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Electrochemical communication between heterotrophically grown *Rhodobacter capsulatus* with electrodes mediated by an osmium redox polymer

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ABSTRACT

The metabolically versatile purple bacteria *Rhodobacter capsulatus* was investigated to check its possible applicability in biofuel cells and electrochemical microbial biosensors. The wild type strain ATCC 17015 and mutant strain 37b4 lacking the lipopolysaccharide capsule was compared for their ability to communicate with electrodes modified with an osmium redox polymer. In this work, aerobic heterotrophically grown *R. capsulatus* were used to screen for efficient cell−electrode communication for later implementation using photoheterotrophically grown bacteria. The bacterial cells embedded in the osmium polymer matrix demonstrated efficient electrical "wiring" with the electrodes and were able to generate a noticeable current with succinate as substrate. Interestingly, at 2 mM succinate the wild type strain showed much better bioelectrocatalytic current generation (4.25 μA/cm^2) than the strain lacking capsule (1.55 μA/cm^2). The wild type strain also exhibited a stable current response for longer time, demonstrating that the bacterial lipopolysaccharide in fact enhances the stability of the polymer matrix layer of the modified electrode. Control experiments with *R. capsulatus* without any mediator did not show any current irrespective of the capsule presence. This demonstrates that development of photosensors and other light driven bioelectrochemical devices could be feasible using *R. capsulatus* and will be at focus for future studies.

1. Introduction

The growing interest in microbial fuel cells (MFCs), for both electricity generation and a wide range of other applications [1] and electrochemical microbial biosensors [2], has during the last decade led to an increased attention in "wiring" microbial cells to electrodes to facilitate the electrochemical communication [3–7]. The bacteria are able to transfer electrons to an electrode mainly by the use of various redox mediators (artificial or produced by bacteria) or using direct electron transfer (DET) via cytochrome rich membranes, electrically conductive pili, or nanowires produced by the bacteria [8,9]. Limited bacterial groups such as *Geobacteraceae* [10,11] and *Shewanellaceae* [12–14] possess the ability to communicate electrochemically with the electrodes without any mediator, thanks to the metabolism and electron conducting structures evolved for use in their natural habitat. These bacteria may, however, not be the most suitable for many applications. Consequently, bacteria that are unable to communicate with the electrodes by themselves have been the focus of research during the last decade [2,7,15,16]. Many attempts have been made to enhance the electron transfer by exploring genetic engineering to provide the bacteria with better electron conducting structures or other enhanced metabolic features [6,17–19]. In addition, research on various other influential factors like anode materials, inoculum sources, substrates, separators, cathode, design, operational parameters etc. have resulted into several advancements, increased performance and widened application range for MFC technology [20–25].

In addition to pure microbial cultures MFC research mainly employs electrochemically enriched biofilms of mixed culture or *G. sulferreducens* from different inoculum sources [5,11,26]. Amongst the widely used bacteria for MFC research, very few like *Shewanella* sp. possess metabolic diversity and facultative oxidant tolerance [27,28]. Furthermore, although research has been conducted with different mixed microbial inoculum sources and pure cultures, very few reports introduced new metabolically versatile microbial catalysts for MFCs and whole cell biosensors [5,26]. Moreover, most of the organisms investigated in MFCs so far, are strictly anaerobic [5], which limits their applicability. Therefore, to speed up the development of MFCs and whole cell electrochemical biosensors, there is a necessity to explore alternative versatile bacteria that might increase the types of microorganisms that can be used as possible catalysts at the bioanode or as biocathode [4].

