

Pore-scale Spectral Induced Polarization (SIP) signatures associated with FeS biomineral transformations

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Abstract

*We measured Spectral Induced Polarization (SIP) signatures in sand columns during (1) FeS biomineralization produced by sulfate reducing bacteria (*D. vulgaris*) under anaerobic conditions, and (2) subsequent biomineral dissolution upon return to an aerobic state. The low-frequency (0.1-10 Hz peak) relaxations produced during biomineralization can be modeled with a Cole-Cole formulation, from which the evolution of the polarization magnitude and relaxation length scale can be estimated. We find that the modeled time constant is consistent with the polarizable elements being biomineral encrusted pores. Evolution of the model parameters is consistent with FeS surface area increases and pore-size reduction during biomineral growth, and subsequent biomineral dissolution (FeS surface area decreases and pore expansion) upon return to the aerobic state. We conclude that SIP signatures are diagnostic of pore-scale geometrical changes associated with FeS biomineralization by sulfate reducing bacteria.*

22 **Introduction**

23 Near surface geophysical measurements are potentially sensitive to microbial processes (see *Atekwana*
24 *et al.*, 2006, for review). Geophysics offers the possibility of observing microbial processes non-
25 invasively, and at larger scales inaccessible with standard microbiological techniques. One microbial
26 process detectable with electrical geophysical methods is iron sulfide (FeS) biomineralization induced
27 by sulfate reducing bacteria (*Williams et al*, 2005; *Ntarlagiannis et al.*, 2005). The potential hence exists
28 to monitor sulfide mineral formation processes in marine environments and wetlands, remediation of
29 mine wastes using wetlands, and biostimulation strategies for the degradation of contaminants.

30 The spectral induced polarization (SIP) method measures the low-frequency (sub-kHz) electrical
31 properties (related to both conduction loss and charge storage) of a porous medium (see *Lesmes and*
32 *Friedman*, 2005, for review). The method was developed for locating buried metallic mineral deposits
33 (e.g. massive FeS mineralization) based on a polarization phenomenon that occurs at the interface
34 between metallic minerals and the pore-filling fluid (*Wong*, 1979). *Williams et al.* (2005) showed that
35 the formation of FeS biominerals under anaerobic sulfate reducing conditions generates measurable (~15
36 mrad) SIP signals with central relaxation frequencies between 0.1-10 Hz. *Ntarlagiannis et al.* (2005)
37 analyzed single frequency data from the same experiment and showed that the polarization magnitude is
38 proportional to the rate of microbial activity. They suggested that changes in the relative strength of
39 polarization and conduction results from a geometric rearrangement of evolved biominerals in pore
40 spaces.

41 In this letter we extend this work by reporting results of a new column experiment whereby we first
42 induce anaerobic FeS biomineralization but then subsequently induce biomineral dissolution by
43 returning the system to an oxic state. We also model the SIP signature of both phases using a Cole-Cole

44 relaxation (*Cole and Cole, 1941*). We show that the SIP signal (and modeled dispersion parameters)
45 generated during biomineralization is fully reversible and exhibits no significant hysteresis.
46 Furthermore, we show that the time constant of the relaxation (and its temporal behavior during
47 biomineral formation and dissolution) is consistent with the source of this polarization being biomineral
48 encrusted pores of the sand matrix, and not the biomineral encrusted bacterial cells themselves.

49 **Methods**

50 The experiment design closely followed that described in *Williams et al. (2005)*. All materials were
51 sterilized using common protocols. Quartzitic sand (grain size for which 50 % of sample is finer (d_{50}) =
52 0.3 mm; porosity = 37 ± 1 %), treated to remove trace organics and/or iron oxides, was packed into a
53 horizontal column (diameter = 0.0317 m; length = 0.203 m) containing six Ag-AgCl electrodes housed
54 in electrolyte-filled ports attached to the column edge. Two Ag-AgCl coils (diameter = 0.0317 m) were
55 emplaced at column ends for current injection. The column was saturated and continually circulated
56 with a growth medium (including lactate, sulfate, iron, minerals and vitamins) and purged with N₂ gas to
57 remove residual air bubbles. A continuous flow rate of ~ 2 pore volumes/day (~0.4 m/day) was
58 established with a peristaltic pump (maintained for the 55 days). Between Days 0-35 (anoxic phase) the
59 column was placed in an anaerobic chamber to facilitate microbial sulfate reduction. The anaerobic
60 chamber was opened to the atmosphere on Day 36 until Day 55 to encourage FeS oxidation and
61 dissolution. The growth medium was still continuously circulated (without sparging with N₂) but exposed
62 to air via a 0.45 µm filter.

