Supplementary Information for
Biocorrosion on nanofilms induces rapid bacterial motions via iron dissolution

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Supplementary Text

Corrosion rates and the suspected abiotic corrosion

As illustrated in Fig. 2B, the linear function equation that best fits the thinning data points – data issued from the optical signal measurements – allowed us to extract a reaction speed, i.e. the corrosion mean rate. Fig. S12 A displays the values of this rate as measured from S. oneidensis WT samples diluted at different initial optical densities \( OD_{initial} \), while nanofilms being not connected to any other metals or any electrical connections. The corrosion mean rate does not depend on \( OD_{initial} \) and, on average, is found equal to 11 +/- 3 nm/h.

In another manipulation (without electrical connection), we allowed the corrosion process to begin and then we processed to a sudden dilution of the sample by addition of liquid medium. We observed that bacterial dilution neither stopped corrosion nor changed the corrosion rate. In other words, once degradation occurs, its rate doesn’t depend on the bacteria concentration, suggesting a corrosion rate not dependent on the number of bacteria at the metal surface.

When nanofilms were connected in series to an ammeter and to a platinum counter-electrode half-immersed from the top of the liquid, we observed that nanofilms degrade much faster with electrical connection than without, as shown in Fig. S12 B. On average, the rate reaches a constant value equal to 17 +/- 3 nm/h. Therefore, the presence of a counter-electrode where only reduction took place increases the corrosion rate.

Interestingly, corrosion rates were measured when nanofilms’ corrosion was triggered by different mutants of S. oneidensis and by E. coli and L. plantarum (without electrical connection). Results, which are displayed in Fig. S12 C, indicate a rate independent of the mutants and similar for E. coli WT and S. oneidensis strains. Only the degradation speed measured on L. plantarum differs significantly from the others. These findings point to a reaction independent of the proteins’ deletion in the external electron transfers (EET), indicating a corrosion mechanism different from the direct iron-to-microbe electron transfer reported on Geobacter species where corrosion pit depth and density strongly depend on the presence of c-cytochromes.\(^1\) The only high rate measured here on L. plantarum is most probably related to the low pH (acidic) environment.

From all these findings, we suspect the corrosion phase on iron nanofilm as not being related to S. oneidensis – as electron donors to the metal or as electron acceptors from the metal as reported in recent studies (see for instance \(^2-5\) – but being purely electrochemically activated; bacteria would only enable the passivity breakdown during the initiation phase. That would explain the similar OCP values of the abiotic corrosion of bulk sheet and of the biocorrosion of nanofilms.

Separated compartments

Using a lab-made PTFE two-compartment cell, bacteria were physically separated from the nanofilm by a sterile semi-permeable membrane (12 kDa of pore size, a size sufficient to permeate small secreted molecules like siderophores and small metabolites). Initial conditions: one compartment was filled with a bacterial suspension (2 ml, initial \( OD_{initial} = 0.03 \)) and the other (the nanofilm compartment) by sterile liquid medium (about 4.5 ml). At each end of experiments, bacterial contamination in the nanofilm compartment was checked by Optical Density measurement of the separated liquid. Experiments were performed three times on S. oneidensis and E. coli WT and no corrosion was observed for S. oneidensis, unlike E. coli. E. coli enabled the corrosion transition at identical \( I_{corr} \) values using or not separated compartments.
**Iron nanofilm processing/preparation**

A thorough cleaning of substrates was critical to ensure a perfectly adherent and homogeneous 10-nanometer thick film. To clean glass coverslips (22 mm²), two protocols were used, both generating the same end result: 1) immersion into an acetone bath with one-minute sonication, then a rinse in isopropanol followed by a second one-minute sonication in an isopropanol bath. Samples were subsequently dried with compressed nitrogen. 2) We manually and actively rubbed the glass substrate with a cotton soaked in CaCO₃ dissolved in 3% RBS. Coverslips were then rinsed with distilled water and dried with compressed nitrogen.

To thermally evaporate titanium (Neyco, 99.99%) and iron (Kurt J. Lesker, 99.95%), we placed the glass substrates onto the sample holder of a Plassys evaporator which is located just above two separated tungsten ME5 Neyco crucibles each containing iron and titanium. A quartz balance provided an indication of coating thickness during deposition. The low deposition rate was adjusted within the range 0.08-0.12 nm/s. The decompression phase took place in a nitrogen atmosphere. Then the coverslips were stored in a clean plastic container together with wipes (Kimtech) and exposed to air for a minimum of one to two weeks before use. Delamination and millimeter failure were observed during the manipulation of a few glass coverslips coated less than two weeks before.

