Supplementary information

Development of Psychrophilic and Extremely Acidophilic Sulfate-Reducing Bacterial Consortium - A Solution Toward Acid Mine Drainage Treatment in Cold Regions

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Table S1. Composition of the nutrient supplements [1] used for enriching microorganism in the batch microcosm experiment.

Trace element solution	
Elements	**g/L**
$FeCl_2,4H_2O$	1.5
$CoCl_2,6H_2O$	0.19
$MnCl_2$, $6H_2O$	0.1
$ZnCl_2$	0.07
H_3BO_3	0.062
Na_2MoO_4, $2H_2O$	0.036
$NiCl_2$, $6H_2O$	0.024
$CuCl_2$, $2H_2O$	0.017
The trace element solution should be added as 1mL/L of the medium	

Vitamin mix solution	
Vitamins	g/L
Biotin	0.001
Ca-Patothenate	0.001
4-aminobenzoic acid	0.005
Pyridoxamine	0.025
Nicotinic acid	0.01
Thiamine	0.002
The vitamin solution should be added as 5 mL/L of the medium	

Vitamin B12 solution	
Vitamin B12 (g/L)	0.005
The vitamin B12 solution should be added as 1 mL/L of the medium	

Table S2. Characterization of water from PL and ARD collection locations

Parameters	PL	AMD
pH	3.3	2.4
Temperature (°C)	13.7	22.1
DO (mg/L)	6.8	4.9
Conductivity (µS/cm)	3448	19817
Sulfate (mg/L)	3059 ± 28	36507 ± 709
Mg (mg/L)	145.2± 1.2	1633.4± 12.4
Fe (mg/L)	63.7± 1.1	2368.5± 36.7
Al (mg/L)	4.6± 0.4	549.6± 42.5
Zn (mg/L)	661.1± 0.5	6082.2± 24.9
Mn (mg/L)	33.6± 0.3	580.2± 2.1

± indicates standard deviation.

Table S3. Changes in pH during the batch microcosm study and stoichiometric total bicarbonate production due to glycerol oxidation coupled to sulfate reduction. The stochiometric calculation was performed following the biochemical reaction[90] coupling sulfate reduction and glycerol oxidation.

Stage	Sample	Bicarbonate (mg/L as HCO_3^-)	Initial pH	Final pH	Change in pH (ΔpH)
1	PA	869.13±4.4	4.5	7.4	2.9
	PN	1022.1±1.2	7	8.1	1.1
	AA	601.1±130.1	4.5	7.2	2.6
	AN	507.5±26.5	7	7.6	0.6
2	PAC1	1139.6±25.9	4.5	7.2	2.7
	PAC2	1212.6±40.8	3	7.1	4.1
	PACS1	370.1±15.8	4.5	6.2	1.7
3	PAC1S1	233.9±1.33	4.5	5.3	0.8
	PAC2S1	111.5±4.5	3	3.6	0.6
	PACS2	425.8±3.9	4.5	6.1	1.6

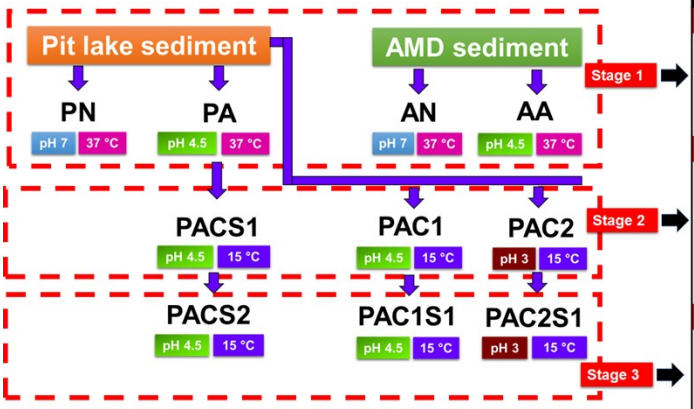
Table S4. Metal removal capacity of the inoculum grown in different condition

