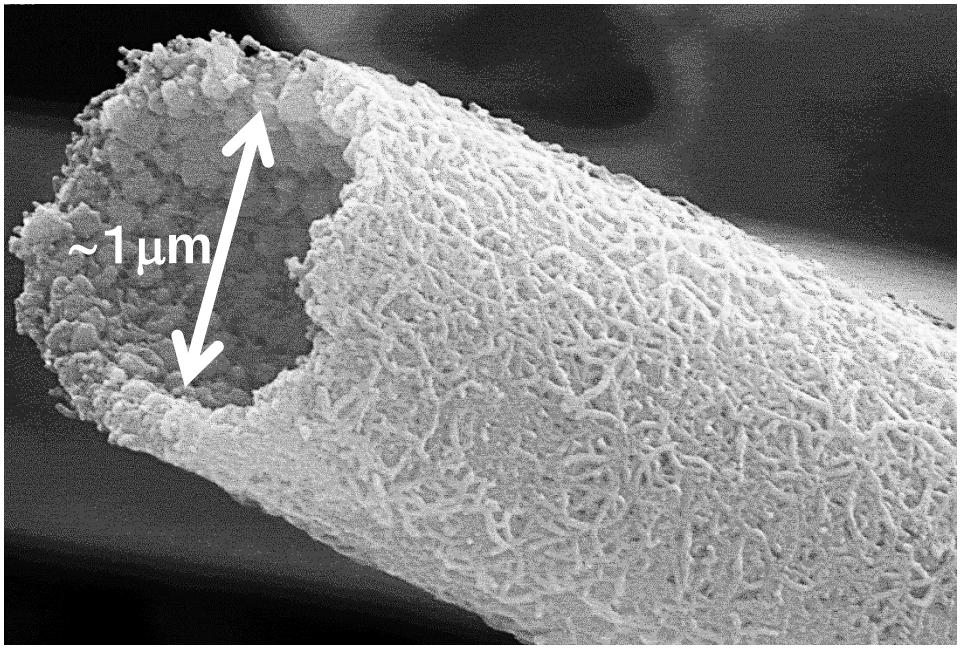
Tatsuki Kunoh, Katsunori Tamura, Hitoshi Kunoh, and Jun Takada
JST-CREST Project, Grad. Sch. Nat. Sci. Technol., Okayama Univ., Japan

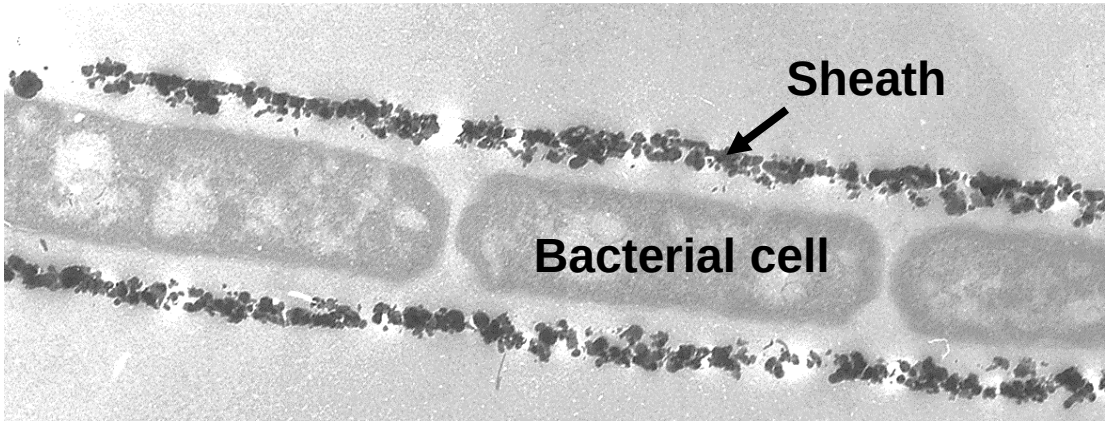
2015.07.21
Barcelona

Ocherous floats and sediments ubiquitous at groundwater-outwelling sites

BIOX enveloping catenulate cells
<genus *Leptothrix*, Fe-oxidizing bacterium>

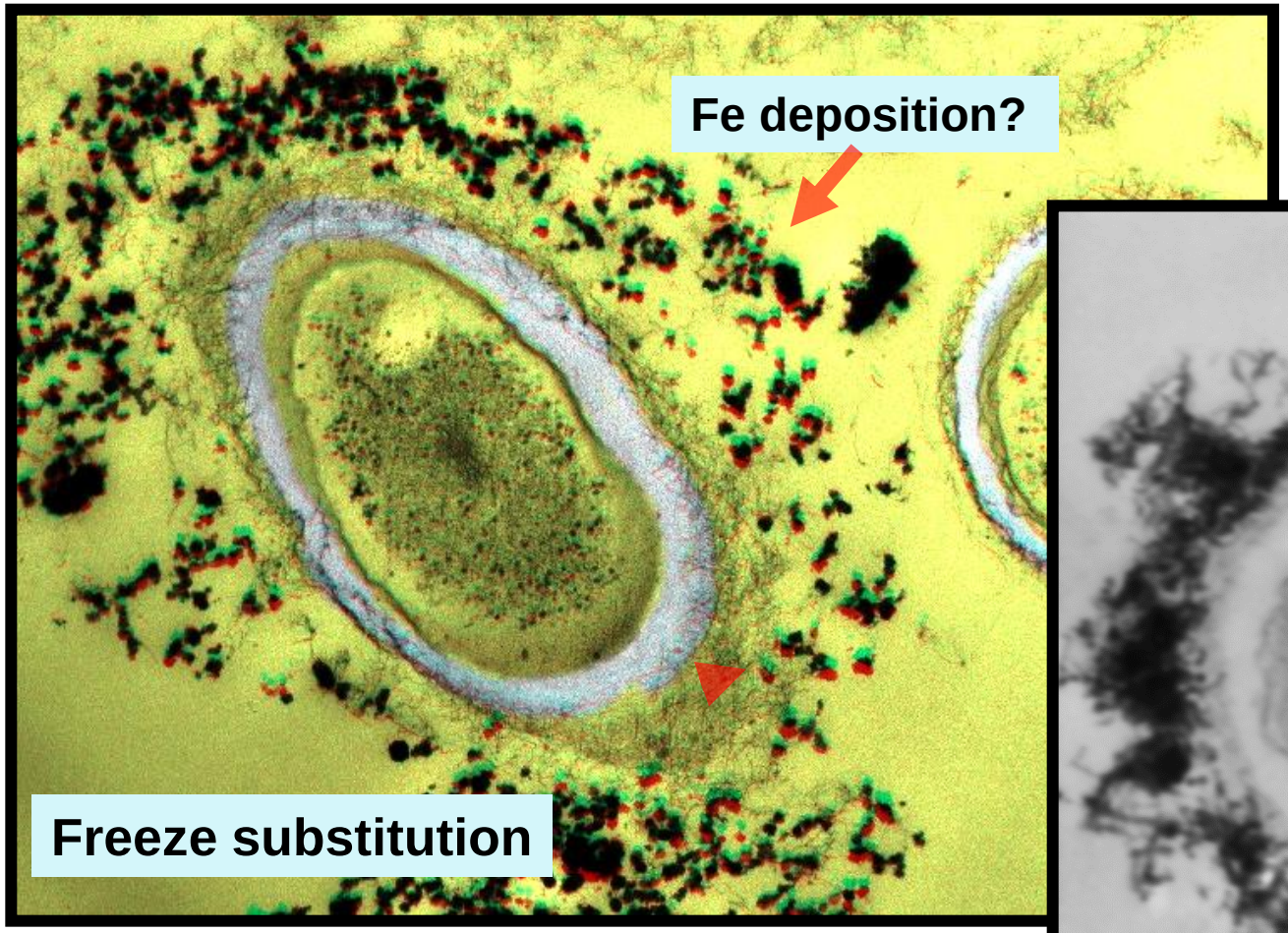


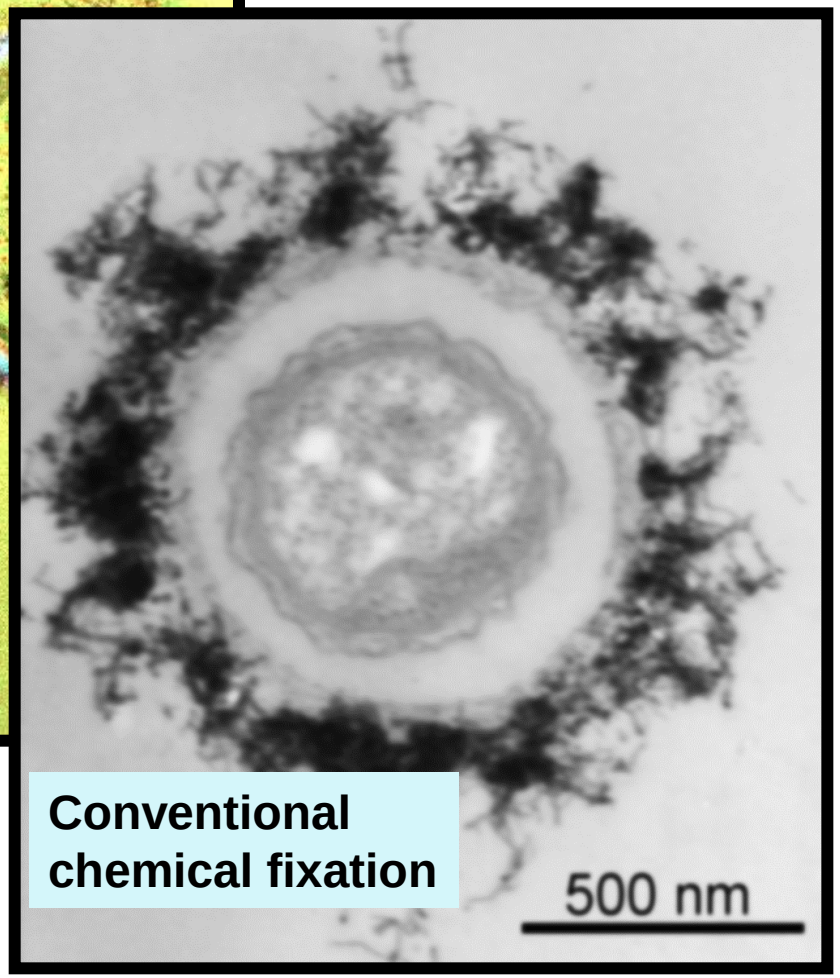




Biogenous Iron Oxides (BIOX)