Titanium is one of the metals commonly deposited to facilitate other metal adhesion. Iron being deposited on the titanium layer, and being capable of forming stable TiFe or TiFe₂ alloys at equilibrium and hot temperature, we cannot exclude the possibility that some iron layers might mix with the titanium film during the deposit. However, measurements of the formation of such an alloy were undetectable by our optical setup. At the beginning of the degradation process, when the signals were accurately measured, kinetic curves and fitted corrosion rates were found to be similar with or without the Ti layer, excluding the presence of such an alloy that would most likely modify the reaction kinetics. However, at the end, when bacterial contribution to the optical signals became relatively high, the low relative precision in the determination of metal thinning might prevent the optical detection of small changes in the completion of metal degradation that would be attributed to the presence of this intermediate layer (TiFe alloy) or simply to the strong adhesion to Ti.

The total number of samples studied in this article is equal to 260, meaning 65 evaporations.

No unexpected or unusually high safety hazards were encountered.

**AFM measurements**

AFM measurements were conducted in liquid Luria Broth culture media at 23°C using the fast-speed approach/retract mode of the Nanowizard III (JPK Instruments AG, Berlin, Germany) placed on a vibration isolation table. We used CSC37 Cr-AU probes (MikroMasch, https://www.spmtips.com/afm-tip-hq-csc37-cr-au) with a nominal value of stiffness around 0.26 N/m, a value checked before each series of scans by measuring the thermal noise. The approach/retract curves were recorded at a constant speed of 100 µm/s on a vertical 500nm extension range, fixing the maximum applied force value to 5 nN. The surface scanning was
128x128 pixels and we applied a linear smoothing post-treatment to the height AFM images in order to remove the non-horizontality of the sample.

No unexpected or unusually high safety hazards were encountered.

XPS measurements

X-Ray Photoelectron Spectrometry (XPS) was achieved using a K-Alpha spectrometer (Thermofisher Scientific), equipped with a monochromated X-ray Source (Al Kα, 1486.6 eV) of 400 μm spot size. The hemispherical analyzer was operated in CAE (Constant Analyzer Energy) mode, with pass energy of 200 eV and a step of 1 eV for the acquisition of survey (wide-scan) spectra, and pass energy of 20 eV and a step of 0.1 eV for the acquisition of narrow spectra. A "dual beam" flood gun was used to neutralize the charge build-up. At each run, four metal-coated glass coverslips (cleaned with a cloth soaked in ethanol) were analyzed and for each sample, 4-5 survey spectra were first recorded at 5 different positions to map the chemical state of the surface and to check its homogeneity. High resolution spectra in the range of the Fe 2p signal were then repeated several times to check the nanofilms’ integrity under vacuum and under the X-ray beam illumination. The chemical state of all detected elements was then determined by fitting the spectra using the casaXPS software. A Shirley type background subtraction was used and the peak areas were normalized using the Scofield sensitivity factors. The peaks were analyzed using mixed Gaussian-Lorentzian curves (70% of Gaussian character) for the case of oxides and an asymmetrical line shape, type LA (1.2,4,8,3)⁸ for metallic Fe. Fitting the multiplet splitting⁹ was done by the software following the procedure given in ⁸, ¹⁰ and establishing area constraints and peak positions. The binding energies were calibrated against the C1s binding energy set at 284.8 eV.

No unexpected or unusually high safety hazards were encountered.

Optical macroscopic measurements

A macroscopic optical setup was mounted using a laser diode source (650 nm of wavelength, Melles Griot) and a photodiode receptor (First Sensor) at the terminals from which we recorded the voltage - high voltage meaning high light level. The diverging light beam emitted from an optical fiber was focalized on the nanofilm while keeping an illuminated area in the order of 1 cm², sufficiently large enough to be projected onto the whole photodiode surface. The vertical light propagated first into the liquid medium and then through the nanofilm coverslip (at the bottom of the sample) before being collected by the photodiode.

No unexpected or unusually high safety hazards were encountered.

Electrical measurements.