Sample	Metal removal (%)				
	Ca	Zn	Mg	Mn	Fe
PN	60.9±1	NA*	46.2±0.5	99.5	NA*
PA	90.8±0.05	99.9	81.4±3.2	82.7±2.9	99.9
AA	55.5±0.3	99.9	48.4±0.5	70.4±0.1	99.9
AN	10.6±0.4	NA*	15.2±0.5	88.2±0.1	NA*
PAC1	49.5±0.4	99.9	6.3±1.1	46±1.7	99.9
PAC2	52.3±4.5	99.9	21.1±2.8	67±1.2	99.9

*NA=Not applicable

Table S5. The richness and diversity of microbial community in PL, different cultures, and subcultures.

Bioreactor	Relative Richness	Shannon (Scaled)	Simpson
PL	1.0	1.63 (0.35)	0.53
PACS1 (day 51)	0.95	2.76 (0.59)	0.85
PACS2 (day 65)	0.64	1.91 (0.45)	0.78
PAC2 (day 25)	0.72	1.87 (0.43)	0.73



Culture name	Inoculum source	Incubation Temperature	Initial pH
STAGE 1			
AMD Neutral **(AN)**	AMD sediment	37 °C	7
AMD Acidic **(AA)**	AMD sediment	37 °C	4.5
Pit Lake Neutral **(PN)**	Pit Lake sediment	37 °C	7
Pit Lake Acidic **(PA)**	Pit Lake sediment	37 °C	4.5
STAGE 2			
Pit Lake Acidic at Cold Temperature, Subculture 1 **(PACS1)**	PA	15 °C	4.5
Pit Lake Acidic at Cold Temperature 1 **(PAC1)**	Pit Lake sediment	15 °C	4.5
Pit Lake Acidic at Cold Temperature 2 **(PAC2)**	Pit Lake sediment	15 °C	3
STAGE 3			
Pit Lake Acidic at Cold Temperature, Subculture 2 **(PACS2)**	PACS1	15 °C	4.5
Pit Lake Acidic at Cold Temperature 1, Subculture 1 **(PAC1S1)**	PAC1	15 °C	4.5
Pit Lake Acidic at Cold Temperature 2, Subculture 1 **(PAC2S1)**	PAC2	15 °C	3

Figure S1. Schematic representation of the enrichment approach of SRB consortium from pit lake and AMD location mine sediments.