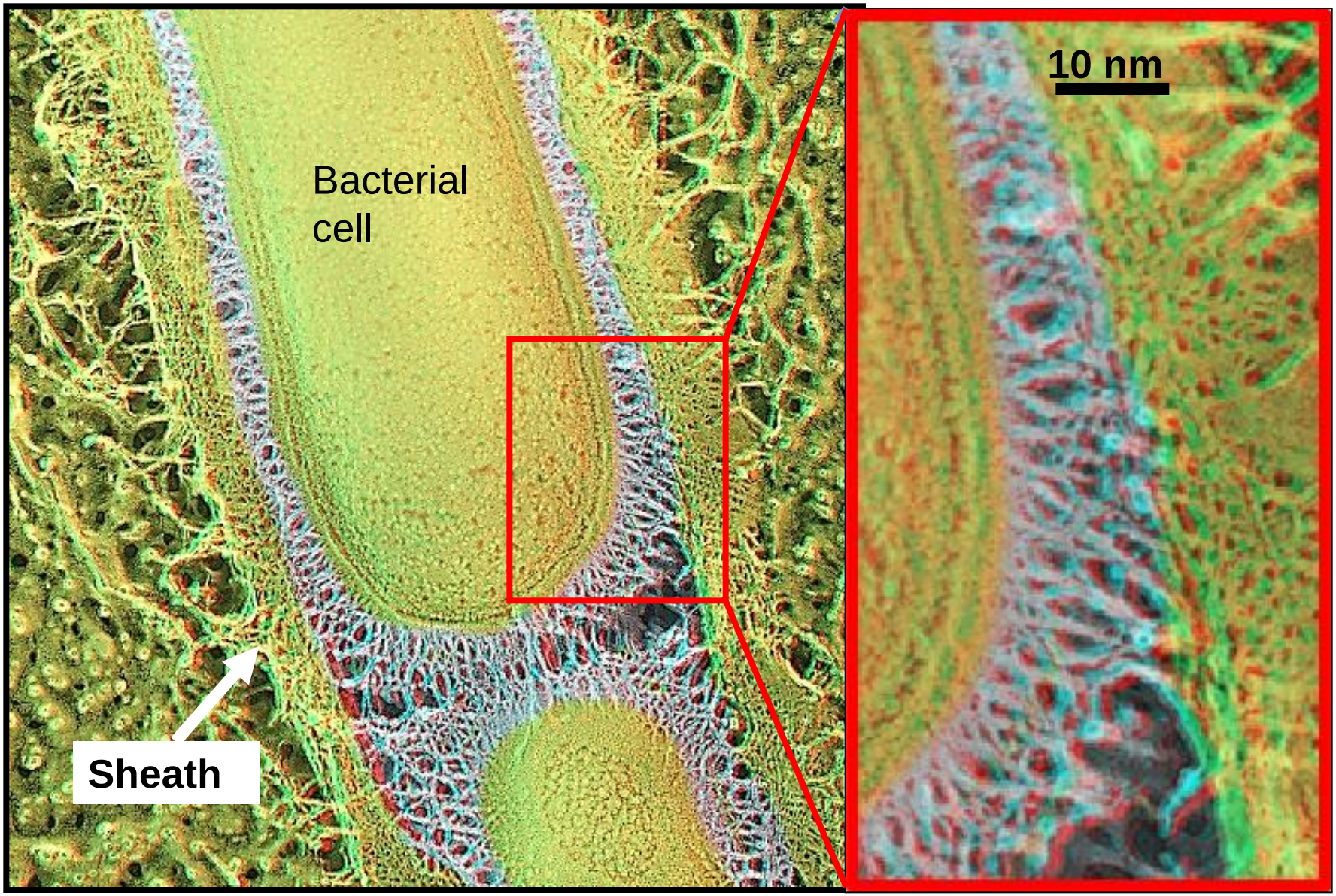
TEM image of bacterium-associated sheaths changes depending on fixation methods





Organic solvents (ethanol or acetone) used for specimen dehydration

Basic skeleton of sheaths composed of fibrillar materials released from bacterial cell (Freeze Fracture Replica) (3dpi)

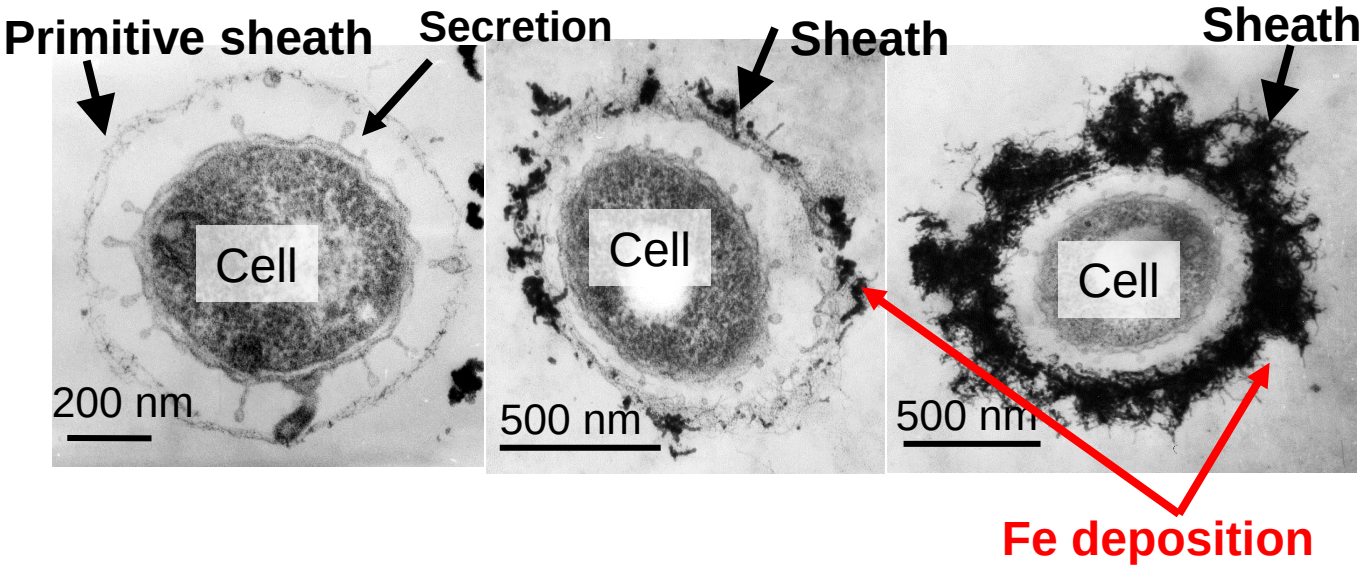


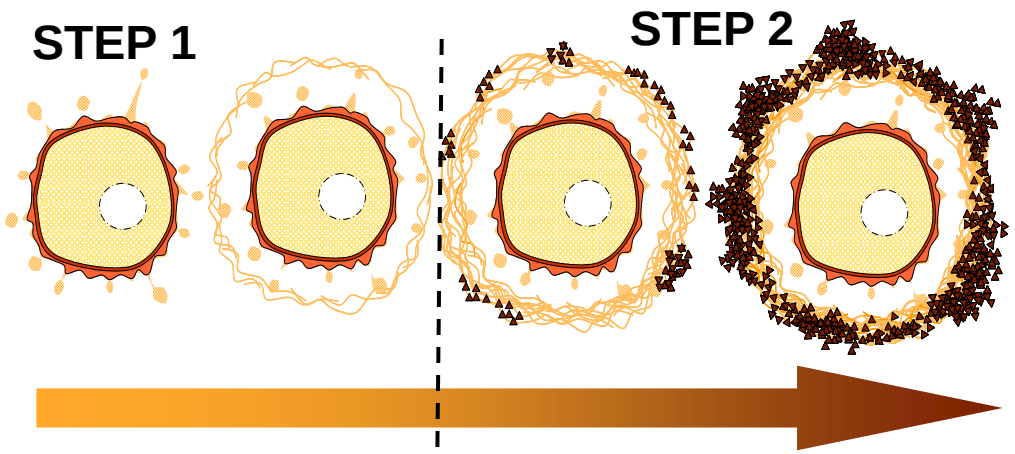
Sheath Formation Steps (OUMS1)

1 day culture

2 day culture

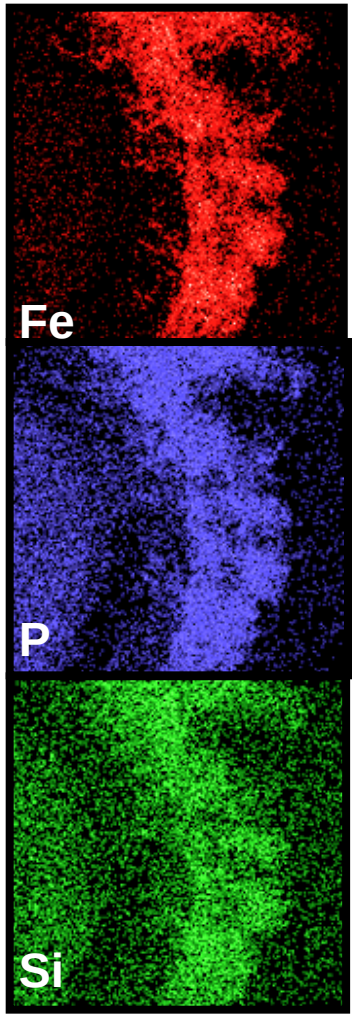
3 day culture





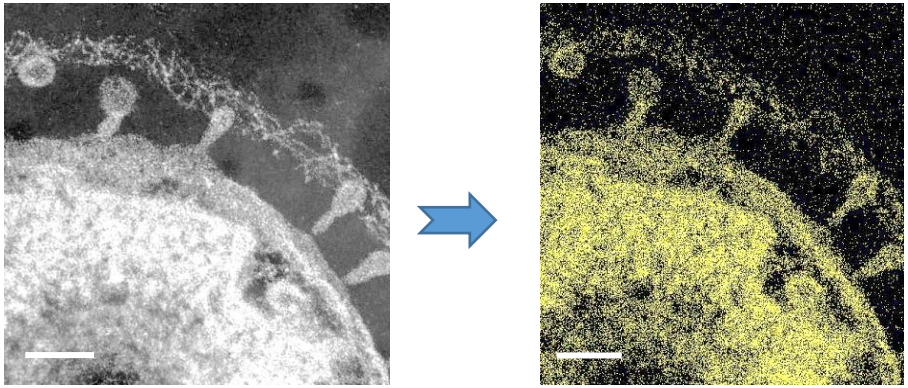
Assembly of bacterial microfibrils (primitive sheath)