Electrical measurements were performed using a standard multimeter (Agilent 34410A, internal resistance of 10¹² Ω). The external wire was connected to the nanofilm through a platinum connector. Platinum and Ag/AgCl counter-electrodes were purchased from Goodfellow and from Pine Research (distributed by Equilabrium – France) respectively. Note: a negative electrical current was measured under our experimental conditions and only its absolute values are represented in this manuscript for clarity. Once complete nanofilm dissolution occurred (optically measured) and OCP measurements were recorded, we noticed the presence of a red species (most likely composed of iron oxides resulting from the nanofilm corrosion) on the porous plug of the Ag-AgCl electrodes altering the exchanges from both sides of the porous plug and incidentally
affecting the post-corrosion OCP values. Additionally, a contact area of the nanofilm with the connector remained present which contributed to the OCP signal as well.

No unexpected or unusually high safety hazards were encountered.

**Microscopic measurements of pH and oxygen (O\textsubscript{2}) concentration.**

Microsensors (Unisense, Denmark) were used to characterize the local environment of bacteria near the nanofilms. Air-saturated (O\textsubscript{2} = 262.78 \textmu mol/l) and Nitrogen-saturated (O\textsubscript{2} = 0 \textmu mol/l) waters were the two calibration points of the oxygen microsensor (Ox-10 model, outside tip dimension = 8-12 \textmu m) while three classical pH solutions (pH = 4, 7 and 10) were used to calibrate the pH sensor (pH-25 model, outside tip dimension = 20-30 \textmu m). Under an inverted optical microscope (Primovert, Zeiss) and using a manual micromanipulator (Märzhäuser Wetzlar, MM33 right), microelectrodes were set at 30-40 micrometers from the glass coverslips placed at the bottom of the Petri dishes. Spacing calibration was previously done thanks to a motorized linear micropositioning stage (M-126-CG1, PI Physik Instrumente).

No unexpected or unusually high safety hazards were encountered.

**Iron Reduction – Ferrozine assay**

After the dilution step (previously described), bacteria were allowed to grow up to \textit{OD}~ 0.3 and were then re-diluted to \textit{OD} = 0.1 into liquid LB supplemented with (7.8 +/- 0.7) mM of soluble iron(III) ammonium citrate (Sigma-Aldrich). Aliquots of 2 ml were prepared and were stored static (without agitation), at room temperature (23°C). After the eppendorf tubes were completely filled and closed, bacteria generated anaerobic conditions within less than 15 minutes. To keep the same conditions for every sample, we did one aliquot per measurement. At each point, bacterial growth was controlled by measuring the \textit{OD}. Then, the aliquot was centrifuged at 12,000 g for 5 min. 100 \mu l of the supernatant was collected and diluted into 0.5 M HCl. Fe(II) and Fe(III) concentrations were determined using ferrozine assay as described in \textsuperscript{11} and calibrated with FeCl\textsubscript{2}. Note that, instead of a microplate reader, a spectrophotometer (GENESYS™ 40/50 visible / UV-visible) was used to read absorbance (optical densities).

No unexpected or unusually high safety hazards were encountered.
Supplementary Figures

