Supplementary Materials: Influence of *Sulfobacillus thermosulfidooxidans* on Initial Attachment and Pyrite Leaching by Thermoacidophilic Archaeon *Acidianus* sp. DSM 29099

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Table S1. Pyrite surface coverage by cells in different growth conditions.

Incubation Time	Exudates of *S. thermosulfidooxidans*^{T}	Iron(III) Ion Supplementation	MAC Medium
Day 3	<1	5.84	<1
Day 6	7.6	29.5	4.3
Day 14	16.1	24.3	13.8

Note: a, numbers are presented in %; b, more than five pyrite grains were imaged and selected for calculation. The mean values are shown.

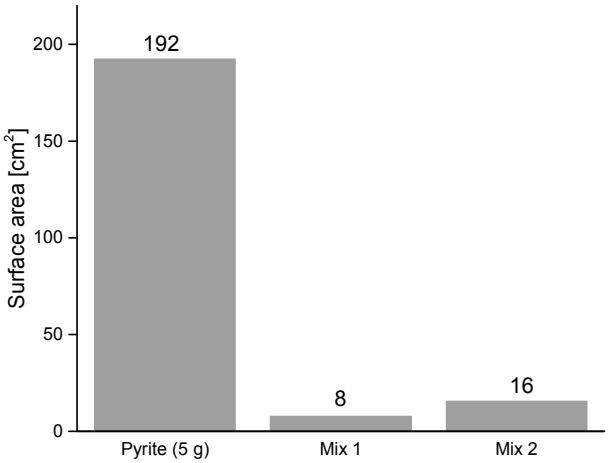


Figure S1. Calculation of the pyrite and cell surface area. Surface area of an *Acidianus* sp. DSM 29099 cell was estimated by $A = 4\pi r^2$ (r = 0.725 μm). Surface area of a *S. thermosulfidooxidans*^{T} cell was estimated by $A = 2\pi r^2 + 2\pi rh$ (r = 0.479 μm, h = 2.5 μm). Surface area of a pyrite grain (200–500 μm) was estimated by $A = 4\pi r^2$ (r = 175 μm). Each gram of pyrite contains ~10^4 grains.

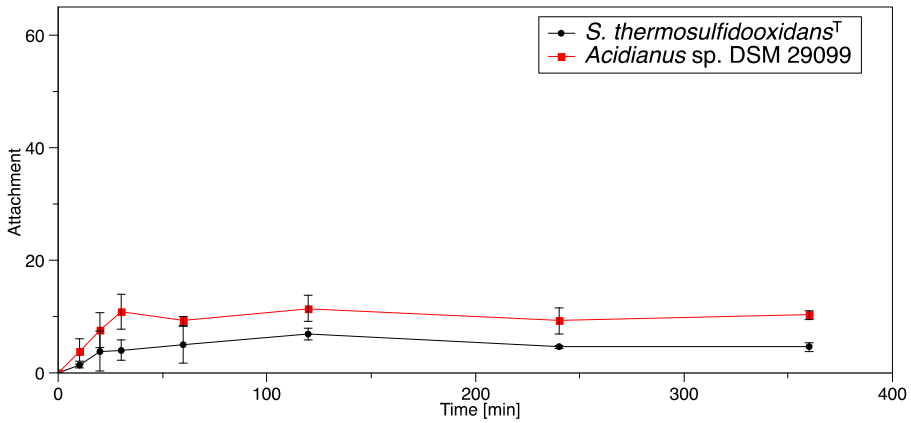
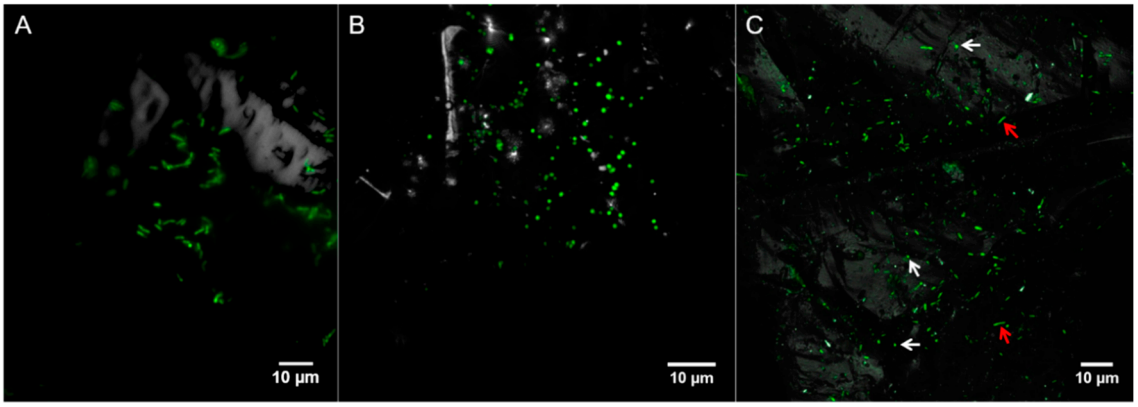