63 Cells of a pure culture of *Desulfovibrio vulgaris* (*D. vulgaris*) were grown and inoculated into the center
64 of the experiment column via syringe on Day 5. Pore water samples for analysis of aqueous
65 geochemistry were extracted by syringe at three locations along the column every 1-2 days following

66 inoculation. We measured Eh, pH, and electrolyte conductivity using bench-top probes. Lactate, acetate,
67 and sulfate were measured with an ion chromatograph. Hydrogen sulfide (H₂S) concentration was
68 estimated using a test kit (HACH HS-WR) immediately after sampling to minimize volatilization of H₂S
69 to the atmosphere. The column solid phase was analyzed upon termination of the experiment at Day 55
70 using scanning electron microscopy (SEM).

71 SIP measurements were collected at 40 equally spaced (in log space) frequencies between 0.1-1000 Hz
72 using a dynamic signal analyzer (see *Slater and Lesmes, 2002* for details). Current was applied via the
73 coil electrodes, and impedance magnitude and phase shift (ϕ) recorded relative to a precision resistor.
74 This measurement was made at three locations along the column (between electrode pairs 1-2, 3-4 and
75 5-6) at 1-3 day intervals. The measurement error for the magnitude is less than 1% whereas the error in
76 ϕ is ~ 0.1 mrad below 100 Hz (*Slater and Lesmes, 2002*).

77 The SIP datasets were modeled with a phenomenological Cole-Cole dispersion expressed in terms of a
78 complex resistivity (ρ^*),

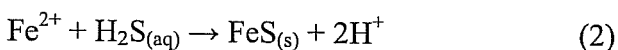
$$79 \quad \rho^* = \rho_0 \left[1 - m \left(\frac{1}{1 + (i\omega\tau)^c} \right) \right], \quad (1)$$

80 where ρ_0 is the dc resistivity, τ is the mean relaxation time associated with the frequency dispersion, c is
81 a shape exponent (0.2-0.8 for unconsolidated soils) and m is the chargeability associated with the
82 interfacial charge storage (*Pelton et al., 1978*). Model parameters were estimated using an algorithm
83 employing a least-squares approach with Marquardt regularization (*Kemna, 2000*). This algorithm
84 automatically nests a second higher-frequency relaxation (usually to model coupling effects in field
85 data). As the SIP frequency range is usually not wide enough to capture the shape of the second
86 relaxation we only report the parameters for the primary relaxation here. We recognize that Cole-Cole

parameter estimation is uncertain and may find local minima in the objective function. However, analysis of parameter uncertainty suggested that τ is the most well resolved parameter (estimated variances being less than 8 % of the parameter estimate).

Results

Figure 1 summarizes the aqueous geochemistry of the system (average values for the samples extracted from the three sampling ports along the column) over the 55 days of the experiment. We define three system states: (1) the anaerobic transition ($t = 14$ -25 days); (2) the anaerobic phase ($t = 26$ -35 days); (3) the aerobic transition ($t = 36$ -45 days). The anaerobic transition represents the onset of biomineralization (following a ~2-3 day lag phase) as evidenced by decreasing sulfate (electron acceptor), decreasing lactate (carbon source), increasing acetate (reaction byproduct) and increasing H_2S (reaction byproduct) due to sulfate reduction. The aqueous H_2S produced from microbial induced sulfate reduction, commonly reacts with any metals in solution forming metal sulfides (solid state). In our column the dominant metal in solution is Fe^{2+} (125 μm inflow concentration) and the precipitating sulfide is FeS ,



The anaerobic phase is characterized by relatively stable, low levels of lactate and sulfate but high levels of acetate and H_2S . This phase represents maximum rates of microbial activity and coincided with maximum visible precipitate formation, interpreted to be $\text{FeS}_{(\text{s})}$. The black precipitate was visible throughout the column by Day 10 and confirmed (via SEM supported with Energy Dispersive X-ray) as FeS biomineralization.

