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Enhanced performance of hexavalent chromium reducing cathodes in the presence of *Shewanella oneidensis* MR-1 and lactate

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Biocathodes for the reduction of the highly toxic hexavalent chromium (Cr(VI)) were investigated using *Shewanella oneidensis* MR-1 (MR-1) as a bio-catalyst and performance was assessed in terms of current production and Cr(VI) reduction. Potentiostatically controlled experiments (-500 mV vs. Ag/AgCl) showed that a mediatorless MR-1 biocathode started up

under aerated conditions in the presence of lactate, received 5.5 and 1.7 times more electrons for Cr(VI) reduction over a 4-hour operating period than controls without lactate and with lactate but without MR-1, respectively. Cr(VI) reduction was also enhanced, with a decrease in concentration over the 4-h operating period of 9 mg/L Cr(VI), compared to only 1 and 3 mg/L respectively in the controls. Riboflavin, an electron shuttle mediator naturally produced by MR-1, was also found to have a positive impact in potentiostatically controlled cathodes. Additionally, a microbial fuel cell (MFC) with MR-1 and lactate present in both anode and cathode produced a maximum current density of 32.5 mA/m^2 (1,000 Ω external load) after receiving a 10 mg/L Cr(VI) addition in the cathode, and cathodic efficiency increased steadily over an 8-day operation period with successive Cr(VI) additions. In conclusion, effective and continuous Cr(VI) reduction with associated current production were achieved when MR-1 and lactate were both present in the biocathodes.

INTRODUCTION

Hexavalent chromium (Cr(VI)) is a highly toxic, mutagenic and carcinogenic substance that is present in the effluent streams of a wide range of industrial processes, including electroplating, leather tanning and wood preserving[1]. It is highly soluble and, because of its long history of use and often its disposal after inappropriate or no treatment, it has become one of the most abundant inorganic contaminants in groundwater. Ideally Cr(VI) should be removed from groundwater in the natural environment, and because of the inherent dangers to health the stringent guideline limit of 50 μg/L has been issued for total chromium concentration in drinking water[1,2]. Removal options include ion-exchange, adsorption and electrodialysis[3]; however in many of these applications chromium keeps its toxic hexavalent state. Reduction of Cr(VI) to the considerably

less toxic trivalent form Cr(III) and its subsequent precipitation at neutral pH could be considered a more effective remediation strategy[4].

A possible method for Cr(VI) reduction using a microbial fuel cell (MFC) has recently been proposed, where Cr(VI) was used as an oxidant in the cathode to generate an electrical energy output[5,6]. At low pH values where H^+ is abundant, cathodic Cr(VI) reduction has been demonstrated at relatively fast rates and without the use of a catalyst[5,6]. In the neutral pH region, however, the reaction kinetics are slower due to low H^+ availability:

$$CrO_4^{2-} + 8H^+ + 3e^- = Cr^{3+} + 4H_2O \quad (1)$$

At neutral pH, Cr(VI) reduction takes place under lower cathode potentials and is also severely inhibited by Cr(III) oxyhydroxide monolayers which form progressively on the cathode surface[7]. It has been shown, however, that efficiency can be enhanced by utilizing Cr(VI)-reducing bacterial biofilms as biocatalysts (biocathodes)[8-11]. In this case, the bacteria used must be able to obtain a metabolic gain from the electron transfer process, and prospective candidates for this task are electrophilic bacteria that have been extensively tested in MFC anodes[12]. Amongst these, *Shewanellae* have a well-studied electron transfer mechanism[13,14] and have received much research attention due to their metal and electrode reducing abilities, as well as their respiratory diversity and adaptability[15]. Studying their behavior at both the anode and cathode in bioelectrochemical systems is therefore important for understanding the electron transfer mechanisms utilized and optimizing the processes involved[16].

Despite their significance, studies on the potential use of Shewanellae in biocathodes remain limited[16,17], and one reason for this could be the metabolic requirement for organic carbon. This requirement appears to contradict the established view that organic carbon use in the cathode must be minimized, in order to prevent oxygen consumption by heterotrophic metabolism that

does not contribute to electrode oxidation[18,19]. While this view is well founded, it is worth studying the effect of the presence or absence of organic carbon, and particularly of lactate which is a primary carbon source for *Shewanellae*, when Cr(VI) is used as the final electron acceptor[20]. The presence of lactate may also offer potential benefits for the biocathode, including the production of new bacterial cells and the production and utilization of bioelectrocatalysts like flavins, which enhance communication between the bacterial cell and the electrode[21,22]. In addition, lactate can act as a Cr(III) chelator and thus prevent the formation of nonconductive Cr(III) products on the electrode surface[7,23].

The aim of this study was to investigate the performance of Cr(VI)-reducing biocathodes when *Shewanella oneidensis* MR-1 (MR-1) is used as a catalyst in the presence or absence of lactate. Both MR-1 biocathodes and the abiotic counterparts were investigated, and results are expressed in terms of the production of an electrical current and of Cr(VI) reduction. Finally the potential role of riboflavin, an electron shuttle produced by MR-1 in the presence of lactate[24], is also discussed in terms of its efficiency in Cr(VI)-reducing cathodes.

EXPERIMENTAL SECTION

MR-1 cultivation. A starter culture of *Shewanella oneidensis* MR-1 (NCIMB 14063) was grown aerobically on plates of tryptone soya agar (pH 7.3, 30 ^{o}C). A colony was then transferred into 200 mL of Luria-Bertani-50 mM phosphate buffer medium (pH 7.0) and incubated in an orbital shaking incubator at 30 ^{o}C until the required optical densities at 600 nm (OD_{600}) were reached: these were 0.2 for potentiostatically controlled biocathode experiments, 0.3 for MFC biocathodes and 0.5 for MFC bioanodes. Cells were harvested by centrifugation (5,000 x g, 4 ^{o}C, 20 min) with the pellet rinsed twice using 100 mM NaCl-50 mM phosphate buffer saline (PBS) solution (pH 7.0) and resuspended in 200 mL of a minimal medium (MM) (pH 7.0) containing

(mM): NaCl (70), KH_2PO_4 (21), K_2HPO_4 (29), $MgCl_2$ (1), NH_4Cl (28), $CaCl_2$ (0.7), before being transferred to the experimental chambers. No vitamins or amino acids were supplemented in the MM but 0.1 % v/v of a trace elements mixture[25] was added.

Chemicals. Na-DL-Lactate (L1375, Sigma Aldrich) and Na-Acetate (S2889, Sigma Aldrich) were dosed in the required volumes to a final 30 mM concentration. The source of chromium was oven-dried potassium dichromate ($K_2Cr_2O_7$) made up as a 4 g/L Cr(VI) stock solution. This solution was added to give an initial concentration of 20 mg/L in potentiostatically controlled cathodes and 10 mg/L in MFC cathodes. Except where the addition of 1 μM riboflavin (R4500, Sigma Aldrich) is mentioned, all experiments were conducted without supplementation of artificial redox mediators. All additions and sampling were carried out under sterile conditions.

Reactor set-up. Dual-chamber electrochemical reactors were made by connecting two 250 mL borosilicate bottles, each with a sidearm tube. A proton exchange membrane (Nafion 117, Ion Power, Inc.) was fixed between the two sidearms using underwater epoxy adhesive, giving an exposed membrane surface area of 4 cm^2. The membrane was pre-treated as described by Kim et al.[26].

A graphite felt electrode (SIGRATHERM®, SGL Carbon Ltd.) with a 20 cm^2 total apparent surface area (TASA) was fitted in each chamber, and connected through 3 mm ∅ graphite rods to a 1 mm copper wire used as the external circuit cable. The wire was first covered with conductive silver epoxy (ITW, Chemtronics®) and then attached to the felt-rod electrode by inserting it through a hole drilled in the graphite rod. To avoid interference from deposits of Cr(III) oxides and hydroxides, new electrodes were used for each experiment after cleaned as described by Chaudhuri and Lovley[27].

After assembly the reactors were autoclaved at 121 ^{o}C for 15 min. 3 M NaCl Ag/AgCl reference electrodes (RE) (RE-5B, BASi, UK) that had been sterilised using absolute ethanol and allowed to dry in air, were inserted in the working electrode (WE) chambers of potentiostatically controlled cells for controlling, and in the anodes of MFCs for monitoring of the electrodes' potential.

When used as MFCs, both chambers were filled with 200 mL of MM, inoculated when indicated. When used as potentiostatically controlled cells the WE chamber was filled with 200 mL of MM, inoculated when indicated, and the counter electrode (CE) chamber with 200 mL of PBS.

All experiments were conducted at room temperature (22±3 ^{o}C) and the reactors were covered with aluminium foil to exclude light. When N_2 or air flushing was required, the gas was first passed through a 0.2 μm filter before entering the reactor. N_2 was introduced from a compressed N_2 cylinder and air (160 mL/min) was supplied by an aquarium pump.

Fuel cell operation. *Potentiostatically controlled cells.* Potentiostatically controlled chronoamperometry experiments were conducted in two phases: in the start-up phase (phase 1) MR-1 was tested for its ability to produce anodic current and in the main phase (phase 2) Cr(VI) was spiked and the reactor was allowed to produce cathodic current for a 4-h period. In phase 1 the WE was maintained at either -500 mV (notated as C(-El)^{ph1}) or +300 mV (notated as C(+El)^{ph1}) (all potentials reported are vs. Ag/AgCl) for 14-18 h after introduction of the MR-1 cell culture into the chamber. WE chambers operated in the presence of lactate, under aerated conditions and without riboflavin or Cr(VI) during phase 1, except if indicated by notation. Phase 2 of most experiments was conducted in the same reactors as phase 1. An exception was made for the reactors notated as (-Lac)^{ph2} or (-Shew)^{ph2}, where phase 2 was lacking the lactate-containing

supernatant of phase 1, or the MR-1 cells respectively. To separate the supernatant from MR-1, the respective WE chamber contents from phase 1 were centrifuged at 5,000 × g for 20 min. In the reactors notated as $(\text{-Lac})^{ph2}$ this centrifugation step was followed by rinsing of the MR-1 pellet twice with PBS solution to remove lactate and metabolites produced in phase 1. The MR-1 pellet was then resuspended in a clean reactor using the original electrodes from phase 1. In the reactors notated as $(\text{-Shew})^{ph2}$ the phase 1 supernatant was additionally filtered through 0.2 μm filters to remove the MR-1 cells, in order to determine whether metabolites released in the medium could have a catalytic effect during Cr(VI) reduction. In the $C(\text{+El})^{ph1}(\text{-Lac+Rbf(1)})^{ph2}$ cell, 1 μM riboflavin was added into the WE chamber before the beginning of phase 2 in order to investigate whether MR-1 could still express electrochemical activity in the absence of lactate but in the presence of its redox mediator. All operations were carried out by applying appropriate sterilization techniques to prevent contamination of the pure culture and the abiotic reactors. A description of the start-up phase, a summary of the experimental conditions but also details on chronoamperometry (CA) and cyclic voltammetry (CV) experiments can be found in Supporting Information (SI).

