Supplementary Information for

Mechanism of Anaerobic Microbial Corrosion Suppression by Mild Negative Cathodic Polarization on Carbon Steel

Marina Shiibashi[+], Xiao Deng[+], Waheed Miran, Akihiro Okamoto*

[+] Equally contributed
* Correspondence: Akihiro.Okamoto@nims.go.jp

This PDF file includes:

Supplementary materials and methods

Composition of WP-LS medium used for cell growth and corrosion experiment

In 980 ml distilled water, following chemicals were dissolved ($CaSO_4$, 1.0 g; NH_4Cl, 1.0 g; KH_2PO_4, 0.5 g; $MgSO_4 \cdot 7H_2O$, 2.0 g; Yeast extract, 1.0 g; Sodium lactate, 2.2 g; Resazurin, 1.0 g), adjust pH to 7.0, then made to anoxic by purging with N_2:CO_2 (80:20, vol/vol) for 20 minutes and autoclave for 15 minutes at 121 ℃. After cooling down of the autoclaved solution, adding 10 ml of filter-sterilized $FeSO_4 \cdot 7H_2O$ (50 g L^{-1}) and ascorbic acid solution (10 g L^{-1}), respectively. The pH of the complete medium was 6.5. The pH observed at the end of corrosion experiments was around 8.0.

Weight loss analysis of corroded carbon steel coupons

Carbon steel electrodes after a 6-day corrosion experiment was used for weight loss determination. The corrosion crust was removed by using aqueous 6 M HCl and 3.5 g L^{-1} hexamethylenetetramine (hexamine). The treated carbon steel coupons were washed in anoxic water and dried before weighting.

Scanning Electron Microscopy (SEM)

SEM sample preparation and observation of iron specimens were conducted as previously reported.[1]

Sulfide Quantification

An aliquot of 0.5 ml of electrolyte was withdrawn from the reactor using a syringe and diluted to 25 ml using anoxic water. Sulfide concentration in the water sample was quantified using the Water Analysis kit No. 53 (Kyoritsu Chemical-Check Lab, Japan). Sulfide concentration in the original electrolyte before dilution was calculated based on the measured values.

Fluorescence Live/Dead Assay

After removing the electrolyte from the reactor via a syringe, cells on the carbon steel electrode were stained for 15 minutes by immersing in 5 ml of stain using the Live/Dead *Bac*Light Bacteria viability kit (ThermoFisher Scientific), washed by immersing in 0.85% NaCl solution, and visualized with a Leica DFC450C epifluorescent microscope.

Gene Expression Analysis

Four parallel reactors were used for corrosion experiments with and without cathodic polarization at −0.5 V. *D. vulgaris* cells in the electrochemical reactor and on carbon steel electrodes were accumulated by vacuum filtration on a 0.22 μm pore-size membrane. RNA was extracted by using All Prep DNA/RNA Mini Kit (Qiagen), purified by an ethanol precipitation method,[2] and dissolved in RNase free water. Gene expression analysis with purified RNA was conducted as described previously.[3] Data were normalized and differentially expressed genes were identified using the CLC Main Workbench (Qiagen). Genes were classified as differentially expressed if the expression ratio change was greater than 1.5-fold, with a *q* value of less than 5%.

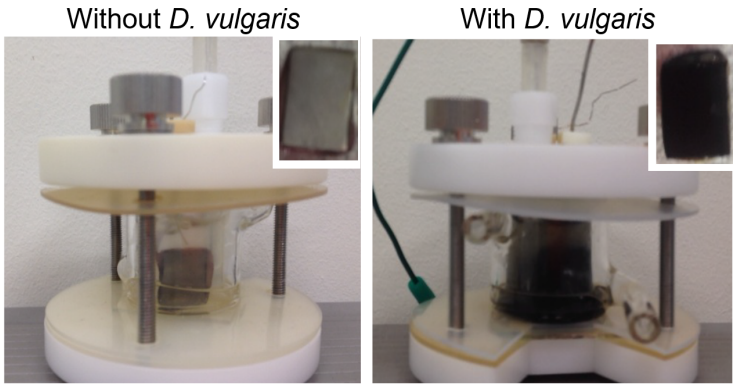

Figure S1. Electrochemical reactors used for the measurement of corrosion currents in the absence and presence of *D. vulgaris* cells. Inset photos are carbon steel electrode after the corrosion experiment.