Deposition of inorganics (mature sheath)



Progressive deposition of Fe, P, and Si

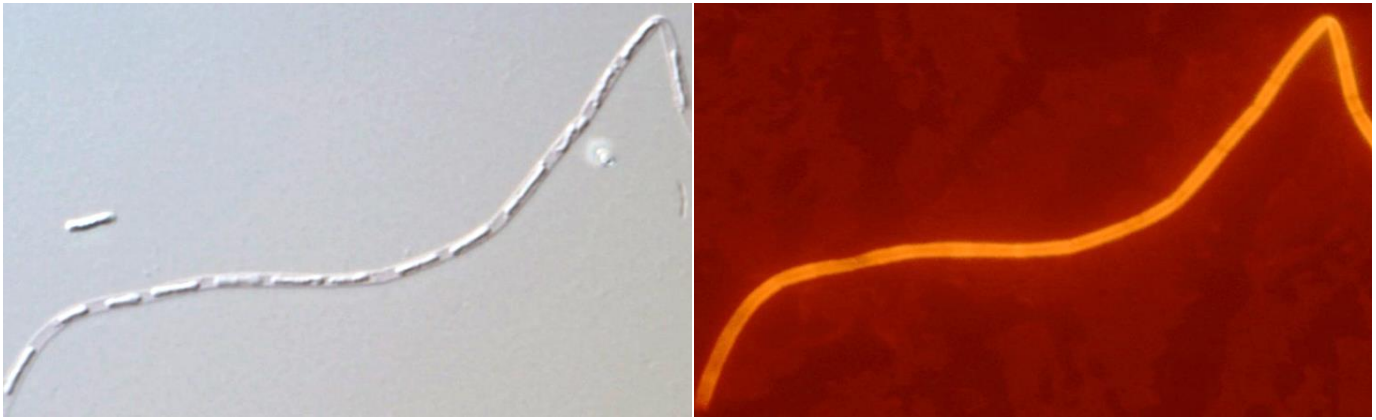
Sheath materials contain organic (saccharic and proteinacious) components of bacterial origin



HAADF STEM

Alkali Bismuth Stain

Polysaccharide

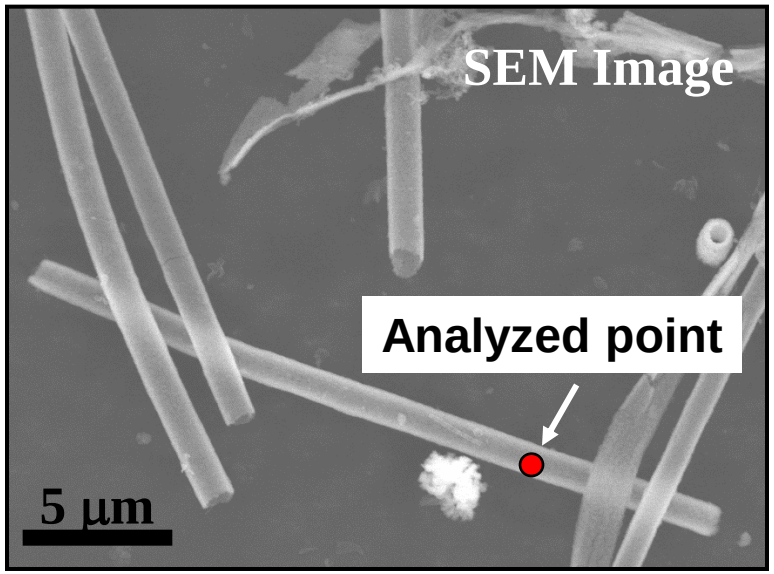


R-phycoerythrin labeled antibody → **-SH group**

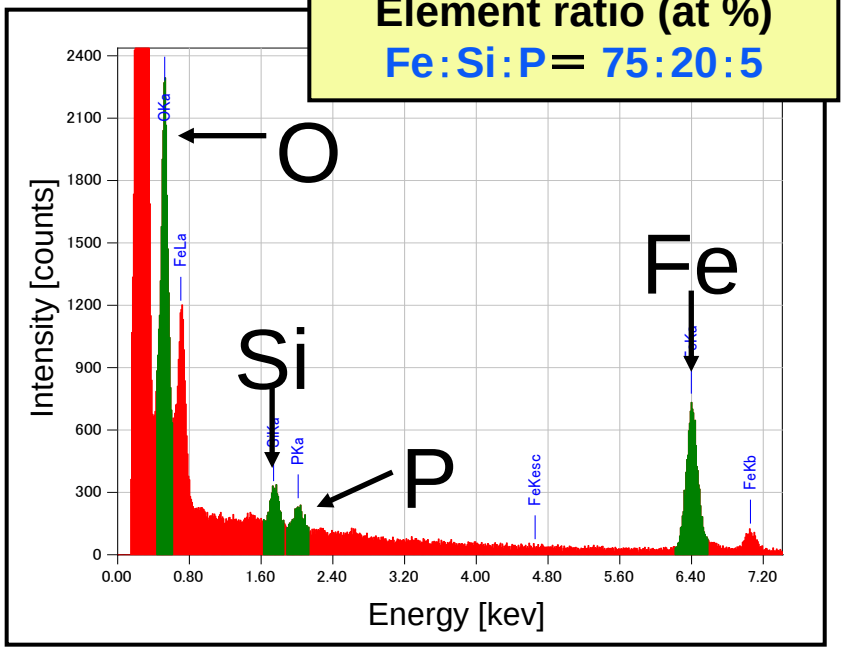
Fluorescein-labeled NH_2 kit → **-NH2 group**

C content：~1 (wt)%

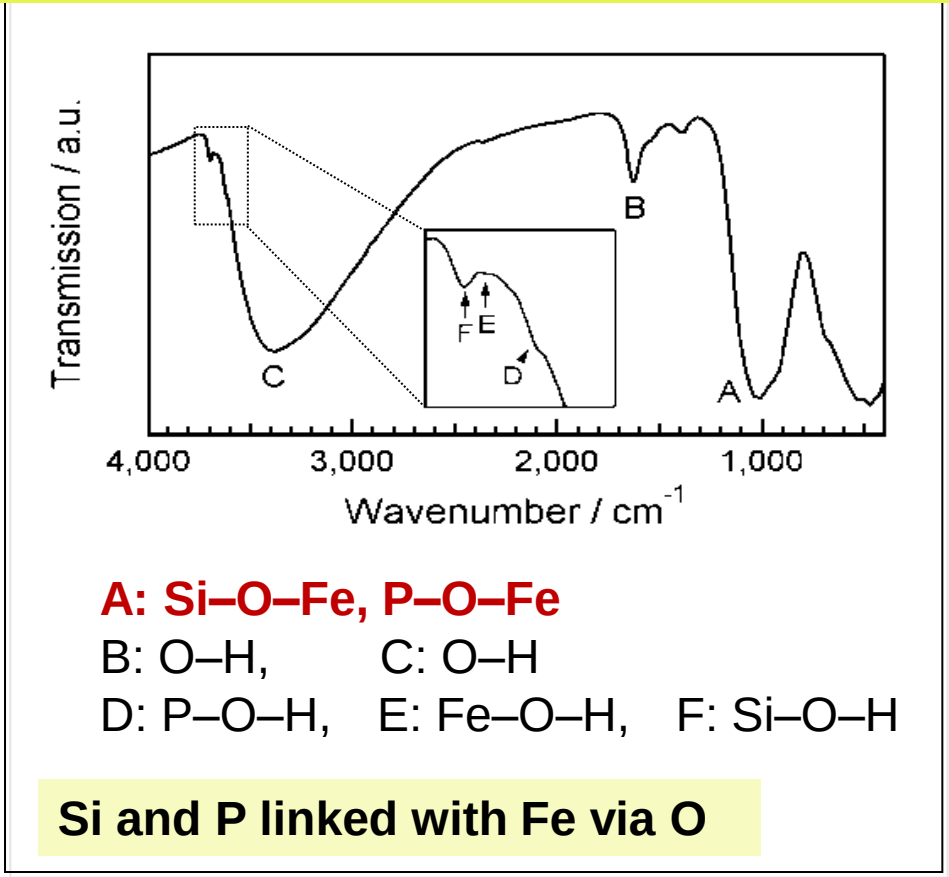
Elemental analysis of BIOX (EDX)



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

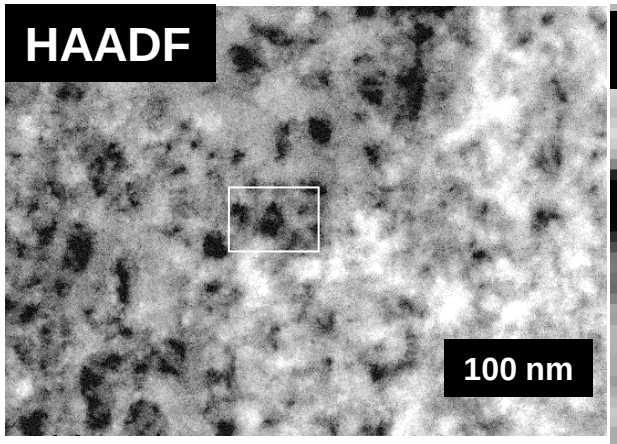
Inorganic elements are mutually linked through chemical connections

