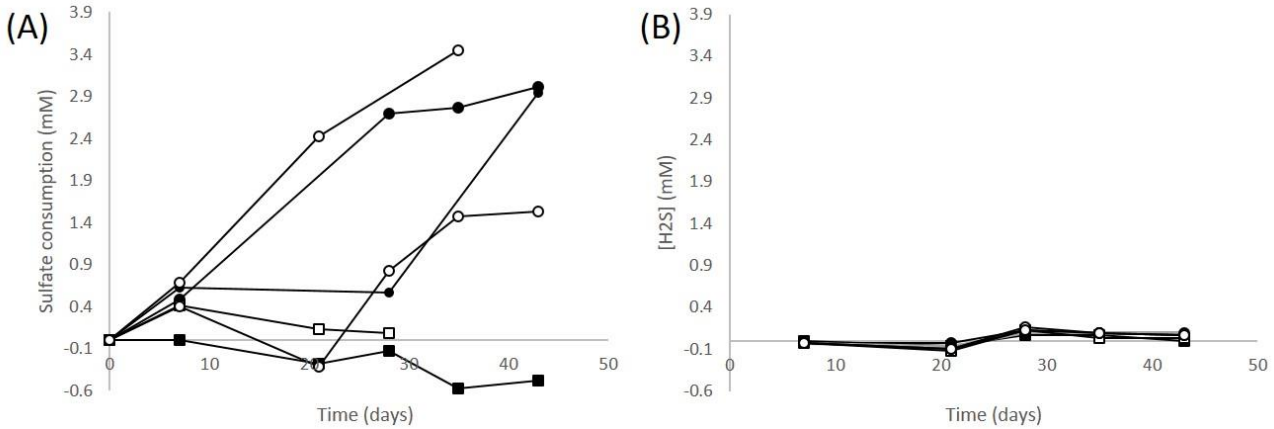
Supplementary Material

On the corrosion of ductile cast iron by sulphate reducing bacteria - implications for long-term nuclear waste repositories

Marc Tamisier*, Florin Musat, Hans-Hermann Richnow, Carsten Vogt, Matthias Schmidt

