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Abiotic syntheses of pyrite: Clues to assess the biogenicity of pyrite spherules

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Supplementary Information

The Supplementary Information includes:

- Methods
- Figures S-1 to S-3
- Supplementary Information References

Methods

Chimney sample from a black smoker at the Trans-Atlantic Geotraverse (TAG)

The natural sample of black smoker described in this study was sampled during the Bicose 2 campaign, operation BIC2-PL01-01, on February 4th 2018 by the Nautile submersible (Cambon, 2018). The chimney was located at 26° 8.223' N - 44° 49.532' W at a depth of 3640 m. The chimney part was placed in a collection box, brought to the surface and directly introduced into anaerobic flask onboard. The sample was stored in an anaerobic flask filled with seawater from the sampling site and the gas phase was replaced by N_2 to limit interaction with O_2. It was stored at 4 °C for 1 year, then the solid phase was vacuum-dried in an anoxic Jacomex™ glove-box under an N_2 atmosphere (<1 ppm O_2). Dry sample was kept at 4 °C in sterile Eppendorf before being analysed in the present study.

Preparation of cell lysates and separation of cellular components

T. kodakarensis KOD1 cultures were prepared as described in Gorlas *et al.* (2018). Cells were grown during 12 h at 85 °C to reach the early stationary phase with final concentrations of 5.10^7 cells mL^{-1}. The cell lysates preparation was conducted in aerobic conditions while the separation of cellular components was carried out in strict anaerobic conditions in an anoxic Jacomex™ glovebox (<10 ppm O_2, Ar atmosphere) (Fig. S-3). The cell lysates were made from 50 mL of *T. kodakarensis* KOD1 culture in a modified Ravot medium (see Gorlas *et al.*, 2018) supplemented with 1g (w/v) elemental sulfur. The remaining elemental sulfur was then removed by sedimentation from the culture for 5 minutes, before recovering the liquid part. The whole culture was centrifuged at 3000 g for 15 min. The supernatant was discarded and the cell pellet was suspended in 5 mL of ultrapure (MilliQ) water, then centrifuged again at 3000 g for 15 min. The supernatant was removed and the cell pellet passed through an ultrasonic bath for 15 min. The cell pellet was then frozen at -20 °C and thawed twice, finally stored at 2 °C until the experiment.

Separation of cellular components was also carried out using 50 mL of *T. kodakarensis* KOD1 culture, which was passed through an ultrasonic bath for 15 min and reintroduced into the glovebox (Fig. S-3). The whole culture was then centrifuged at 3000 g for 15 min directly in the glovebox to separate the supernatant and the intracellular material from the cell envelopes. The liquid part (supernatant + intracellular material) was finally filtrated at 0.2 µm to avoid any envelopes residues. The cell envelopes were resuspended in the synthesis medium before mineralization experiments.

Mineralisation protocol in anoxic conditions

Each synthesis was carried out under strictly anaerobic conditions in an anoxic JacomexTM glove box (<10 ppm O_2, Ar atmosphere). The syntheses were conducted at 85 °C for 96 hr in titanium reaction bombs filled with 10 mL of a Ravot medium traditionally used for Thermococcales growth, however devoided of organic matter (named "Ravot mineral medium"). The Ravot mineral medium contains, per litre of distilled water: 1 g NH_4Cl, 0.2 g $MgCl_2.6H_2O$, 0.1g $CaCl_2.2H_2O$, 0.1 g KCl, 0.83 g, 20g NaCl, 3 g PIPES, 0.001 g resazurin and Na_2S (1.3 mM) to reduce the medium. The pH was adjusted to 7 before autoclaving. After autoclaving, 5 ml of 6 % (w/v) K_2HPO_4 solution and 5 ml of 6 % (w/v) KH_2PO_4 solution were added aseptically. The medium was then supplemented with colloidal sulfur (20 mM) from a solution at 10% (m/v), ferrous iron ($FeSO_4$ 5mM) and the various organic compounds (with the exception of the negative control): the KOD1 lysates, the KOD1 envelopes, yeast extract (1g/L) and tryptone (1g/L), or graphitic carbon (2g/L). In the case of KOD1 intracellular material synthesis, 10 mL of the mixture of supernatant and intracellular material was used directly. Colloidal sulfur (30 g, Sigma-Aldrich, product number 13825) was vigorously mixed in 350 mL distilled water and centrifuged at 3000 g for 5 minutes. The pellet was rinsed 10 times with MilliQ water, resuspended in 10 % final MilliQ water, and filtered through 0.45 µm polycarbonate filters before use. The syntheses were performed in triplicates (graphite, yeast extract + tryptone, negative control) or duplicates (lysates, envelopes and intracellular content). Each synthesis (triplicates and duplicates) were observed by SEM, but we selected one sample of each for the FIB sections, TEM and STXM analyses.