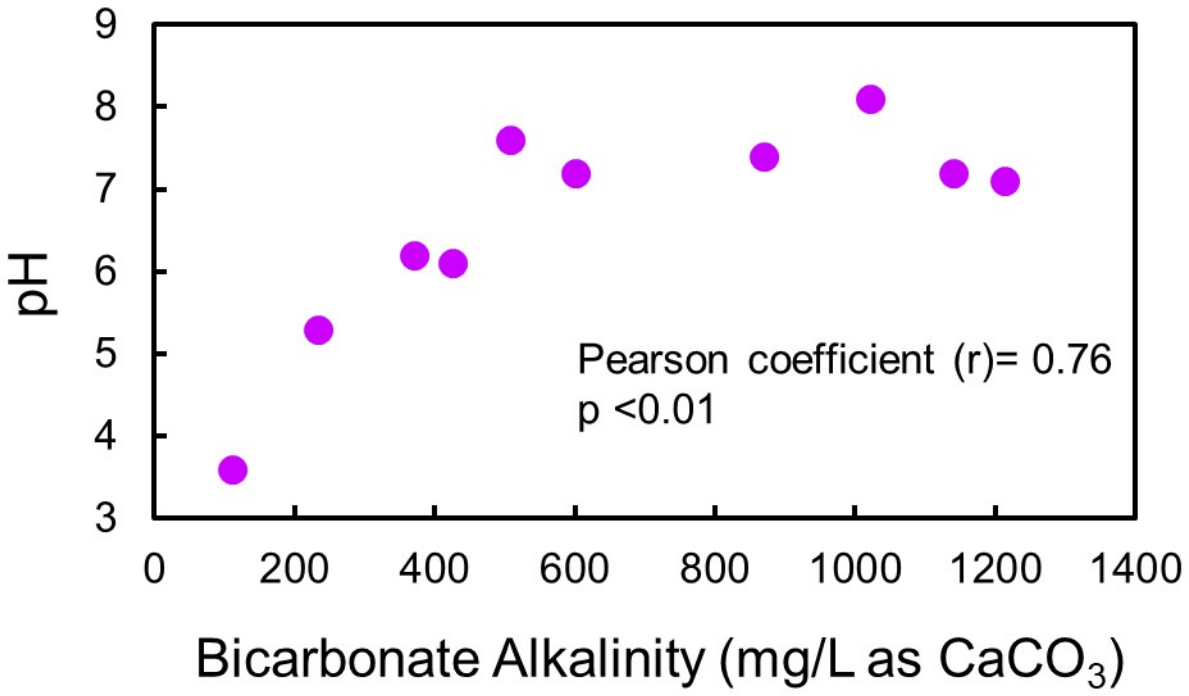


Figure S2. Analysis of the Pearson coefficient (r) and p between pH and bicarbonate alkalinity produced during enriching the microbial culture in batch bioreactors at varying pH and temperatures.

