

Sulfur reduction in acid rock drainage environments

Anna P. Florentino^{1,2}; Jan Weijma²; Alfons J. M. Stams^{1,3}; Irene Sánchez-Andrea^{1*}

¹Laboratory of Microbiology, Wageningen University, Stippeneng 4, 6708 WE, Wageningen, The Netherlands.

²Sub-department of Environmental Technology, Wageningen University, Bornse Weilanden 9, 6708 WG, Wageningen, The Netherlands.

³CEB-Centre of Biological Engineering, University of Minho, Campus de Gualtar, 4710-057, Braga, Portugal.

*Corresponding author. Phone/Fax: +31 317 483115 14 / +31 317 483829.

Email: irene.sanchezandrea@wur.nl

Keywords: Sulfur reduction; metal precipitation; acidic; extreme; Tinto River; *Desulfurella*.

Number of pages: 10

Supplementary figures caption

Figure S1 - Experimental flow diagram.

Figure S2 – Colonies of the strain TR1 grown at pH 4, with acetate as electron donor and colloidal sulphur as electron acceptor.

Figure S3 - Effect of temperature on the growth rate of strain TR1 growing at pH 6 and on 5mM acetate. The analyses were carried out in biological duplicates and the results averaged.

Figure S4 - Effect of pH on the growth rate of strain TR1 growing at 30°C and on 5mM acetate. The analyses were carried out in biological duplicates and the results averaged.

Supplementary tables caption

Table S1 – Phylogenetic affiliation of sequences in the sediment mixture from Tinto River used as inoculum. OTU clustering criterium was 98% identity.

Table S2 – Averaged sulfide production (mM) from replicate enrichments incubated at different pH and with different electron donors.