The aerobic transition represents the return of the system to oxic conditions. Oxidation of FeS over a ~10 day period (Day 36-45) is supported by previous laboratory studies confirming rapid rates of pyrrhotite ($\text{Fe}_{(1-x)}\text{S}$, $0.0 < x < 0.125$) oxidation at atmospheric concentrations of O_2 and at 22° C, being

~100 times those measured for pyrite (FeS_2) (Nicholson and Scharer, 1994). The return of H_2S to levels comparable to the start of the experiment is consistent with decreased sulfate reduction rates by *D. vulgaris* (and possibly chemical oxidation of sulfide). However, sulfate does not rebound completely, suggesting the continuation of sulfate-consuming anaerobic conditions in parts of the column. This is consistent with the known tolerance of *D. vulgaris* in microaerophilic (even in aerobic) conditions, it being likely that cells survived the aerobic transition, albeit with reduced activity. The aerobic transition was directly visible from the disappearance (dissolution) of the black FeS precipitate during this period. Continually low levels of lactate and the removal of acetate during the aerobic transition may reflect consumption by aerobic microbes unintentionally introduced to the column upon exposure to air.

In this letter we present only the results for electrode pair 5-6, which exhibited the most pronounced geoelectrical changes. A full analysis of the spatial variability in the geoelectrical data, and its correlation with spatially variable biomineral formation, will be presented in a forthcoming paper. Consistent with the results of Williams *et al.* (2005), FeS biomineralization results in the development of a dispersive relaxation. Figure 2 is a plot of resistivity magnitude ($|\rho|$) and phase (ϕ) as a function of frequency at $t = 21$ days (anaerobic transition) illustrating this dispersion and showing the fit of Equation 1. The data are truncated at 100 Hz as above this frequency ϕ is less than the instrument accuracy (0.1 mrad). The open squares/circles are the measurements, and the lines depict the dispersion model fit. The model fits the data well (RMS error between model and data = 9 %) except below 0.1 Hz (most obvious in the $|\rho|$ term due to the fine plotting scale), which may suggest the presence of a very low frequency relaxation just captured in this dataset. The modeled time constant for the primary relaxation is 0.22 s (see caption in Figure 2 for other model parameters).

The temporal character of the SIP signature of this biomineralization process is demonstrated for the anaerobic transition and aerobic transition stages in Figure 3a and 3b respectively. Data points where the

$\varphi < 0.1$ mrad (instrument accuracy) are again omitted. Figure 3c shows the temporal evolution of the estimated dispersion model parameters $m_n = m/\rho_0$ (being the polarization strength e.g. *Lesmes and Frye, 2001*) and τ obtained for these datasets. Note that a satisfactory model could not be fit to the Day 14 dataset due to the very small polarization peak (~ 1.5 mrad). The anaerobic transition is characterized by an increase in the dispersion strength (i.e. increase in phase peak in data and m_n from model) and shift in phase peak to higher frequencies (decrease in τ from model). The aerobic transition shows essentially the reverse behavior as evidenced in the data (Figure 3b) and the modeled parameters (Figure 3c).

Post experimental destructive analysis followed by SEM imaging indicated the presence of biominerals (Figure 4a) and biofilms on quartz grains (Figure 4b). Energy dispersive microscopy (EDS) analysis showed that both biominerals and biofilms are composed of FeS. The FeS encrusted cells (Figure 4a) show the characteristic curved rod shape of *D. vulgaris* with an average size of $0.44 \pm 0.06 \mu\text{m}$ and $1.93 \pm 0.15 \mu\text{m}$ (based on three SEM images) in the short and long axis, respectively.

Discussion

The SIP response of FeS biomineralization can be modeled with a Cole-Cole model with τ in the range of 0.2-0.4 s (Figure 3c). We find that the SIP response that develops during the transition to sulfate reducing conditions and biomineral formation (anaerobic transition) is reversible, exhibiting no significant evidence of hysteresis, upon biomineral dissolution during the return to oxic conditions (aerobic transition). This suggests that the SIP method could be used to monitor and track not only variable rates, but also variable directions, of microbial-mineral transformations in environments where redox conditions fluctuate.