Scanning electron microscopy coupled with energy dispersive X-ray spectroscopy

Minerals were investigated using Scanning Electron Microscopy (SEM) coupled with Energy-Dispersive X-ray Spectroscopy (EDXS). Samples were dried in an anoxic Jacomex^{TM} glove box (<10 ppm O_2, Ar atmosphere). Sample dry powders (natural chimney and experimental syntheses) were deposited on a carbon tape and carbon-metallised. SEM-EDXS data were collected at IMPMC, MNHN Electron Microscopy and Microanalysis Technical Platform, with a GEMINI ZEISS^{TM} Ultra55 Field Emission Gun Scanning Electron Microscope equipped with two Bruker™ XFlash silicon drift detectors in antagonist positions for EDXS. Both images and EDXS data were collected using an acceleration voltage of 15 kV at a working distance of 12 mm and 300 pA probe current.

Sample preparation by focused ion beam

Focused ion beam (FIB) foils (20µm * 5µm * 100nm) were extracted from a pyrite of TAG sample and from pyrite synthetized in presence of KOD1 lysates, KOD1 envelopes, KOD1 YE + tryptone or graphitic carbon syntheses, never exposed to high acceleration voltage using a FEI Strata DB 235 (IEMN, Lille, France). Milling at low gallium ion currents allowed minimizing common artefacts including local gallium implantation, mixing of components, redeposition of the sputtered material on the sample surface and significant changes in the speciation of carbon-based polymers (Bernard *et al.*, 2009; Schiffbauer and Xiao, 2009).

Transmission electron microscopy

FIB sections of the samples were analysed using a JEOL JEM-2100F at IMPMC, equipped with a field emission gun (FEG) operating at 200kV. Identification of minerals was done by selected-area electron diffraction (SAED) and energy-dispersive X-ray spectroscopy (EDXS) in Scanning TEM (STEM) mode.

Scanning transmission X-ray microscopy

Scanning transmission X-ray microscopy (STXM) analyses were performed on FIB sections to document the carbon speciation of the organic compounds trapped within the pyrite using the HERMES STXM beamline at the synchrotron SOLEIL (Saint-Aubin, France - Belkhou *et al.*, 2015; Swaraj *et al.*, 2017). We did the energy calibration using the well-resolved 3p Rydberg peak of gaseous CO_2 at 294.96eV for the C K-edge. X-ray absorption near edge structure (XANES) hypercube data (stacks) were collected with a spatial resolution of 100 nm at energy increments of 0.1eV over the carbon (270–340eV) absorption range with a dwell time of less than 1ms per pixel to prevent irradiation damage (Wang *et al.*, 2009). Stack alignments and extraction of XANES spectra were done using the using the Hyperspy python-based package (De La Peña *et al.*, 2018). Data was normalized using the QUANTORXS freeware (Le Guillou *et al.*, 2018). A spectral parameter ($[C]_{STXM}$) was extracted from spectra collected at the C-K edge to provide a non-calibrated estimation of the concentration in C. The $[C]_{STXM}$ values reported here correspond to the ratio between the carbon quantity (estimated from the area of the spectra from 280 to 291.5 eV after subtraction of a power law fitting the pre-edge region - see Le Guillou *et al.*, 2018 for details) and the absorption at 280 eV (before substraction of the power law). By construction, the $[C]_{STXM}$ values are proportional to the atomic concentration of carbon in the pyrites, but given that no calibration was conducted, these values only provide qualitative information rather than absolute concentrations.

