

Electronic Supplementary Information

Cisplatin-induced elongation of *Shewanella oneidensis* MR-1 cells improves microbe-electrode interactions for use in microbial fuel cells†

Sunil A. Patil,* Kamil Górecki, Cecilia Hägerhäll and Lo Gorton*

1. Results

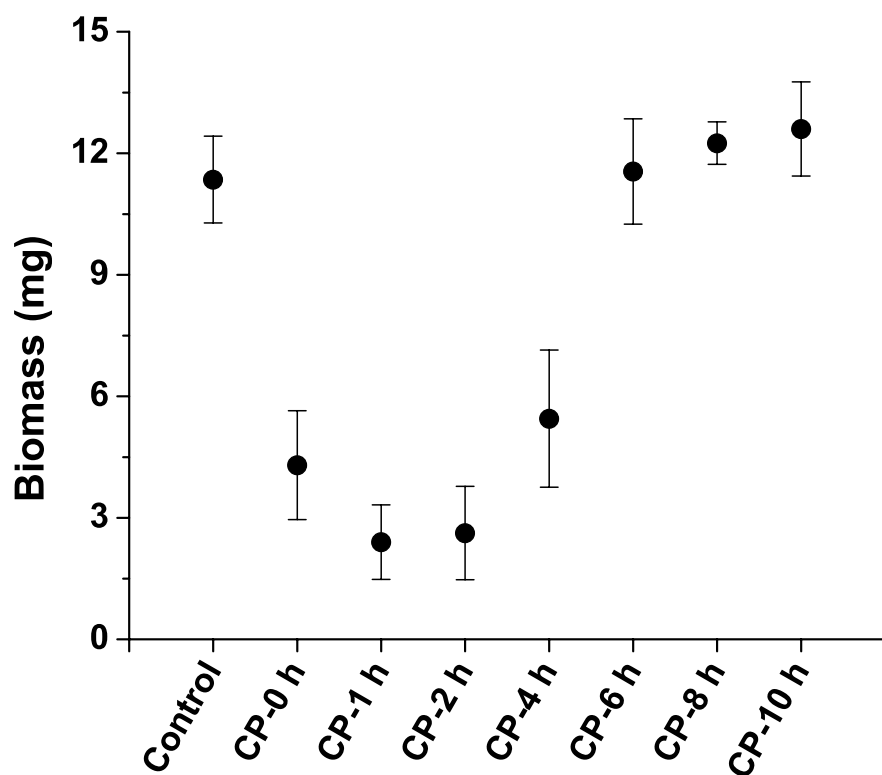


Fig. S1 The biomass of *S. oneidensis* MR-1 (estimated as dry weight of the cells after 24 h of incubation) obtained from 15 mL of LB medium containing 10 ppm cisplatin (CP). The cisplatin was added to the LB medium at different time intervals (starting from 0 h up to 10 h) during incubation. Control represents the biomass of the normal cells (grown without cisplatin).