In addition to the MR-1 experiments, seven abiotic control experiments were conducted with the WE chambers containing: only MM solution (AC), MM with 30 mM lactate (AC+Lac), MM with 30 mM lactate and 0.25, 1 or 5 μM riboflavin (AC+Lac+Rbf(0.25), AC+Lac+Rbf(1) or AC+Lac+Rbf(5) respectively), MM with 1 μM riboflavin (AC+Rbf(1)), and MM with 30 mM acetate (AC+Acet).

Microbial fuel cells. Four MFCs were assembled and tested, each with different cathode conditions. The cathode of the MFC notated as “None” only contained MM and 10 mg/L Cr(VI), while the other three cathodes additionally contained: 30 mM lactate (“+Lac”), 30 mM lactate

and 1 μM riboflavin ("+Lac+Rbf"), and 30 mM lactate with MR-1 ("+Lac+Shew"). Details on the MFC start-up can be found in SI.

Drops in potential across the 1,000 Ω resistors and anodic potentials vs. Ag/AgCl RE were recorded every 1 min using a datalogger (DT 505, DataTaker®). Polarization curves for "+Lac+Shew" were obtained by repeating this experiment up to the first Cr(VI) spiking step and allowing open circuit voltage to evolve after spiking. Current values were then obtained by closing the circuit using external resistors, with resistances gradually decreasing from 16,300 Ω to 7 Ω. Sufficient time was allowed between each resistor change in order to achieve a relatively stable current production.

Analytical methods and calculations. For analysis of Cr(VI) and acids the chamber contents were sampled and then centrifuged at 20,800 × g for 3 min. The concentration of dissolved Cr(VI) was determined according to Standard Method 3500-Cr B[28]. Lactic and acetic acids were measured using Ion Chromatography (882 Compact IC Plus, Metrohm) with an ion-exclusion column (Metrosep Organic Acids, 250/7.8 mm, Metrohm). A 0.5 mM H_2SO_4 solution was used as eluent and flow rate was set at 0.6 mL/min.

In MFC experiments, current was calculated from the voltage drop across the external resistor according to Ohm's law. Current density, power density and charge produced were calculated according to Logan et al.[29], while cathodic efficiency was calculated based on the fraction of electrons transferred to Cr(VI) from the cathode. Formulas are presented in SI.

RESULTS AND DISCUSSION

Potentiostatically controlled cells. ***Electrode fouling.*** Initially, cathodic current produced by the potentiostatically controlled cells was measured under abiotic conditions and in the absence of lactate. In the abiotic cathode AC, current showed a high initial response but dropped rapidly

(Figure 1a), leaving 19 mg/L Cr(VI) still in solution at the end of the 4-h operation period. Two consecutive runs were then performed in AC, using the same electrode which was cleaned with ultra-pure water between successive Cr(VI) additions (spikings), but replacing the catholyte each time. It can be seen in Figure 1a that the initial current response dropped sharply from 820×10^{-3} in the 1st run to less than 100×10^{-3} A/m^2 in the 2nd and 3rd runs. A recovery of the initial current response was observed in the 4th run, however, after the electrode was cleaned using sequential alkaline, acid and ultra-pure water washing[27]. The decrease in the current response observed in this set of experiments and the recovery after electrode cleaning indicated the formation and accumulation of inhibitory substances on the electrode surface. This inhibitory effect has also been observed when Cr(VI) was tested as a corrosion inhibitor on carbon, copper and platinum electrodes[7,23]. According to these studies, the reason for this inhibitory effect was the formation of non-conductive Cr(III) oxyhydroxide monolayers on the electrode surface at neutral and near neutral pH, which prevent further reduction of Cr(VI).

Process enhancement in the presence of lactate and riboflavin. By supplying 30 mM of lactate in the control experiment AC+Lac, the initial current peak increased when compared to that of AC (Figure 1b). However, the fall in current to background levels in AC+Lac was also quick and 17 mg/L Cr(VI) were still left in solution at the end of the 4-h operation period (Figure 2a). The higher initial current response in AC+Lac, compared to that in AC, can be partially explained by the ability of Cr(III) to form insoluble chelates with organic ligands[30,31], which may delay or even prevent the deactivation of the electrode's active sites, depending on the initial Cr(VI) and ligand concentration. While electrode deactivation was more apparent in the absence of lactate, increasing lactate concentrations in the cathode generally prolonged current production and Cr(VI) reduction in abiotic cathode MFCs (SI, MFC experiments for studying the effect of

lactate in abiotic cathodes and Figure S1). A possible mechanism for this enhancement could involve more effective neutralisation of the metal's positive electrical charges with higher lactate concentrations and a more effective reduction of its affinity to the negatively charged cathode. Lactate-Cr(III) chelates can precipitate and potentially be removed from the solution at neutral pH, however separation with other organic ligands like malate and citrate will not be possible as they form soluble Cr(III)-complexes[30,31]. Acetate can also form insoluble Cr(III) complexes[31], but when acetate was supplied instead of lactate (AC+Acet), cathodic performance showed no difference from that of AC (Figures 1b, 2a and 3a).

Filtered supernatant ($C(+El)^{ph1}(-Shew)^{ph2}$ and $C(-El)^{ph1}(-Shew)^{ph2}$) and MM containing lactate and riboflavin (AC+Lac+Rbf(0.25,1,5)) showed enhanced performance (Figures 1-3) as a result of the presence of redox mediators. Riboflavin concentration was also important, with up to 10.1 mg/L Cr(VI) reduced and 10.7 Coulombs produced when 5 μM riboflavin was supplied. In the absence of lactate (AC+Rbf(1)), however, the catalytic activity of riboflavin could not be expressed due to the rapid fouling of the electrode by Cr(III) hydroxides like $Cr(OH)^{2+}$, $Cr(OH)_2^{+}$ and $Cr(OH)_3$ present at near neutral pH. Although riboflavin has also been demonstrated to be capable of forming chelates with metal cations[22], the concentrations of riboflavin used (up to 5 μM) would be sufficient for the chelation of less than 0.26 mg/L (5 μM) of chromium ion assuming a 1:1 molar chelation ratio.

CV experiments at the end of the 4-h experimental period showed that AC+Lac+Rbf(1) gave a clear catalytic current at low applied potentials (<-400 mV) with similar patterns also observed in the filtered supernatants $C(+El)^{ph1}(-Shew)^{ph2}$ and $C(-El)^{ph1}(-Shew)^{ph2}$ (Figure 4a). This indicates that even in the absence of active cells, compounds released in the medium are responsible for

Cr(VI) electroreduction; and is in agreement with previous studies where compounds released in the medium by *Shewanella* were found to be able to catalyse oxygen electroreduction[17,32,33].

Cathodic current was minimal in all potentiostatically controlled cells which did not contain lactate, although MR-1 and/or riboflavin were present in some cases (Figure 4b). By comparing the CV graphs for $C(+El)^{ph1}$, $C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}$, AC+Lac+Rbf(1) and AC+Rbf(1), it is clear that the cathodic catalytic current produced in $C(+El)^{ph1}$ and AC+Lac+Rbf(1) could be suppressed in the absence of lactate ($C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}$, AC+Rbf(1)). Cathodic current was also suppressed by the end of the operation period in the abiotic experiment with 30 mM lactate but without MR-1 or riboflavin (AC+Lac) (Figure 4c). The above results indicate that the effective expression of cathodic current under the conditions described, required the presence of both lactate and redox mediators.

Effect of MR-1 pre-treatment conditions on biocathode performance. Oxygen enhanced anodic current production during phase 1 (SI, Figure S2a), probably due to the higher energy obtained by MR-1 during complete lactate oxidation under aerated conditions[34,35]. A thicker biofilm that could be visually observed in this instance and a presumably higher production of redox mediators which exhibited (anodic) current onsets at potentials similar to the midpoint potentials of flavins[22] (Figure S3), are also possible reasons for this enhanced performance. Furthermore, MR-1 demonstrated the ability to enhance electroreduction of its own soluble metabolic products even when lactate was present (flask experiment description and Figures S4 and S5) and it is possible that these products could later participate in reduction-oxidation cycles with Cr(VI) as the final electron acceptor. From a metabolic point of view this possibility is remarkable, considering the interconnected reduction pathways for Cr(VI) and flavins[21,36].

When MR-1 was continuously supplied with oxygen in the presence of lactate and a poised electrode at +300 mV during phase 1 ($C(+El)^{ph1}$), dosing with Cr(VI) gave a lower initial current response than in the absence of MR-1 (Figure 1b,c). Current was sustained for a longer time in $C(+El)^{ph1}$, however, probably due to the bacterial energy-gaining processes and the abiotic redox cycles promoted by electron shuttle mediators. The charge produced in $C(+El)^{ph1}$ by the end of the 4-h period was 5.5 and 1.7 times more than in AC (no lactate) and AC+Lac (30 mM lactate) (Figure 3a). Also the current produced in $C(+El)^{ph1}$ at the end of the experiment (52×10^{-3} A/m^2) was higher than that in AC (1×10^{-3} A/m^2) and AC+Lac (9×10^{-3} A/m^2), despite the fact that $C(+El)^{ph1}$ had a considerably lower Cr(VI) concentration (11 mg/L Cr(VI)) compared to AC (19 mg/L Cr(VI)) and AC+Lac (17 mg/L Cr(VI)) (Figure 2).

