

SUPPLEMENTAL ONLINE MATERIAL

SUPPLEMENTAL RESULTS

Supplemental Table 1. Number of cells, amount of carbon assimilated, measured analyte (H₂S, Fe³⁺, Fe²⁺, and SO₄²⁻) where applicable, and calculated amounts of energy available per mol electron transferred during the specified times in cultures of *Acidianus* sp. DS80 grown at 80°C in base salts medium (pH 3.0) with CO₂ as the carbon source, H₂ as electron donor, and S^o as electron acceptor.

Substrate H ₂ / S ^o (e ⁻ donor/acceptor)							
Time	Cells	CO ₂ (fmol)	H ₂ S (M)	Fe ³⁺ (M)	Fe ²⁺ (M)	SO ₄ ²⁻ (M)	ΔG (kJ (mol e ⁻) ⁻¹)
0	2.66 x 10 ⁵	4.07 x 10 ⁶	2.95 x 10 ⁻⁵	N/A	N/A	N/A	-28.20
24	2.66 x 10 ⁵	4.10 x 10 ⁶	2.95 x 10 ⁻⁵	N/A	N/A	N/A	-28.20
48	1.10 x 10 ⁶	4.74 x 10 ⁶	5.61 x 10 ⁻⁵	N/A	N/A	N/A	-27.25
72	2.76 x 10 ⁶	6.15 x 10 ⁶	1.18 x 10 ⁻⁴	N/A	N/A	N/A	-26.15
96	4.56 x 10 ⁶	1.14 x 10 ⁷	2.57 x 10 ⁻⁴	N/A	N/A	N/A	-25.01
120	5.73 x 10 ⁶	1.49 x 10 ⁷	4.62 x 10 ⁻⁴	N/A	N/A	N/A	-24.15
144	7.59 x 10 ⁶	2.09 x 10 ⁷	7.35 x 10 ⁻⁴	N/A	N/A	N/A	-23.47
168	8.07 x 10 ⁶	2.07 x 10 ⁷	9.46 x 10 ⁻⁴	N/A	N/A	N/A	-23.10
192	7.70 x 10 ⁶	2.04 x 10 ⁷	1.08 x 10 ⁻³	N/A	N/A	N/A	-22.90

Abbreviations: N/A, Not Applicable.

34 **Supplemental Table 2.** Number of cells, amount of carbon assimilated, measured analyte (H₂S,
 35 Fe³⁺, Fe²⁺, and SO₄²⁻) where applicable, and calculated amounts of energy available per mol
 36 electron transferred during the specified times in cultures of *Acidianus* sp. DS80 grown at 80°C
 37 in base salts medium (pH 3.0) with CO₂ as the carbon source, S^o as the electron donor, and Fe³⁺
 38 as the electron acceptor.
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Substrate S ^o / Fe ³⁺ (e ⁻ donor/acceptor)							
Time	Cells	CO ₂ (fmol)	H ₂ S (M)	Fe ³⁺ (M)	Fe ²⁺ (M)	SO ₄ ²⁻ (M) ¹	ΔG (kJ (mol e ⁻) ⁻¹)
0	2.04 x 10 ⁵	4.21 x 10 ⁶	N/A	9.25 x 10 ⁻³	B.D.	0.00	-41.83
24	2.04 x 10 ⁵	4.33 x 10 ⁶	N/A	9.24 x 10 ⁻³	1.00 x 10 ⁻⁵	1.67 x 10 ⁻⁶	-82.95
48	2.10 x 10 ⁵	4.32 x 10 ⁶	N/A	9.22 x 10 ⁻³	2.88 x 10 ⁻⁵	4.81 x 10 ⁻⁶	-79.84
72	2.46 x 10 ⁵	4.21 x 10 ⁶	N/A	9.18 x 10 ⁻³	7.48 x 10 ⁻⁵	1.25 x 10 ⁻⁵	-77.02
96	2.57 x 10 ⁶	6.67 x 10 ⁶	N/A	8.36 x 10 ⁻³	8.96 x 10 ⁻⁴	1.49 x 10 ⁻⁴	-69.46
120	4.62 x 10 ⁶	1.29 x 10 ⁷	N/A	7.01 x 10 ⁻³	2.24 x 10 ⁻³	3.73 x 10 ⁻⁴	-66.24
144	6.14 x 10 ⁶	1.98 x 10 ⁷	N/A	5.57 x 10 ⁻³	3.68 x 10 ⁻³	6.14 x 10 ⁻⁴	-64.06
168	6.87 x 10 ⁶	2.49 x 10 ⁷	N/A	4.45 x 10 ⁻³	4.81 x 10 ⁻³	8.01 x 10 ⁻⁴	-63.53
192	8.09 x 10 ⁶	2.73 x 10 ⁷	N/A	2.55 x 10 ⁻³	6.70 x 10 ⁻³	1.12 x 10 ⁻³	-59.88
216	1.01 x 10 ⁷	3.47 x 10 ⁷	N/A	1.85 x 10 ⁻³	7.40 x 10 ⁻³	1.23 x 10 ⁻³	-58.61
240	1.03 x 10 ⁷	3.10 x 10 ⁷	N/A	1.11 x 10 ⁻³	8.14 x 10 ⁻³	1.36 x 10 ⁻³	-56.81