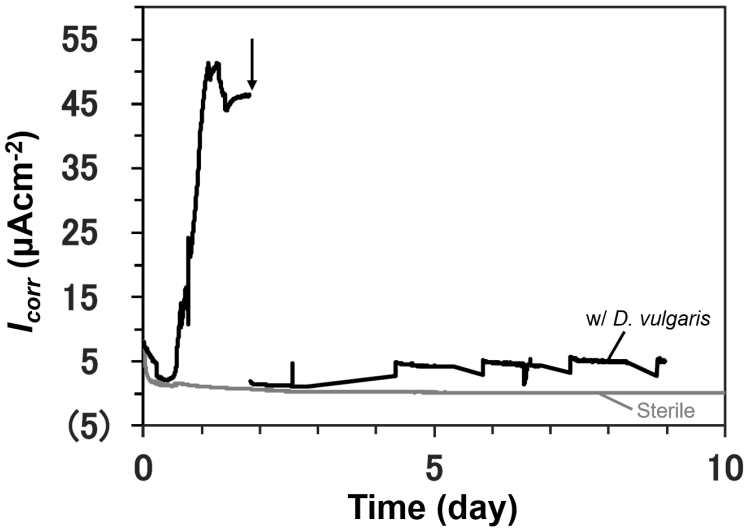

Figure S2. Electrochemical measurement of corrosion current density (I_{corr}) and the suppression effect by potential poising at −0.5 V in the presence and absence of *D. vulgaris* cells. At timing indicated by the black arrow, I_{corr} measurement was ceased and −0.5 V was poised to the electrode for 12 h.