$C(+El\text{-}Air)^{ph1}$ showed lower cathodic activity during phase 2, probably as a result of the limited electrophilic activity of the MR-1 cells during phase 1. MR-1 obtained less energy from lactate during phase 1 in this instance and thus cells occupying the electrode surface might have become less active. However, process was enhanced upon addition of riboflavin ($C(+El\text{-}Air+Rbf(1))^{ph1}$), when 4 mg/L more Cr(VI) was reduced (Figure 2b) and 1.9 times more charge was produced (Figure 3a) compared to $C(+El\text{-}Air)^{ph1}$. Although cathodic Cr(VI) reduction was observed in $C(+El\text{-}Air+Cr(VI))^{ph1}$, the charge produced was still lower than that of $C(+El)^{ph1}$ and $C(-El)^{ph1}$ (Figure 3a). This process enhancement seen in $C(+El)^{ph1}$ and $C(-El)^{ph1}$ (aerobic pre-treatment) compared to $C(+El\text{-}Air)^{ph1}$ (anaerobic pre-treatment) and $C(+El\text{-}Air+Cr(VI))^{ph1}$ (anaerobic pre-treatment with Cr(VI)) indicates that cathodic electrochemical activity can be enhanced when oxygen is supplied during the pre-treatment step. The charge produced in both $C(+El\text{-}Air)^{ph1}$ and $C(+El\text{-}Air+Cr(VI))^{ph1}$ during the 4-h test period was similar to that in the abiotic AC+Lac (Figure 3a). The current in $C(+El\text{-}Air)^{ph1}$ and $C(+El\text{-}Air+Cr(VI))^{ph1}$ (32×10^{-3} A/m^2 and 40×10^{-3} A/m^2)

was around 4 times that in AC+Lac towards the end of test, however, despite the lower Cr(VI) concentrations in the MR-1 containing chambers (Figure 2).

C(+El)^{ph1} produced 15 % more charge than that produced by C(-El)^{ph1} by the end of the 4-h operation period and C(+El)^{ph1}(-Shew)^{ph2} produced 32 % more charge than C(-El)^{ph1}(-Shew)^{ph2} (Figure 3a). This can be linked to the enhanced anodic current showed in the CV tests after phase 1, in the experiments where both oxygen and the electrode were supplied as the electron acceptors during phase 1, compared to the experiments where the electron acceptor was more limited (C(-El)^{ph1}, C(-El)^{ph1}(-Lac)^{ph2}, C(-El)^{ph1}(-Shew)^{ph2}, C(+El-Air)^{ph1}) (SI, Figure S3). The CV experiments provide further evidence that the pre-treatment conditions play an important role in the catalytic activity of MR-1 (Figure 4c). The CV results showed that MR-1 had an active catalytic role in C(+El)^{ph1}, as it provided cathodic current over a broader potential range, not only when compared to abiotic AC+Lac+Rbf(1) (Figure 4b), but also to C(-El)^{ph1} and C(+El-Air)^{ph1} (Figure 4c). Catalytic activity of the biocatalyst over a broad potential range is important for power-producing applications such as MFCs, where imposed overpotentials will increase the energy losses of the system.

Microbial fuel cells. The experiments described above showed that an improved catalytic activity of MR-1 could be achieved by manipulation of culture conditions: this demonstration, however, was carried out under energy-consuming potentiostatic control. The efficiency of power-producing devices such as MFCs is restricted by internal resistances and potential losses[37] and therefore the conditions applied in the potentiostatically controlled experiments needed to be verified in MFCs. As the primary purpose in this instance was to compare the different conditions, the same simple reactor configuration was used, which had a relatively small ion exchange membrane area and low electrode TASA to volume ratio. In addition, all MFC anodes

were operated under aerated conditions and had comparable anodic potentials under closed circuit conditions throughout operation: -447±10 mV in "None", -446±17 mV in "+Lac", -450±13 mV in "+Lac+Rbf" and -432±28 mV in "+Lac+Shew".

MFC "None" which operated without lactate and MR-1 in the cathode produced an initial current of 84 μA; the system stopped operating within the first few hours, however, although there was still 9 mg/L Cr(VI) available (Figure 5a). This behaviour was similar to that of the potentiostatically controlled cathodes without lactate, as already discussed previously. MFC "+Lac" which contained 30 mM lactate in the cathode achieved an initial current of 145 μA, which dropped to background levels (4 μA; around 0.3 mg/L/day of Cr(VI) reduced assuming 100 % cathodic efficiency) by the end of the 48h operation period, with 43 % of Cr(VI) still in solution. MFC "+Lac+Rbf", with an additional 1 μM riboflavin, produced an initial current of 153 μA. Its performance was not significantly better than that of "+Lac", however, as the addition of riboflavin only enhanced the process at low applied potentials (Figure 4a) which are difficult to achieve in MFCs. Also, only a negligible increase in current production was observed after a further increase in Cr(VI) concentration to 10 mg/L in both "+Lac" and "+Lac+Rbf" (Figure 5a). This indicates the dominating effect of Cr(III) precipitates, which finally managed to form and deactivate the cathodes.

On the other hand, despite the lower initial value of 65 μA (32.5 mA/m^2 TASA), current was produced by MFC "+Lac+Shew" (MR-1 biocathode) after every Cr(VI) addition (Figure 5b). The maximum current produced by "+Lac+Shew" occurred after the first addition and was comparable to the 46.6 mA/m^2 observed by Tandukar et al.[11], who used a mixed culture biocathode with a similar configuration (electrode TASA to working volume ratio: 16 m^2/m^3 [vs. 340-9,600 m^2/m^3 in other studies[8-10]], $Cr(VI)_0$: 22 mg/L, $R_{ext.}$: 1,000 Ω). Current production in

"+Lac+Shew" responded immediately to every Cr(VI) addition even though air was continuously supplied to the anode: this clearly indicates that Cr(VI) has an advantage over oxygen for electroreduction when organic carbon is provided in the biocathode. In total, 3.5 mg Cr(VI) were reduced by the cathode electrode of "+Lac+Shew" by the end of the MFC operation, compared to the maximum Cr(VI) reduction of around 1.2 mg Cr(VI) by "+Lac" (Figure 5c). In the absence of an electrode, Cr(VI) reduction was considerably slowed down after the first 3-4 days, regardless of the biomass concentration and the presence or absence of lactate (Figure 5d; for Cr(VI) reducing flask experiments description, see SI). In the "+Lac+Shew" MFC a similar plateau was observed for the pathways other than electrode oxidation, which accounted for only 1.1 mg Cr(VI) reduced within the last 240 h. The relatively slow decrease of the Cr(VI) bio-electroreduction rate in "+Lac+Shew" on one hand, along with the rapidly decreasing rate of Cr(VI) reduction due to lactate utilization and other pathways on the other hand, were the reasons why an increase in cathodic efficiency was observed, from 14 % (1^{st} addition) to 64 % (5^{th} addition) and to 81 % when the cathode was switched to potentiostatic control at -500 mV (6^{th} addition) (Figure 5e). By the end of operation, Cr(VI) bio-electroreduction accounted for around 46 % of the total Cr(VI) reduction in "+Lac+Shew", clearly showing that MR-1 could utilise both the electrode and lactate as an electron donor.

While it is well established that mixed culture biocatalysts will yield higher current and power densities than pure cultures[38], the "+Lac+Shew" MFC produced a maximum of 172 nW/cm^2 (Figure 5f). However, its Cr(VI) reducing performance was considerably higher compared to the performance of the only other study which investigated Cr(VI) reduction in an MFC biocathode with MR-1 as the biocatalyst[16]. In contrast to the current study, the biocathodes used in these experiments were supplied with fumarate as an electron acceptor in a biocathode inoculated with

MR-1 as part of a pre-treatment step. When the electron acceptor was switched to Cr(VI), the overall cathodic efficiency in the system was around 67 %, which decreased to around 42 % in the last 3 days of a 9-day operation period. According to the authors, this cathodic efficiency decrease was due to the unavoidable Cr(VI) reduction by other electron sources (presumably dead cells and succinate, the metabolic by-product of fumarate), towards the end of the operation period. It is possible that electrode fouling by Cr(III) hydroxides over time was one of the reasons for this metabolic shift when MR-1 started utilising electron donors other than the electrode. In the present study, although the external resistor was 100 times higher (1,000 Ω vs. 10 Ω) compared to that used by Hsu et al.[16], the electrode in the "+Lac+Shew" cathode reduced considerably higher amount of Cr(VI) than the one in Hsu et al.[16], i.e. 1,750 mg/m^2 in 8 days vs. an estimated 87 mg/m^2 in 9 days. Considering the total Cr(VI) reduction in the biocathodes, i.e. Cr(VI) reduced by electrical current and other pathways, around 5,000 mg/m^2 of Cr(VI) were reduced in "+Lac+Shew" in 8 days of MFC operation, whereas only around 130 mg/m^2 of Cr(VI) reduction was achieved in 9 days in Hsu et al.[16]. Overall, the abiotic cathode in Hsu et al.[16] performed equally or better than many *Shewanella* strains, reducing around 139 mg/m^2 Cr(VI) in 9 days vs. 104 mg/m^2 in the MR-4 cathode and 130 mg/m^2 in the MR-1, PV-4 and ANA-3 cathodes. On the contrary, MR-1 in our study exhibited a clear enhancement of the process and the biocathode clearly outperformed the abiotic cathode. By adding lactate along with MR-1 pre-treated under the conditions described, the electrocatalytic activity and Cr(VI) reducing ability of the cathode were considerably increased and this is the first study to demonstrate this process enhancement.