40 ¹Calculated by subtracting SO₄²⁻ in cultures from SO₄²⁻ in abiotic controls.

41 Abbreviations: N/A, Not Applicable; B.D., Below Detection

42 Detection limits for Fe²⁺ and SO₄²⁻ are 0.3 and 5.0 μM, respectively.

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65 **Supplemental Table 3.** Number of cells, amount of carbon assimilated, measured analyte (H₂S,
66 Fe³⁺, Fe²⁺, and SO₄²⁻) where applicable, and calculated amounts of energy available per mol
67 electron transferred during the specified times in cultures of *Acidianus* sp. DS80 grown at 80°C
68 in base salts medium (pH 3.0) with CO₂ as the carbon source, H₂ as the electron donor, and Fe³⁺
69 as the electron acceptor.
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Substrate H ₂ / Fe ³⁺ (e ⁻ donor/acceptor)							
Time	Cells	CO ₂ (fmol)	H ₂ S (M)	Fe ³⁺ (M)	Fe ²⁺ (M)	SO ₄ ²⁻ (M) ¹	ΔG (kJ (mol e ⁻) ⁻¹)
0	1.85 x 10 ⁵	4.28 x 10 ⁶	N/A	9.25 x 10 ⁻³	B.D.	B/D	-68.56
24	1.95 x 10 ⁵	4.33 x 10 ⁶	N/A	9.25 x 10 ⁻³	3.17 x 10 ⁻⁶	B/D	-110.41
48	2.08 x 10 ⁵	4.21 x 10 ⁶	N/A	9.24 x 10 ⁻³	7.50 x 10 ⁻⁶	B/D	-107.88
72	2.08 x 10 ⁵	4.17 x 10 ⁶	N/A	9.24 x 10 ⁻³	1.60 x 10 ⁻⁵	B/D	-105.65
96	3.17 x 10 ⁵	4.19 x 10 ⁶	N/A	4.60 x 10 ⁻³	3.63 x 10 ⁻⁵	B/D	-101.15
120	8.17 x 10 ⁵	4.57 x 10 ⁶	N/A	9.10 x 10 ⁻³	1.56 x 10 ⁻⁴	B/D	-98.92
144	1.22 x 10 ⁶	5.21 x 10 ⁶	N/A	8.84 x 10 ⁻³	4.09 x 10 ⁻⁴	B/D	-96.01
168	2.33 x 10 ⁶	7.35 x 10 ⁶	N/A	8.40 x 10 ⁻³	8.58 x 10 ⁻⁴	B/D	-93.70
192	4.05 x 10 ⁶	8.82 x 10 ⁶	N/A	7.82 x 10 ⁻³	1.43 x 10 ⁻³	B/D	-92.00
216	8.59 x 10 ⁶	1.55 x 10 ⁷	N/A	6.63 x 10 ⁻³	2.62 x 10 ⁻³	B/D	-89.75
240	1.39 x 10 ⁷	2.47 x 10 ⁷	N/A	4.73 x 10 ⁻³	4.52 x 10 ⁻³	B/D	-87.16
264	1.40 x 10 ⁷	2.82 x 10 ⁷	N/A	4.19 x 10 ⁻³	5.06 x 10 ⁻³	B/D	-86.47

71 ¹Calculated by subtracting SO₄²⁻ in cultures from SO₄²⁻ in abiotic controls.