Figure S1. Some physical properties of iron nanofilms. (A) Optical Density of nanofilms as a function of their thickness, iron being thermally evaporated on different glass coverslips without titanium adhesion prelayer. Optical density was directly measured using a commercial spectrophotometer (Genesys 50, Thermoscientific) at a wavelength of 633nm and without liquid. When considering the best linear fit (blue line), the slope provides an experimental value of the absorption coefficient equal to $\alpha = (9.7 \pm 0.4) \times 10^5 \text{ cm}^{-1}$. This value is close to the optical constant reported in the literature: $6.1 \times 10^5 \text{ cm}^{-1}$.\textsuperscript{12-13} Note that surprisingly no change in slope is observed when the iron thickness values reach a few nanometers, which represent the typical thickness of iron oxide overlayers. In this case, the absorption coefficient is expected to be at least ten times smaller than the Fe(0) value, for example $\alpha = 29202 \text{ cm}^{-1}$ for the Fe$_3$O$_4$ magnetite.\textsuperscript{14} (B) Dependence of the electrical resistivity over the iron nanofilm thickness. Electrical resistivity of iron nanofilms was monitored by a four-point electrical setup without liquid. Four point electrodes were aligned and separated by two millimeters from each other. Current was applied through outer probes and voltage was measured across two inner probes. Resistivity data (pink-filled circles) were deduced from sheet resistance values applying corrective factors 0.943 due to geometry and 4.53 due to ultrathin thickness as described in \textsuperscript{15}. The blue curve corresponds to the Fuchs-Sondheimer function that best fits the experimental data;\textsuperscript{16} three parameters ($mfp$, $\rho$, $p$) were adjusted. The model provides a mean free path of $mfp = (2.1\pm 0.3) \text{ nm}$, which is consistent with the value calculated from the Drude model equal to 3 nm. The value of bulk resistivity extracted...
from the fit was equal to $\rho = 0.20 \pm 0.01 \mu\Omega.m$ which corresponds to two times the value expected for bulk Fe(0): 0.10 $\mu\Omega.m$. A similar difference was already observed in ultrathin films. The negative fitted value of the last parameter $p$ ($p = -3.8$) corresponding to the fraction of electron specularly scattered from the surface, was thought to be due to the presence of oxygen atoms and of an oxide-metal rugose interface. The irregular interface was thought to be responsible for the electrons scattering. (C) Mapping of nanofilm surface detected by Atomic Force Microscopy where isotropic irregularities and grainy texture were observed. Figure S1 C represents a typical reconstructed image of the scanned surface height (128x128 pixels) in which brightness of each pixel varies with height according to the height scale bar. At each pixel, height is extracted from an approach/retract curve – that is, force-distance curve –, height corresponding to the contact point position. Similar topography images with disordered small irregularities were obtained at different locations on a nanofilm. (D) Profile of the surface height along the white dashed line on Fig. S1 C displays nanofilm roughness. Figure S1 D illustrates a height profile along one axis – along the x dashed white line in Fig. S1 C – and reveals a height dispersion smaller than 0.6 nm over the micrometer horizontal distance; when averaged over the whole image scanned surface, the root mean square roughness equals to 0.4 nm, a value equivalent to the size of few atoms for an image area of 1 x 1 micrometers$^2$. These values are typical of those obtained from metals thermally deposited at low evaporation rates (around 0.1 nm/s). Nanometric roughness and homogeneity of nanofilms could prevent pinning of corrosion pits as pitting initiation was found strongly dependent on the metal surface condition and usually related to chemical and physical surface inhomogeneities or defects.
Figure S2. Spectra of X-ray Photoelectron Spectrometry (XPS). Under X-ray irradiation, core electrons are ejected from these surface atoms, captured, and counted according to their energy. (A) Wide-scan spectra of different samples indicated the presence of oxygen (50-70%), iron (20-35%) and carbon (15-25%). Identical results were obtained over different parts of the nanofilms surface demonstrating their chemical uniformity. The presence of carbon elements is attributed to surface contamination as commonly reported for most samples exposed to the atmosphere.26 (B) Figure S2 B shows further detailed analyses of the iron region Fe2p revealing the presence of Fe(0) (10-20%), FeII (25-30%) and FeIII (50-60%). It clearly suggests that the surface iron overlayer is mainly composed of different oxides/hydroxides/oxyhydroxides, a result confirmed by the presence of oxygen. Note that the oxide grows and covers the surface quite rapidly when exposed to air, being detected on samples evaporated 4 hours before the XPS analysis. We also noticed the fragility of these freshly evaporated samples because they are subject to millimetric hole formation during their manipulation. In contrast, nanofilm samples that were left alone for more than a week after evaporation are mechanically stable. XPS analysis indicates a reduced fraction of Fe(0) compared to the other Fe states, suggesting the existence of a slow oxide growth. (C) Finally, ion etching enabled us to determine the chemical composition of the inner layers. As illustrated in Fig. S2 C, underneath the iron oxide and carbon layer appears a single state, the pure Fe(0) metal. These results are in agreement with 27,28 in which similar nanofilms were studied. Oxidized iron nanolayers overlaying the Fe(0) metal were found non-crystalline and about 3 nm thick. Interestingly, though having a different chemical composition, a similar thin oxidized overlayer (1-3 nm) protects stainless steels from corrosion and is considered to be passive and responsible for their outstanding corrosion resistance.29 Pure iron metal, however, exhibits a passive oxidized layer that is not stable enough to maintain the passivation state for prolonged time periods.30