Freguia et al.[17] have reported that stored carbon within *Shewanella* cells was sufficient to inhibit cathodic oxygen electroreduction, despite the fact that no organic carbon was provided

during the experiment. This observation is in line with the rapid current decrease seen in the C(-El)^{ph1} experiments during phase 1, even in the absence of lactate (SI, Figure S2b). Whilst MR-1 can cause low redox conditions and inhibit oxygen electroreduction within a few hours when lactate is available, this did not seem to be the case for Cr(VI). MR-1 used both lactate and the electrode as the electron donor for Cr(VI) reduction, and cathodic efficiency increased over time.

Current is believed to be enhanced in three ways when lactate was present in the biocathode: (i) Cr(III)-lactate interaction delayed the deactivation of the electrode, (ii) electron shuttle mediators produced during phase 1 mediated electrons from the electrode to Cr(VI) and promoted indirect Cr(VI) reduction, and (iii) the presence of lactate and redox mediators produced during phase 1 enabled MR-1 to be actively involved in the electrode oxidation process and drive direct or indirect Cr(VI) reduction.

Practical implications. The addition of lactate in a Cr(VI) reducing MFC cathode considerably increased the operating life and the Cr(VI) reducing ability of the electrode, which are of critical importance for the implementation of the MFC technology in Cr(VI) remediation. In the presence of MR-1, the Cr(VI) reducing ability of the electrode was further increased by around 3 times compared to the abiotic control, and in total, Cr(VI)-wastewater bioremediation was considerably enhanced by simultaneously providing two separate electron sources to the bacteria; lactate and the poised electrode. As a result, the electrode accounted for almost the same amount of Cr(VI) reduced by MR-1 and lactate alone, indicating that bio-electroreduction and MFC technology could be considered in addition to conventional Cr(VI) bioremediation.

Furthermore, the demonstrated capability of MR-1 to operate both in the anode and the cathode could offer more flexibility in applying the MFC technology for Cr(VI) remediation. In this occasion, microbes, lactate and redox mediators needed in the cathode could be provided by

recirculating anodic effluent to the cathode. The reverse action is also possible to allow COD removal and Cr(VI) polishing of the cathodic effluent: however, further research would be required to elucidate to what extent and under what conditions (e.g. biomass density in the anode), low levels of Cr(VI) present in the cathodic effluent affect the established redox conditions in the anode.

ASSOCIATED CONTENT

Supporting Information

Description of chronoamperometry (CA) and cyclic voltammetry (CV) experiments, description of the start-up phase in potentiostatically controlled experiments, Tables S1-S2, MFC start-up description, calculations, MFC experiments for studying the effect of lactate in abiotic cathodes, Figures S1-S5, electrochemical activity of MR-1 during phase 1 of potentiostatically controlled cells, flask experiment description and Cr(VI) reducing flask experiments description. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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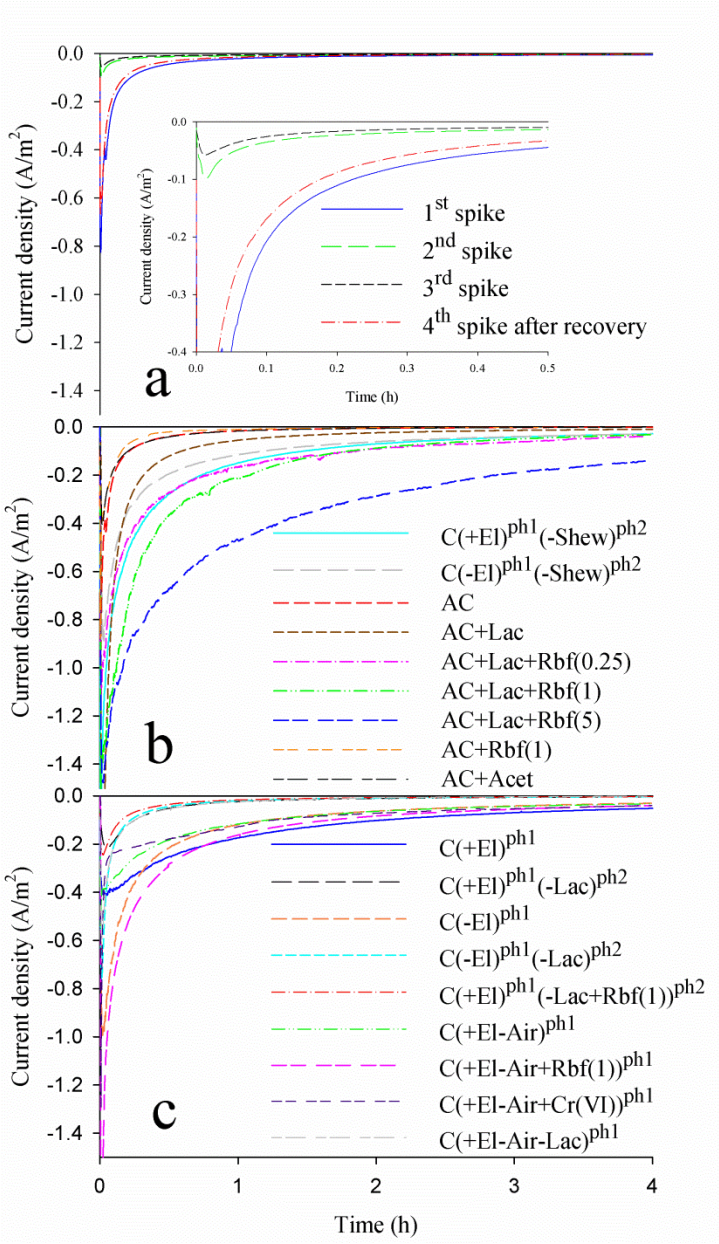


Figure 1. Chronoamperometry profiles at -500 mV for the potentiostatically controlled cathodes. (a) AC experiments (inset: zoom in for the first 0.5 h); (b) experiments without MR-1 cells; (c) experiments with MR-1 cells.

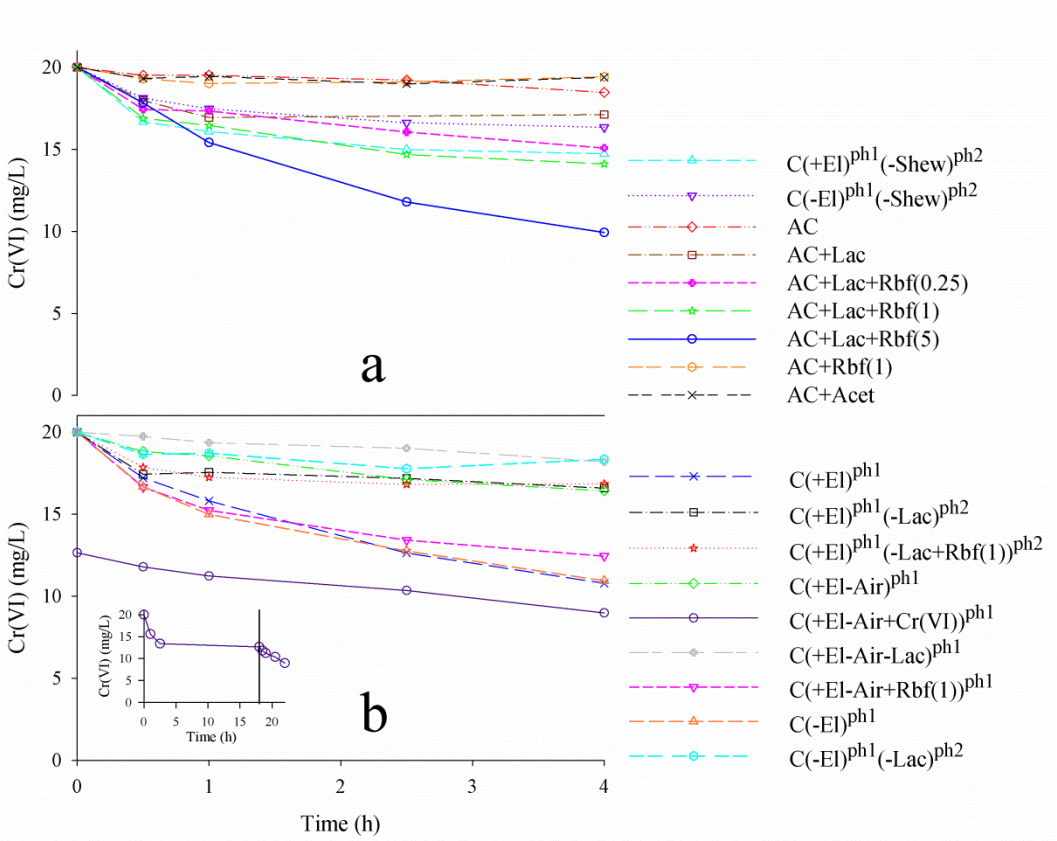


Figure 2. Soluble Cr(VI) concentration in the potentiostatically controlled cathodes. (a) experiments without MR-1 cells; (b) experiments with MR-1 cells (inset: C(+El-Air+Cr(VI))^{ph1} throughout both phases 1 and 2, with phase 2 start indicated by the vertical solid line).