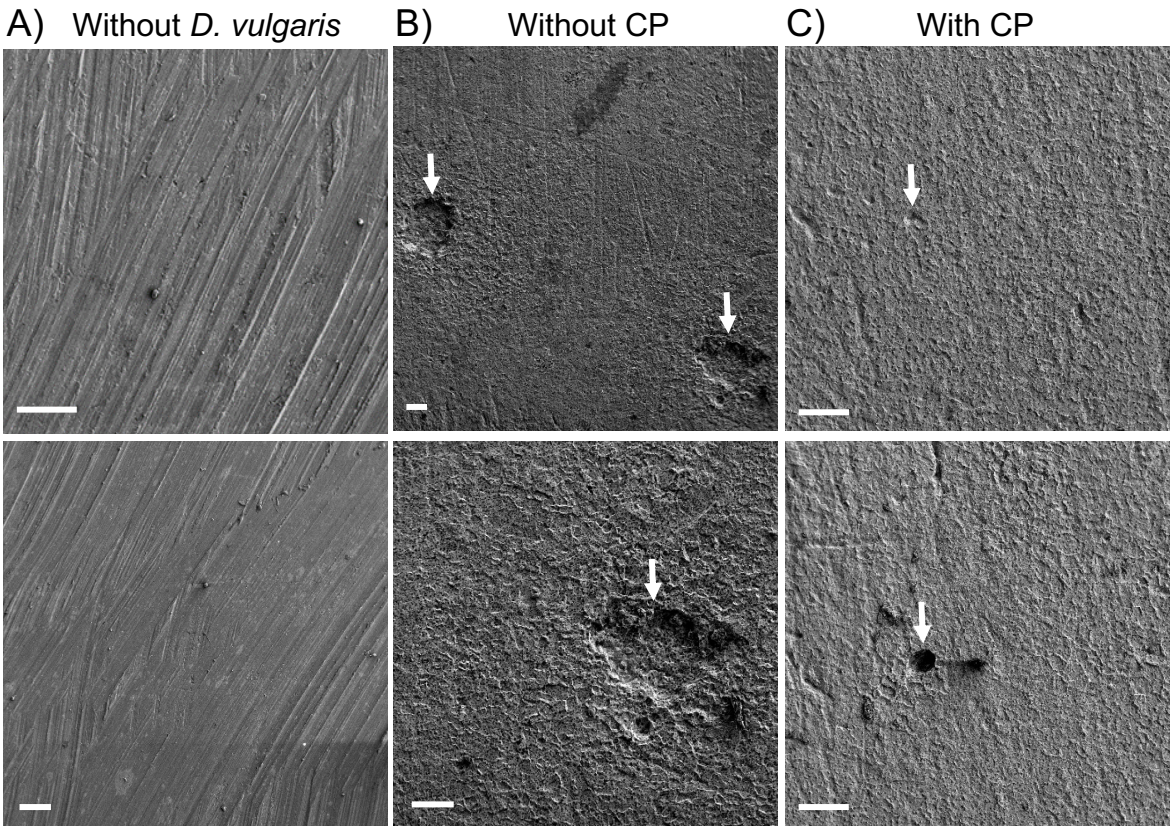

Figure S3. Scanning electron microscopy images of the carbon steel electrode surface to check the pitting severity at the end of a 6-day corrosion experiment. Coupons were treated with HCl-Hexamine mixture to remove the corrosion crust. SEM images of (A) Sterile control, (B) without CP, and (C) with CP at −0.5 V. Weight loss was 15.4 mg and 7.6 mg for the sample of pane B and C, respectively. Arrows indicate the pits on carbon steel surface. Scale bar, 100 μm.

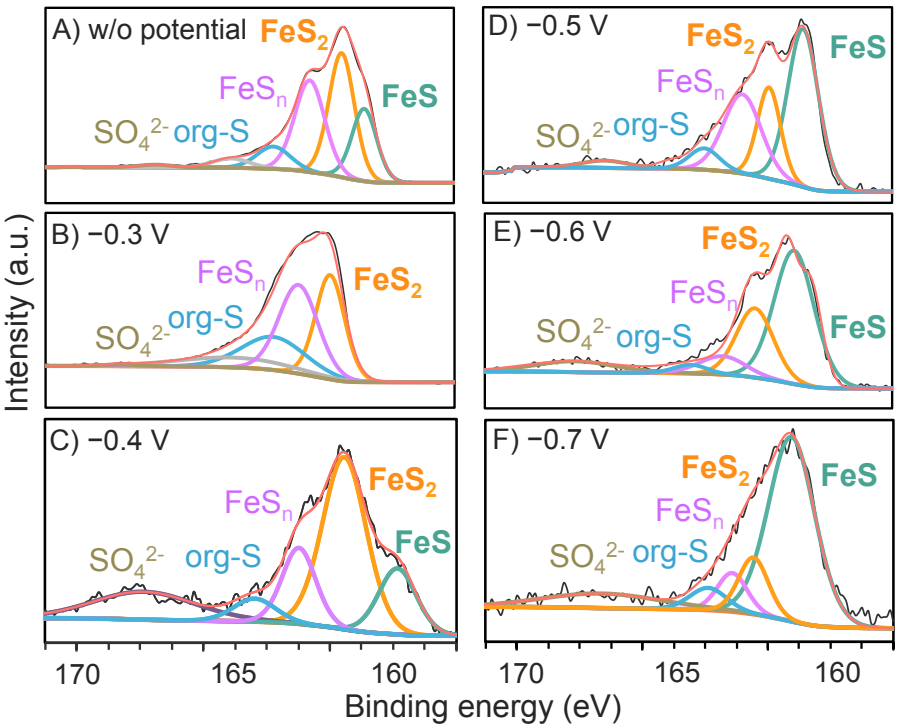

Figure S4. High-resolution S 2*p* core-level X-ray photoelectron spectroscopy spectra of iron sulfides on the surface of iron electrode without (A) and with potential poising at −0.3 V (B), −0.4 V (C), −0.5 V (D), −0.6 V (E), and −0.7 V (F).

