# From laboratory towards industrial operation: biomarkers for acidophilic metabolic activity in bioleaching systems

## 8. Supplementary Materials

**Table S1.** Primers designed for *At. thiooxidans* (ATT), *At. ferrooxidans* (AFE), *L. ferriphilum* (LII), and *Sulfobacillus sp* CBAR13 (STO) and used for real time quantitative PCR (RT-qPCR) analysis.

| Gene | Primer | Sequence (5' to 3') | Efficiency (E) | Annealing T° (°C) |
|---|---|---|---|---|
| *rbcL1* | ATT-rbcL-1 F | TCAAGGAGTACCGCCACACAT | 1.9 | 60 |
| | ATT-rbcL-1 R | TAAGCAGGTCAGTCCAGACCGT | | |
| *rbcL2* | ATT-rbcL-2 F | ATACACTGCTTTCGTGCTGGC | 1.9 | 60 |
| | ATT-rbcL-2 R | CAGAAATGATCGGTGTAGTGGG | | |
| *csoS2* | ATT-csoS2 F | TATCTGGTACGCAACTGGGC | 1.9 | 60 |
| | ATT-csoS2 R | CCATCGCCACTTTCTGAGGT | | |
| *csoS3* | ATT-csoS3 F | ACCAATGCCACCATGCTTTG | 1.9 | 60 |
| | ATT-csoS3 R | AGCCTGCTCGGTAAAGTCAC | | |
| *csoS4* | ATT-csoS4 F | CTACGGGTGTTGTCTGATGC | 1.9 | 60 |
| | ATT-csoS4 R | GCCGAACCTGCTACCGTAAAC | | |
| 16S | ATT-16S F | GGGTGCTAATANCGCCTGCT | 1.9 | 60 |
| | ATT-16S R | TTGTGCGGGCCCCCGTCAAT | | |
| *gyrA* | ATT-gyrA F | GCTTTGTTCGATCTGGTGCCT | 1.8 | 60 |
| | ATT-gyrA R | ACCACTGCGGTATGCTTCAA | | |
| *rbcL-1* | AFE-rbcL-1 F | AATCTTTGGCGATGATGCGGTA | 2.0 | 60 |
| | AFE-rbcL-1 R | GGACTGTCTTTCGCTGCGTT | | |
| *rbcL-2* | AFE-rbcL-2 F | CTACTACAAGGGGCGCGCATAC | 2.0 | 60 |
| | AFE-rbcL-2 R | CGTTACCAACCAGCGAGGTG | | |
| *alaS* | AFE-alaS F | CGGAGTACCGGTATTGATCA | 1.9 | 60 |
| | AFE-alaS R | CGTCCAGTCATTCCTTTACC | | |
| *treZ* | LII-treZ F | GAAACCCGGCCGTTTCTCTA | 2.0 | 60 |
| | LII-treZ R | GAACGCGGCGAACTCTTTTT | | |
| *treY* | LII-treY F | GATCAGCCTTCCGATCCTCG | 2.1 | 60 |
| | LII-treY R | CTCGAGTTCCCGGTTTTCCA | | |
| *treX* | LII-treX F | GGTCGGAGAAAATCCCCGTT | 2.3 | 60 |
| | LII-treX R | TGCCGCATGTTCAGGGTATT | | |
| *lamB* | LII-lamB F | TGAACAGTTCGGCCTTCTCC | 1.9 | 60 |
| | LII-lamB R | AGCAGAACTTCCGGTTCCAC | | |
| *galU* | LII-galU F | TTTTGAAAGCCACGGTGCAG | 2.0 | 60 |
| | LII-galU R | TTTCCGCGGATGGAGAAGTC | | |
| *gadAB* | LII-gadAB F | GATGATTCGCTTTCCGCCAC | 2.0 | 60 |
| | LII-gadAB R | GGTTTCCGTCGAGTTCCAGT | | |
| *nifH* | LII-nifH F | TGCGACCCCAAGGCTGATTC | 2.1 | 60 |
| | LII-nifH R | GCCCTCCAGATTCCACGCAA | | |
| *amt2* | LII-amt F | CCGTGGATCTCGATGAACAT | 2.0 | 60 |
| | LII-amt R | ACCACGATATTCAGGGCGAT | | |
| *glnA* | LII-glnA F | GGAAGTGCATCATCATGAGG | 1.9 | 60 |
| | LII glnA R | CATATCCGCCTTTCTGGAAG | | |
| *glnB* | LII glnB F | GTCTCGGGCTACACGATCAT | 2.0 | 60 |
| | LII glnB R | AACACCACACCGGAATACCG | | |
| *alaS* | LII-alaS F | TACCCGGAGCTTAGAACATC | 2.0 | 60 |
| | LII-alaS R | TATCGAGCGGAAATCCATGC | | |
| 16S | LII-16S F | TACAAGCTTCCGCTCCTG | 1.8 | 60 |
| | LII-16S R | CCGGGCAAAAGTGGTTTACA | | |
| *carb* | STO-carb F | ATGGCGTCGTGTCTCACTTT | 1.9 | 60 |
| | STO-carb R | GCGGCAAAACGGTCTTGTAA | | |
| *cytbdl* | STO-cytbdl F | CTCCTGGGTCGCAAGTCATT | 2.0 | 60 |
| | STO-cytbdl R | TGTAGGATCCCCTCCCGTTT | | |
| *sulf* | STO-sulf F | GCAAGTTCATCCCGCTTCAA | 1.9 | 60 |
| | STO-sulf R | TAACCGACCGGAATGGTCAC | | |
| *chypll* | STO-chypll F | CTTGGCCCACGTTTCATTCG | 2.0 | 60 |
| | STO-chypll R | TGGGTAGTCGGACACTTCCA | | |
| *tehy* | STO-tehy F | AGTCCTTTTGCACGCGTAGT | 2.0 | 60 |
| | STO-tehy R | ACTTCGAAAGCCGCATTGTG | | |
| *fadp* | STO-fadp F | GGCAACAACGTCAGCTTCAA | 2.0 | 60 |
| | STO-fadp R | CCAATCGCCACACATTACGC | | |
| *gyrA* | STO-gyrA F* | CGGATTTACCGGGTCAAGGT | 2.0 | 60 |
| | STO-gyrA R* | ATGCGCTCATCAGCTTCCAT | | |