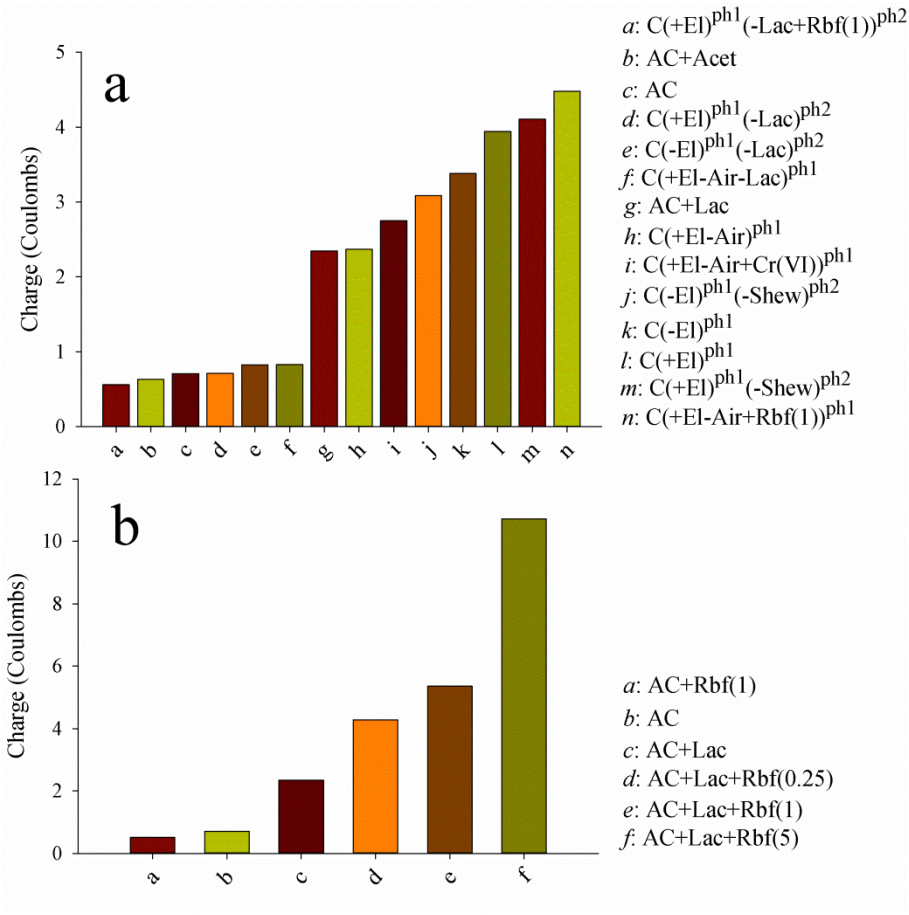


Figure 3. Charge produced after the 4-h operation period. (a) comparison of MR-1 with abiotic controls; (b) comparison of abiotic experiments with riboflavin (AC+Lac+Rbf(0.25,1,5) and AC+Rbf(1)), with AC and AC+Lac.

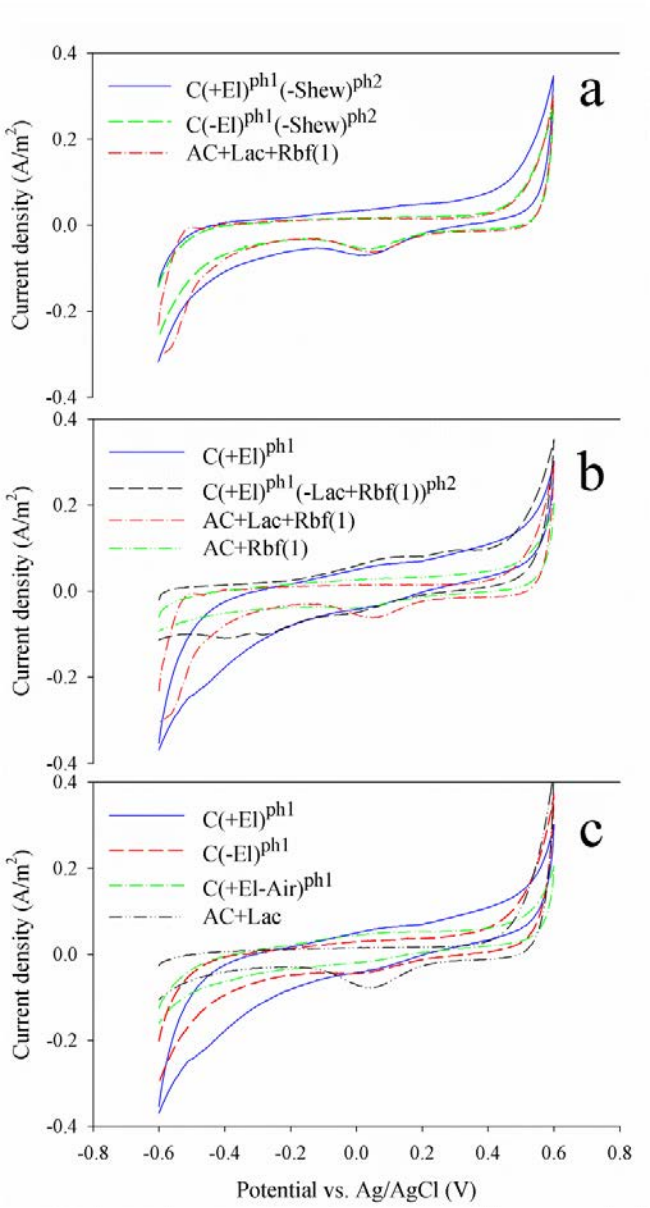


Figure 4. Cyclic voltammograms at the end of phase 2. Scan rate is 5 mV/sec. (a) comparison of the filtered supernatants $C(+El)^{ph1}(-Shew)^{ph2}$ and $C(-El)^{ph1}(-Shew)^{ph2}$ with AC+Lac+Rbf(1); (b) comparison between $C(+El)^{ph1}$, $C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}$, AC+Lac+Rbf(1) and AC+Rbf(1), showing the importance of lactate on the expression of catalytic activity; (c) comparison between $C(+El)^{ph1}$, $C(-El)^{ph1}$, $C(+El\text{-}Air)^{ph1}$ and AC+Lac, showing the importance of pre-treatment conditions.

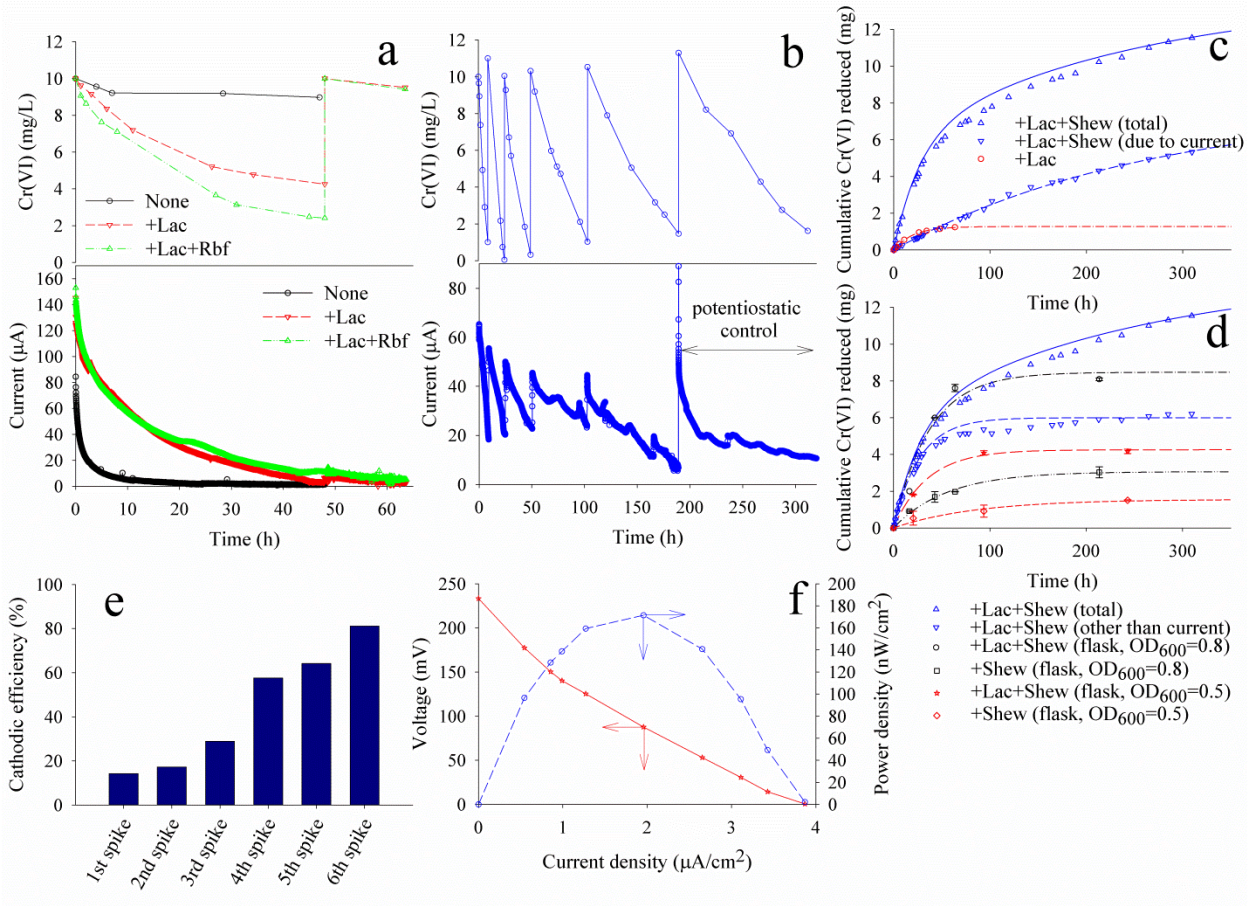
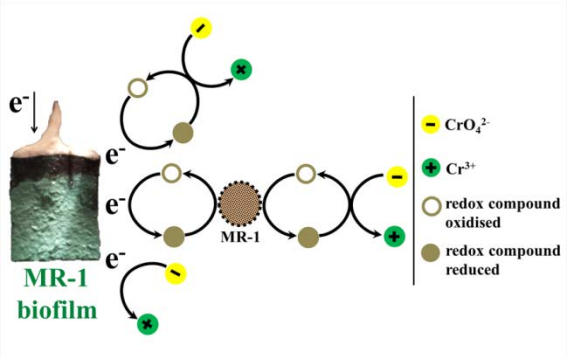


Figure 5. Parameters during MFC operation. (a) current production and Cr(VI) concentration in MFCs with abiotic cathodes; (b) current production and Cr(VI) concentration in the biocathode MFC; (c) comparison of the Cr(VI) reduced in the abiotic lactate MFC with that in the biocathode MFC due to current (equations of the graphs are given in SI, Table S2); (d) comparison of the Cr(VI) reduced in the flasks with that in the biocathode MFC due to pathways other than electrode oxidation (planktonic MR-1 in the "Lac+Shew" MFC had OD_{600}= 0.5 at time 0; equations of the graphs are given in SI, Table S2; error bars represent the min and max of the measurements); (e) cathodic efficiency of the biocathode MFC during MFC operation (spikes 1-5) and during potentiostatic control of the cathode at -500 mV (6^{th} spike); (f) polarization curves of the biocathode MFC after the first Cr(VI) spike.