Although purely a phenomenological approach, the Cole-Cole modeling affords insights into the polarization mechanism associated with this process. It is recognized that τ is inversely related to some

measure of the length scale over which ions in the electrical double layer at the mineral-fluid interface are displaced. *Schwarz (1962)* provides a model for the polarization of a particle in a uniform electrical field, based on tangential ionic fluxes, whereby the time constant of the relaxation is related to the particle radius (r),

$$\tau = \frac{r^2}{2D}, \quad (3)$$

where D is the surface ionic diffusion coefficient (in m^2s^{-1}). Values of D are uncertain but are often considered to be an order of magnitude smaller than the diffusion coefficient for the bulk solution ($\sim 10^{-9} \text{m}^2\text{s}^{-1}$).

Tarasov and Titov (2007) performed SIP measurements on sands with a well defined, narrow grain size distribution and estimated D as $\sim 3 \times 10^{-9} \text{m}^2\text{s}^{-1}$. Table 1 shows the equivalent polarizable particle diameter for the two end members of the modeled τ range shown in Figure 3c based on this value of D . The estimated diameters (0.07-0.10 mm) are almost two orders of magnitude larger than the long axis (approaching three orders of magnitude larger than the short axis) of the FeS encrusted, *D. vulgaris* biominerals (Fig 4a). Despite the simplicity of the model (the tubular biominerals in Fig 4a are not spherical particles, uncertainty in the magnitude of D), this discrepancy suggests that the dispersion between 0.1-100 Hz is not the relaxation associated with polarized biomineral encrusted bacteria themselves. We recognize the uncertainty inherent in the parameter estimates, but stress that we could reach similar conclusions using the raw data alone (i.e. the magnitude of the phase peak [in place of m_n] and $1/f_c$ [f_c being the critical relaxation frequency] in place of τ).

The particle size estimated from Equation 3 is close to (about 3 times smaller than) the d_{50} of the quartzitic sand particles used in this study (Table 1). We therefore suggest that the SIP relaxation observed here (and by *Williams et al., 2005*) likely results from the formation of FeS biofilms

(coagulation of individual biominerals) on the mineral surface of pores in the sand matrix. An example of this FeS biofilm forming on a quartzitic sand grain is shown in Figure 4b. We thus interpret the basic polarizable element to be the biomineral encrusted pores, having similar dimensions to the grains.

This polarizable pore model can conceptually explain the change in τ and m_n (or from the data, $1/f_c$ and magnitude of phase peak) observed during the anaerobic, and subsequent aerobic, transitions. The variation in m_n is consistent with this model as m_n is a measure of the amount of polarizable surface area (*Lesmes and Friedman, 2005*). The increase in m_n during the anaerobic transition is consistent with an increase in FeS-fluid interfacial area resulting from biomineralization growth within the pore space. The reverse behavior during the subsequent aerobic transition is then expected given the subsequent biomineral dissolution and loss of FeS-pore fluid interfacial area. The decrease in τ (and hence r) during the anaerobic transition is consistent with a shift to a smaller average polarizable pore size as the FeS lining the pores thickens. The reverse behavior is then observed in the aerobic transition as FeS dissolution starts at the biomineral-fluid interface and progressively enlarges the pores back to their original sizes. We note that such direct relation between τ and m_n could conceivably break down if FeS mineralization continued such that pores became so extensively filled with FeS that FeS-fluid interfacial area started to decline. Visual observations of the column (and flow rates) suggest that such extensive biomineralization was avoided.

We conclude that SIP signatures are likely diagnostic of pore-scale geometrical changes associated with FeS biomineralization in pores by sulfate reducing bacteria. These signatures appear reversible (within the accuracy of the measurement) following subsequent biomineral dissolution. The estimates of polarizable element (i.e. biomineral-modified pore) size are uncertain, although the opportunity to reduce this uncertainty may exist using Bayesian estimation procedures (*Chen et al., 2007*). Our work

198 highlights the opportunity that exists to improve understanding of biogeochemical processes using
199 geophysical measurements that could be deployed from boreholes or the ground surface.

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204 modeling algorithm used here.

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245 **Table captions**

246 Table 1: Comparison of SIP estimated radii (r) of polarizable spheres with sand grain size from sieve
247 analysis and biomineral size from SEM imaging.

248 **Figure captions**

249 Figure 1: Aqueous geochemistry of column effluent. Anaerobic and aerobic transitions when SIP data
250 were collected are shaded.