In this work we have investigated the metabolically versatile α-proteobacterium *Rhodobacter capsulatus* [29,30] and tested its capability as a microbial catalyst for MFC and microbial biosensor. *R. capsulatus* is amongst the most nutritionally versatile non-sulfur purple bacteria [31], which have the capacity to grow rapidly under

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either anaerobic photosynthetic conditions or aerobic dark conditions [30,32]. *R. capsulatus* has previously been utilized for anoxygenic hydrogen production [33,34], but this bacterium has never been tried in any electrochemical application. In this study, we used heterotrophically grown *R. capsulatus* under aerobic conditions and tested its ability to communicate electrochemically with osmium (Os) redox polymer modified electrodes. *R. capsulatus* is a Gram-negative organism with an outer and an inner membrane, with a thin peptidoglycan cell wall in between. In addition, wild type *R. capsulatus* possesses an outer capsule of slimy lipopolysaccharides [35,36]. The exact composition of the lipopolysaccharide layer varies among different *R. capsulatus* strains [37], but the capsule presence as such may interfere with the polymer used for communication with the electrode and increase the distance from the outer membrane surface to the electrode surface. Therefore, the mutant strain 37b4 that does not produce any lipopolysaccharide capsule [38] was compared to the wild type *R. capsulatus*.

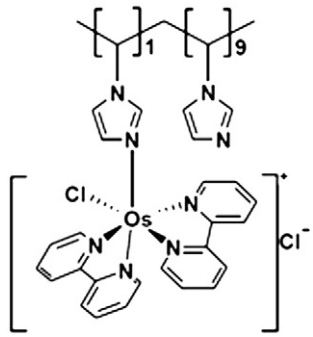
The use of flexible Os redox polymers in biofuel cells (BFCs) or biosensors has gained attention due to the efficient electron shuttling properties, polymeric structure, a stable adsorption in addition to the possibility to lead to the formation of multiple layers of both immobilized enzymes and microbial cells on the electrode surface [6,39–41]. Our earlier studies with such polymeric mediators showed an enhanced electron transfer between different microbial cells and electrodes [7,15,17,18]. Therefore, the current research is a logical continuation of previous work from our group involving the electrochemical communication between different bacteria and electrodes using Os polymers. For this study, an Os polymer with a high positive redox potential (0.176 V vs. SCE; 0.221 V vs. Ag|AgCl, sat. KCl) was chosen, because of its proven ability of efficient wiring of enzymes to electrodes [42,43]. This Os polymer has a high redox potential and a short length of the side chains, where the $Os^{3+/2+}$ functionalities are located at their ends. In addition, the advantage of using high redox potential polymers is that they efficiently compete with O_2 as electron acceptor [18].

Herein, the electrochemical communication of the wild type and capsule lacking mutant strains of *R. capsulatus* with Os redox polymer modified electrodes was investigated using cyclic voltammetry (CV) measurements. Additionally, the current response characteristics of the bacterial cell modified Os polymer graphite electrodes were evaluated for succinate as substrate in flow analysis mode. Succinate was chosen as this substrate can be used both for heterotrophic and phototrophic *R. capsulatus* cells. The succinate:quinone oxidoreductase enzyme has not been biochemically characterized in *R. capsulatus*, but primary sequence comparison reveal it to be similar to that of *Paracoccus denitrificans* [44] and thus belonging to class 1 [45]. Furthermore, the ability of *R. capsulatus* to communicate with the electrode by itself through a DET mode was also investigated in potentiostatically controlled half-cell set-ups. Besides, the electron transfer efficiency of the Os polymer was compared with that of a soluble mediator, hexacyanoferrate. The stability of the current response for *R. capsulatus* with Os polymer modified electrodes was carefully considered throughout the experiments. Both gold electrodes protected by a thiol based self assembled monolayer as well as graphite electrodes were used in line with previous investigations [7,18].

2. Experimental

2.1. Chemicals

$[Os(2,2'\text{-bipyridine})_2\text{-poly(N-vinylimidazole)}_{10}Cl]^{2+/+}$ (poly[$Os(bpy)_2(PVI)_{10}Cl]^{2+/+}$) of E° equal to +176 mV vs. SCE (sat. KCl)/+221 mV vs. Ag|AgCl (sat. KCl) ("Scheme 1") was synthesized as reported in [46]. The weight-average molecular weight of the poly(vinylimidazole) as determined by viscometry in ethanol was 10,000 g/mol [47]. All chemicals were purchased from Sigma–Aldrich/Merck and were of either research or analytical grade. All aqueous solutions were prepared by using water purified and deionized (18 MΩ) with a Milli-Q system (Millipore, Bedford, MA, USA).