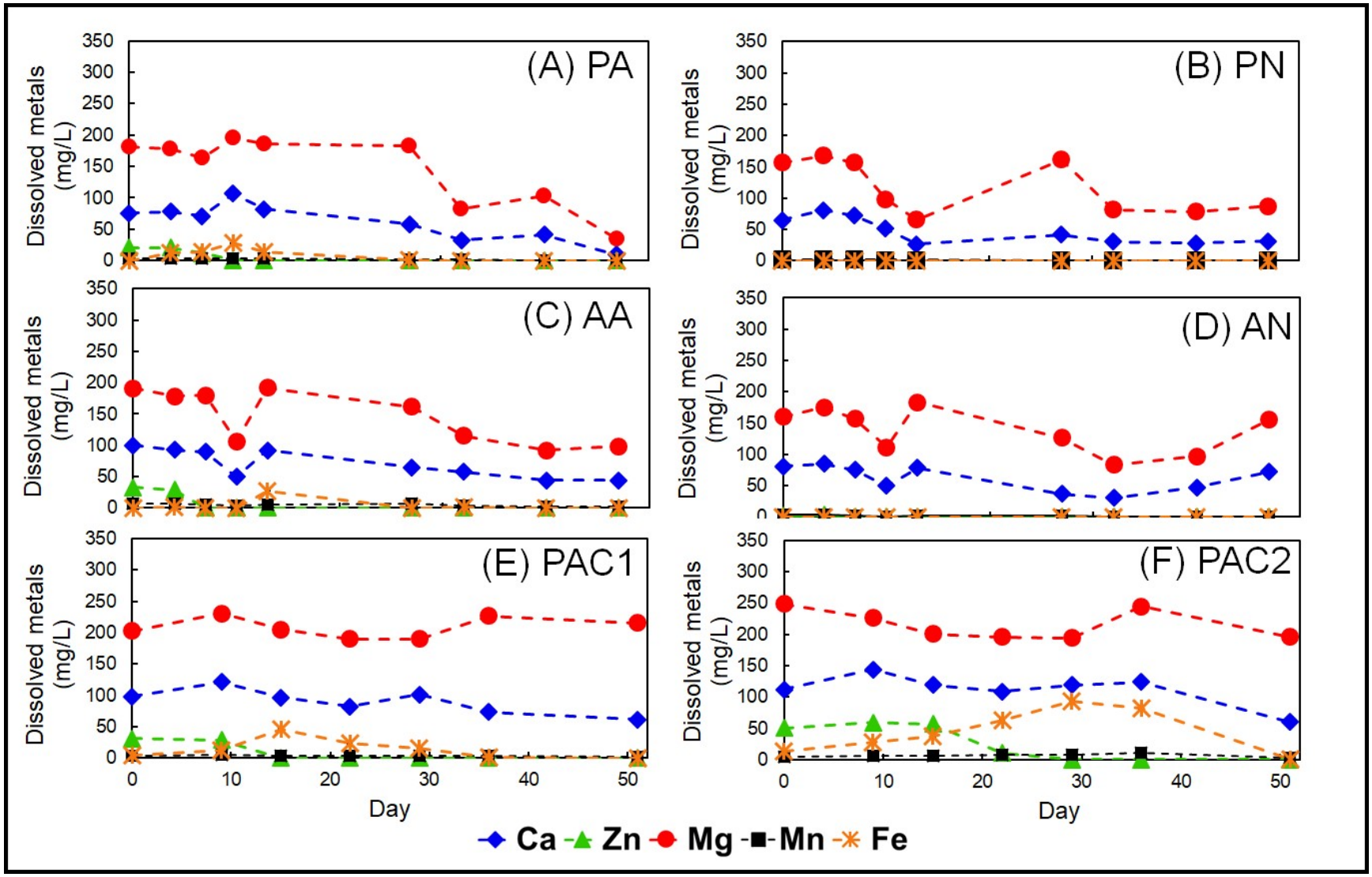


Figure S3. Profile of residual dissolved metals in the media used for enrichment