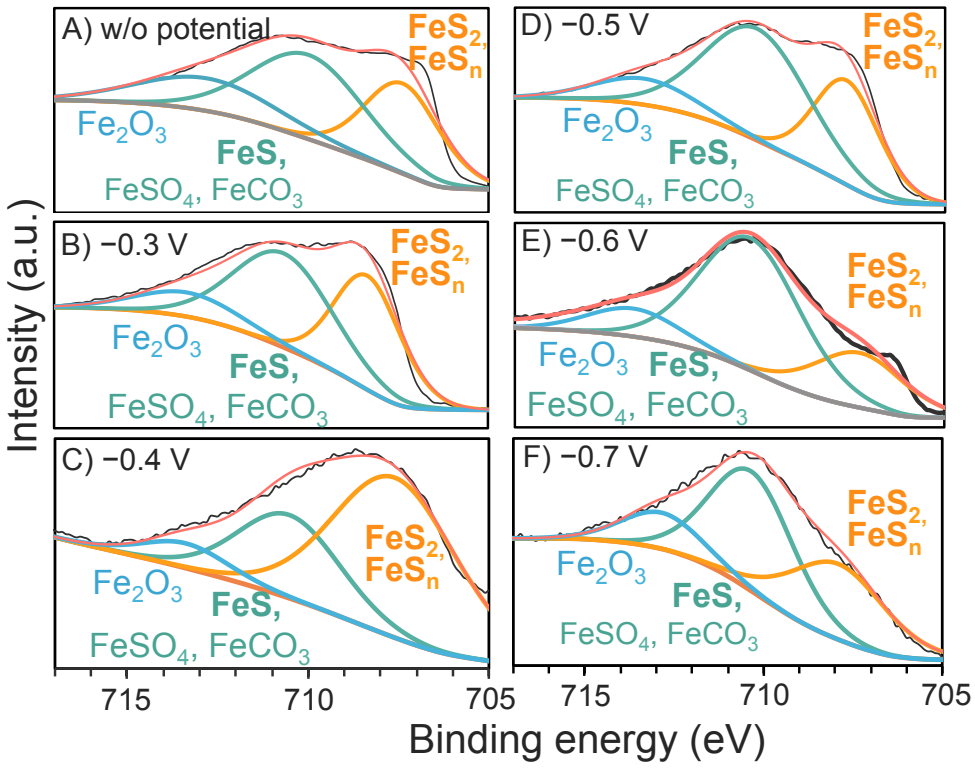

Figure S5. High-resolution Fe 2*p* core-level X-ray photoelectron spectroscopy spectra of iron sulfide on the surface of iron electrode without (A) and with potential poising at −0.3 V (B), −0.4 V (C), −0.5 V (D), −0.6 V (E), and −0.7 V (F).

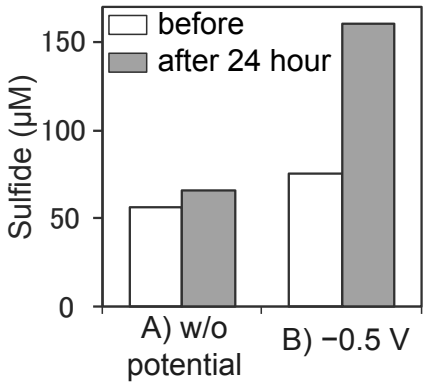

Figure S6. Change of sulfide concentration in two electrochemical reactors equipped with iron electrodes without and with potential poising at −0.5 V for 24 h.