Supplementary Figures

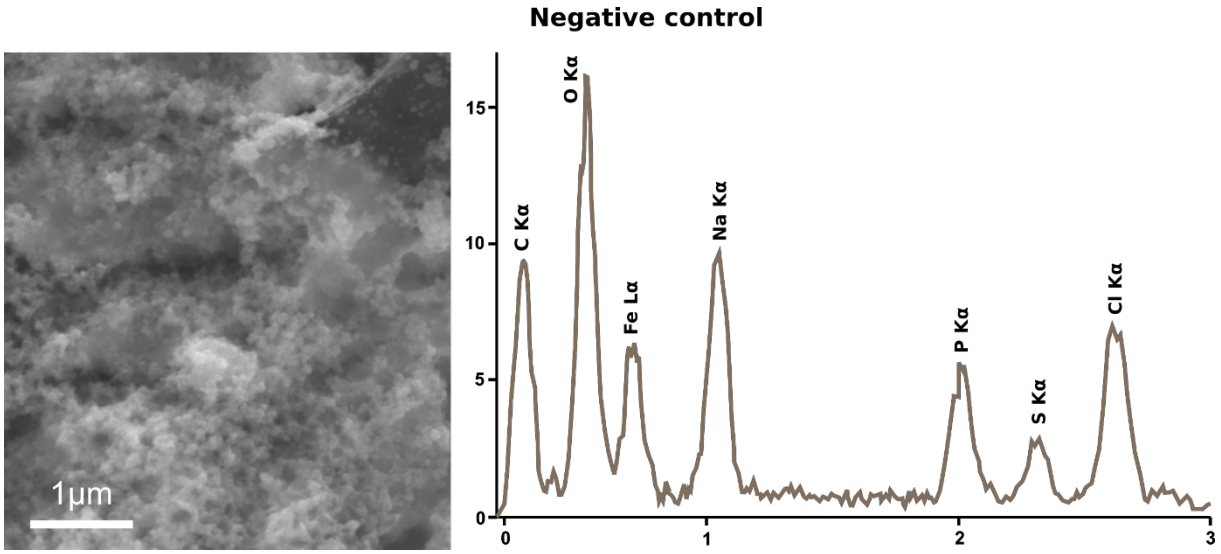

Figure S-1 EDXS spectrum of the material observed in the negative control (in absence of organic material).