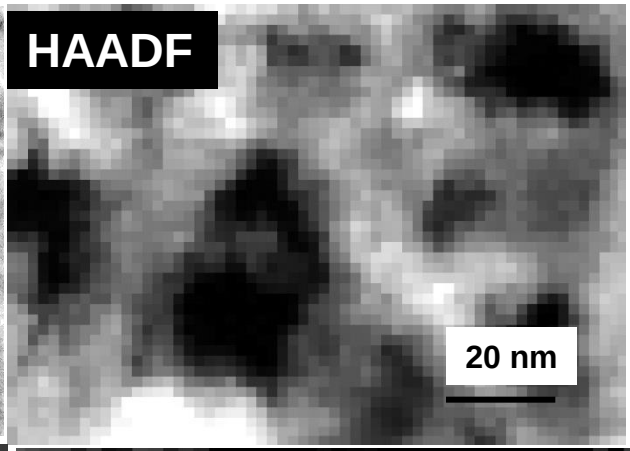
Elemental composition of groundwater

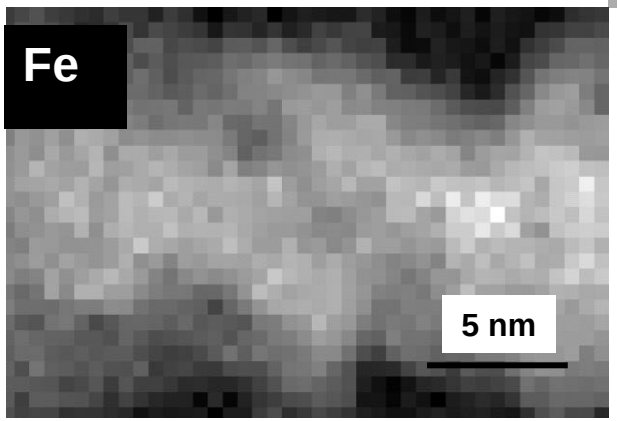
Fe:Si:P = 13:14:1 （wt. %）

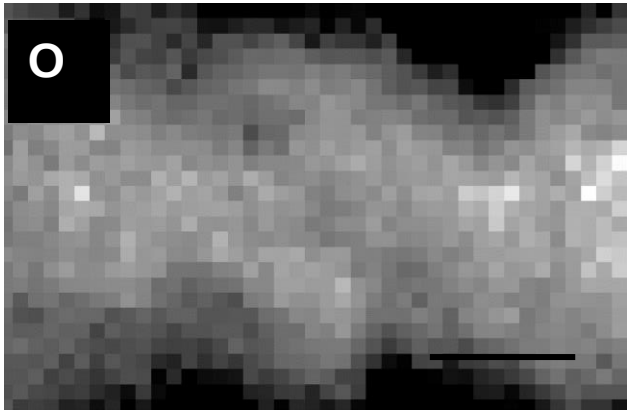
Ca, Na, K : minor elements

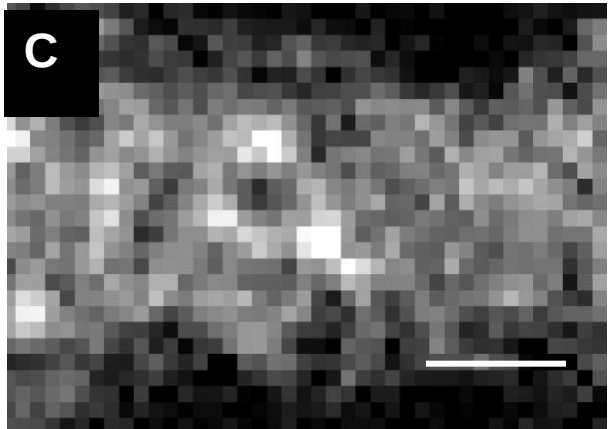
Distribution of Fe, C, and O in Sheath Texture（EELS）

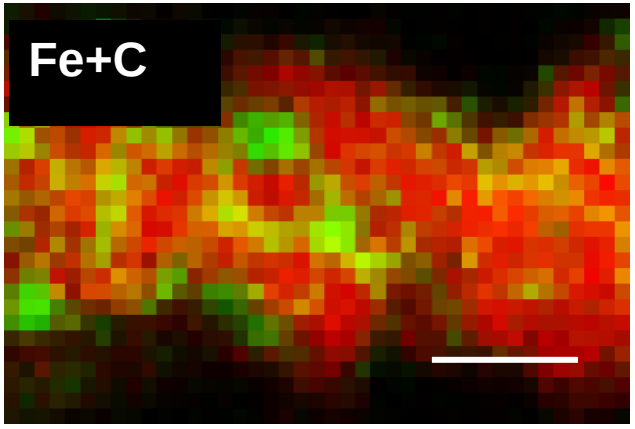












Identical localization of Fe and O

↓

Iron oxides?

Basic Sheath Texture

⇩

Organic/inorganic Hybrid Material

Fe-oxidizing bacteria are often used to remove Fe from groundwater at water purifying plants

Tones of Fe complexes on the reservoir bottom →
Disposed by landfill

Are they waste materials?

Amazing, interesting functions of BIOX for industrial application

1. Pigment (glaze) for pottery
2. Cell culture (cell affinity)
3. Enhancer of catalytic activity
4. Anode material of Lithium-ion battery
5. Plant protectant
6. Plant growth enhancer

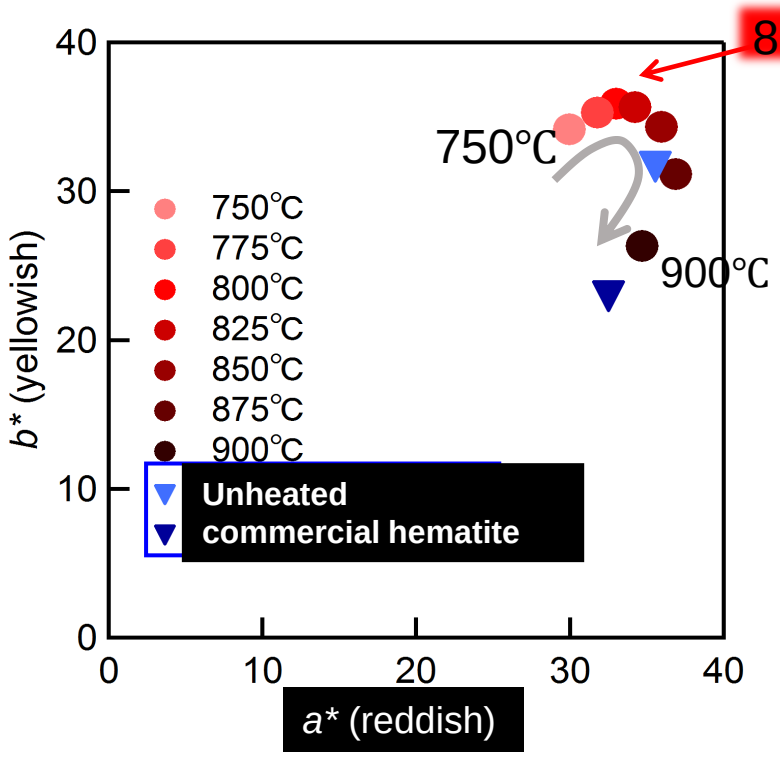
Ceramic artists always seek bright color pigments (glazes)

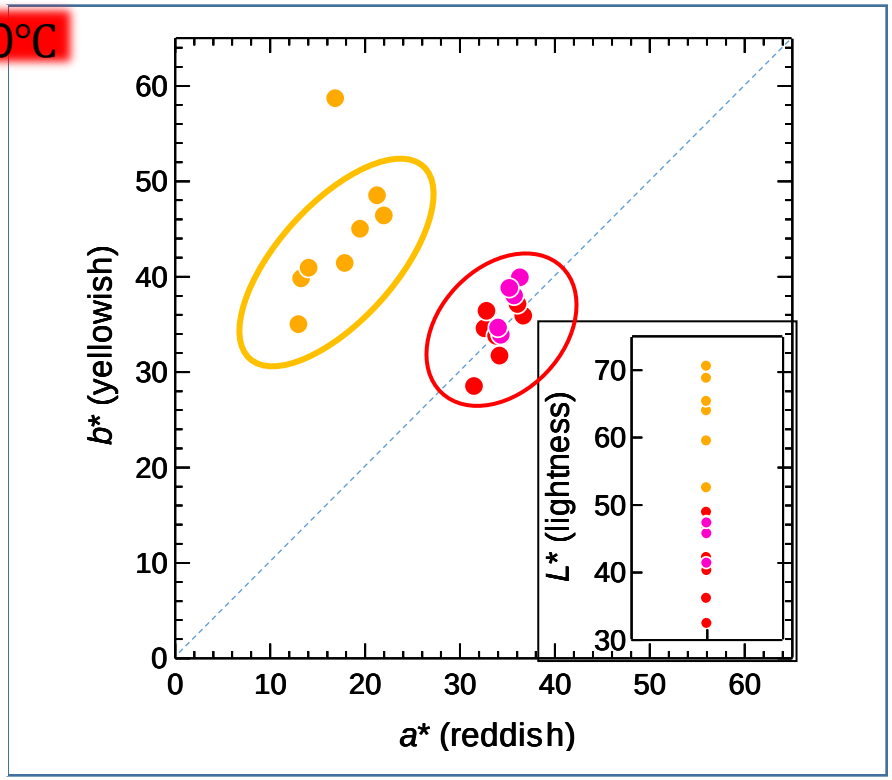
Color Change of Heated or Cultured BIOX

Natural BIOX

Culture at low pH and with CO_2

unheated **700℃** **900℃** **1000℃**



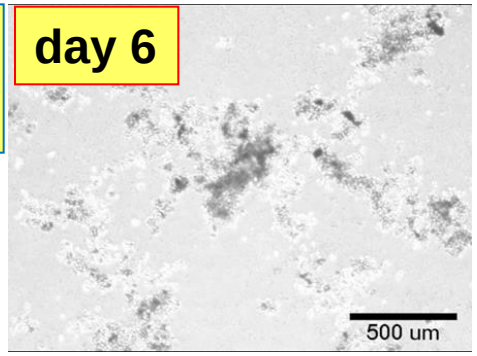


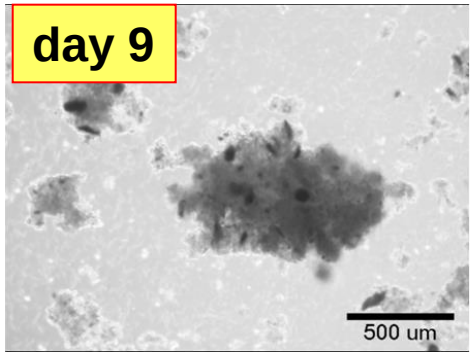
Hashimoto H. et al. **(2012)**. *Dyes and Pigments*, **95**, 639-643,