251 Figure 2: Spectral Induced Polarization signature of FeS biomineralization at 21 days showing both
252 phase (ϕ) and magnitude ($|\rho|$) response. The fit of the data to a Cole-Cole model is also shown. The
253 Cole-Cole parameters for this model are $\rho_0 = 39.1$ Ohm m; $m = 0.025$; $\tau = 0.22$ s; $c = 0.76$. Open
254 symbols = measured data; Solid lines = model fit; The x-axis is truncated at 100 Hz as the response
255 between 100-1000 Hz is below the 0.1 mrad instrument accuracy. The rms misfit between the model and
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257 Figure 3: Measured phase ($-\phi$) data as a function of time (a) Anaerobic transition between 14-25 days
258 (b) Aerobic transition between 36-45 days. The data are truncated at a value of -0.1 mrad (the accuracy
259 of the instrument). (c) Variation of modeled Cole-Cole parameters as a function of time (black circles =
260 τ ; open circles = m_n)

261 Figure 4: Examples of scanning electron microscope (SEM) images captured upon destructive analysis
262 of the column at the end of the experiment ($t = 55$ days) (a) FeS encrusted *D. vulgaris* biomineral The
263 approximate dimensions of these elongate, tube-like biominerals (average dimensions based on three

264 cells captured in three separate images) are $1.93 \pm 0.15 \mu\text{m}$ by $0.44 \pm 0.06 \mu\text{m}$. (b) Quartz sand particle
265 encrusted with FeS biomineralization.

266

Table 1: Comparison of SIP estimated radii (r) of polarizable spheres with sand grain size from sieve analysis and biomineral size from SEM imaging.

D^* (m^2s^{-1})	τ (s)	$2r^{\#}$ (mm)	Sand	Biomineral [†]	
			$d_{50}^{\S}$ (mm)	long axis (mm)	short axis (mm)
3.00E-09	0.4	0.10	0.30	0.0019	0.0004
3.00E-09	0.2	0.07		$\pm 0.0001^{\ddagger}$	$\pm 0.0001^{\ddagger}$

*after Tarasov & Titov (2007); [#] calculated from Equation 3; [§] from sieve analysis,

[†] from SEM images; [‡] calculated from standard deviation of measurements on three biominerals

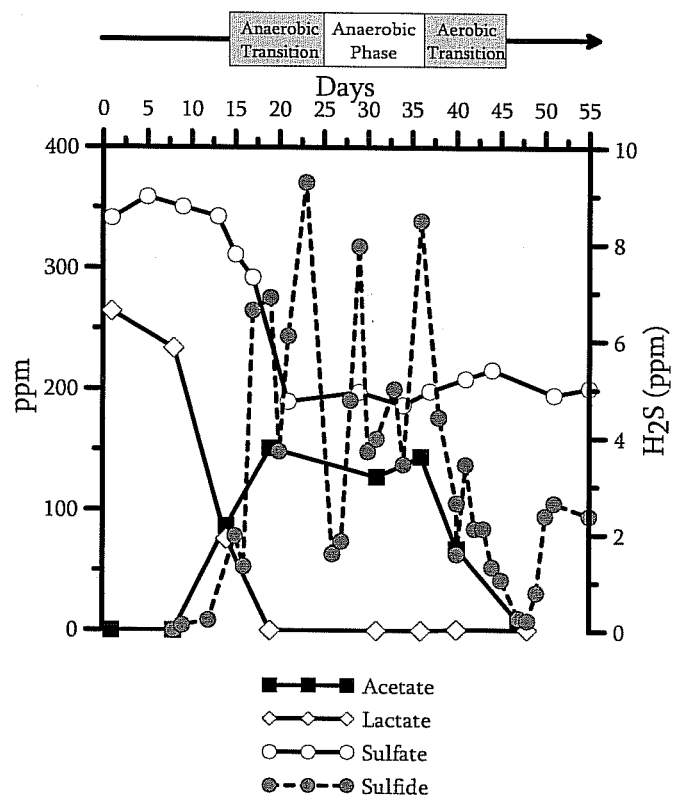


Figure 1: Aqueous geochemistry of column effluent. Anaerobic and aerobic transitions when SIP data were collected are shaded. Lactate, acetate and sulfate are plotted on the left y-axis where sulfide is plotted on the right y-axis.