Abstract art.

Supporting information

Enhanced performance of hexavalent chromium reducing cathodes in the presence of *Shewanella oneidensis* MR-1 and lactate

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Contents:

15) Cr(VI) reducing flask experiments description (p.21)

Description of chronoamperometry (CA) and cyclic voltammetry (CV) experiments

For CA and CV experiments, the cells operated in a three-electrode configuration with a potentiostat (PG580, Uniscan Instruments Ltd., UK) controlling the potential difference between the WE and the RE. The potentiostat was interfaced to a computer and UiEchem™ software was used to record the current every 4 seconds in CA and every 1 second in CV experiments. CV analysis was conducted for each set of experiments, to test the electrocatalytic activities of the WE chambers. CVs were performed from -600 mV to +600 mV (all potentials reported are vs. Ag/AgCl), with a scanning rate of 5 mV/sec under quiescent, N_2-flushed conditions. CV experiments were repeated until consistent results were obtained (normally after 2 to 5 times). The WE chambers were stirred at 250 rpm during CA and between CV experiments, and both chambers were stirred during MFC operation.

Description of the start-up phase in potentiostatically controlled experiments

A number of operational conditions were tested during phase 1, in which MR-1 was allowed to produce the metabolites needed for electron transfer and to form a biofilm. At the end of phase 1, CV tests were conducted and then the potentiostat potential was set at -500 mV so that the electrode could act as the electron donor for Cr(VI) reduction. All WE chambers except that in $C(+El\text{-}Air+Cr(VI))^{ph1}$ were flushed with N_2 for one extra hour between phases 1 and 2, and current was allowed to stabilise to background levels at -500 mV. This was normally less than 7×10^{-3} A/m^2, and the value recorded was deducted from the result. This stabilisation step was omitted in $C(+El\text{-}Air+Cr(VI))^{ph1}$, however, as Cr(VI) was present from phase 1 in this reactor.

Table S1. Experimental conditions applied in potentiostatically controlled experiments

Cell #	Phase 1	Phase 2 (-500 mV, Cr(VI) and N_2)
C(+El)^{ph1}	30 mM lactate, air, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (20 mM lactate)
C(+El)^{ph1}(-Lac)^{ph2}	30 mM lactate, air, 14 h, +300 mV (common phase 1 for reactors C(+El)^{ph1}(-Lac)^{ph2} and C(+El)^{ph1}(-Shew)^{ph2})	MR-1 from phase 1 resuspended in another reactor with the same electrode (no lactate)
C(+El)^{ph1}(-Shew)^{ph2}		supernatant of phase 1 filtered (0.2 μm) and resuspended in another reactor (17 mM lactate)
C(-El)^{ph1}	30 mM lactate, air, 14 h, -500 mV	MR-1 in the same reactor as in phase 1 (25 mM lactate)
C(-El)^{ph1}(-Lac)^{ph2}	30 mM lactate, air, 14 h, -500 mV (common phase 1 for reactors C(-El)^{ph1}(-Lac)^{ph2} and C(-El)^{ph1}(-Shew)^{ph2})	MR-1 from phase 1 resuspended in another reactor with the same electrode (no lactate)
C(-El)^{ph1}(-Shew)^{ph2}		supernatant of phase 1 filtered (0.2 μm) and resuspended in another reactor (23 mM lactate)
C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}	30 mM lactate, air, 14 h, +300 mV	MR-1 from phase 1 resuspended in another reactor with the same electrode and 1 μM riboflavin (no lactate)
C(+El-Air)^{ph1}	30 mM lactate, N_2, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (28 mM lactate)
C(+El-Air+Rbf(1))^{ph1}	30 mM lactate, 1 μM riboflavin, N_2, 14 h, +300 mV	MR-1 in the same reactor as in phase 1 (28 mM lactate)
C(+El-Air+Cr(VI))^{ph1}	30 mM lactate, Cr(VI), N_2, 18 h, +300 mV	MR-1 in the same reactor as in phase 1 (25 mM lactate)
C(+El-Air-Lac)^{ph1}	N_2, 14 h (no lactate), +300 mV	MR-1 in the same reactor as in phase 1 (no lactate)
AC	n/a	MM only (abiotic)
AC+Lac	n/a	MM + 30 mM lactate (abiotic)

AC+Lac+Rbf(0.25, 1 or 5)	n/a	MM + 30 mM lactate + 0.25, 1 or 5 μM riboflavin (abiotic)
AC+Rbf(1)	n/a	MM + 1 μM riboflavin (abiotic)
AC+Acet	n/a	MM + 30 mM acetate (abiotic)

MFC start-up description

Anodes in MFCs “None”, “+Lac” and “+Lac+Rbf” were inoculated with MR-1 and left overnight with air continuously flushed (160 mL/min), until the anodic potential under open circuit conditions dropped and stabilised at around -470 mV. In the cathode N_2 was flushed overnight and then continuously during the experiment. After stabilisation, the circuits were closed using 1,000 Ω external resistors and Cr(VI) was spiked (time zero). In the bioanodes, air continued to be flushed for the duration of the experiment and lactate was supplied at intervals to ensure electron donor availability and to promote stable anodic potentials.

The MFC experiment “+Lac+Shew” was conducted in two phases. In the start-up phase 1, chamber 1 (Ch1) of +Lac+Shew contained 30 mM lactate and was inoculated with MR-1. MR-1 was then allowed to produce anodic current (1,000 Ω external resistor) for 3 d, while air was continuously flushed into both the anode (Ch1) and the abiotic cathode (Ch2) which only contained MM. After phase 1, Ch2 was inoculated with MR-1 and lactate was added in both chambers to give a 30 mM initial concentration. Ch1 was then continuously flushed with N_2 and the system was allowed to equilibrate for 2 h until the current dropped to background levels (<4 μA). At this point Cr(VI) was spiked into Ch1 to give an initial concentration of 10 mg/L, while Ch2 continued to be flushed with air to test whether Cr(VI) has an advantage over oxygen as an electron acceptor when both MR-1 and lactate are present. From then on, current flow was reversed, with Ch1 acting as the biocathode and Ch2 as the bioanode. Additional doses of 10 mg/L Cr(VI) were given at intervals to ensure the presence of Cr(VI) in the cathode at all times.

Calculations

Current (A) and current density (A/m^2) were calculated according to (1) and (2), respectively:

$$I=E_{cell}/R_{ext} \quad (1)$$

$$I_{cat}=I/A_{cat} \quad (2)$$

where *I*: current (A); E_{cell}: voltage drop across R_{ext} (V), recorded at a fixed time interval; R_{ext}: external resistor (Ω); I_{cat}: current density (A/m^2); A_{cat}: total apparent surface area of the cathode (m^2).

Power (W) and power density (W/m^2) were calculated according to (3) and (4), respectively:

$$P=E_{cell}^2/R_{ext} \quad (3)$$

$$P_{cat}=E_{cell}^2/(R_{ext}A_{cat}) \quad (4)$$

The charge transferred from the cathode electrode was calculated based on current measurements at fixed time intervals, as:

$$C_1=\Sigma I\Delta t \quad (5)$$

where C_1: the proportion of charge (Coulombs) transferred to Cr(VI) by the electrode; *I*: current (A) recorded at a fixed time interval Δt; Δt: time interval (s).

The total amount of charge transferred to Cr(VI), assuming complete reduction of Cr(VI) to Cr(III), was calculated as follows:

$$C_2=nFv\Delta[Cr(VI)]/M \quad (6)$$

where C_2: total charge (Coulombs) transferred to Cr(VI) for complete Cr(VI) to Cr(III) reduction; n: number of moles of electrons transferred per mole of Cr(VI) reduced to Cr(III), 3 moles/mol of Cr(VI); F: Faraday constant, 96,485.3 Coulombs/mol of electrons; v: catholyte volume (L); $\Delta[Cr(VI)]$: Cr(VI) concentration reduction at a given time interval (g/L); M: atomic weight of Cr, 52 g/mol of Cr.

Cathodic efficiency (CE, %) was calculated as below:

$$CE=C_1/C_2\times100\% \quad (7)$$

The cumulative mass of Cr(VI) (g) reduced due to current was calculated according to the cumulative electrical charge produced, as below:

$$\Sigma Cr(VI)=M\Sigma I\Delta t/(nF) \quad (8)$$

MFC experiments for studying the effect of lactate in abiotic cathodes

To study the effect of lactate in Cr(VI) reducing abiotic cathodes, 4 lactate concentrations were studied, i.e. 0, 2, 20 and 200 mM. The same reactor design described in the "Experimental Section" was used and all experiments were ran in triplicates. Anode effluent (15 mL) from an MFC initially inoculated with anaerobic sludge, was used to inoculate each of the 3 MFC anodes which were connected with the cathodes through 1,000 Ω external loads. Then, the MFC reactors were let operate with aerated cathodes until current stabilised at around 120 ± 4 μA for more than 3 d. The catholyte was then replaced with an anaerobic phosphate buffer (50 mM, pH 7) which also contained Na-DL-Lactate at the concentrations mentioned (0, 2, 20, 200 mM). To keep the solution's ionic strength at similar levels, NaCl was added to give a final Na^+ concentration of 200 mM in all cathodes. The experimental period started by adding 10 mg-Cr(VI)/L in the cathodes and connecting the two chambers with the external load of 1,000 Ω. N_2 was continuously flushed in both chambers and all experiments were ran at room temperature (22±3 ^oC). To ensure that the anode was not considerably affecting the cathode's performance, acetate was supplied in the anodes before the start of each experiment to increase acetate concentration at around 40 mM. In addition, the anodic potential and pH were monitored; the anodic potential remained at -542 mV ± 20 mV vs. Ag/AgCl under closed circuit conditions, while anodic pH increased during start-up and remained at around 7.9 ± 0.2 throughout operation in all experiments. Results are presented in Figure S1.