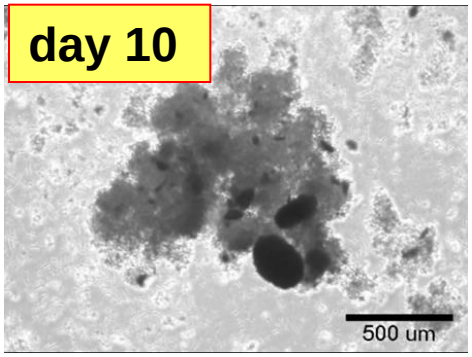
BIOX heated at 800 C gives an ideal reddish glaze for ceramic works

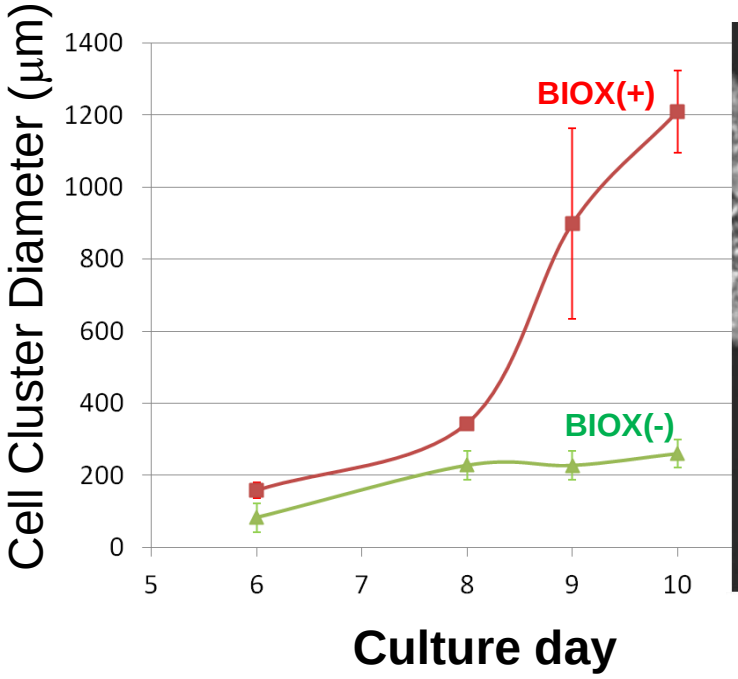
BIOX promotes 3D growth of human cells in culture

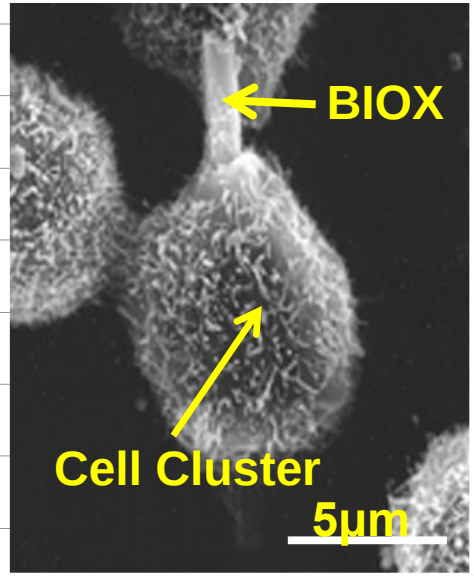
Breast Cancer Cell

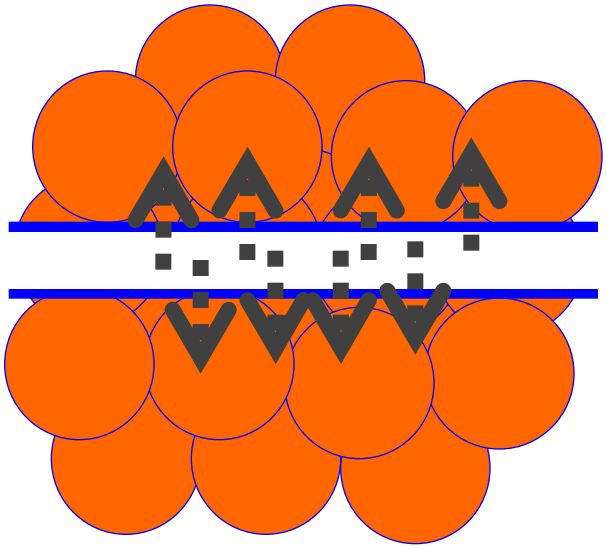








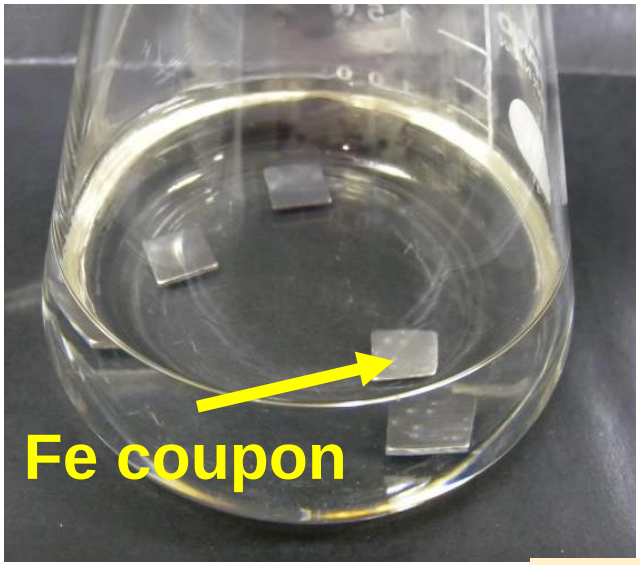




3D cell cluster: useful for production of specific proteins; drug screening

BIOX microtubule: route of O_2/nutrients

Si content in medium affects Fe/Si ratio of BIOX



Fe source:
3 Fe coupons / 100 ml SGP medium

Si contents: 0~300 ppm

Si=0 ppm	BIOX Atomic %	Fe:Si=100: 0
Si=100 ppm		60:40
Si=300 ppm		50:50

Learning from this result, we attempted to synthesize new Fe oxides linked with varied amounts of Si by artificial techniques