72 Abbreviations: N/A, Not Applicable. B.D., Below Detection

73 Detection limits for Fe²⁺ and SO₄²⁻ are 0.3 and 5.0 μM respectively.
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96 **Supplemental Table 4.** Number of cells, amount of carbon assimilated, and measured analyte
 97 (H₂S, Fe³⁺, Fe²⁺, and SO₄²⁻) where applicable during the specified times in cultures of *Acidianus*
 98 sp. DS80 grown at 80°C in base salts medium (pH 3.0) with CO₂ as sole carbon source.
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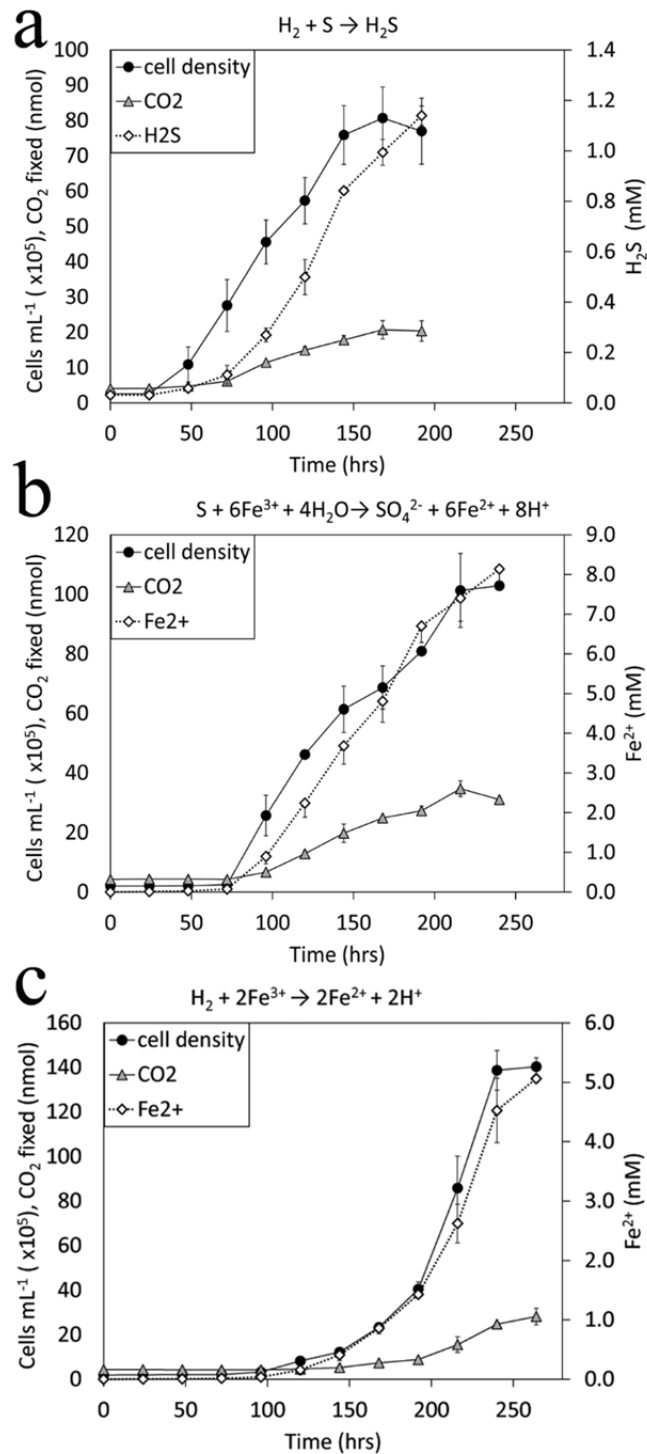
Substrate H ₂ /S ⁰ /Fe ³⁺						
Time	Cells	CO ₂ (fmol)	H ₂ S (M)	Fe ³⁺ (M)	Fe ²⁺ (M)	SO ₄ ²⁻ (M) ¹
0	2.57 x 10 ⁵	4.07 x 10 ⁶	6.72 x 10 ⁻⁸	8.83 x 10 ⁻³	2.08 x 10 ⁻⁵	B/D
24	2.57 x 10 ⁵	4.07 x 10 ⁶	6.72 x 10 ⁻⁸	8.18 x 10 ⁻³	4.76 x 10 ⁻⁴	B/D
48	6.83 x 10 ⁵	4.56 x 10 ⁶	1.88 x 10 ⁻⁵	7.54 x 10 ⁻³	1.04 x 10 ⁻³	B/D
72	2.27 x 10 ⁶	5.97 x 10 ⁶	1.33 x 10 ⁻⁴	4.06 x 10 ⁻³	2.76 x 10 ⁻³	B/D
96	3.44 x 10 ⁶	7.19 x 10 ⁶	3.35 x 10 ⁻⁴	2.37 x 10 ⁻³	2.28 x 10 ⁻³	B/D
120	5.82 x 10 ⁶	1.43 x 10 ⁷	5.85 x 10 ⁻⁴	1.26 x 10 ⁻⁴	4.78 x 10 ⁻⁴	B/D
144	7.05 x 10 ⁶	1.73 x 10 ⁶	1.27 x 10 ⁻³	4.48 x 10 ⁻⁶	3.72 x 10 ⁻⁵	B/D
168	7.52 x 10 ⁶	2.01 x 10 ⁶	1.41 x 10 ⁻³	6.98 x 10 ⁻⁶	1.31 x 10 ⁻⁵	B/D