**Table S2.** RNA quantity and quality obtained from bioleaching column test and industrial bioleaching strip samples.

| **Samples** | **RNA (ng/uL) (*range)** | **260/230 ratio (*range)** | **260/230 ratio (*range)** |
|---|---|---|---|
| Col 1.: control | 76 - 146* | 1.8 - 2.0* | 0.7 - 0.9* |
| Col 2.: $O_2$ and $CO_2$ availability | 48 - 160* | 1.7 - 2.0* | 0.4 - 2.2* |
| Col 3.: $N_2$ and $NH_4^+$ availability | 52 - 114* | 1.8 - 2.0* | 0.3 - 1.9* |
| Col 4.: osmotic stress ($SO_4^{2-}$ impurity) | 66 - 131* | 1.7 - 2.0* | 0.7 - 1.2* |
| S-410-W0-D | 30.1 | 1.55 | 0.7 |
| S-410-W0-M | 49.4 | 1.78 | 1.5 |
| S-410-W2-D | 26.5 | 1.68 | 1.1 |
| S-410-W2-M | 7.8 | 1.77 | 1.4 |
| S-410-W4-D | 173 | 1.64 | 0.7 |
| S-410-W4-M | 47.6 | 1.61 | 1.1 |
| S-132-D | 317.8 | - | - |
| S-327-D | 184.6 | - | - |
| S-413-D | 36.9 | - | - |
| S-414-D | 106.8 | - | - |
| S-405-D | 133 | 1.9 | 1.7 |
| S-405-B | 109 | 1.9 | 0.4 |
| S-405-M | 153 | 2.0 | 0.4 |
| S-405-D | 133 | 1.9 | 1.7 |
| S-405-B | 109 | 1.9 | 0.4 |
| S-405-M | 153 | 2.0 | 0.4 |

**Table S3**. Estimated *p-values* for multiple regression models of the *At. thiooxidans*, *L. ferriphilum* IESL-25, and *L. ferriphilum* type strain DSM 14647 growing at different $CO_2$ and $SO_4^{2-}$ levels.