Figure S1

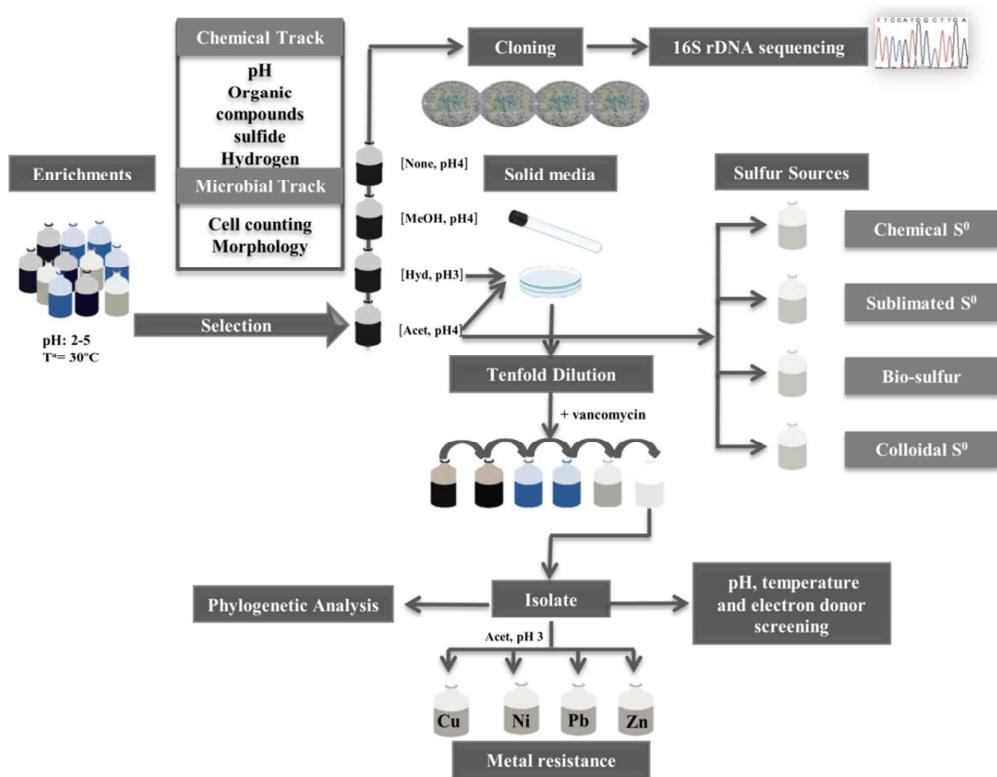


Figure S2



Figure S3

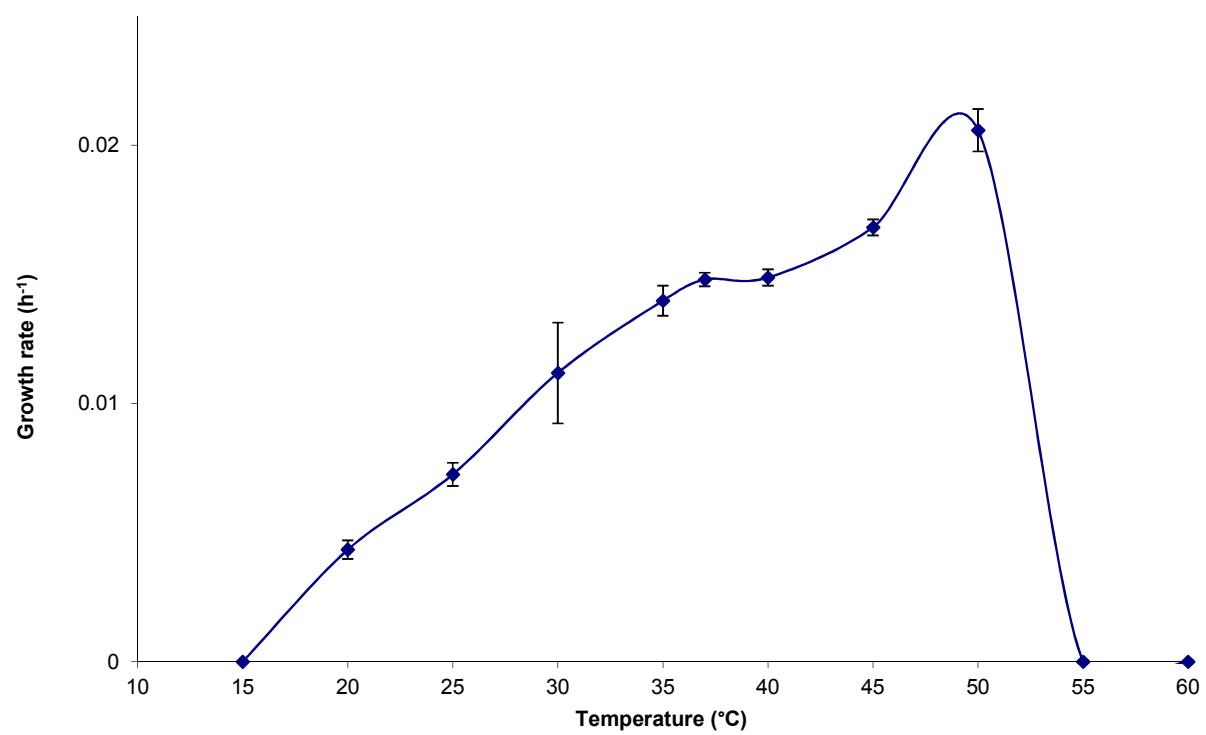


Figure S4

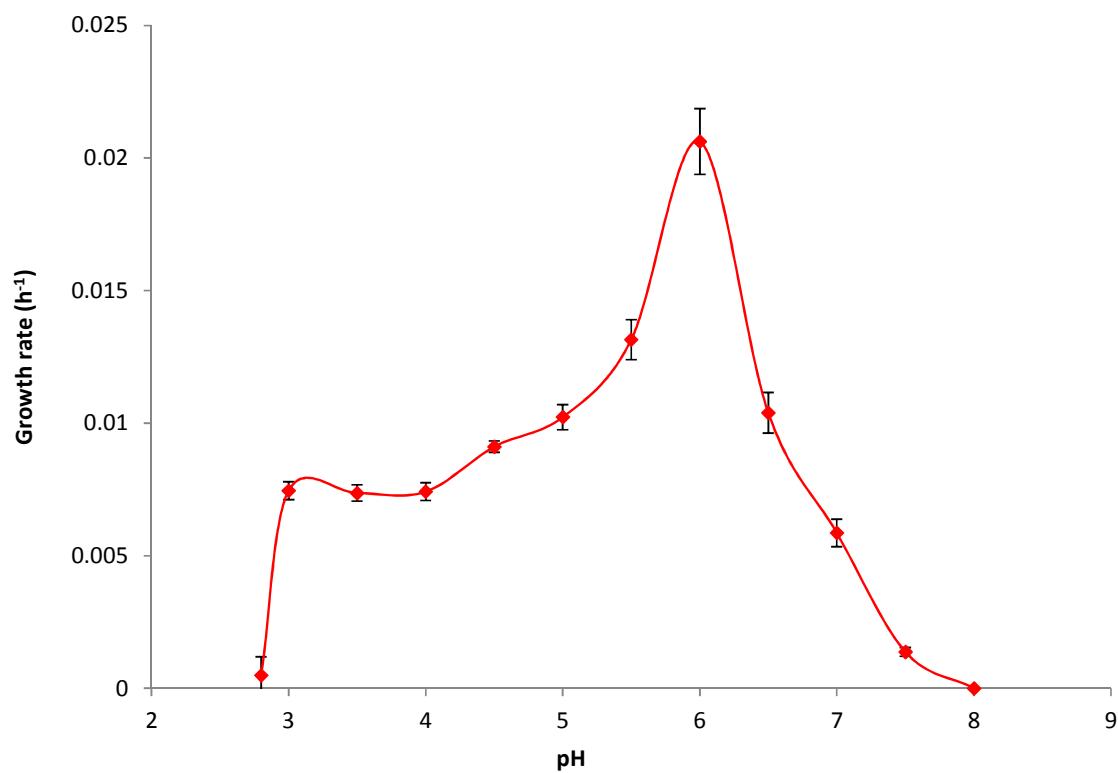


Table S1

Nº of OTUs	Phylum	Class	Order	Family	Genus
2	<i>Acidobacteria</i>	<i>Acidobacteria</i>	<i>Acidobacteriales</i>	<i>Acidobacteriaceae</i>	<i>Acidobacterium</i>
16					<i>Granulicella</i>
1					<i>Telmatobacter</i>
22					uncultured
1	<i>Actinobacteria</i>	<i>Acidimicrobia</i>	<i>Acidimicrobiales</i>	<i>Acidimicrobiaceae</i>	<i>Ferrimicrobium</i>
1					uncultured
2					uncultured
1		<i>Actinobacteria</i>	<i>Micrococcales</i>	<i>Cellulomonadaceae</i>	<i>Cellulomonas</i>
1				<i>Intrasporangiaceae</i>	<i>Janibacter</i>
1				<i>Micrococcaceae</i>	<i>Arthrobacter</i>
1			PeM15		
1			<i>Propionibacteriales</i>	<i>Propionibacteriaceae</i>	<i>Propionibacterium</i>