¹Calculated by subtracting SO₄²⁻ in cultures from SO₄²⁻ in abiotic controls.

Abbreviations: B.D., Below Detection

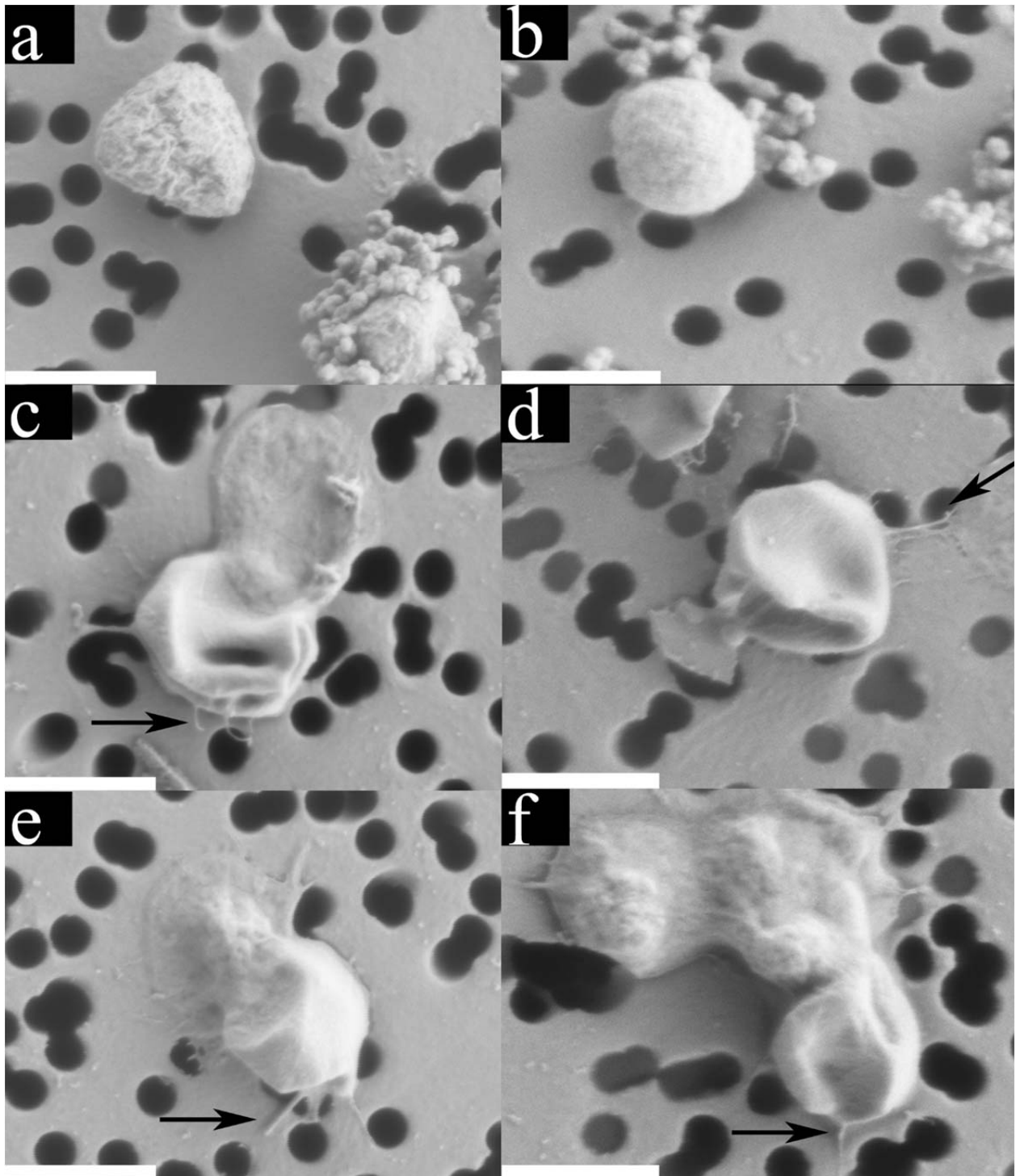
Detection limits for SO₄²⁻ is 5 μM respectively.