Figure S3. Temporal degradation of a nanofilm in the presence of bacteria (red-filled circles), as observed by optical microscopy. Initial $OD = 0.07$. Figure S3 displays a time variation of the mean grey value (per pixel) together with two images recorded at the beginning and at the end of the experiment. The first dark image contrasts to the last bright image, which reveals without ambiguity the nanofilm corrosion in between, hence validating our approach. Under these experimental illumination and detection conditions, the mean grey value increases from 80 up to 235, a value close to 255 (Black = 0 and white = 255 pixel values of an 8 bit grayscale image). The nonmonotonic increase suggests the existence of a sudden corrosion onset. Moreover, images of intermediate brightness (not shown) present uniform backgrounds indicative of a uniform and homogeneous corrosion. Corrosion above the micron scale would imply localized brightness surrounded by dark areas and consequently, a standard deviation much higher than the low standard deviations represented by the error bars in Figure S3. The standard deviation only increases from 4 to 12 grey levels as it does in samples where bacteria grew without metal deposit, suggesting an increase correlated to the growing number of bacteria (dark features) rather than to degradation heterogeneity. Without bacteria (blue-filled circles), the mean grey value remains constant, meaning no degradation.
Figure S4. Open Circuit Potential measured during the nanofilm degradation when connecting the iron nanofilm to a voltmeter and to a Ag/AgCl reference electrode (as a counter-electrode). A sudden drop from -200 mV to -600 mV appeared at the beginning of the corrosion process (red-filled circles). Initial $OD = 0.03$. Other constant electrical signals were measured on an Fe nanofilm immersed into the abiotic liquid medium without bacteria (blue-filled circles). All the electrical measurements were done on iron nanofilms evaporated directly on glass coverslips (without Ti layer).
Figure S5. Electrical signals measured during the bulk iron degradation and using a Ag/AgCl counter-electrode. Two bulk iron sheets (0.1 mm-thick, GoodFellow, Iron 99.5%) were each immersed into two separate abiotic LB media at time $t = 0$ without bacteria. The temporal variation of the OCP (Open-Circuit Potential) measured on the first sample is shown in (A-B). Horizontal time axes are expressed in second (A) and minute (B) units to display the short and long-time variations. Two kinetics were observed: the OCP values initially dropped to $-300$ mV when connecting the electrical circuit to the iron sheet (contact) (A) and then decreased to $-642$ mV after one or two minutes (A-B). (C) The electric current resulting from the bulk iron degradation of the second sheet was measured over a period of three days. The absolute values, displayed in Fig S5 C, are of the order of $15 \mu$A. Data suggest a rapid instability of the oxide covering the bulk metal and a corrosion starting quasi-immediately after immersion.
Figure S6. Temporal variations of bacterial surface density (per unit area) as functions of time measured by processing the digital images recorded by optical microscopy on eleven samples. Images were treated and analyzed using Python programs. Bacteria were extracted from the background by searching features of variable pixel intensity and of given size or area. Among the different applied methods, all rendering the same results, the segmentation and binarization techniques described in allowed us to unambiguously determine the area covered by bacteria. Densities $\sigma$ (red-filled circles) were calculated by counting the total surface areas occupied by bacteria per unit area. For each sample, corrosion onset $t_{\text{onset}}$, determined by analyzing simultaneously variations of the mean grey values, is marked by a semi-transparent red disk. On each plot, all the coordinate points $(t_{\text{onset}}, \sigma_{\text{onset}})$ determined from imaging of all samples are also marked by the black-filled circles. Three plots (4., 5., 6.) display data obtained from the iron nanofilms directly evaporated on glass without an adhesive Titanium layer. A titanium layer was deposited under the iron layer of the other samples. Plot (2.), initial $OD = 0.03$; the others, $OD = 0.07$. 