→ Expected to create novel functional materials

Si contents in synthesized 2-line ferrihydrites affects crystallinity

Artificial synthesis protocol

$Fe(NO_3)_3 \cdot 9H_2O$ +

m-$Na_2SiO_3 \cdot 9H_2O$

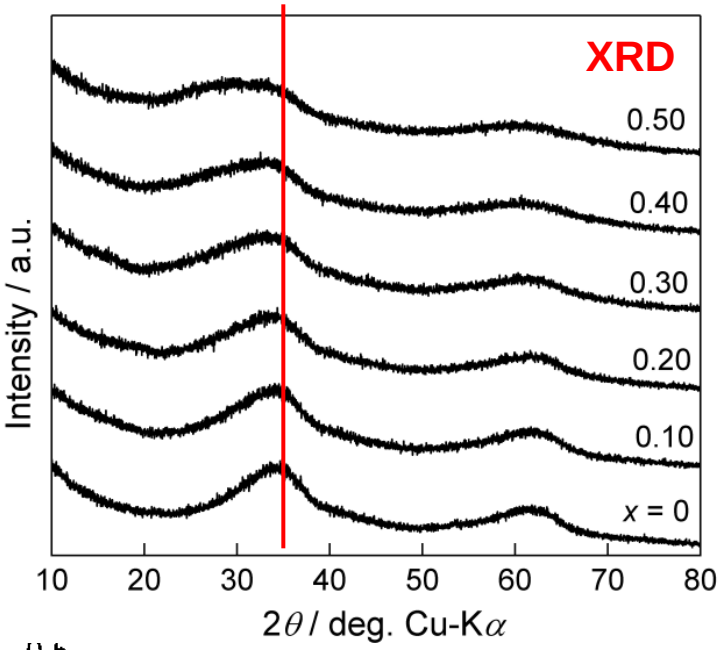
Starting solution
Si/(Fe+SI) = 0~0.7

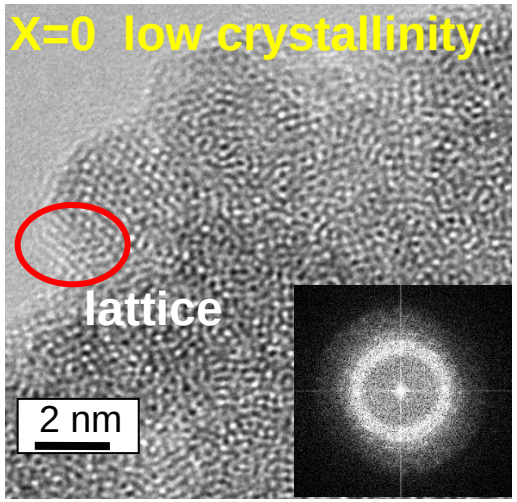
pH adjusted to 10 with aqua ammonium

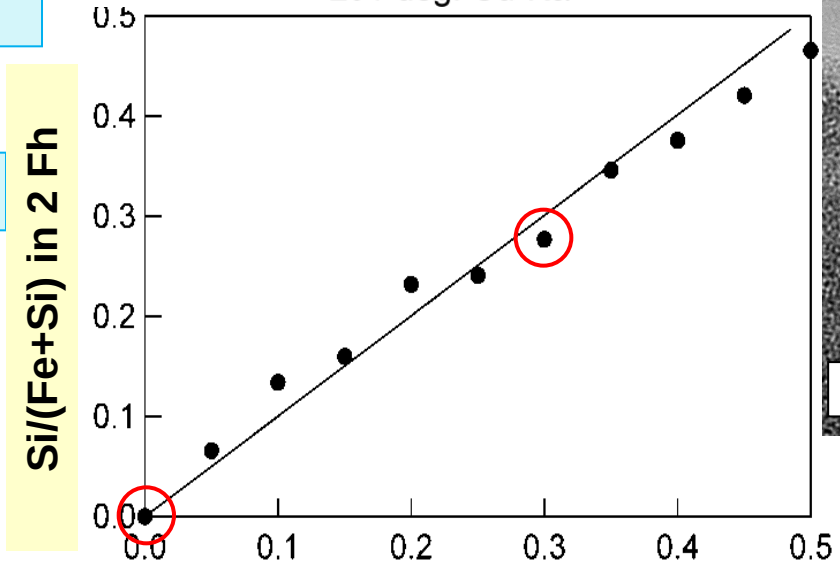
Reaction at room temp

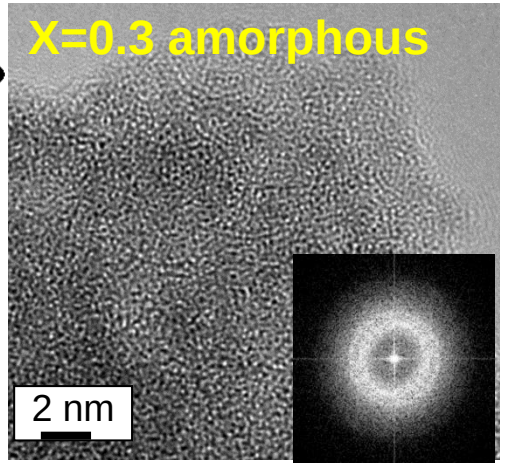
Washing with UPW

Vacuum dried

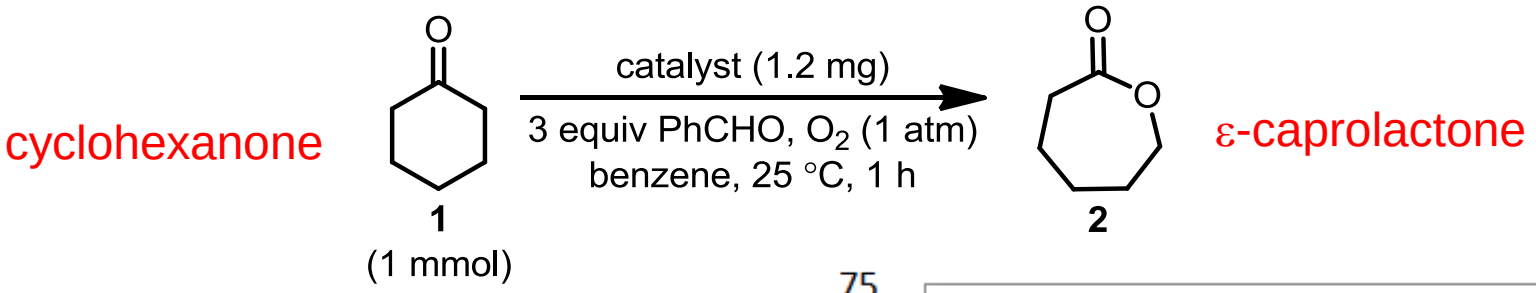






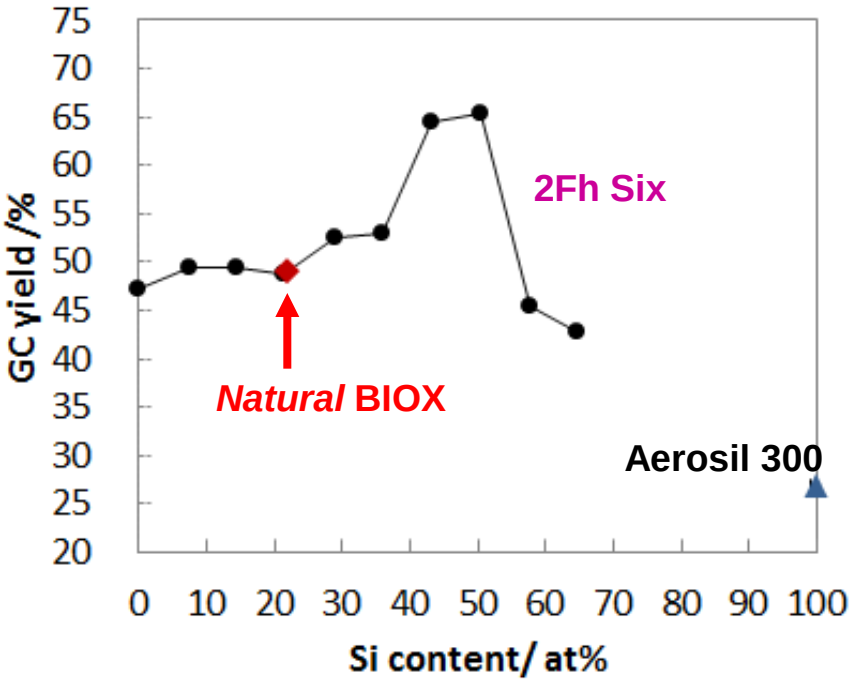


Synthesized BIOX-analogous 2-Fh with varied contents of Si promotes chemical reaction (Catalytic effects)



entry	catalyst	Si content x (at %) [a]	yield of **2** (%) [b]
1	***L*-BIOX (Joyo)**	22	49
2	**2Fh Si-x**	0	47
3		7.4	49
4		14.4	50
5		21.0	49
6		28.9	52
7		36.0	53
8		43.2	65
9		50.4	65
10		57.6	45
11		64.8	43
12	**Aerosil 300**	100	27

[a] Si/(Fe + Si) x 100 at %. [b] Determined by GC analysis. Average values.



- ✓ **Si content 40-50 at % showed maximum yield of 2.**
- ✓ **Aerosil 300 gave the lowest yield of 2.**

Conclusion

1. **Basic texture of BIOX produced by *Leptothrix* is composed of unique, ingenious organic/inorganic hybrid materials**
2. **BIOX materials have diverse, attractive functions for industrial purposes (the respective mechanisms are still in investigation)**
3. **Basic knowledge of BIOX (structure, component, formation process etc.) benefits creation of novel functional materials for human life**

Acknowledgement

<Okayama University>

Professor Mikio Takano (Metal chemistry)
Dr. Tomoko Suzuki (Cytology/microbiology)
Dr. Hideki Hashimoto (Material science)
Emeritus Professor Tomonori Shiraishi (Plant pathology/physiology)
Professor Kazuhiro Toyoda (Plant pathology)

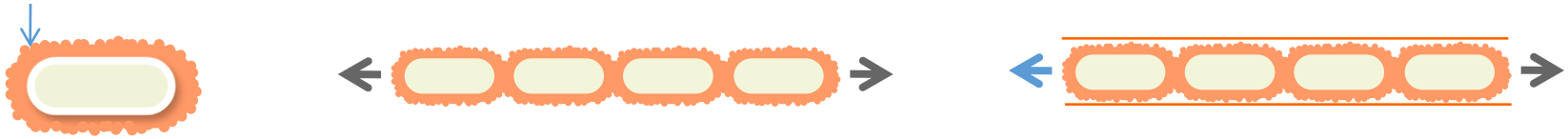
<Kyoto University>

Professor John Heuser (Bacteriology/microscopy)
Dr. Nobuhiro Morone (Bacteriology/microscopy)

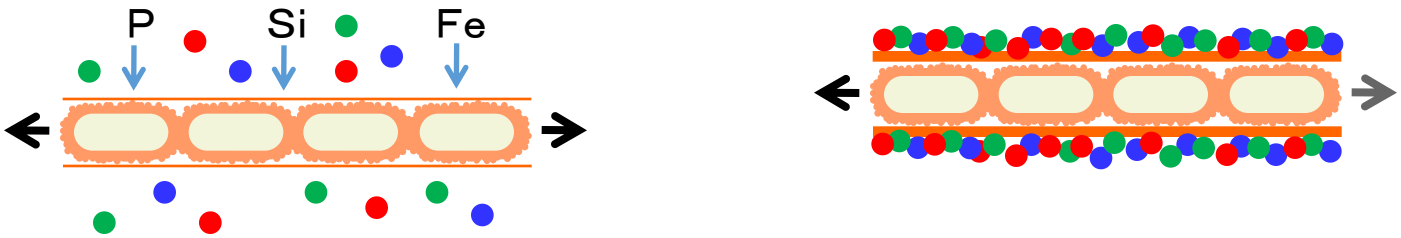
Thank you for your attention

Model of Sheath Formation and Hollowing Procedure

Bacterial Cell → Cell Division→Linked cells → Formation of Initial Sheath



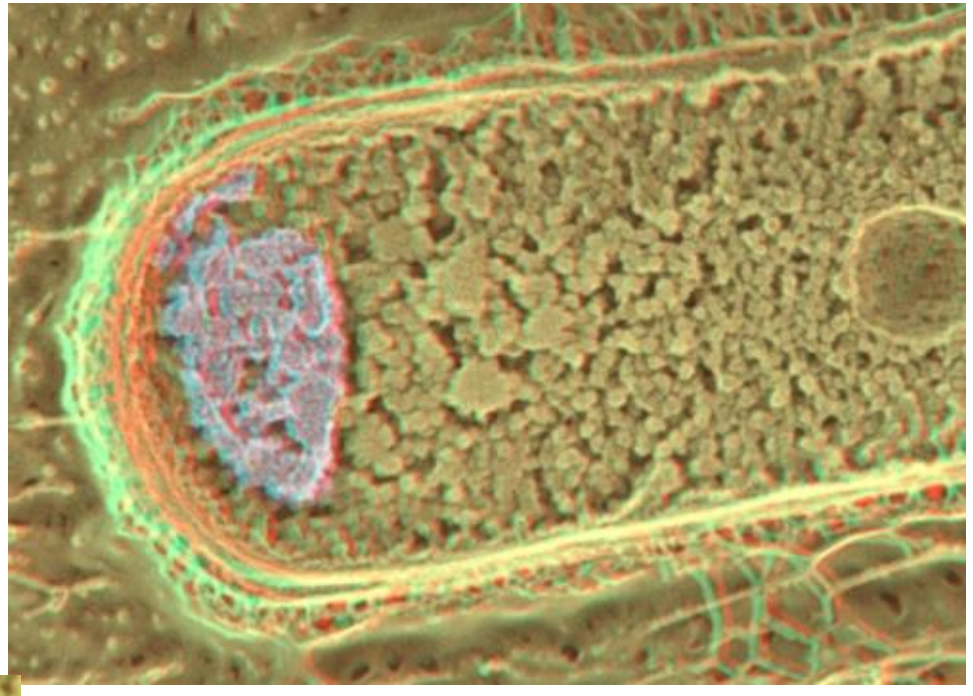
Deposition of Aquatic Inorganics → Sheath Elongation