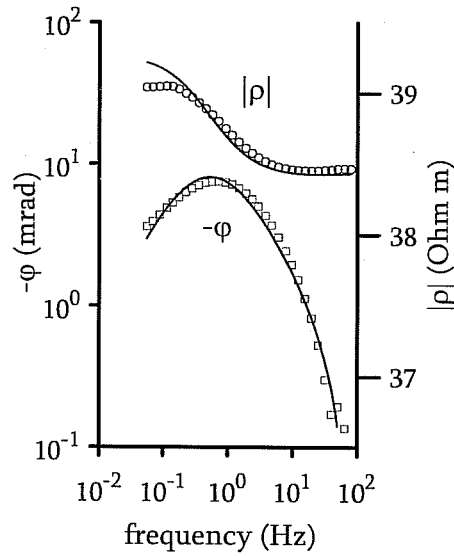


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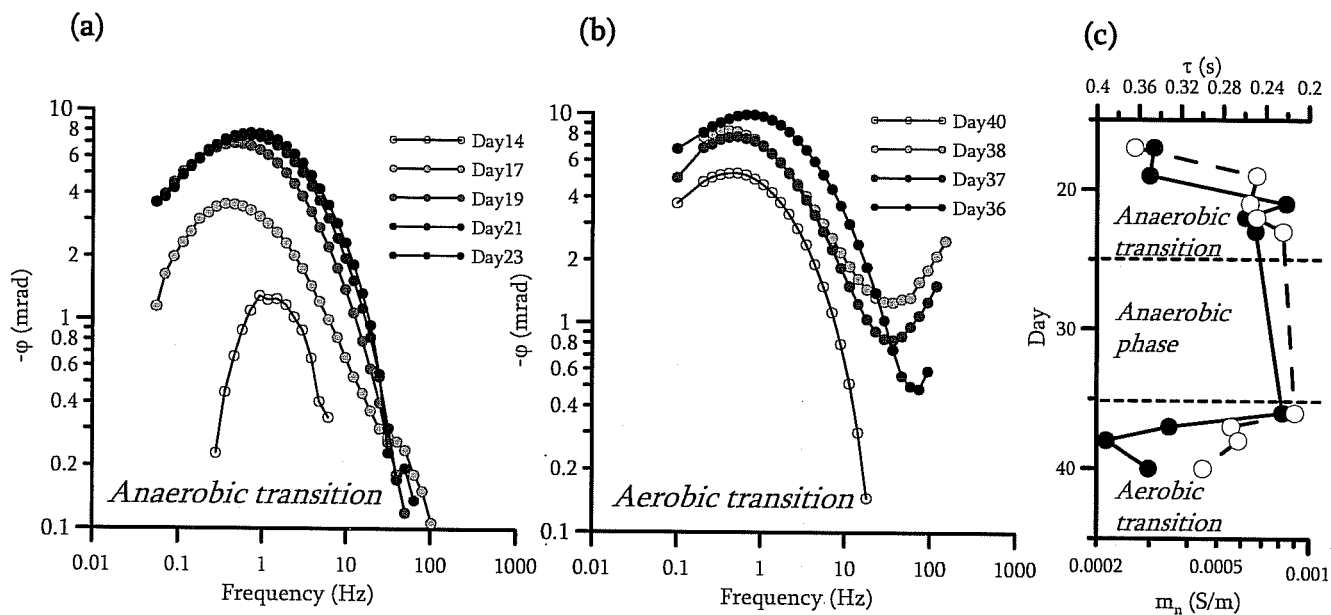


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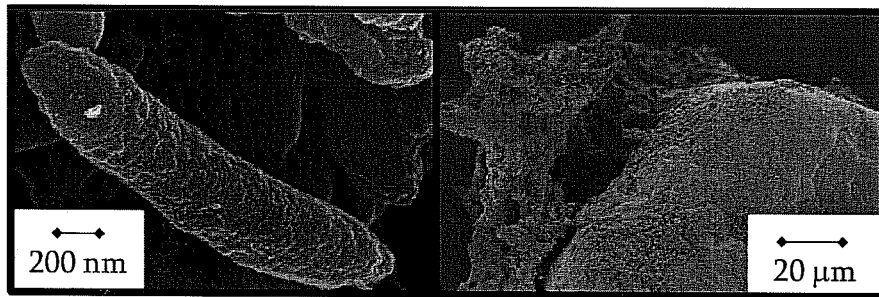


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