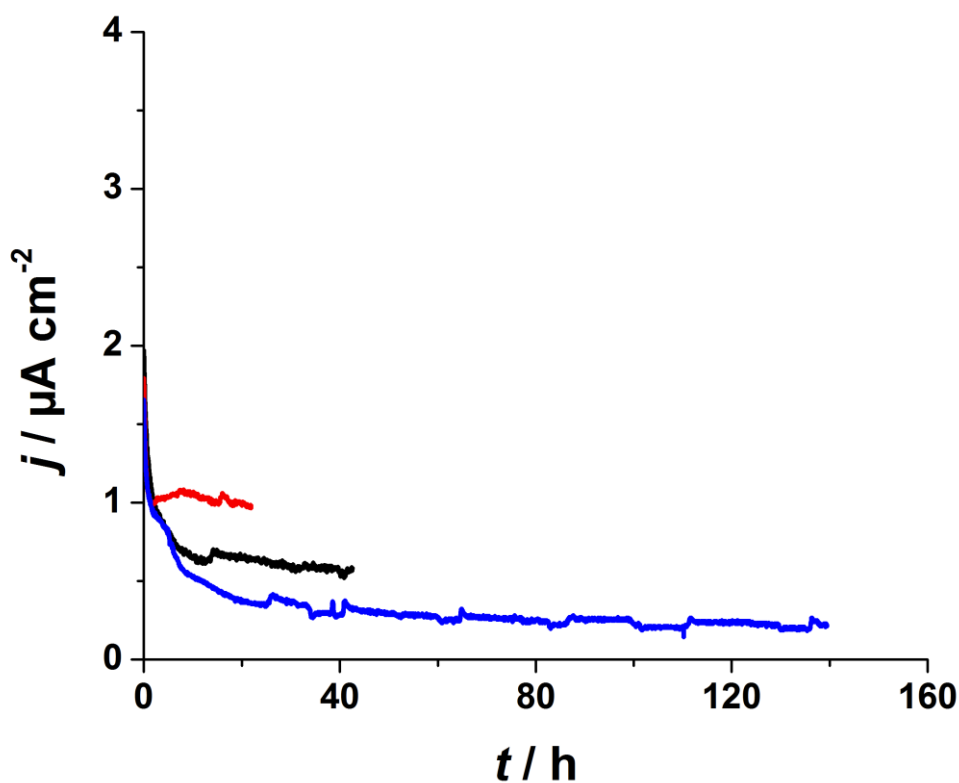


Fig. S2 Potentiostatically-controlled chronoamperometric measurements with the bioelectrochemical set-ups hosting minimal medium with 10 ppm cisplatin (added directly to the set-up) at an applied potential of 0.2 V (vs. Ag|AgCl). Data is shown for three replicates.

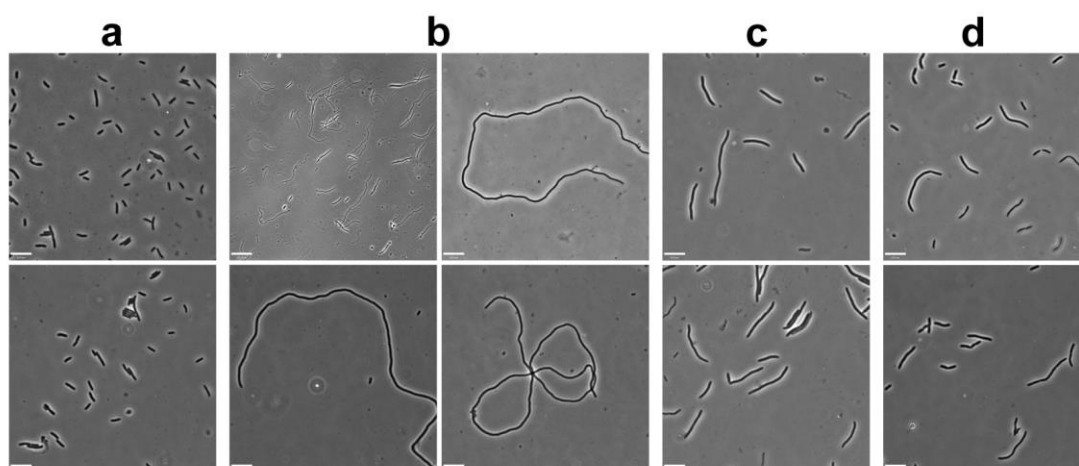


Fig. S3 Phase contrast microscopy images of untreated (a) and cisplatin-treated (b, c and d for CP-0 h, CP-4 h and CP-8 h respectively) *S. oneidensis* MR-1 cells (scale bar: 8 μ m, if not mentioned otherwise) from the spent medium after

chronoamperometric measurements. The scale bar for first image in panel b is 100 μm . Please refer fig. S4 provided below for a clear view of this image.