Figure S2. Non-specific attachment of *Acidianus* sp. DSM 29099 and *S. thermosulfidooxidans*^{T} cells in MAC medium (without pyrite). Cells were cultivated at 65 and 45 °C, respectively.



FigureS3. Morphology of biofilm cells of *S. thermosulfidooxidans*^{T} (**A**); *Acidianus* sp. DSM 29099 (**B**) and mixed cultures of *Acidianus* sp. DSM 29099 and *S. thermosulfidooxidans*^{T} on pyrite (**C**) under CLSM. Red arrows indicate cells of *S. thermosulfidooxidans*^{T}; white arrows indicate cells of *Acidianus* sp. DSM 29099.

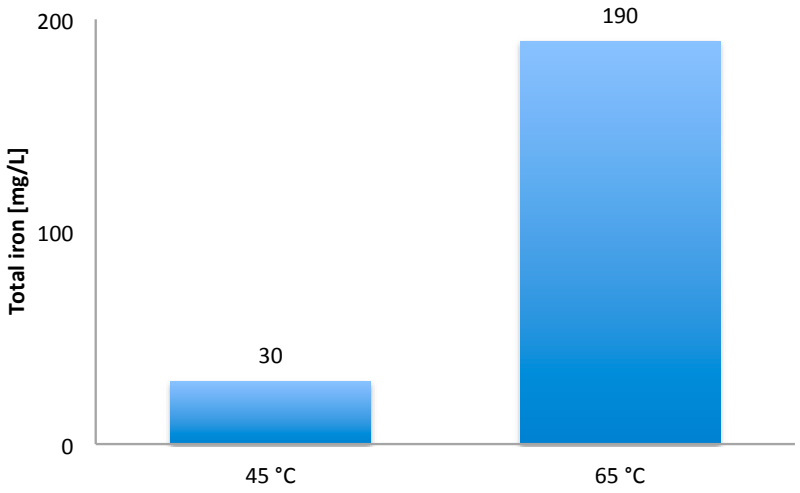


Figure S4. Abiotic controls of pyrite leaching at 65 and 45 °C after 21 days. Total iron concentration is shown.

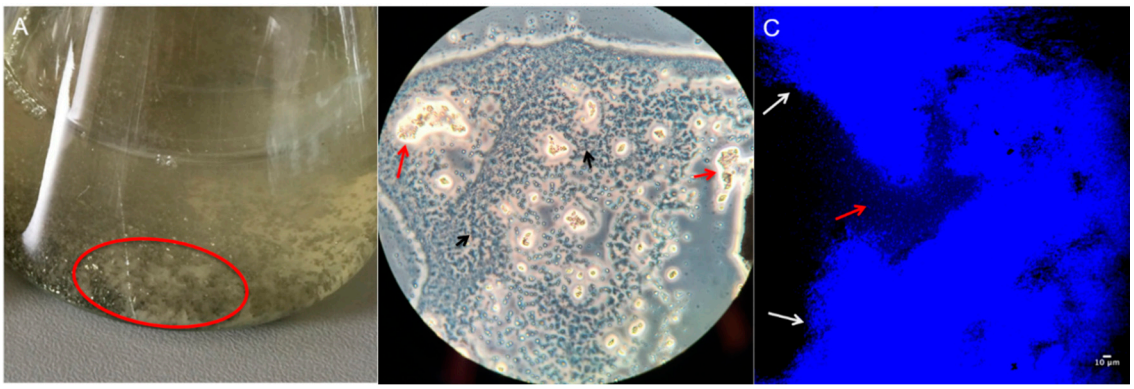


Figure S5. Floc formation by pure culture of *Acidianus* sp. DSM 29099 grown on pyrite at 45 °C. (**A**) Flocs formed in the bottom of an Erlenmeyer flask after settling the culture for ~5 min; (**B**) flocs observed under light microscope (400×). Black arrows indicate cells; red arrows indicate crystal structures; (**C**) flocs observed under CLSM after staining samples with DAPI. White arrows indicate big cell flocs and red arrow indicates individual cells within the boundary of flocs.