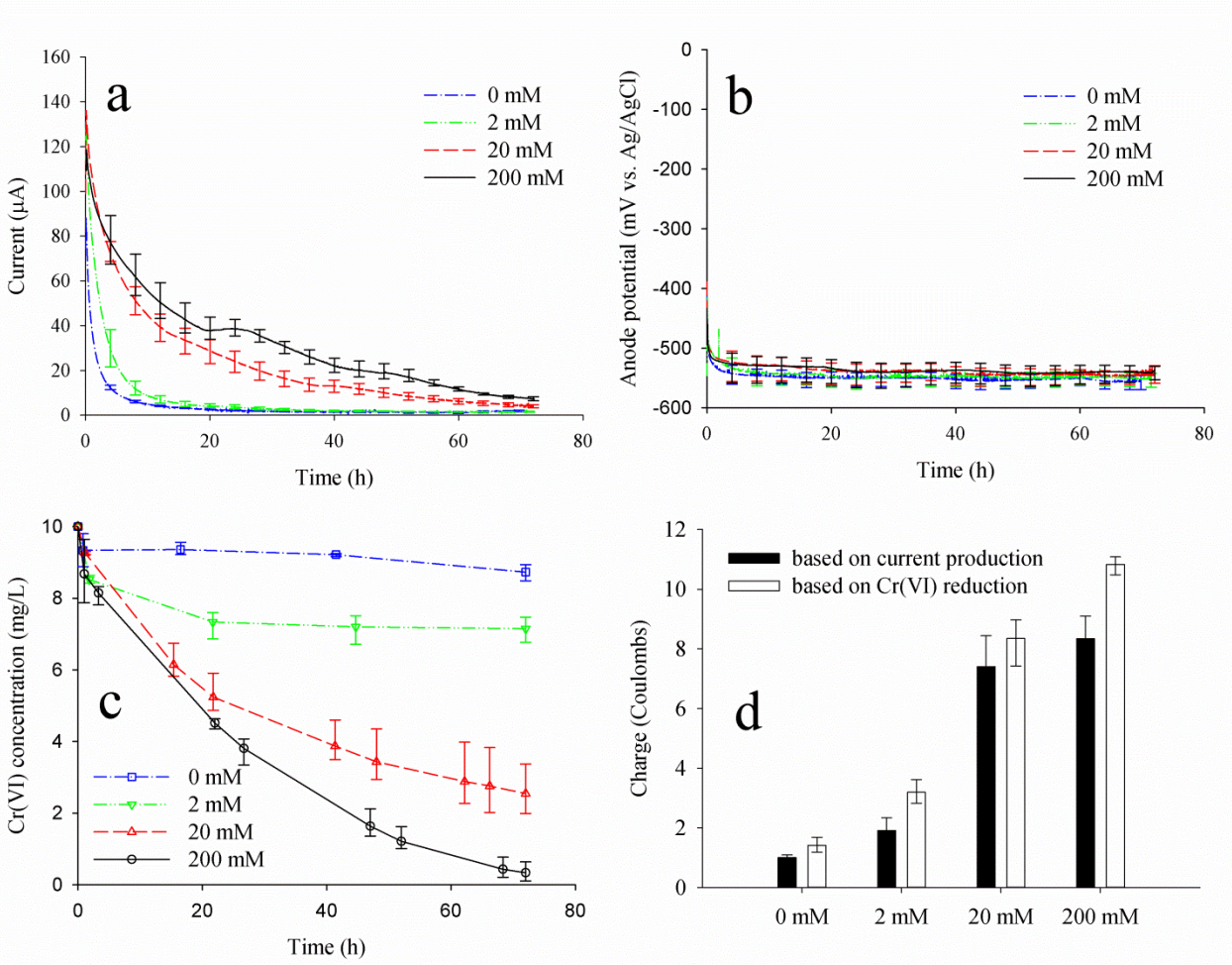

Figure S1. The effect of different lactate concentrations present in the cathode at pH 7. Values reported are average of triplicate experiments and error bars represent the min and max of the measurements from the three reactors running in parallel. (a) current evolution ($R_{ext.}$= 1,000 Ω) (error bars added every 4 h); (b) anode potentials (error bars added every 4 h); (c) Cr(VI) remaining in solution; (d) charge produced by the end of the 72 h operation period (charge calculated based on both current production and Cr(VI) reduction).

Electrochemical activity of MR-1 during phase 1 of potentiostatically controlled cells.

When MR-1 was kept under anaerobic conditions without any external redox mediator addition ($C(+El\text{-}Air)^{ph1}$), lactate consumption caused a current increase during the first 3 h which then stabilised at a level of 40×10^{-3} A/m^2 (Figure S2a). Air flushing ($C(+El)^{ph1}$ reactors with air) had a positive effect, with anodic current production constantly increasing and reaching 270×10^{-3} A/m^2 by the end of phase 1. Enhancement of anodic current by redox mediators was confirmed when a 7-fold higher value (compared to that of $C(+El\text{-}Air)^{ph1}$) was produced and remained stable when 1 μM of riboflavin was added under anaerobic conditions ($C(+El\text{-}Air+Rbf(1))^{ph1}$). On the other hand, anodic current remained close to background levels (around 20×10^{-3} A/m^2) when lactate was not supplied to MR-1 ($C(+El\text{-}Air\text{-}Lac)^{ph1}$). The presence of 20 mg/L Cr(VI) ($C(+El\text{-}Air+Cr(VI))^{ph1}$) totally inhibited anodic current production, and this was reflected in the high open circuit potential observed after phase 1 (+150 mV vs. -350 mV in $C(+El\text{-}Air)^{ph1}$, -400 mV in $C(+El)^{ph1}$ and -430 mV in $C(+El\text{-}Air+Rbf(1))^{ph1}$ under N_2 flushing anaerobic conditions). When MR-1 was aerated and the electrode was poised at -500 mV ($C(-El)^{ph1}$, $C(-El)^{ph1}(-Lac)^{ph2}$, $C(-El)^{ph1}(-Shew)^{ph2}$), oxygen electroreduction was inhibited, as a result of the heterotrophic oxygen consumption (Figure S2b).

CV experiments at the end of phase 1 showed the onset of anodic current at -0.45 to -0.40 mV in the presence of MR-1 (Figure S3). Also, poising the electrode at +300 mV under aerated conditions during phase 1 ($C(+El)^{ph1}$, $C(+El)^{ph1}(-Lac)^{ph2}$, $C(+El)^{ph1}(-Shew)^{ph2}$, $C(+El)^{ph1}(-Lac+Rbf(1))^{ph2}$) gave CV graphs with higher anodic current than when the electrode was poised at -500 mV under aeration ($C(-El)^{ph1}$, $C(-El)^{ph1}(-Lac)^{ph2}$, $C(-El)^{ph1}(-Shew)^{ph2}$) or when phase 1 was kept anaerobic ($C(+El\text{-}Air)^{ph1}$) (Figure S3b).

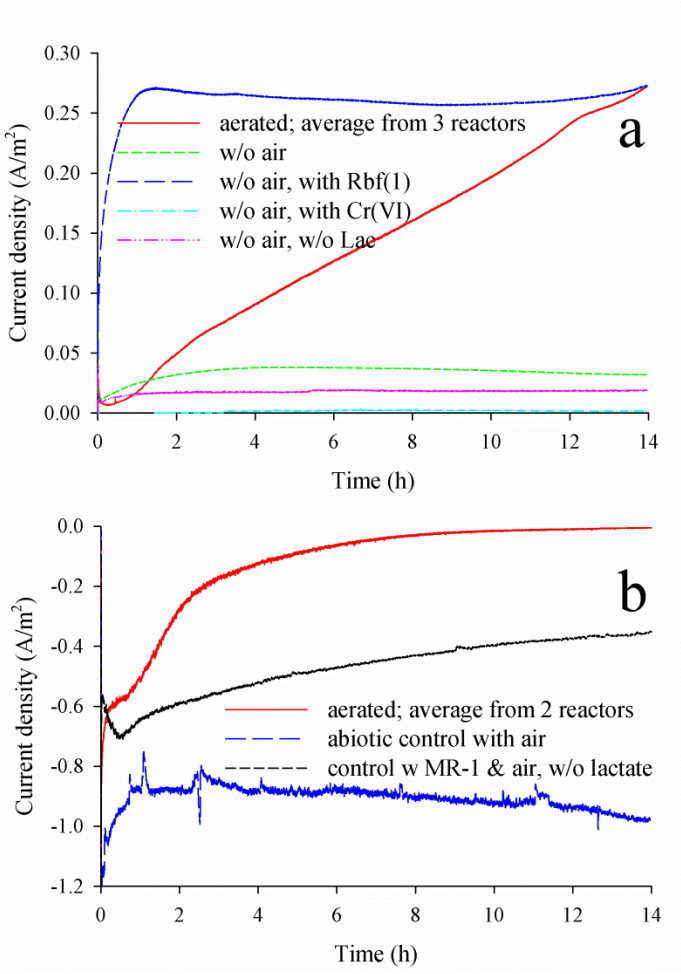

Figure S2. Chronoamperometry profiles at +300 mV (a) and at -500 mV (b) during phase 1 of the potentiostatically controlled experiments.