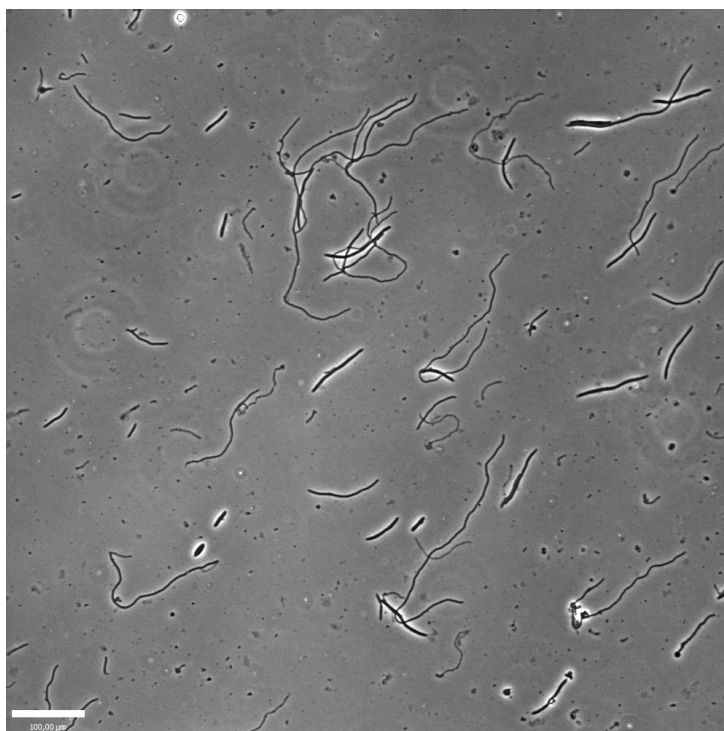


Fig. S4 Phase contrast microscopy image of cisplatin treated (CP-0 h) *S. oneidensis* MR-1 cells (Scale bar: 100 μm)

2. Materials and methods

2.1 Microbial strain and inoculum preparation

Shewanella oneidensis MR-1 (LMG 19005/ATCC 700550) was obtained from Belgian Coordinated Collections of Microorganisms/Laboratorium voor Microbiologie (BCCMTM/LMG, Ghent University, Belgium). It was grown and maintained on tryptone soya agar as recommended by the provider. A well-isolated microbial colony from tryptone soya agar was inoculated in 15 mL of Luria-Bertani (LB) broth (in 50 mL tubes) and incubated aerobically at 30 ± 2 °C, while shaking at 150 rpm (Orbital incubator shaker (GyromaxTM, Amerex Instruments Inc. Lafayette, CA) for 24 h during inoculum preparation. The cell mass grown in LB medium was then harvested by centrifuging the broth at 4000 rpm for 15 min. Subsequently, the cell mass was washed in 50 mM PIPES (piperazine-N,N'-bis(2-ethanesulfonic acid)) buffer (pH 7.4) again centrifuging as before and was used for microscopic and other analyses. The cell mass obtained by the same procedure was also used as the

inoculum in the bioelectrochemical reactor hosting minimal (M1) growth medium (see composition in section 2.3). The procedure described above was followed throughout to maintain the consistency in microbial inoculum preparation with and without cis-platin for start-up as well as for subsequent medium replenishments in bioelectrochemical experiments.