*** Correspondence:** Corresponding author: marc.tamisier@ufz.de

Supplementary Figures

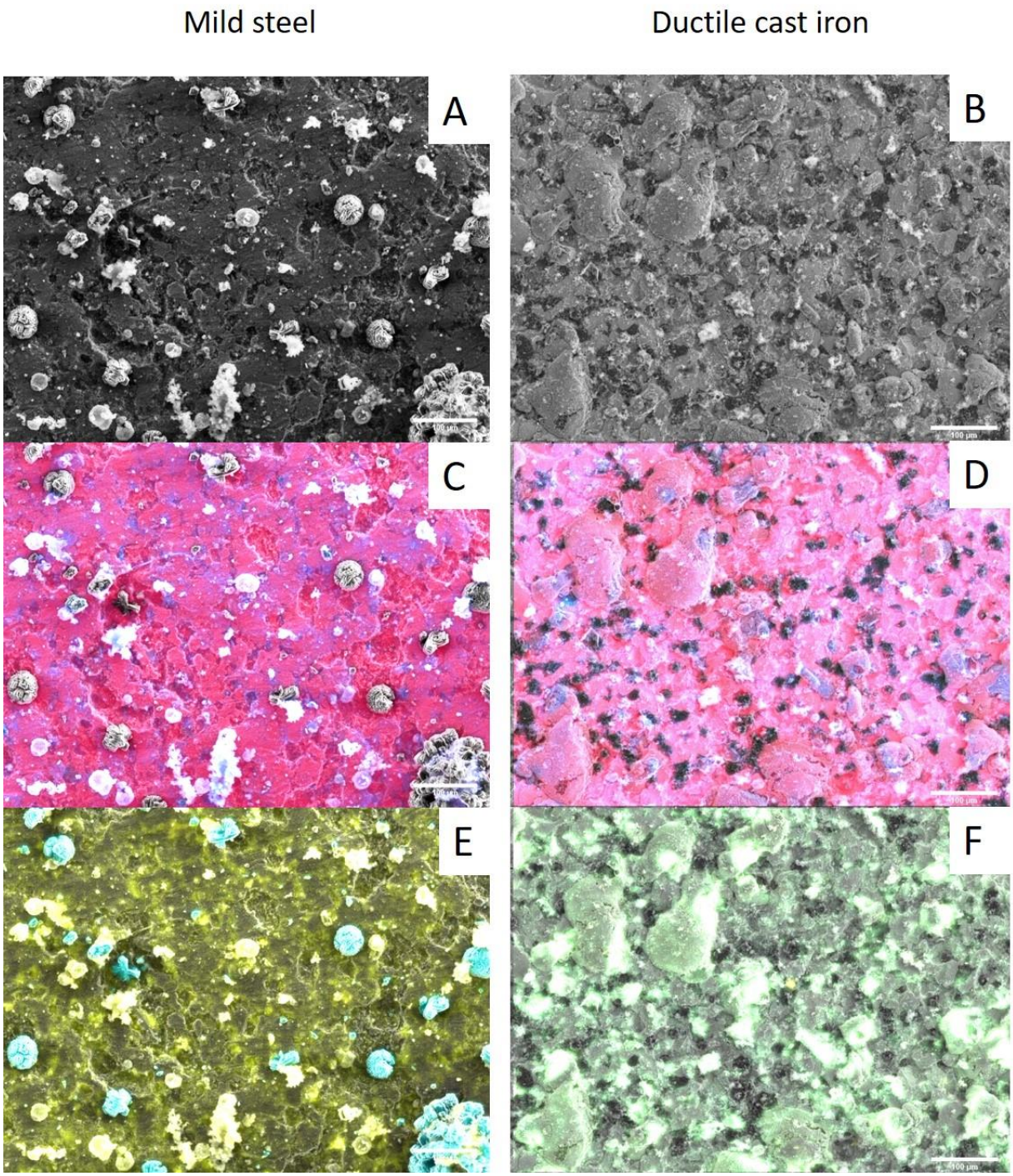


Supplementary figure S1: Sulphate and sulphide profiles during corrosive growth of the sulphate reducing bacterium *D. corrodens* strain IS4.

(A) Sulphate consumption in culture with ductile cast iron corroded by the sulphate reducing bacteria IS4 (●), mild steel corroded by the sulphate reducing bacteria IS4 (○) and abiotic controls, ductile cast Iron (■), mild steel (□)

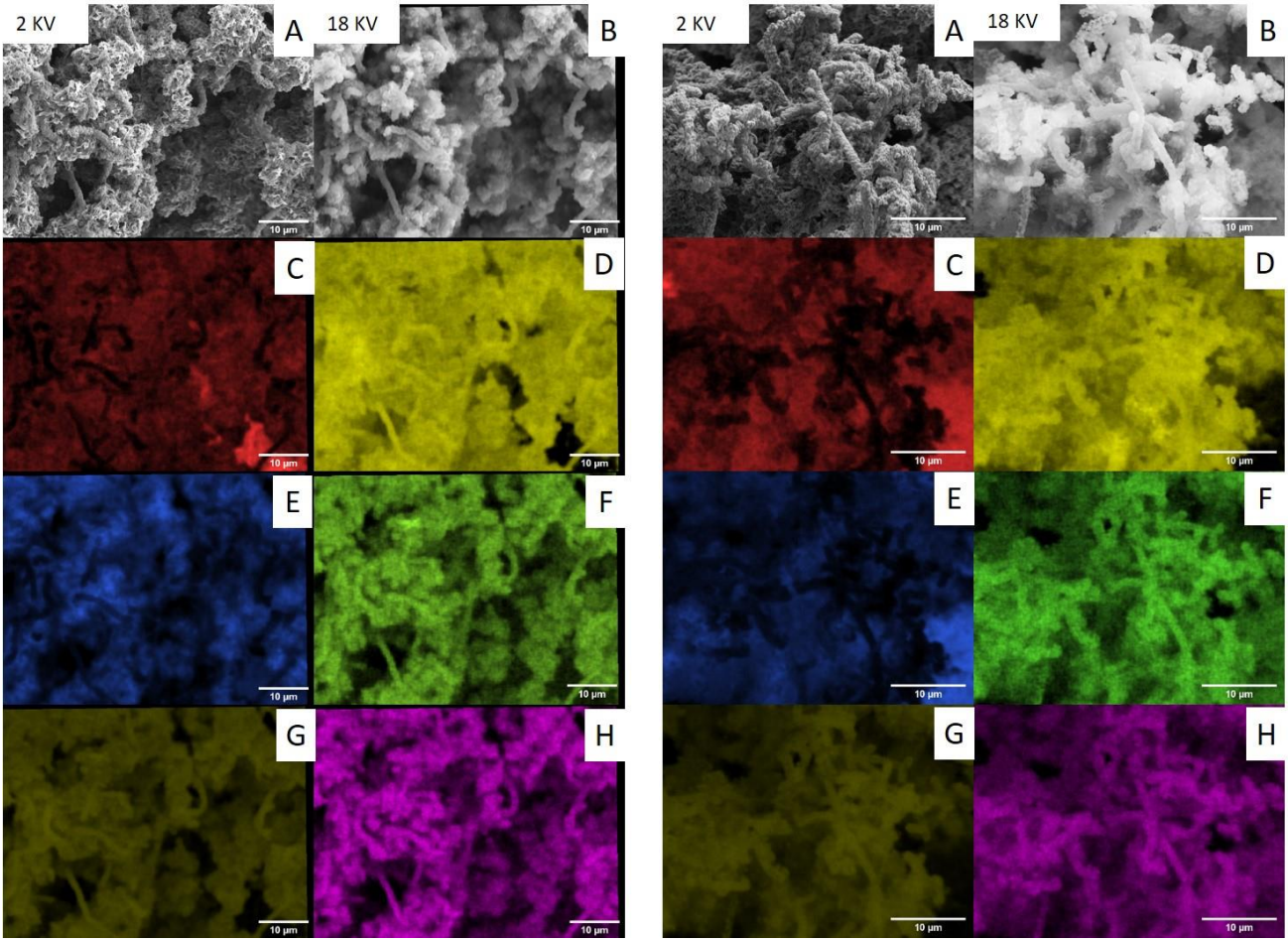
(B) H_2S concentration in culture with ductile cast iron corroded by IS4 (●), mild steel corroded by IS4 (○) and abiotic controls, ductile cast iron (■), mild steel (□)