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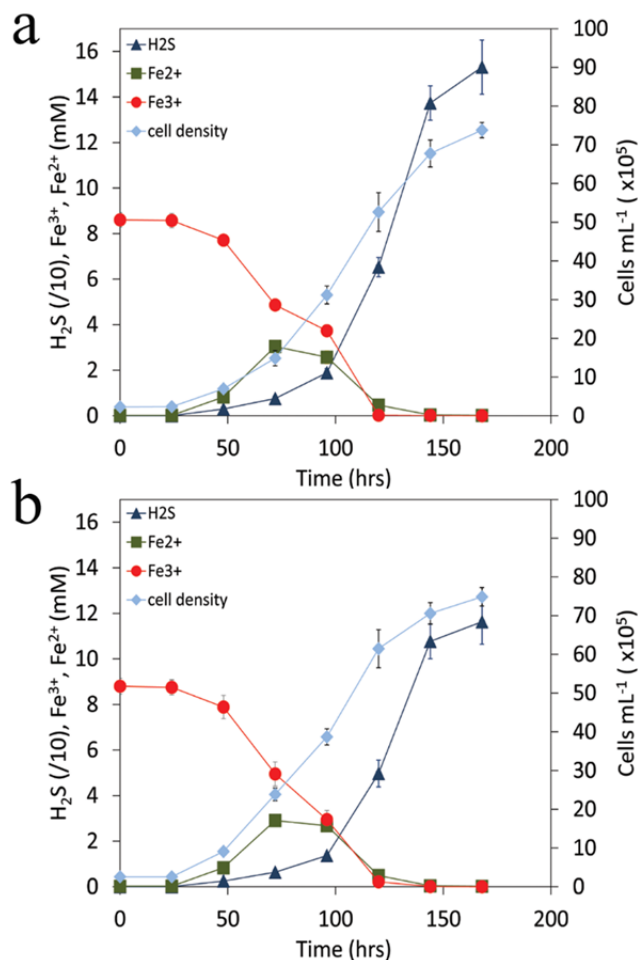
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 129 **Supplemental Figure 1.** Growth kinetics and substrate transformation activities of cultures of
 130 DS80 grown with H_2/S° (a), S°/Fe^{3+} (as ferric sulfate) (b), or H_2/Fe^{3+} (as ferric sulfate) (c). All
 131 cultures were provided with CO_2 as a carbon source. Error bars reflect the standard deviation of
 132 measurements made on three separate cultures.

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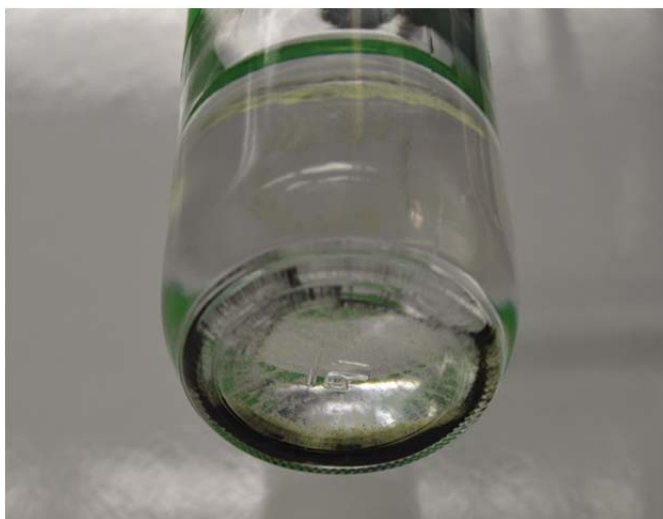
Supplemental Figure 2. Field emission scanning electron micrographs (FE SEMs) of cells grown with H_2/S° (a,b), S°/Fe^{3+} (c,d), and H_2/Fe^{3+} (e,f) with arrows denoting pilin-like structures where present. Scale bars represent 1000 nm.