| **Strain** | **Response** | **Transformation** | **p values** | | | | | | | | **Regression Equation** |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | **Continuous predictors** | | | | **Interactions** | | **Categorical variables** | | |
| | | | **Time (h)** | **Time (h) * Time (h)** | **$SO_4^{2-}$ (g $L^{-1}$)** | **$CO_2$ (ppm)** | **Time (h) * $SO_4^{2-}$ (g $L^{-1}$) (Time(h) * [$SO_4^{2-}$])** | **Time (h) * $CO_2$ (g $L^{-1}$) (Time(h) * [$CO_2$])** | **$SO_4^{2-}$ (H, M, L)*** | **$CO_2$ (H, L)**** | |
| *At. thiooxidans* IESL-33 (Fig. 2) | Cell number (cell $mL^{-1}$) | square root | <0.0001 | | | | | <0.0001 | | 0.049 | L cel mL-1^0.5 = 14784273 + 260633 Time (h)<br>H cel mL-1^0.5 = 10426501 + 429367 Time (h) |
| | pH | | <0.0001 | | | | | | | <0.01 | L pH = 2.8462 - 0.00946 Time (h)<br>H pH = 2.7537 - 0.00946 Time (h) |
| | | | | | | | | | | | |
| *L. ferriphilum* DSM 14647 (Fig. 7) | $\log_{10}$ (Cell number (cell $mL^{-1}$)) | square root | <0.0001 | 0.001 | <0.0001 | | 0.006 | | | | 2.50 log cell mL-1^0.5 = 2.5661 + 0.00986 Time (h) - 0.000092 Time (h)$^2$<br>25.00 log cell mL-1^0.5 = 2.3832 + 0.01231 Time (h) - 0.000092 Time (h)$^2$<br>50.00 log cell mL-1^0.5 = 2.4076 + 0.01203 Time (h) - 0.000092 Time (h)$^2$ |
| | Fe II (g $L^{-1}$) | | <0.0001 | | 0.936 | | (=0.02) | | | | L Fe+2 (g/L) = 3.461 - 0.07799 Time (h)<br>M Fe+2 (g/L) = 3.540 - 0.06888 Time (h)<br>H Fe+2 (g/L) = 3.444 - 0.05642 Time (h) |
| | | | | | | | | | | | |
| *L. ferriphilum* IESL-25 (Fig. 8) | $\log_{10}$ (Cell number (cell $mL^{-1}$)) | square root | <0.0001 | 0.012 | | | | | | | log cell mL-1^0.5 = 2.55066 + 0.009258 Time (h) - 0.000093 SO42- 0.000048 Time (h)$^2$ |
| | Fe II (g $L^{-1}$) | | <0.0001 | | | | (<0.0001) | | 0.025 | | L Fe 2+ = 3.413 - 0.08750 Time (h)<br>M Fe 2+ = 4.020 - 0.08743 Time (h)<br>H Fe 2+ = 3.679 - 0.05548 Time (h) |

* L, M and H represent SO42- concentration of 2.5, 25 and 50 g L-1, respectively.

** L and H represent CO2 levels of 50 and 500 ppm, respectively

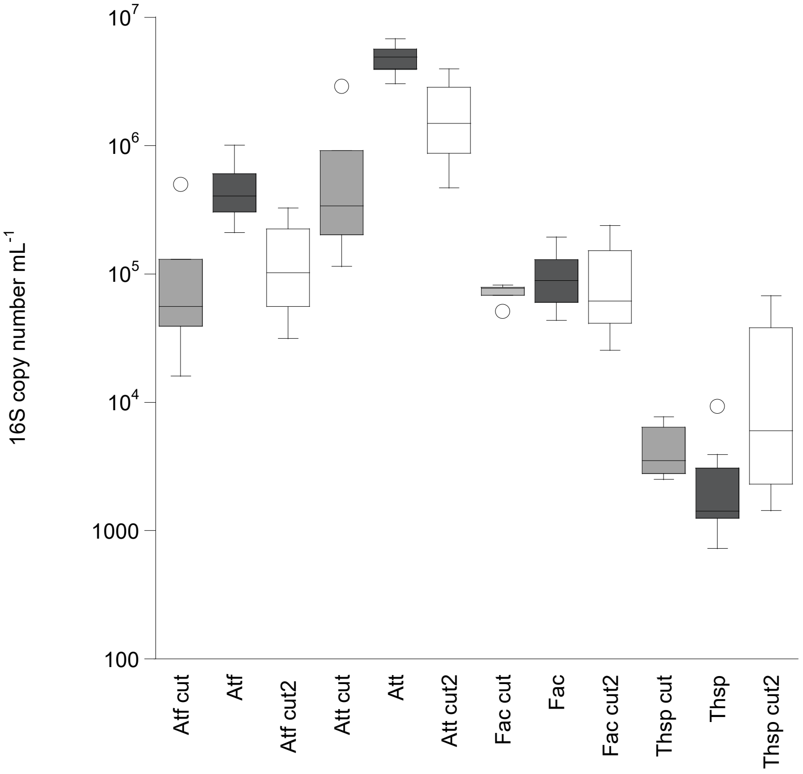

**Figure S1**. Copy numbers of 16S rRNA by real-time PCR analysis of autotrophic (Atf and Att) and heterotrophic (Fac and Thsp) microorganisms in column experiment with on/off air feeding. Cut = air feeding interrupted. Atf: *Acidithiobacillus ferrooxidans*; Att: *Acdithiobacillus thiooxidans*; Fac: *Ferroplasma acidiphilum*; Thsp: *Thermoplasma* sp.