(C) Data corresponding to dataset 2 in table S1.



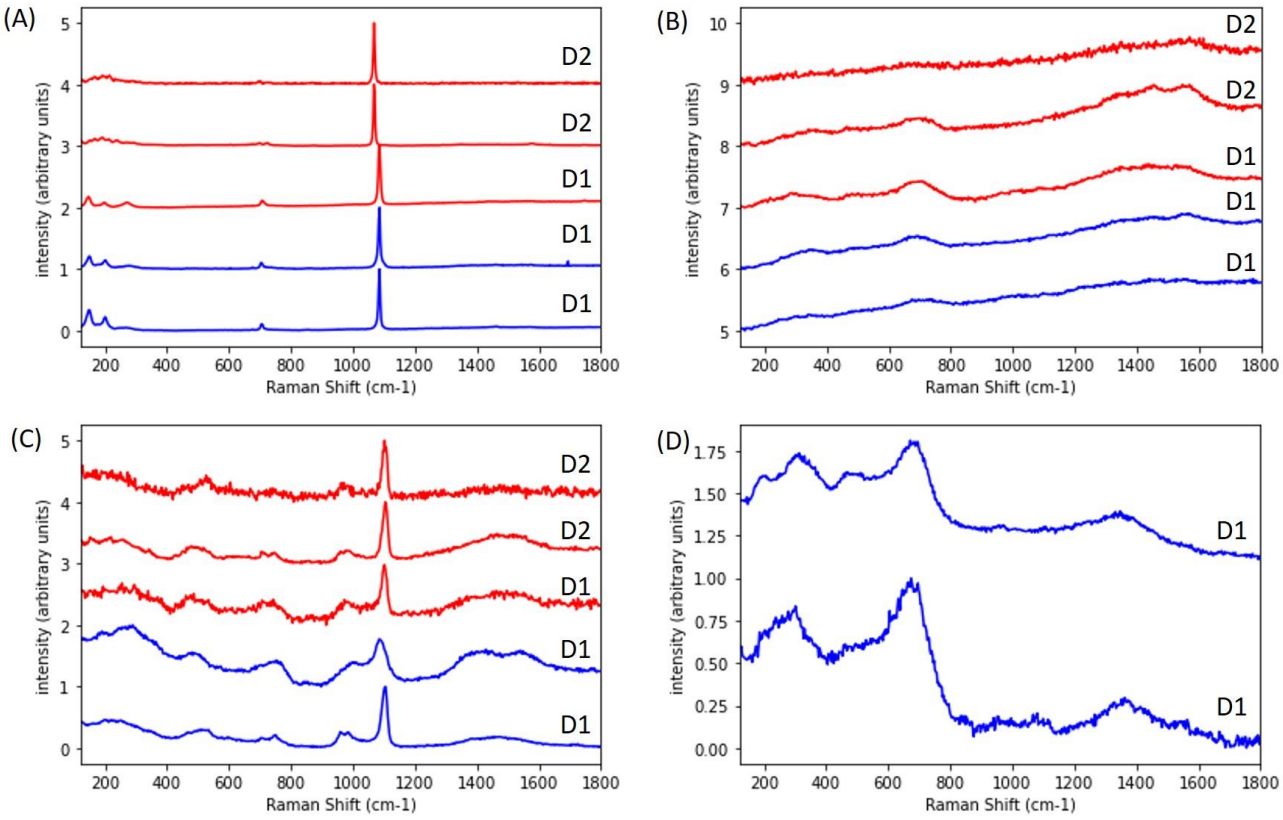
Supplementary figure S2. SEM (18kV) and SEM-EDX of Ductile cast iron and mild steel, abiotic controls after 2 days of incubation. EDX at 18 kV for the mild steel (A) and ductile cast iron (B). EDX with overlay of iron (red) and sulphur (blue) (pink = presence of iron and sulphur) for the mild steel (C) and ductile cast iron (B), overlay of calcium (yellow) and phosphorus (turqoise) (green = presence of calcium and phosphorus) for the mild steel (E) and ductile cast iron (F).