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 141 **Supplemental Figure 3.** Growth kinetics and substrate transformation activities of cultures of
 142 DS80 grown with H₂/S⁰/Fe³⁺ (as ferric sulfate). The inoculum used in experiments presented in
 143 the “a” panel was grown with S⁰/Fe³⁺ (as ferric sulfate) whereas the inoculum used in
 144 experiments presented in the “b” panel was grown with H₂/Fe³⁺ (as ferric sulfate). All cultures
 145 were provided with CO₂ as a carbon source. Error bars reflect the standard deviation of
 146 measurements made on three separate cultures.

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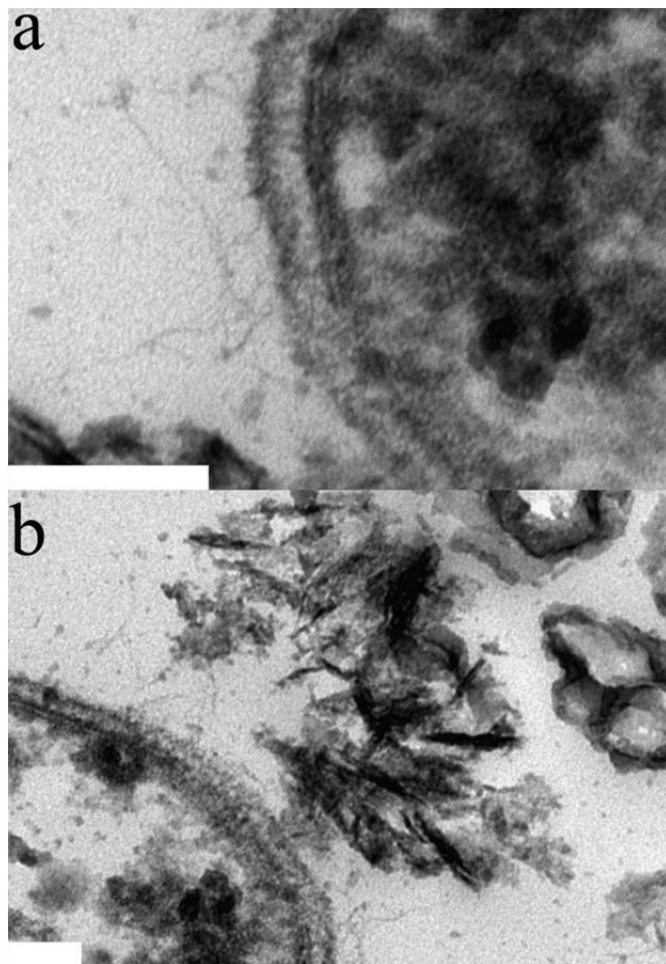
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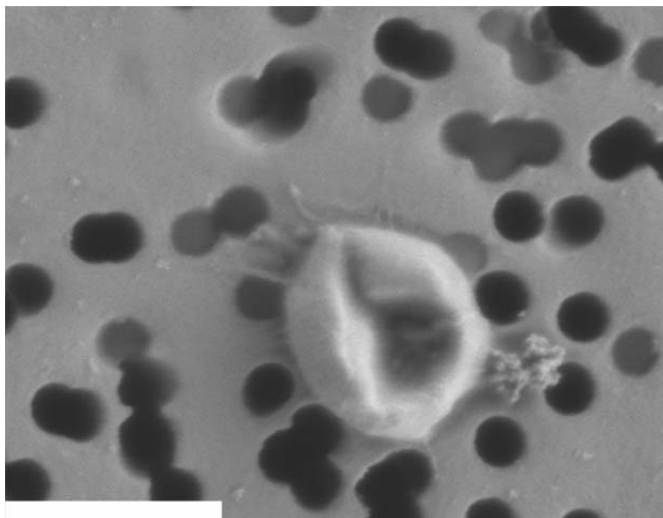
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Supplemental Figure 4. Insoluble, black iron sulfide precipitates produced during microbial growth in medium containing $\text{H}_2/\text{S}^\ominus/\text{Fe}^{3+}$.



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179 **Supplemental Figure 5.** Thin section transmission electron micrographs (TEMs) of cells grown
180 with the $\text{H}_2/\text{Fe}^{3+}$ redox couple (a). Scale bars represent 100 nm. Both images show the presence
181 of a precipitate of unknown composition (b).

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191 **Supplemental Figure 6.** Field emission scanning electron micrograph (FE SEM) of cells grown
192 with H₂/ferrihydrite. Scale bar represent 1000 nm.
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