Figure S7. Cells’ dynamics and sessility analysis. (A) Visualization of cells or any immobile part for long periods of time. The first image on the left side was obtained by processing the image recorded at an initial moment, noted $t=0$ sec. The first and second images correspond to the first and last images of a 50-picture sequence lasting 2.2 seconds. In the first, bacteria detected by the program appear white-colored and simply overlaid onto the initial image. As can be seen, all cells have been detected. The second was reconstructed differently: only pixel sites occupied by the same immobile bacteria within the 50 previous images appear white-colored. Once a pixel grayscale level changed from one picture to another (due to bacterial motion) its grayscale level was converted to a zero background level. By comparing the pixel values from one image to the next in the sequence and annealing successively their level, the result (second image) contains only white-colored pixel sites in which no motion was detected during the entire sequence analysis; the rest of pixels were considered as background and transparent. Therefore, the binary overlaying image only enhances the contrast of cells or any part that remained immobile during the 50 previous images i.e. during 2.2 seconds. Similar enhancement was applied to three other images recorded at 5 min, 15 min and 1 hour after the initial moment. (B) Quantitative analysis of bacterial immobility based on a localization function. To quantify temporal distribution of displacements that extended from milliseconds to hours, we analyzed how bacteria moved from initial sites or equivalently how bacteria or part of bacteria continued to reside on these initial sites. The relative total area of initial sites that were still occupied at any time $t$ after the initial moment ($t_{\text{initial}}=0$) is termed the localization function; the function equals 1 or 0 when initial sites are all still occupied (no motion at all) or abandoned, respectively. For practical purposes, we restricted our analysis to the first seconds that followed the initial moment. The functions analyzed on the two samples of Fig. 4B are plotted here versus time. The reference sample where bacteria resided on a glass substrate is considered on the left-hand graph (green-filled circles). The different curves in this plot were calculated starting from different initial states i.e. from states recorded at different incubation times. Thus the upper curve describes the situation in which bacteria were initially localized on the surface 10 minutes after bacterial inoculation. Progressively, the function decreases at varied characteristic times attributed to multiple bacterial motions. Within 150 ms, an initial rapid decrease from 1 to 0.8 was recorded, followed by a slowing decay between 0.8 and 0.5. By decomposing the motions observed on the images’ sequence, we attribute the first decrease to local fast runs of bacteria swimming in the liquid near the glass surface within the 20 μm depth of observation field; we deduced a 20%
fraction of running bacteria. The second part of the curve deals therefore with non-running cells, the cells that seemed immobile long-term. Two processes contribute to the decreasing function: 1) a progressive thinning of each localized site area induced by local small displacements and attributed to constrained thermal motions on the surface 2) sudden extinctions of large areas due to large motions of bacteria like removal/detachment or large reorientation. The function reached the 0.5 value after only a few seconds; this being the largest value observed when analyzing localization persistence at different incubation times, meaning the earliest surface bacteria remained localized and motionless for the longest period of time. Surprisingly, after 1 hour 20 minutes (1 h 20) of incubation, the curves superimposed and it became difficult to separate the contribution of each kind of motion to the ratio. Bacteria became more concentrated at long incubation times and subject to thermal motions. The other graph on the right-hand side (red-filled circles) displays the temporal variation function when bacteria were in contact with a metal nanofilm. The different initial states are indicated according to their temporal proximity to the corrosion onset – not to be confused with the incubation time. During the initiation phase (negative time), the curves were similar to the previous recorded on samples without metal, pointing to the absence of any specific interactions or affinities with the substrate that would have modified bacteria localization and dynamics during this phase. Similarly, after the corrosion process (more than one hour after the onset), the curve converged with the curve recorded before the onset i.e. to a hypothetical unique curve as previously noted. The only differences clearly observed were in the first minutes and ten minutes just after the corrosion onset. The function rapidly decreased and reached about 0.8 and 0.2, just 0.1 and 1 second after the initial state, respectively. Therefore, as seen in Movie S1, each bacterium was highly dynamic and all local motions were rapid; no cells were motionless meaning a complete and immediate delocalization. Their instantaneous velocities are discussed in the main text.
Figure S8. Kinetics of oxygen (O$_2$) depletion measured using a microelectrode on four samples: the medium itself without bacteria and three bacterial cultures of different initial concentrations ($OD_{initial} = 0.003$, 0.03 and 0.58). As shown in the insert (scale bar = 20 $\mu$m), the microelectrode was lowered to the bacteria located at the sample bottom (at 20-50 micrometers from the surface). Bacteria consumed dissolved oxygen (O$_2$) and generated an anaerobic environment after a certain time period.$^{34}$ The different times, $t_{anaero}$ marked by green arrows indicate when oxygen was locally fully-depleted.