Scheme 1. Structural representation of the osmium redox polymer.

2.2. Microbial growth conditions and inoculum preparation

R. capsulatus strains, ATCC 17015 (wild type) and 37b4 (capsule lacking mutant) were purchased from DSMZ – Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (Braunschweig, Germany). These strains were grown and maintained on minimal peptone yeast extract medium (MPYE) agar plates [29]. A single, well isolated colony was used for inoculum preparation throughout the study. For inoculum preparation, the bacterial cells were grown aerobically in 10 mL of MPYE broth (in 50 mL baffled E-flasks at 200 rpm) for 20 h at 30 ± 2 °C by transferring 1 mL of cell suspension prepared by suspending an isolated colony into the saline (0.85% NaCl). The cells grown in MPYE were harvested in the early stationary growth phase by centrifuging the broth at 4000 rpm for 10 min. Further, the cells were washed once in 20 mM MOPS (3-morpholinopropanesulfonic acid) buffer (pH 7.4), centrifuged again as before, resuspended in the same buffer to adjust cell density to 1 g/mL (wet weight) [48] and used immediately for electrochemical experiments (CV and amperometric flow mode measurements).

For batch mode chronoamperometry (CA) experiments the cells harvested from the growth medium were transferred to 200 mL of synthetic 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; $NaH_2PO_4 \cdot H_2O$ 0.6 g/L; NaCl 5.8 g/L; mineral solution 10 mL/L; vitamin solution 10 mL/L; amino acid solution 10 mL/L [49]. The substrate used was 10 mM succinate (disodium salt). After incubation of flasks at 30 ± 2 °C (500 mL baffled E-flasks, aerobically while shaking at 200 rpm for 72 h) the cells were harvested and used as an inoculum in potentiostatic half-cell set ups hosting a similar synthetic medium. These experiments were performed to check the capability of *R. capsulatus* to communicate with an electrode without any mediator.

2.3. Preparation of the electrodes modified with bacteria

Either gold (BAS, West Lafayette, IN, USA) or spectrographic graphite rods (Ringsdorff Werke GmbH, Bonn, Germany, type RW001, 3.05 mm diameter and 13% porosity) were used as working electrodes. The procedure explained by Coman et al. [18] was followed to prepare the modified working electrodes. Polycrystalline gold electrodes (projected surface area; 0.02 cm^2) were electrochemically cleaned by cycling in 0.1 M NaOH between 0 and −1000 mV vs. NHE, followed by mechanical polishing on Microcloth (Buehler, Lake Bluff, IL) in an aqueous alumina UF slurry (1 and 0.1 μm, Struers, Copenhagen, Denmark). The electrodes were rinsed with water, ultrasonicated for 5 min in Milli-Q water, followed by cycling in 0.5 M H_2SO_4 between 0 and +1950 mV vs. NHE, and finally rinsed with Milli-Q water. Formation of a self-assembled monolayer (SAM) of aldrithiol at the electrode surface was done by immersing the electrode in a saturated aqueous solution of

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the thiol for 2 h (at room temperature). In the following step, a 5 μL portion of Os redox polymer solution (10 mg/mL in Milli-Q water) was spread over the surface of the modified gold electrode and water was allowed to evaporate at room temperature for 30 min. Finally, a 5 μL portion of *R. capsulatus* suspension in 20 mM MOPS buffer (pH 7.4) was placed on the modified electrode surface. The droplet was allowed to gently dry at room temperature. Before use, a dialysis membrane (Spectrum Laboratories Inc., Rancho Dominguez, CA, USA, molecular mass cutoff: 6000–8000) was used to trap the bacterial cells onto the surface to prepare a permselective membrane electrode [50]. The dialysis membrane (presoaked in buffer) was pressed onto the electrode and fixed tightly to the electrode with a rubber O-ring and Parafilm.