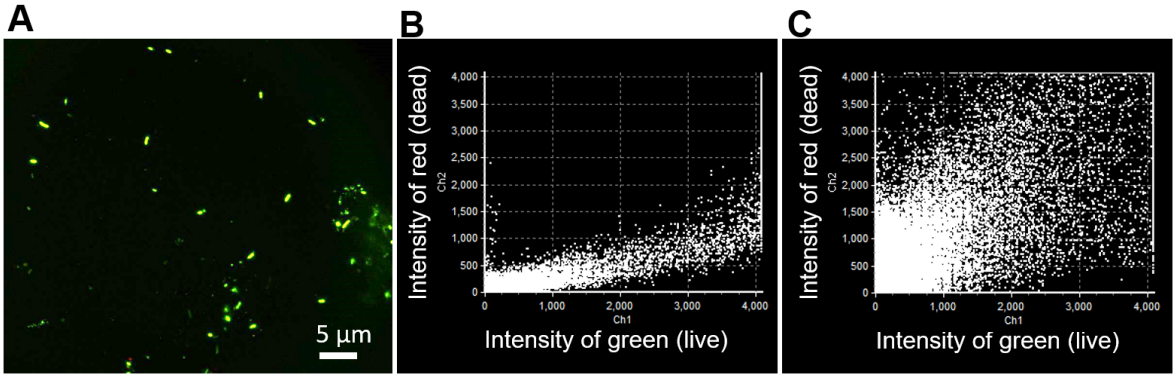

Figure S7. Live/dead assay of *D. vulgaris* cells on the surface of a carbon steel electrode after −0.5 V poising. (A) Fluorescent image of live (green fluorescent) and dead (red fluorescent) cells on the surface of carbon steel. (B) Distribution of red and green fluorescence intensities analyzed for panel A. (C) Distribution of red and green fluorescence intensities of a standard reference sample which contained 1:1 live:dead cell mixture.

Table S1. Number of up-regulated and down-regulated genes in 22 clusters of orthologous groups (COGs) in *D. vulgaris* cells under a corrosion suppression condition by −0.5 V poising versus the without (w/o) potential poising condition.

	COG functional category	−0.5 V vs. w/o potential, up	−0.5 V vs. w/o potential, down
i	Translation, ribosomal structure and biogenesis	2	10
ii	RNA processing and modification	0	0
iii	Transcription	0	11
iv	DNA replication, recombination and repair	1	2
v	Cell division and chromosome partitioning	0	2
vi	Defense mechanisms	0	2
vii	Posttranslational modification, protein turnover, chaperones	0	14
viii	Cell envelope biogenesis, outer membrane	0	8
ix	Cell motility and secretion	0	7
x	Signal transduction mechanisms	0	15
xi	Extracellular structures	0	0
xii	Intracellular trafficking, secretion, and vesicular transport	0	2
xiii	Energy production and conversion	1	17
xiv	Carbohydrate transport and metabolism	0	3
xv	Amino acid transport and metabolism	1	9
xvi	Nucleotide transport and metabolism	0	5
xvii	Coenzyme metabolism	1	11
xvii	Lipid metabolism	0	2
xix	Inorganic ion transport and metabolism	0	7
xx	Secondary metabolites biosynthesis, transport and catabolism	0	0
xxi	General function prediction only	3	15
xxii	Function unknown	3	9
	Overall	12	151

SI References

1. Deng, X.; Dohmae, N.; Kaksonen, A. H.; Okamoto, A., Biogenic iron sulfide nanoparticles to enable extracellular electron uptake in sulfate-reducing bacteria. *Angew. Chem. In. Ed.* **2020**, *59*, 5995-5999
2. OpenWetWare contributors, Ethanol precipitation of nucleic acids, https://openwetware.org/mediawiki/index.php?title=Ethanol_precipitation_of_nucleic_acids &oldid=611420 (Page Version ID: 611420)
3. Caffrey, S. M.; Park, H. S.; Been, J.; Gordon, P.; Sensen, C. W.; Voordouw, G., Gene expression by the sulfate-reducing bacterium *Desulfovibrio vulgaris* Hildenborough grown on an iron electrode under cathodic protection conditions. *Appl. Environ. Microbiol.* **2008,** *74*, (8), 2404-2413.