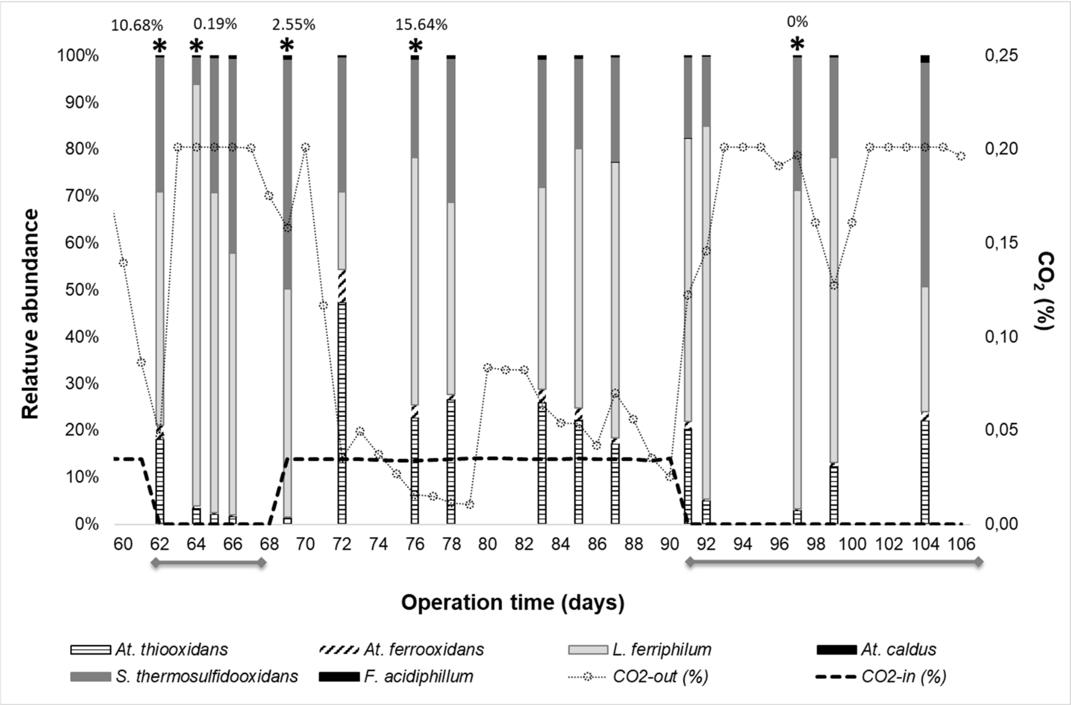

**Figure S2**. Characterization of col.2 community in response to input-airflow interventions. Samples used in the transcriptional analysis are indicated with an asterisk and their $O_2$ levels (%). Horizontal bars on the below the x-axis indicate the two periods when input-airflow was inter-rupted in the column (days 62 to 68 and days 91 to 104). The input ($CO_2$-in) and output ($CO_2$-out) $CO_2$ levels are shown.

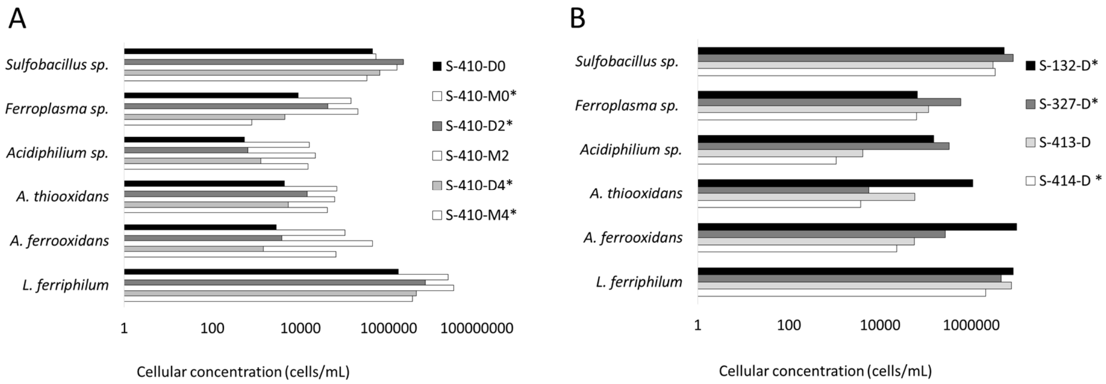

**Figure S3**. Community characterization of the industrial strips S-410 (A) and S132, 327, 413, 414 (B) of MEL bioleaching heap responding to aeration interventions. Samples that were used in the transcriptional analysis are indicated with an asterisk (*). A) 0, 2, 4 correspond to the three samplings performed biweekly before (0) and after (2, 4) aeration intervention in strip 410. M are samples taken on the base of the fourth lift. D is a drop (base of the first lift).

**Figure S4**. Ammonium level measured in industrial PLS solutions vs. the age of the heap expressed as the day after the start of irrigation.