In case of graphite electrodes, the material was polished on wet emery paper (Tufbak, Durite, P1200) and subsequently carefully rinsed with Milli-Q water and dried. Then 5 μL of Os polymer solution (10 mg/mL in Milli-Q water) was spread onto the entire active surface of the electrode (0.0731 cm^2). The electrode was dried at room temperature for 10–15 min and then 5 μL of the cell suspension was spread onto the surface and further gently dried until a layer was formed on the surface (10–15 min).

2.4. Measurement set-ups

The amperometric measurements were done in flow modes at room temperature (22 ± 2 °C) with disodium succinate as electron donor for the *R. capsulatus* modified electrode mounted in a standard three electrode flow through electrochemical wall jet cell containing the working electrode (graphite electrode modified with Os polymer and cells) as well as a Pt counter and an Ag|AgCl (0.1 M KCl) (Beta Sensor AB, Södra Sandby, Sweden) reference electrode controlled with a small potentiostat (Zäta Elektronik, Höör, Sweden). The procedure explained elsewhere was followed to perform flow mode experiments [18]. The modified electrode was press fitted into a Teflon holder and inserted into the wall jet cell and kept at a constant distance (ca. 1 mm) from the inlet nozzle. The flow rate of the solutions was maintained at 0.5 mL/min with a peristaltic pump Minipuls 2 (Gilson, Villiers-le-Bel, France). The response currents were recorded on a strip chart recorder (Kipp & Zonen, Delft, The Netherlands). The injector was an electrically controlled six-port valve (Rheodyne, Cotati, CA, USA), and the injection loop volume was 50 μL. The MOPS buffer (20 mM MOPS, 0.1 M KCl; pH 7.4) used as electrolyte throughout the study, was carefully degassed under vacuum prior to experiments. The reported results are based on three equivalently prepared electrodes and the relative standard deviation was always less than 10%.

To test the competition theory, the efficiency of the Os polymer was also compared with a freely diffusing mediator, hexacyanoferrate (III) (0.236 V vs. Ag|AgCl; sat. KCl) by continuously pumping 2 mM hexacyanoferrate (III) solution prepared in 20 mM MOPS buffer with 2 mM succinate into the flow cell. Furthermore, the stability of the Os polymer modified graphite electrode with the *R. capsulatus* cells was checked by monitoring the current response with continuous pumping of 2 mM succinate with flow buffer (20 mM MOPS) for a few hours through the flow cell.

CV measurements were performed using an AUTOLAB PGSTAT 30 (EcoChemie, Utrecht, The Netherlands) equipped with GPES 4.9 software with a three-electrode configuration using an Ag|AgCl reference electrode (sat. KCl, Sensortechnik Meinsberg, Germany) and a counter electrode made of a platinum foil. These experiments were performed with both gold and graphite as working electrodes. In case of modified gold electrodes, a SCE (sat. KCl) was used as reference electrode. If not stated otherwise, these measurements were performed at a scan rate of 2 mV s^{-1}. All CV experiments were performed under anoxic conditions (to exclude possible competition by O_2 as electron acceptor), which were achieved by sparging argon for 20 min in the buffer solution. Furthermore, the headspace of the solution was also sparged with argon during the CV measurements. All reported data are based on at least three independent replicates of each experiment.