Data from dataset 5 and dataset 3 (table S1)



Supplementary figure S3. SEM (2kV and 18kV) (panel A and B) and SEM-EDX of mild steel corroded by the sulphate reducer IS4. Iron (red) (panel C), calcium (yellow) (panal D), sulphur (blue) (panel E), magnesium (green) (panel F), phosphorus (brown) (panel G), carbon (purple) (panel H).

Data from Dataset 5 (table S1) and Dataset 6



Supplementary figure S4. Raman spectra obtained on long incubation (>36 days) corrosion crust surface. Red spectra correspond to the ductile cast iron and the blue spectra to the mild steel. D1 = from samples in dataset 1 (table S1); D2 = from samples in dataset 2 (table S1).

Supplementary Tables

Supplementary Table S1: Presentation of the data. The different experiments and corresponding samples are described

Data set	samples	Time corroded (days)	Valid for analysis (reason if No)
Data set 1	Ductile cast iron 1	36 (abiotic control)	yes
	Ductile cast iron 2	36	yes
	Ductile cast iron 3	36	yes
	Mild steel 1	36 (abiotic control)	yes
	Mild steel 2	36	yes
	Mild steel 3	36	yes
Data set 2	Ductile cast iron 1	43 (abiotic control)	yes
	Ductile cast iron 2	43	yes
	Ductile cast iron 3	43	yes
	Mild steel 1	43 (abiotic control)	yes
	Mild steel 2	43	Yes
	Mild steel 3	43	Yes (But earlier stage of corrosion than the replicates, see figure S1)
Data set 3	Ductile cast iron 1	2 (abiotic control)	yes
	Ductile cast iron 2	2	Yes

	Ductile cast iron 3	4	yes
	Ductile cast iron 4	6	yes
	Ductile cast iron 5	8	Yes
	Ductile cast iron 6	8 (abiotic control)	yes
	Ductile cast iron 1	2 (abiotic control)	yes
	Ductile cast iron 2	2	yes
	Ductile cast iron 3	4	yes
	Ductile cast iron 4	6	yes
	Ductile cast iron 5	8	yes
	Ductile cast iron 6	8 (abiotic control)	yes
	Mild steel 1	2 (abiotic control)	No (no sulphate consumption in data set for the type of steel)
	Mild steel 2	2	No (no sulphate consumption in data set for the type of steel)
	Mild steel 3	4	No (no sulphate consumption in data set for the type of steel)
	Mild steel 4	6	No (no sulphate consumption in data set for the type of steel)
	Mild steel 5	8	No (no sulphate consumption in data set for the type of steel)
	Mild steel 6	8 (abiotic control)	No (no sulphate consumption in data set for the type of steel)

Data set 4	Ductile cast iron 1	2 (abiotic control)	No (no sulphate consumption in data set for the type of steel)
	Ductile cast iron 2	2	No (no sulphate consumption in data set for the type of steel)
	Ductile cast iron 3	4	No (no sulphate consumption in data set for the type of steel)
	Ductile cast iron 4	6	No (no sulphate consumption in data set for the type of steel)
	Ductile cast iron 5	6 (abiotic control)	No (no sulphate consumption in data set for the type of steel)
	Mild steel 1	2 (abiotic control)	No (no sulphate consumption in data set for the type of steel)
	Mild steel 2	2	No (no sulphate consumption in data set for the type of steel)
	Mild Steel 3	4	No (no sulphate consumption in data set for the type of steel)
	Mild steel 4	6	No (no sulphate consumption in data set for the type of steel)
	Mild steel 5	6 (abiotic control)	No (no sulphate consumption in data set for the type of steel)
Data set 5	Ductile cast iron 1	2 (abiotic control)	yes
	Ductile cast iron 2	2	yes
	Mild steel 1	2 (abiotic control)	Yes
	Mild steel 2	2	yes
	Mild Steel 3	4	yes
	Mild steel 4	6	yes