2.2 Cisplatin experiments

In order to find suitable conditions for cisplatin-modification of *S. oneidensis* a series of preliminary experiments were conducted with different cisplatin concentrations ranging from 1 ppm up to 500 ppm. After inoculation of the LB medium with the microbial culture, different time points during incubation (0 h, 4 h and 8 h) were selected for the addition of the drug to find out an optimum concentration that does not inhibit growth of biomass but that induces cell elongation in nutrient rich medium. On the basis of microscopic observations (for cell elongation) and dry biomass estimations (for cell growth inhibition) after incubation for 24 h, cisplatin at a concentration of 10 ppm was chosen for subsequent studies. Addition of 10-ppm cisplatin at different time intervals (starting from 0 h up to 10 h) during incubation of the LB medium containing the microbial inoculum was subsequently executed. After 24 h of incubation, the growth inhibition (estimated as dry weight of biomass) and the cell elongation effect (by phase contrast microscopy) were analyzed as mentioned above. Similar analyses were also extended to untreated cell growth (without cisplatin addition) that acted as controls. The cisplatin addition at initial stages of growth, in particular at 0 h, 1 h and 2 h, severely affected the cell growth (Fig. S1). However, the biomass and cell appearance after cisplatin addition at and beyond 6 h was in the similar range as that of the untreated control cells without any addition indicating that addition of cisplatin after 6 h does not affect the onward cell growth. However, addition of cisplatin at initial stages of incubation (up to 2 h) resulted in a maximum elongation of the cells in comparison to its addition at later stages (after 6 h). On the basis of these observations, microbial inocula obtained with cisplatin addition at 0 h, 4 h and 8 h along with untreated cells without any additions were chosen for bioelectrochemical experiments and other analyses. The biomass data and microscopic observations are based on at least four independent replicates.

2.3 Bioelectrochemical set-up and experiments

The potential values specified in this article refer to the Ag|AgCl (sat. KCl) reference electrode. All bioelectrochemical experiments were conducted under strictly anoxic conditions under potentiostatic control (PalmSens, Palm Instruments BV, Utrecht, The Netherlands). These experiments were carried out using a conventional three-electrode arrangement. The working electrode was a graphite rod (type RW I, Ringsdorff Werke GmbH, Bonn, Germany). Similar graphite rods were employed as counter electrodes, whereas the reference electrode was an Ag|AgCl electrode (sat. KCl, Sortortechnik Meinsberg, Germany). Water-jacketed glass vessels (capacity: 100 mL) connected to a thermostat (RM6, Lauda Dr. R. Wobser GMBH & Co. KG Germany) that allowed the maintenance of the optimum growth temperature ($30 \pm 2^\circ\text{C}$) acted as electrochemical cells. These electrochemical set-ups hosted the microbial inoculum and 20 mL of M1 medium (composed of PIPES buffer 15.1 g/L; NaOH 3.0 g/L; NH_4Cl 1.5 g/L; KCl 0.1 g/L; $\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$ 0.6 g/L; NaCl 5.8 g/L; mineral solution 10 mL/L¹; vitamin solution¹ 10 mL/L and amino acid solution² 10 mL/L). Sodium lactate (18 mM) served as the carbon source. Before use, the reactor and growth medium were purged with nitrogen for at least 25-30 min to ensure anaerobic conditions.

The bioelectrocatalytic current generation with untreated cells and cisplatin-treated cells was investigated using semi-batch chronoamperometry (CA) experiments at an applied potential of +0.2 V with regular medium replenishments. The substrate-containing medium was continuously stirred during all CA experiments. The maximum current from each semi-batch experiment was considered for calculating current densities with respect to the projected surface area of the electrodes. All reported electrochemical data are based on at least three independent replicates of both untreated and cisplatin-treated cells. Cyclic voltammetry (CV) was applied during substrate turnover and substrate non-turnover conditions to get insights into the electron transfer mechanisms by these cells.

2.5 Microscopy

Phase contrast microscopy

The morphology of the *S. oneidensis* MR-1 cells grown in LB medium with different cis-platin concentrations (during initial screening experiments) was observed with a Zeiss AxioImager.Z1 phase contrast microscope (Carl Zeiss Microscopy GmbH, Göttingen, Germany). The cells grown in LB medium were directly used for these

observations. The same microscope was also used to observe the *S. oneidensis* MR-1 cells from the used M1 medium from bioelectrochemical reactors after CA experiments with untreated as well as cisplatin-treated cells.