1		<i>Coriobacteriia</i>	<i>Coriobacteriales</i>	<i>Coriobacteriaceae</i>	uncultured
2	<i>Bacteroidetes</i>	<i>Bacteroidia</i>	<i>Bacteroidales</i>	<i>Porphyromonadaceae</i>	<i>Paludibacter</i>

1				<i>Prevotellaceae</i>	<i>Prevotella</i>
1		<i>Flavobacteria</i>	<i>Flavobacteriales</i>	<i>Flavobacteriaceae</i>	<i>Sufflavidibacter</i>
1	Candidate division				
		TM7			
5	<i>Cyanobacteria</i>	<i>Chloroplast</i>			
15	<i>Firmicutes</i>	<i>Bacilli</i>	<i>Bacillales</i>	<i>Alicyclobacillaceae</i>	<i>Alicyclobacillus</i>
22				<i>Bacillaceae</i>	<i>Bacillus</i>
1				<i>Paenibacillaceae</i>	<i>Brevibacillus</i>
2					<i>Cohnella</i>
2					<i>Oxalophagus</i>
15					<i>Paenibacillus</i>
3					<i>uncultured</i>
2			<i>Planococcaceae</i>		<i>Incertae Sedis</i>
1					<i>Rummeliibacillus</i>
2					<i>Sporosarcina</i>
1			<i>Staphylococcaceae</i>		<i>Staphylococcus</i>