of SRB community from pit lake sediment.

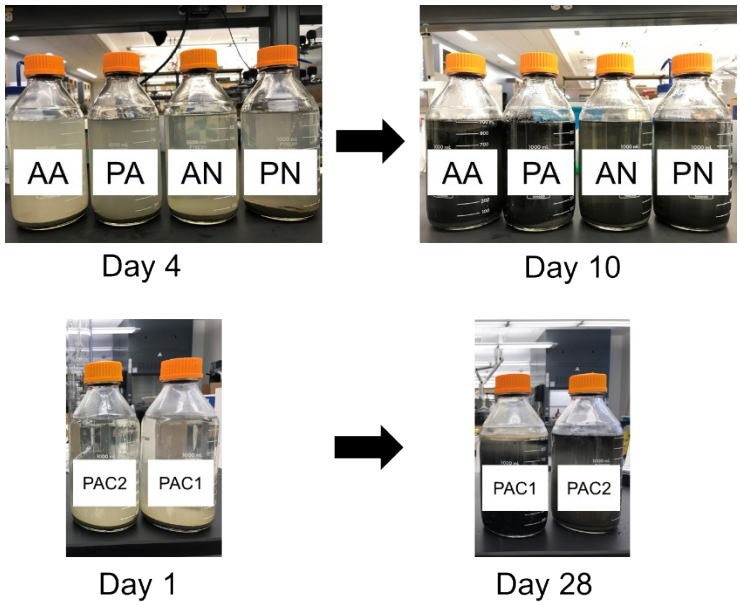
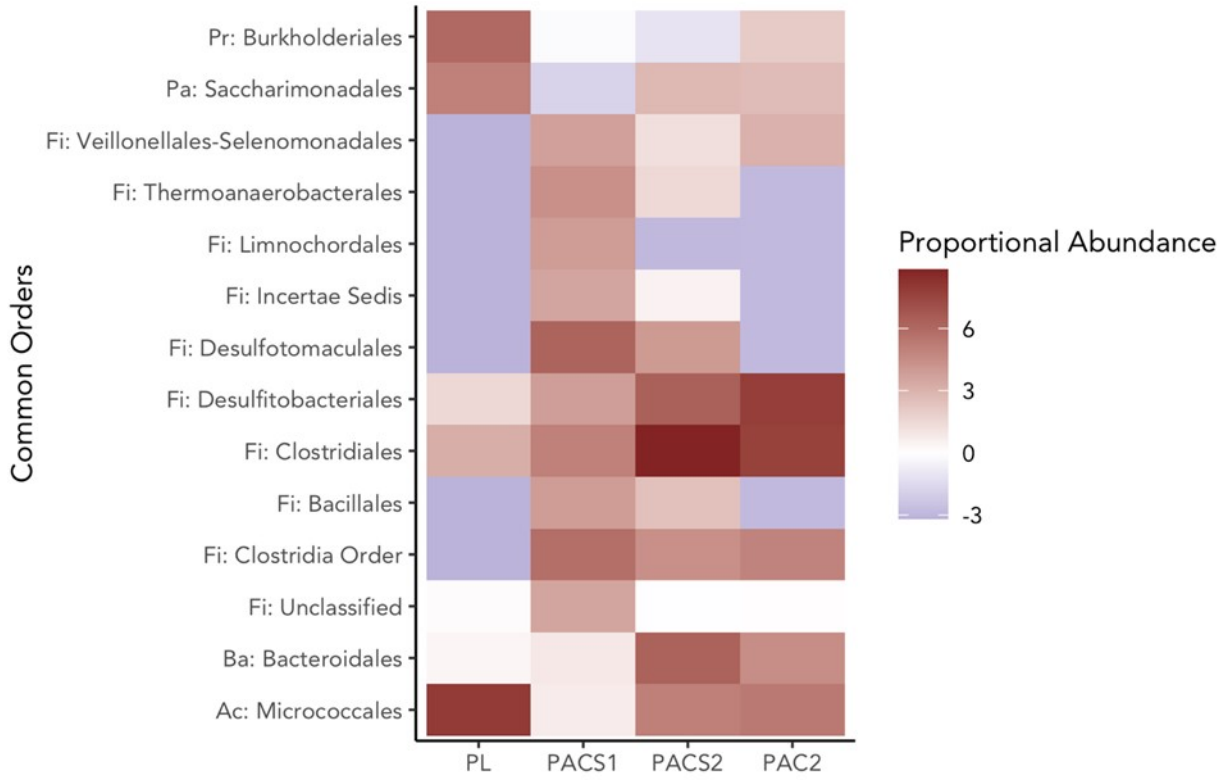