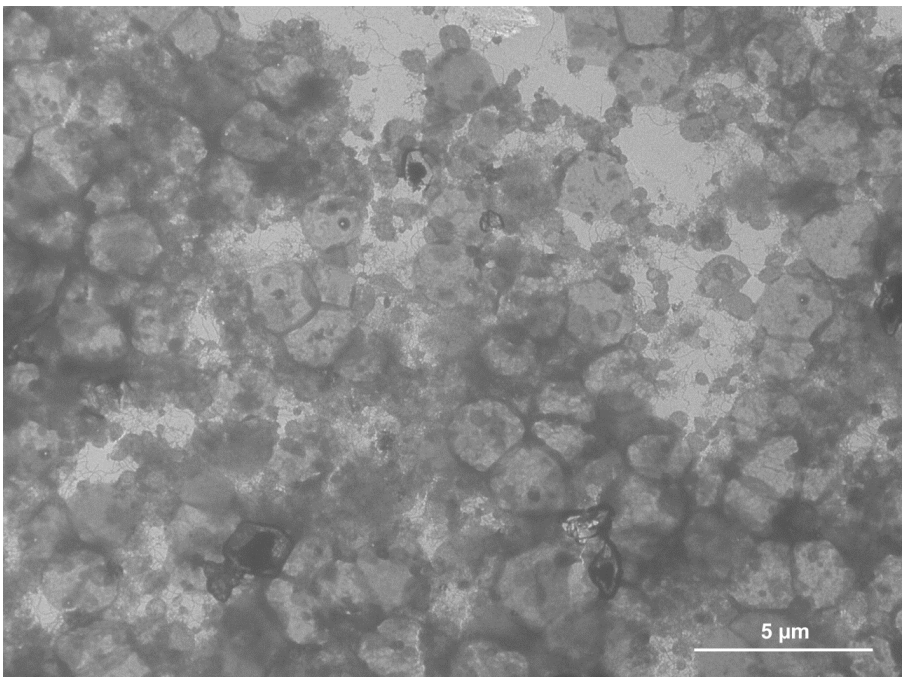

Figure S-2 TEM observation of the cell envelopes after the separation protocol. The envelopes appear translucent (emptied of their intracellular contents) while the overall integrity of the envelopes is preserved. Numerous flagella and vesicles are also visible.

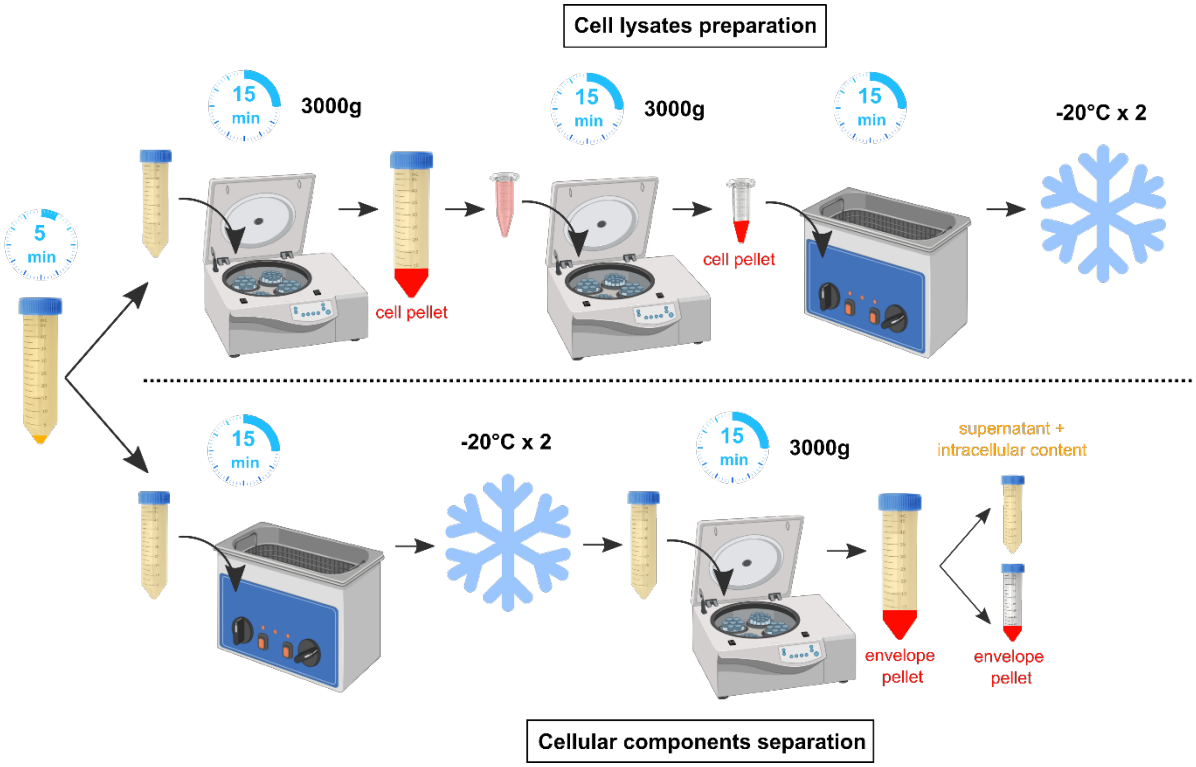

Figure S-3 Diagram of the cell lysate preparation and cell components separation protocol.

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