1				uncultured
34	<i>Clostridia</i>	<i>Clostridiales</i>	<i>Clostridiaceae</i>	<i>Clostridium</i>
1			Family XVIII <i>Incertae Sedis</i>	uncultured
1			<i>Lachnospiraceae</i>	<i>Epulopiscium</i>
1				<i>Incertae Sedis</i>
2				uncultured
1			<i>Peptococcaceae</i>	<i>Desulfitobacterium</i>
11				<i>Desulfosporosinus</i>
2				<i>Desulfotomaculum</i>
1				<i>Desulfurispora</i>
48			<i>Peptostreptococcaceae</i>	<i>Incertae Sedis</i>
8				uncultured
1			<i>Ruminococcaceae</i>	<i>Incertae Sedis</i>
1				<i>Ruminococcus</i>
1				<i>Sporobacter</i>
4				uncultured

1		<i>Thermoanaerobacterales</i>	<i>Thermoanaerobacteraceae</i>	<i>Caldanaerobius</i>
1			<i>Thermodesulfobiaceae</i>	<i>Thermodesulfobium</i>
3	<i>Erysipelotrichi</i>	<i>Erysipelotrichales</i>	<i>Erysipelotrichaceae</i>	<i>Turicibacter</i>
1	<i>Negativicutes</i>	<i>Selenomonadales</i>	<i>Veillonellaceae</i>	
1				<i>Pelosinus</i>
3	OPB54			
1	<i>Nitrospirae</i>	<i>Nitospira</i>	<i>Nitrospirales</i>	<i>Nitospiraceae</i>
2	<i>Proteobacteria</i>	<i>Alphaproteobacteria</i>	<i>Rhodospirillales</i>	<i>Acetobacteraceae</i>
1				<i>Acidiphilium</i>
1				<i>Rhodovastum</i>
1	<i>Betaproteobacteria</i>	<i>Burkholderiales</i>	<i>Burkholderiaceae</i>	<i>Burkholderia</i>
1				<i>Cupriavidus</i>
6		<i>Nitrosomonadales</i>	<i>Gallionellaceae</i>	<i>Gallionella</i>
1		TRA3-20		
4	<i>Deltaproteobacteria</i>	<i>Bdellovibrionales</i>	<i>Bacteriovoracaceae</i>	uncultured
2		<i>Desulfurellales</i>	<i>Desulfurellaceae</i>	<i>Desulfurella</i>
6		<i>Syntrophobacterales</i>	<i>Syntrophobacteraceae</i>	<i>Syntrophobacter</i>

1	<i>Epsilonproteobacteria</i>	<i>Campylobacterales</i>	<i>Campylobacteraceae</i>	<i>Arcobacter</i>
30	<i>Gammaproteobacteria</i>	<i>Acidithiobacillales</i>	<i>Acidithiobacillaceae</i>	<i>Acidithiobacillus</i>
1			<i>KCM-B-112</i>	
1		<i>Chromatiales</i>	<i>Chromatiaceae</i>	<i>Nitrosococcus</i>
1		<i>Legionellales</i>	<i>Legionellaceae</i>	<i>Legionella</i>
2		<i>Oceanospirillales</i>	<i>Halomonadaceae</i>	<i>Halomonas</i>
2		<i>Xanthomonadales</i>	uncultured	
2			<i>Xanthomonadaceae</i>	<i>Metallibacterium</i>
12	No Relative			

Table S2

pH	Electron donors added				
	Acetate	Glycerol	Hydrogen	Methanol	No donor
2	-	-	-	-	-
3	8.7 (± 0.8)	1.9 (± 0.1)	10.9 (± 0.9)	1.5 (± 0.1)	1.5 (± 0.3)
4	16.4 (± 1.2)	2.3 (± 0.5)	10.5 (± 0.5)	1.3 (± 0.1)	1.5 (± 0.2)
5	8.11 (± 0.6)	1.1 (± 0.2)	6.9 (± 0.3)	1.6 (± 0.2)	1.7 (± 0.2)