Figure S4. The enrichment of the SRB consortia from sediment. The growth of SRB caused the blackening of the media due to the formation of iron sulfide precipitates.



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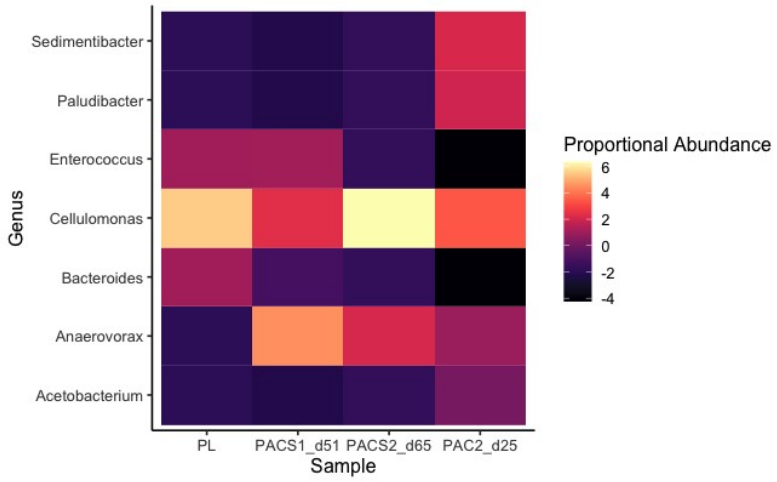
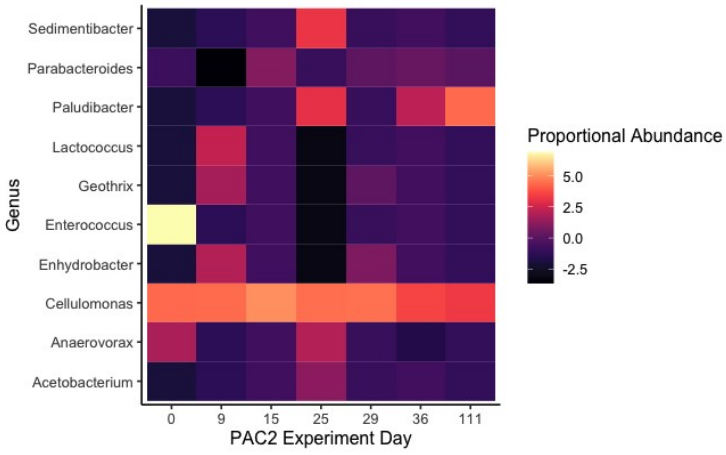
72 **Figure S5.** The Major orders of Bacteria present in samples. Orders are preceded by phylum
73 abbreviations: Pr = *Proteobacteria*, Pa=*Patescibacteria*, Fi = *Firmicutes*, Ba=*Bacteroidetes*,
74 Ac=*Actinobacteria*. Proportional abundances reflect a fold level difference from the geometric
75 mean abundance of each sample.