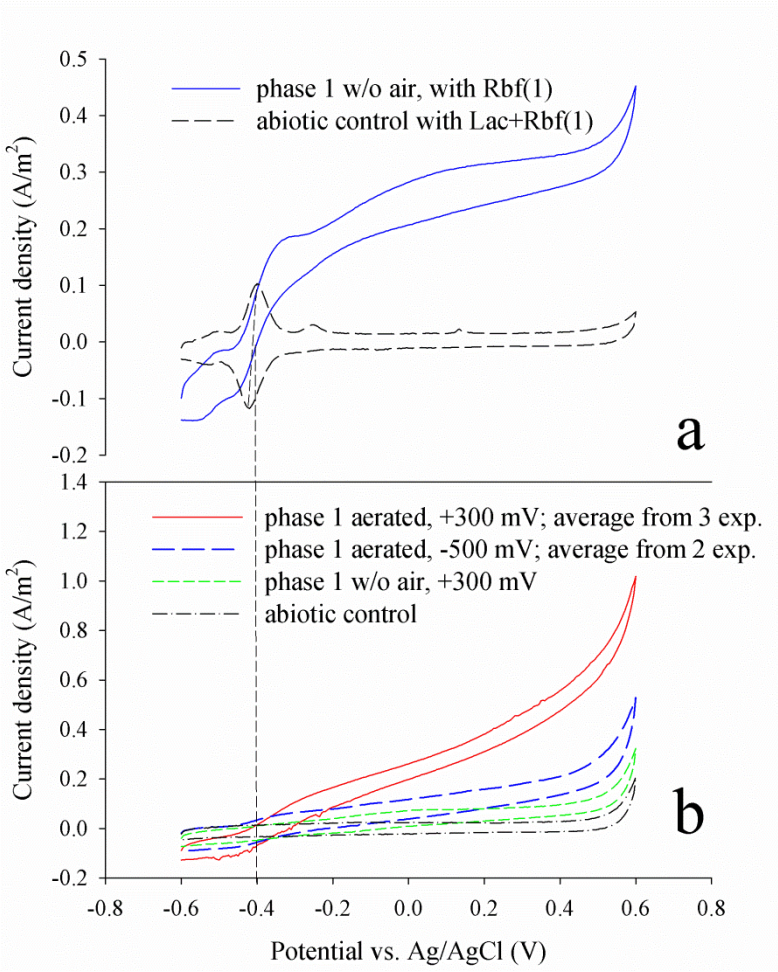

Figure S3. Cyclic voltammograms at the end of phase 1 of the potentiostatically controlled experiments. Scan rate is 5 mV/sec. (a) experiments with riboflavin addition; (b) mediatorless experiments with MR-1 under different pre-treatment conditions.

Flask experiment description

After initial growth in Luria-Bertani-50 mM phosphate buffer medium (pH 7.0), MR-1 was harvested by centrifugation (5,000 x g, 4 ^{o}C, 20 min), rinsed twice with 100 mM NaCl-50 mM phosphate buffer saline (PBS) solution (pH 7.0) and resuspended in 2 × 100 mL of the minimal medium which was amended to contain 30 mM Na-Lactate. The MR-1 containing flasks were then incubated in an orbital shaker incubator (25 ^{o}C, 200 rpm) for 24 h. After 24 h, the flasks' content was centrifuged and then the supernatant was filter-sterilized (0.2 μM pore size) and added into the working electrode chamber of a potentiostatically controlled cell for cyclic voltammetry and chronoamperometry analysis. Also, MR-1 was harvested and resuspended in 200 mL of fresh minimal medium. Both media were flushed with N_2 for 30 minutes before start and continuously afterwards and stirring was applied at 250 rpm only during the chronoamperometry experiment.

Chronoamperometry experiment at -500 mV vs. Ag/AgCl (Figure S4) showed initial current production in the absence of oxygen, which reached around 160 × 10^{-3} A/m^2 during the first 30 minutes of operation and remained considerably stable for 30 minutes afterwards. This initial current shows that soluble compounds released in the medium by MR-1 remained in the oxidized form by the end of the agitation period, during which oxygen was constantly supplied. When MR-1 (1 mL) was added, an increased current production was observed which reached 300 × 10^{-3} A/m^2 within the next hour and remained almost stable for 1 hour afterwards. This increased current production upon MR-1 addition indicated the bio-catalysed reduction of soluble compounds released in the medium by MR-1 during flask growth, and probably also of membrane-associated electroactive compounds added in the medium with MR-1. It should be

noted that this bio-electro-catalysis observed was independent of the presence of 4 mM lactate in the solution, clearly showing that MR-1 can utilise the electrode as an electron donor to reduce these compounds even when lactate is present. Finally current started decreasing by the end of the operation period, indicating complete reduction of the electron acceptor.

The cyclic voltammogram before MR-1 addition (Figure S5) revealed a clear cathodic current which became more apparent at electrode potentials lower than -200 mV vs. Ag/AgCl and which had an increasing rate which reached its maximum at -430 mV vs. Ag/AgCl. After MR-1 addition and reduction of the potential electron acceptors present in solution, the cyclic voltammograms clearly revealed two formal potentials, at -20 mV vs. Ag/AgCl and at -430 mV vs. Ag/AgCl. While the later can be directly attributed to the production of riboflavin and mediated electron transfer, the former, which also had a broad potential window, could be attributed to membrane-related proteins responsible for direct electron transfer.

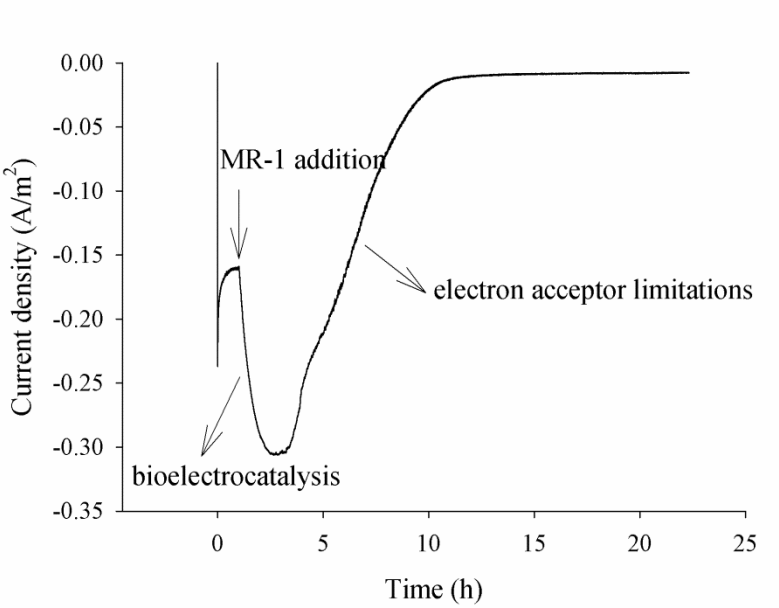

Figure S4. Chronoamperometry profile (-500 mV vs. Ag/AgCl) of the flask's filtered supernatant- effect of MR-1 addition.

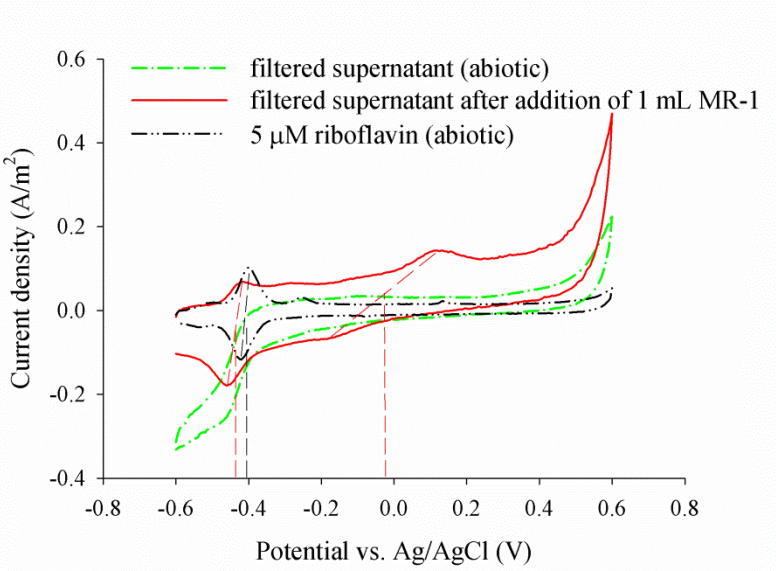

Figure S5. Cyclic voltammograms of the filtered supernatant before and after MR-1 addition and chronoamperometry experiment. Scan rate is 5 mV/sec.

Table S2. Regression parameters of the graphs presented in Figures 5c,d

Figure	Cell #	Equation	R^2
5c	+Lac	1.278*(1-exp(-0.051*x))	0.999
5c	+Lac+Shew (due to current)	7.847*(1-exp(-0.004*x))	0.998
5c,d	+Lac+Shew (total)	6*(1-exp(-0.037*x))+7.847*(1-exp(-0.004*x))	0.987
5d	+Lac+Shew (other than current)	6*(1-exp(-0.037*x))	0.975
5d	+Lac+Shew (flask, OD_{600}=0.8)	8.476*(1-exp(-0.027*x))	0.956
5d	+Shew (flask, OD_{600}=0.8)	3.064*(1-exp(-0.018*x))	0.991
5d	+Lac+Shew (flask, OD_{600}=0.5)	4.254*(1-exp(-0.028*x))	0.997
5d	+Shew (flask, OD_{600}=0.5)	1.557*(1-exp(-0.012*x))	0.929

Cr(VI) reducing flask experiments description

To investigate the ability of MR-1 for lactate-utilizing Cr(VI) reduction, four sets of flask experiments were conducted, each one in duplicate. Two of these sets (one with 30 mM lactate and one without lactate) contained the biomass of MR-1 present in planktonic form in "+Lac+Shew" right before the first Cr(VI) addition (OD_{600}= 0.5 in the 200 mL cathode) and another two sets (one with 60 mM lactate and one without lactate) contained higher biomass concentration (OD_{600}= 0.8). For these experiments, MR-1 was initially grown aerobically in 250 mL Erlenmeyer flasks with LB medium (pH 7.4) for 24 h, centrifuged and washed twice in a 100 mM NaCl- 50 mM phosphate buffer solution (pH 7.0). After washing, MR-1 was resuspended in the flasks to achieve the desired biomass concentration using the minimal medium described in the experimental section. The content of each flask was amended to contain adequate concentrations of lactate at all times and Cr(VI) was supplied each time after depletion, using the same stock solution described in the experimental section. The content of each flask was flushed with filter-sterilised oxygen-free N_2 for 20 min before the first Cr(VI) addition and for 5 min after each Cr(VI) addition. After each Cr(VI) addition, flasks were closed using rubber stoppers and incubated at 22±1 ^{o}C in an orbital shaking incubator (200 rpm). Appropriate sterilisation techniques were followed at all times to avoid contamination of the pure culture.