Scanning electron microscopy (SEM)

SEM (JEOL JSM-5600LV, Tokyo, Japan) was used to observe the morphology of *S. oneidensis* MR-1 cells. The cells were grown according to the protocol explained in the section inoculum preparation. For SEM analysis, the samples were prepared according to the following protocol. Firstly, the cell mass was suspended in the same buffer. Then, 200 μL of the cell suspension was passed through the black isopore membrane filters (0.2 μm , GTPB) (Millipore Ireland B.V., County Cork, Ireland) fixed in the syringe filter holders (13 mm) (Sartorius AG, Göttingen, Germany). Subsequently, these filters with cells were placed in a 2.5% glutaraldehyde solution (prepared in a 0.1 M sodium cacodylate buffer, pH 7.2) and stored overnight at 4 °C for fixation. After washing them with the same buffer, these samples were then dehydrated by using a graded ethanol series (once with 50%, 70% and 96%, and thrice with 100% for 10 min each step) with a gentle periodic agitation. Before SEM observation, all samples were critical point dried using a Bal-Tec CPD-030 Critical Point Dryer (Bal-Tec AG, Balzers, Liechtenstein) and sputter coated with Au-Pd using a SC7640 Sputter Coater (Polaron, Newhaven, UK).

SEM analysis was also used to observe the electrodes after CA experiments with all above-mentioned microbial inocula and thus to check the biofilm formation as well as to analyze their growth pattern on the electrodes. A similar protocol as explained above, starting from fixation to sputter coating was applied for these sample preparations.

2.6 Riboflavin estimation

The procedure explained by Roy et al.³ was used for estimating the amount of riboflavin produced by untreated and cisplatin-treated *S. oneidensis* MR-1 cells in the CA experiments. The spent M1 medium samples from the bioelectrochemical reactors during medium replenishments were collected and clarified by filtration (0.2 μm). A standard curve for riboflavin (in the range of 0 nM to 2.4 μM) was obtained by exciting standard solutions and measuring the fluorescence at 520 nm using a spectrofluorometer (Fluoromax-2, Horiba Scientific, New Jersey, USA). The

concentration of riboflavin in the filtrates from the different samples was estimated by determining the correlation between the observed fluorescence at a similar wavelength to the standard curve correlating the observed fluorescence at a similar wavelength to the standard curve.

2.7 DNA and protein content

S. oneidensis MR-1 cells obtained without and with cisplatin were suspended in 2.5 ml of 50 mM MOPS (3-(N-morpholino)propanesulfonic acid) buffer (pH 7.4) and treated with 1% sodium dodecyl sulfate (SDS) for 1 h in 37°C to dissolve the membranes. The DNA content was estimated by using DNA stain dye Hoechst 34580 (Invitrogen Ltd., Paisley, UK). Binding of the dye to the minor grooves changes its fluorescence maximum from 510 nm to 440 nm. 0.1 µg/ml dye was dissolved in 20 mM bis-tris-propane-HCl buffer containing 2 M NaCl. 2 µl of the samples were mixed with 2.8 ml of the dye solution and the fluorescence intensities were compared to the standard curve (*E. coli* JM109 chromosomal DNA of known concentration was used as standard). The total protein content was measured by BCA (bicinchoninic acid) assay (bovine serum albumin was used as standard).

3. References

1. R. M. Atlas, *Handbook of Microbiological Media, 3rd edition*, CRC Press LLC, New York, 2004.
2. O. Bretschger, A. Obraztsova, C. A. Sturm, I. S. Chang, Y. A. Gorby, S. B. Reed, D. E. Culley, C. L. Reardon, S. Barua, M. F. Romine, J. Zhou, A. S. Beliaev, R. Bouhenni, D. Saffarini, F. Mansfeld, B.-H. Kim, J. K. Fredrickson and K. H. Neilson, *Appl. Environ. Microbiol.*, 2007, **73**, 7003-7012.
3. J. N. Roy, H. R. Luckarift, C. Lau, A. Falase, K. E. Garcia, L. K. Ista, P. Chellamuthu, R. P. Ramasamy, V. Gadhamshetty, G. Wanger, Y. A. Gorby, K. H. Neilson, O. Bretschger, G. R. Johnson and P. Atanassov, *RSC Advances*, 2012, **2**, 10020-10027.