	Mild steel 5	6 (abiotic control)	yes
Data set 6	Ductile cast iron 1	4	No (no sulphate consumption in data set for the type of steel)
	Ductile cast iron 2	6	No (no sulphate consumption in data set for the type of steel)
	Ductile cast iron 2	6 (abiotic control)	No (no sulphate consumption in data set for the type of steel)
	Mild steel 1	2 (abiotic control)	Yes
	Mild steel 2	2	yes
	Mild Steel 3	4	yes
	Mild steel 4	6	No (Crust forming structure not seen anywhere else)
	Mild steel 5	6 (abiotic control)	yes
Data set 7	Mild steel 1	2 (abiotic control)	Yes
	Mild steel 2	2	yes
	Mild Steel 3	4	yes
	Mild steel 4	6	yes
	Mild steel 5	6 (abiotic control)	yes

Data at 8 days were not repeated neither used in the analysis since the time point of the early corrosion stages were conducted for inspection of the impact of the graphite incrustation. For analysis of the early formation of crusts the time points at day 2, 4 and 6 were better to observe onset of crust formation due to lower extension of crust.

Supplementary Table S2: Comparative corrosion by IS4 after 1 week.

Data set	Culture type	Sulphate consumption first week (mM)	Amount of iron corroded by *Desulfopila corrodens*, first week (mM)
Data set 1	Ductile cast iron, culture 1	0.86	3.44
	Ductile cast iron, culture 2	1.75	7.0
	Mild steel culture 3	1.06	4.24
	Mild steel, culture 4	0.93	3.72
Data set 2	Ductile cast iron, culture 1	0.49	1.96
	Ductile cast iron, culture 2	0.63	2.52
	Mild steel, culture 3	0.68	2.72
	Mild steel, culture 4	0.40	1.6

*Iron corrosion calculated from the sulphate consumption, 1 mM of sulphate consumed correspond to 4 mM of Fe(0) (reaction 1, main text)

Value in blue may be biased (see figure S1)

Supplementary table S3. List of standard Gibbs energy of formation.

Compound	ΔG_f^0	References
$H_2S_{(aq)}$	-27,83	Widdel and Musat, 2019
$H_2O_{(l)}$	-237,141	Ning et al., 2014
$H_{2(g)}$	0	Widdel and Musat, 2019
$Fe(0)_{(s)}$	0	Ning et al., 2014
$FeS_{(s)}$	-100,07	Ning et al., 2014
$SO_4^{2-}{}_{(aq)}$	-744	Ayala-Luis et al., 2008
$HCO_3^-{}_{(aq)}$	-586,8	Azoulay et al., 2012
$FeCO_{3(s)}$	-680,71	Bénézeth et al., 2009
$HO^-{}_{(aq)}$	-157,3	Ayala-Luis et al., 2008

References:

Ayala-Luis, K. B., Koch, B. C., and Hansen, H. C. B. (2008). The standard gibbs energy of formation of Fe(II)Fe(III) hydroxide sulfate green rust. *Clays Clay Miner.* 56, 633–644. doi: 10.1346/CCMN.2008.0560604.

Azoulay, I., Rémazeilles, C., and Refait, P. (2012). Determination of standard Gibbs free energy of formation of chukanovite and Pourbaix diagrams of iron in carbonated media. *Corros. Sci.* 58, 229–236. doi: 10.1016/j.corsci.2012.01.033.

Bénézeth, P., Dandurand, J. L., and Harrichoury, J. C. (2009). Solubility product of siderite (FeCO3) as a function of temperature (25-250 °C). *Chem. Geol.* 265, 3–12. doi: 10.1016/j.chemgeo.2009.03.015.

Ning, J., Zheng, Y., Young, D., Brown, B., and Nešić, S. (2014). Thermodynamic study of hydrogen sulfide corrosion of mild steel. *Corrosion* 70, 375–389. doi: 10.5006/0951.

Widdel, F., and Musat, F (2019). "Energetic and Other Quantitative Aspects of Microbial Hydrocarbon Utilization: An Introduction" in *Aerobic Utilization of Hydrocarbons, Oils, and Lipids*. Ed. F. Rojo (Cham: Sringer International Publishing), 33-72. doi: 10.1007/978-3-319-50418-6_2