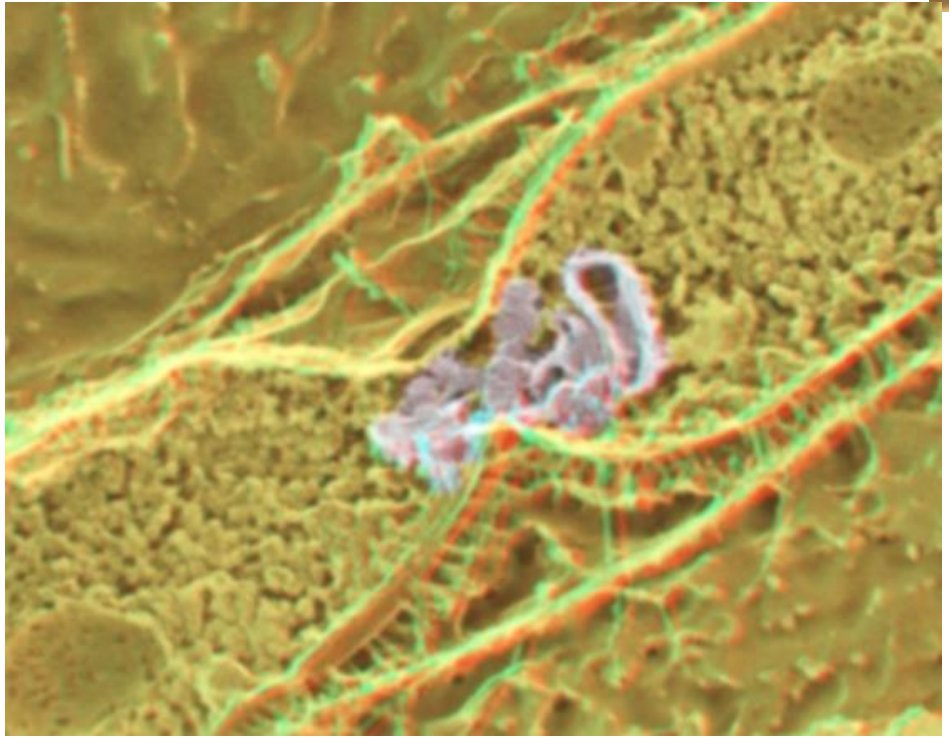


Isolation of Cells (Solo Existence) → Death/Degeneration of Isolated Cells

Autolysis / Disappearance

Freeze Fracture Replica
(Acetone- or ethanol-dehydration is unneccesary





Numerous fibrils arising from outer membrane of cell

(1 dpi)

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)

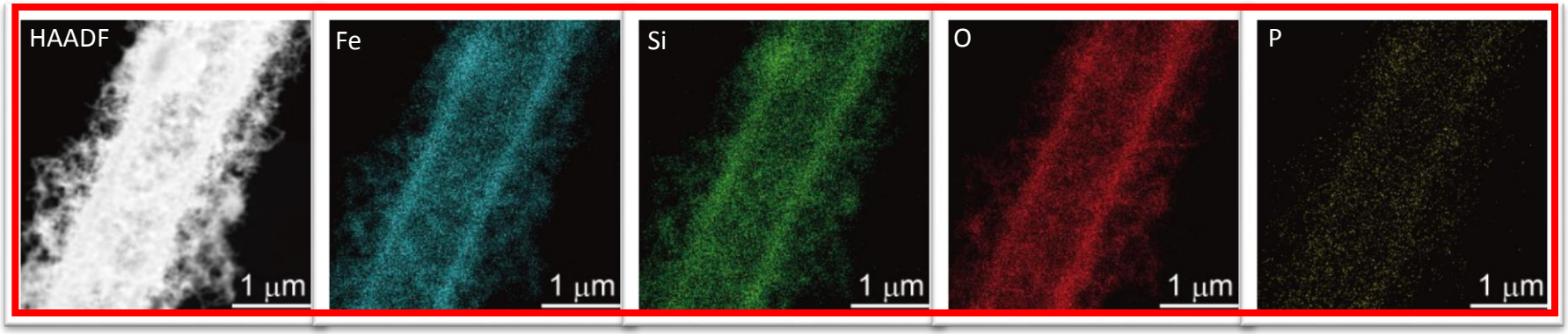
→ Chemically fixed with acetone-mixed fixative at -200C

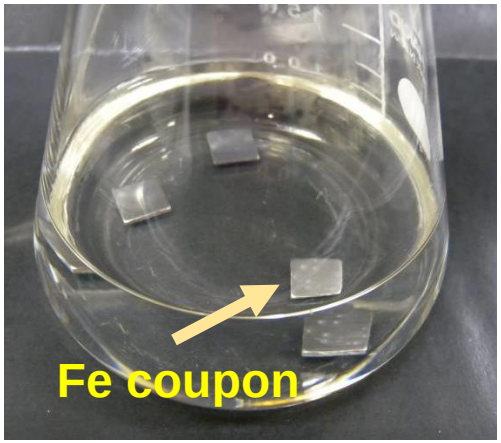
→ Gradually back to room temperature

→ Same as the chemical fixation

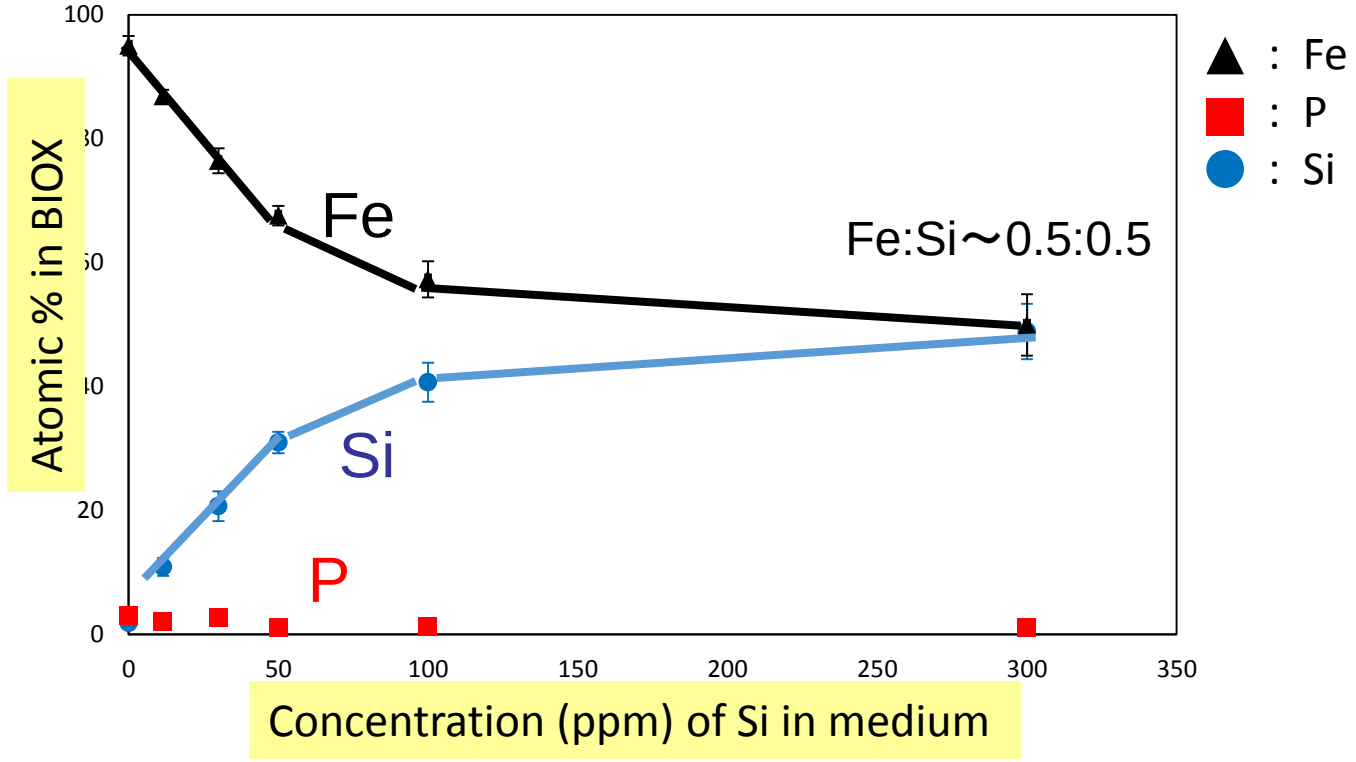
Si content in medium affects Fe/Si ratio of BIOX

300 ppm Si in medium





Si content in medium changes from 0 to 300ppm



★ Minerals, 3, 247-257, (2014)

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	
	[^3H]DPM	[^{14}C]Ura	[^3H]Thy	[^{14}C]Gal	[^3H]DPM	[^{14}C]Ura	[^3H]GlN	[^{14}C]Ura	[^3H]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	
	[^3H]DPM	[^{14}C]Ura	[^3H]Thy	[^{14}C]Gal	[^3H]DPM	[^{14}C]Ura	[^3H]GlN	[^{14}C]Ura	[^3H]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.