Figure S9. Delay in the corrosion onset upon addition of soluble Fe(III). Supplementing the medium with Fe(III) ammonium citrate strongly delayed the corrosion event ($t_{\text{onset}}$, red-filled circles, right vertical axis) without perturbing the bacterial $O_2$ consumption ($t_{\text{anaero}}$, green-filled circles, left vertical axis). The initial $OD$ value ($= 0.03$) was kept constant. Dashed lines represent linear (red) and constant (green) fits.
Figure S10. Kinetics of iron(III) reduction. Thanks to ferrozine assay, iron (II) concentrations in samples from three different bacterial species were measured at incubation times ranging from 6 to 100 h. Each sample initially contained (7.8 +/- 0.7) millimolars of iron(III) citrate with initial OD = 0.10. The data sets of S. oneidensis MR-1 and its three mutants (previously described) were plotted in the first row (green-filled circles). For each strain, the reduction rate $k_{\text{reduc}}$ was extracted from the slope of the best affine fit (green line), leading to: (0.68 +/- 0.01) mM/h, S. oneidensis WT; (0.041 +/- 0.001) mM/h, ΔmtrA/ΔmtrD; (0.064 +/- 0.001) mM/h, ΔmtrC/ΔomcA/ΔmtrF; (0.89 +/- 0.02) mM/h, ΔmxD. These values are in agreement with those reported in the literature. Iron reduction was related to the lifestyle of Shewanella. In the second row, plots render measurements performed on E. coli and L. plantarum samples (grey-filled circles) with the best affine fits (grey lines) leading to $k_{\text{reduc}}$ (0.011 +/- 0.001) mM/h, E. coli and (0.0098 +/- 0.0004) mM/h, L. plantarum. Similar iron (II) concentration measurements were done on sterile culture media as controls and are plotted in the third row (black filled circle). LB culture medium was used to culture S. oneidensis and E. coli, and MRS was used to culture L. plantarum, both being set at nearly neutral pH (6 – 7.5). As L. plantarum acidified its environment, a control of iron III reduction under acidic conditions (pH=4.15) was also performed. Here, residual iron II molecules (0.05-0.1-0.2 mM) were found over large periods of time.
Figure S11. Temporal variations of local pH as measured using a microelectrode positioned at 20-50 µm from the bottom glass coverslip surface, a situation similar to the previous case (local oxygen levels on Figure S6). Five plots are presented, three data series recorded from three different bacterial species, *S. oneidensis* (green-filled circles), *E. coli* and *L. plantarum* (gray-filled circles) and two from two different culture media without bacteria (black-filled circles). The latter pH values remain constant over time with values around 6.5 +/- 0.05 for LB and 6.35 +/- 0.02 for MRS. A constant value was also observed for *E. coli* samples with a mean pH value of 6.37 +/- 0.7. Furthermore, *S. oneidensis* samples exhibited a slow pH increase from 6.35 to 6.81 over a 6-hour incubation period with a mean value of 6.54+/-.07. Conversely, a clear decrease was measured on *L. plantarum* samples from 6.65 to 4.12 over 21 hours most likely due to its fermentative metabolism which tends to acidify the culture media. The initial OD was 0.03 for each bacterial samples.
Figure S12. Corrosion rates. Kinetics of nanofilm thinning were adjusted by linear decreasing functions in order to obtain corrosion rate values (straight line in Fig. 2B). (A) S. oneidensis WT. A log-lin plot showing corrosion rate values independent of initial bacterial concentration or of optical density $OD_{\text{initial}}$. On average, the rate was equal to 11 $+$/− 3 nm/h (Mean and Standard Deviation illustrated by the horizontal-colored line and the color-filled rectangle respectively). (B) S. oneidensis WT. Corrosion rate vs. Initial OD. Connection of the iron nanofilm to a Platinum counter-electrode via an external electrical circuit induces faster thinning than without electrical connection. On average, the rate became equal to 17 $+$/− 3 nm/h. (C) Corrosion rate values were extracted from kinetics measurements of the three mutants of S. oneidensis (ΔmtrA/ΔmtrD, mtrC/ΔomcA/ΔmtrF and ΔmxD, red-filled circles) and of the two bacterial species (E. coli and L. plantarum, gray-filled circles). S. oneidensis, as noted alone, refers to the WT strain. The initial $OD$ was set to 0.03 for each bacterial sample.
Legend for Movie S1 (separate file). A one-looped and slow motion movie showing six sequences of 50 images recorded before and during the corrosion of the same sample (t\text{onset} = 1 \text{ h 40}). Microscopic observations of the nanofilm surface were performed within the 20 µm depth observation field. Brightness of the grayscale images was kept constant by image processing (thresholding and renormalization)\textsuperscript{31} in order to facilitate the bacteria observation. In this sample, bacterial influx, quantified by non-monotonic variation of the surface density illustrated in the right plot Fig. 3B, was clearly seen less than ten minutes after the corrosion onset. As observed on all samples, bacteria exhibited rapid motions after t\text{onset}, likely due to iron release. (Image scale given in Fig. 3A). Initial $OD = 0.07$. 

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