(A)



(B)

Figure S6. Distribution of fermentative bacteria from PL sediments to enriched inoculum through different stages (A), and as a function of incubation time in stage one (B).

Figure S7. Distribution of the microorganisms involved in the sulfur cycle in PL, PAC2, PACS1, and PACS2.

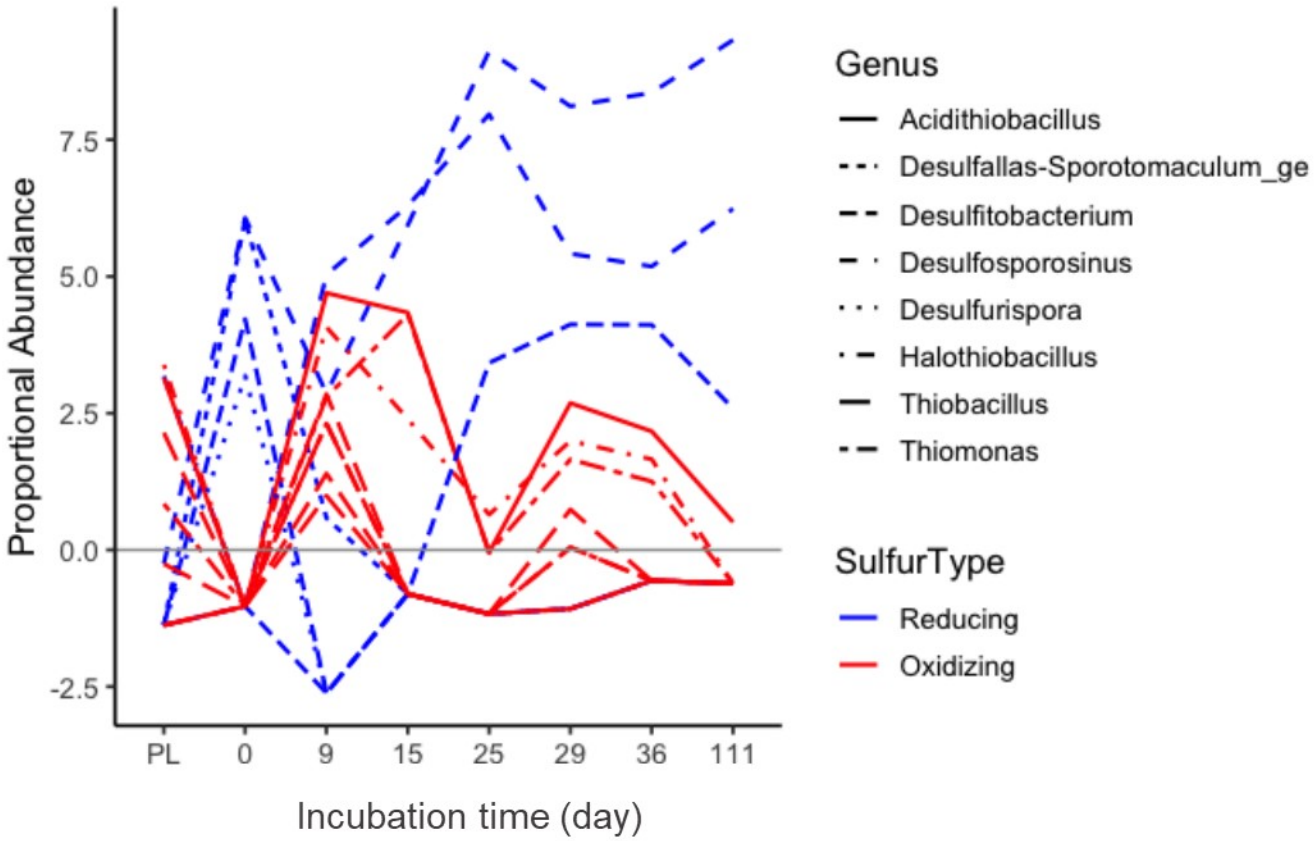


Figure S8. Profile of acidophilic sulfur-oxidizing bacteria and sulfate-reducing bacteria throughout the enrichment period in PAC2.

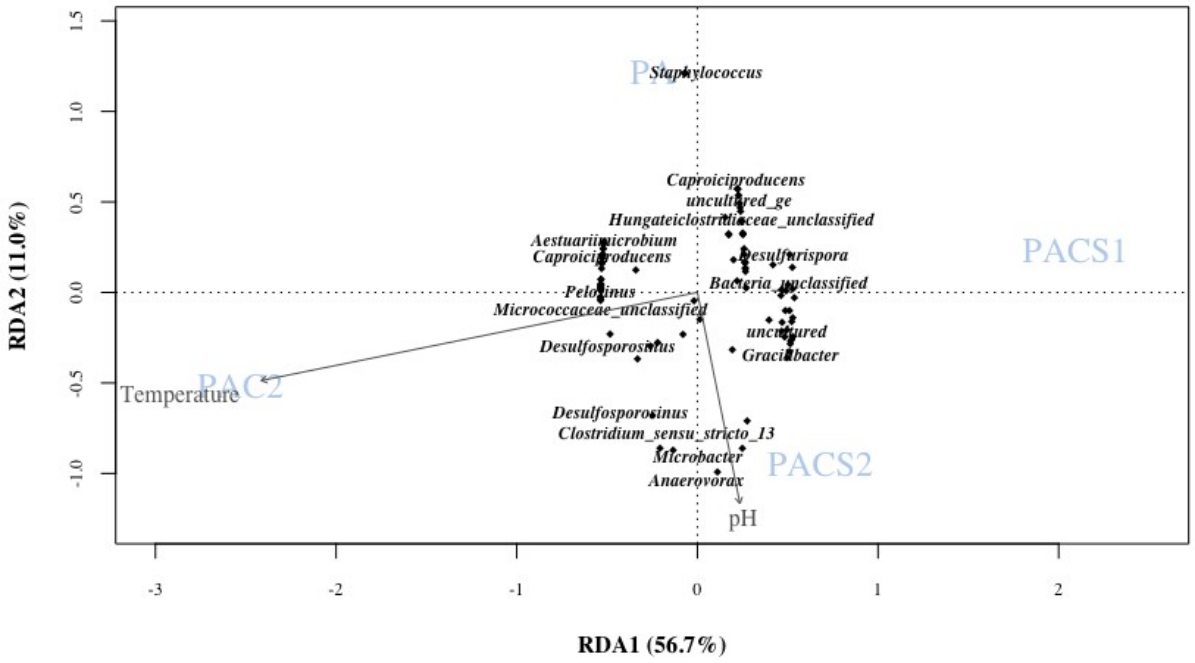


Figure S9. Redundancy analysis between initial pH and incubation temperature, and enriched microorganisms for all the bioreactors in stage one to three.

Reference.

1. F. Widdel and N. Pfennig, *Archives of Microbiology*, 1981, **129**, 395-400.