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

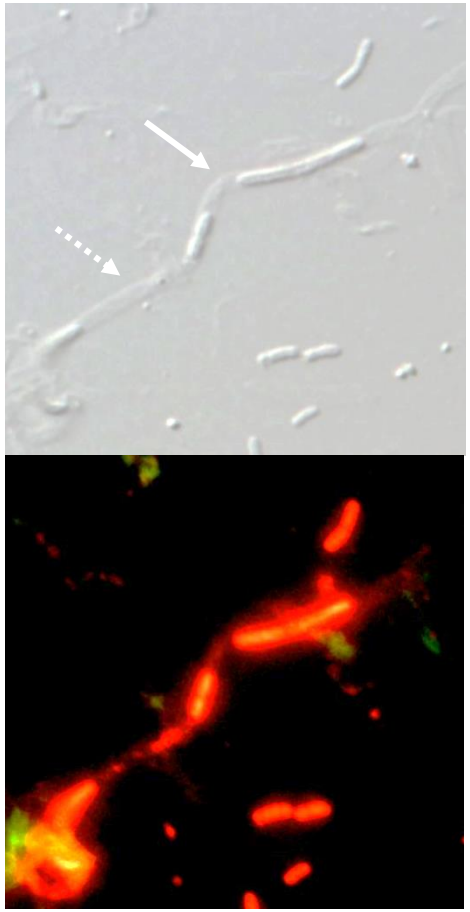
Freeze Fracture/Replica proved

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

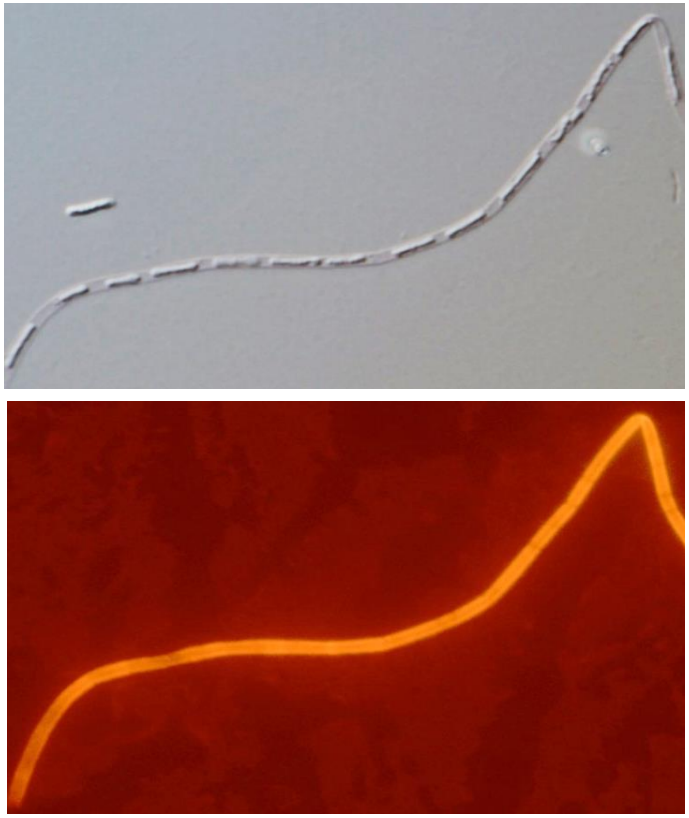
¥

Sheath contains protein

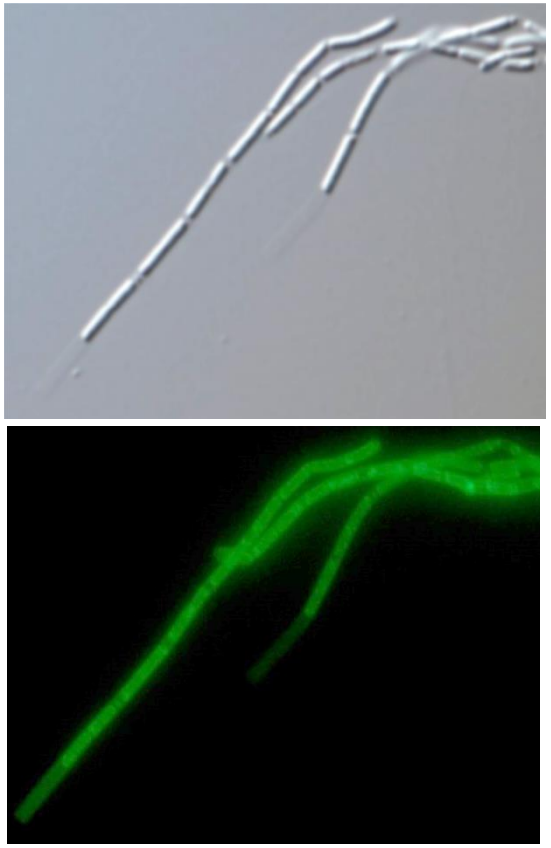
OUMS1 2-3 dpi



amino acids detection
（Ruby)



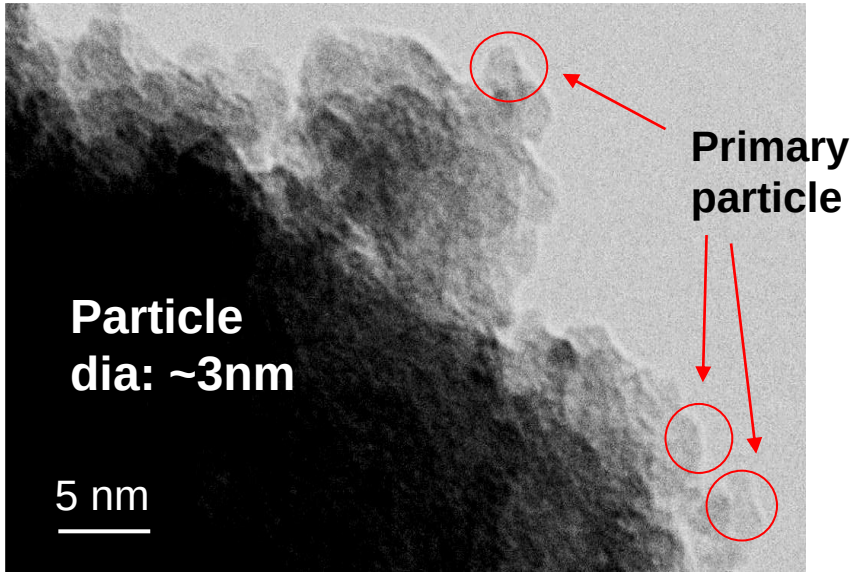
-SH Detection
(R-phycoerythrin
labeled antibody

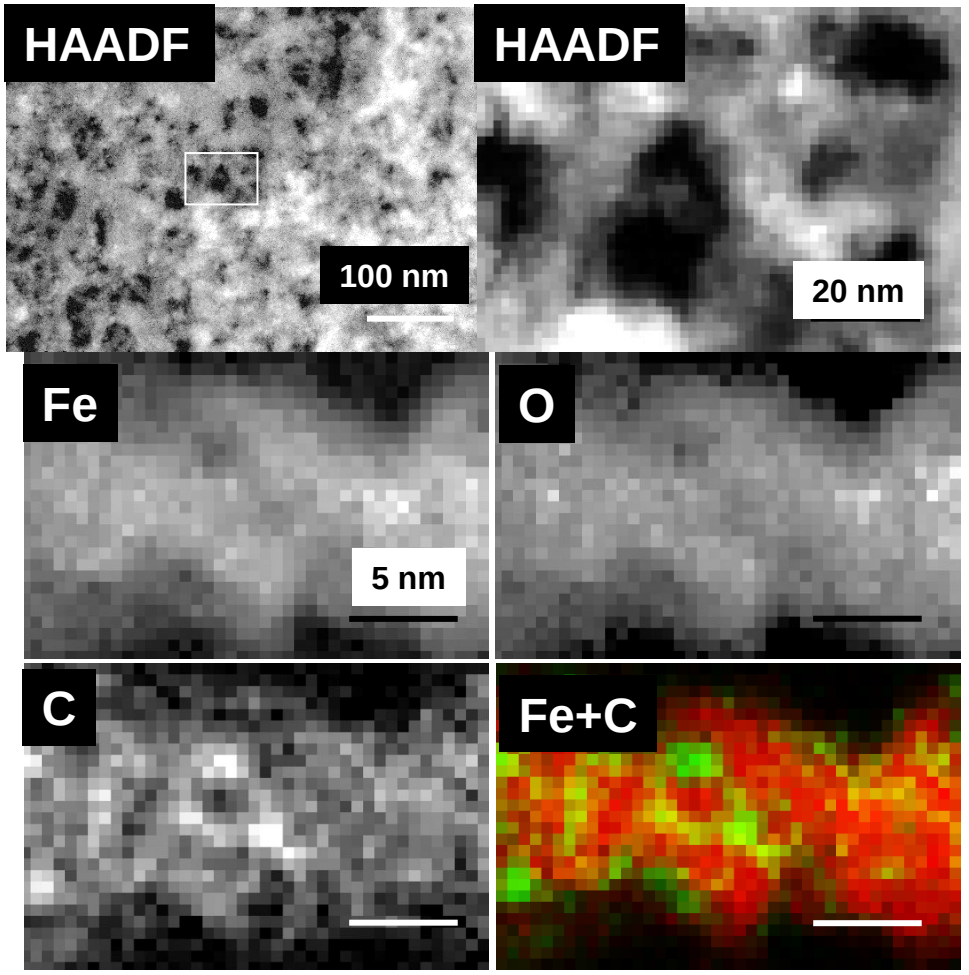


-NH_2 Detection
(Fluorescein-labeled
NH_2 kit）

BIOX contains saccharic and proteinacious materials of bacterial origin

Distribution of Fe, C, and O in Sheath Texture （EELS）





Basic Sheath Texture: Organic/inorganic Hybrid

Identical localization of Fe and O → Iron oxides?

Inconsistent localization of Fe and C

→ C and Fe irregularly connected?