Batch mode CA experiments with succinate were performed to check the ability of *R. capsulatus* to communicate with the electrode by itself without the use of any mediator/Os polymer using optimum growth temperature of 30 ± 2 °C at anoxic conditions. These experiments were executed with a three-electrode configuration using bare graphite rods as working as well as counter electrodes and an Ag|AgCl reference electrode (sat. KCl). The working electrode was constantly polarized at positive potential of + 0.2 V (vs. Ag|AgCl) to check the ability of the bacteria to utilize the electrode as an electron acceptor. The sealed, water jacketed glass vessels, connected to a thermostat (Paratherm U2 electronic, Julabo, Schwarzwald, Germany) were employed as electrochemical cells that allowed maintenance of strictly anoxic conditions as well as temperature control. These CA experiments performed with bare graphite electrode demonstrated the incompetence of *R. capsulatus* to communicate with the electrode without any mediator (data not shown).

3. Results and discussion

The electrochemical behavior of the used Os polymer was characterized using CV. The CV response of the Os polymer adsorbed on the surface of an aldrithiol modified gold electrode obtained in 20 mM MOPS buffer at pH 7.4 with and without bacterial cells is depicted in Fig. 1. As can be seen in the CV (Fig. 1A), the Os polymer shows a pair of well-defined redox peak for the Os redox center in the polymer. The E°-value of the Os polymer was evaluated by taking the mean value of the anodic and cathodic peak potentials of the CV and was found to be + 180 mV vs. SCE (sat. KCl), a value in close agreement with previous results [43,46]. In comparison to the electrode modified with only Os polymer, the adsorption of bacterial cells into the polymer matrix retards the redox response of the Os center of the polymer as displayed by the drastic decrease in the peak current values in the CV (Fig. 1B). The decrease in the current response in the presence of bacterial cells is attributed to the fact that the cationic Os polymers form strong electrostatic complexes with the anionic cell wall coating of the viable bacterial cells. As a consequence, the mobility of the individual redox units get hampered that leads to the lowering of the current response, which is well in accordance with previous investigations with bacterial cells and redox enzymes [18,43]. Furthermore, it is also reasonable that the interaction between bacterial cells and Os polymer reduces the mobility of the segments of

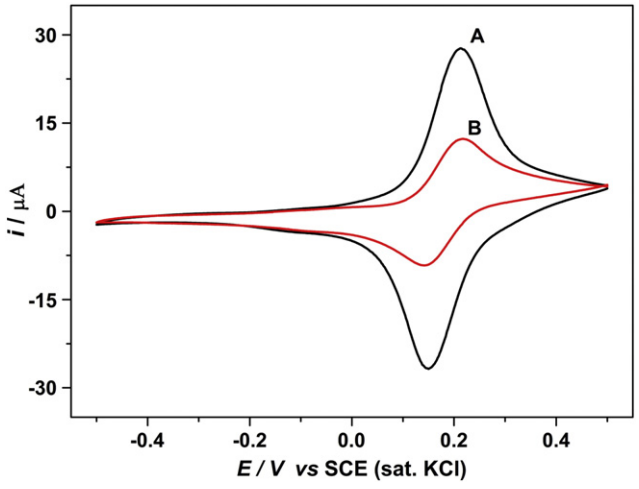

Fig. 1. Cyclic voltammograms of the Os polymer adsorbed at an aldrithiol modified gold electrode, in the (A) absence and (B) presence of bacterial cells. Experiments were performed in a 20 mM MOPS buffer (0.1 M KCl) at pH 7.4. The scan rate was 50 mV s^{-1}.

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the Os polymer and thus the flow of electrons between the $Os^{3+/2+}$ redox centers (electron self-exchange) of the polymer resulting in lowering of current response as observed in the CV. However, as can be seen in Fig. 1B, the bacterial cells do not influence the redox potential of the Os polymer.

Further CV measurements with Os polymer modified graphite electrodes exhibited similar electrochemical behavior with and without wild type *R. capsulatus* cells (Fig. 2B and C). Fig. 2 depicts the comparative CVs with bare graphite (A), Os polymer modified graphite (B), Os polymer + cell modified graphite (C) in 20 mM MOPS buffer and Os polymer + cell modified graphite in 20 mM MOPS buffer with 2 mM succinate (D) at 2 mV/s scan rate. In comparison to the CV response observed in previous studies with *Gluconobacter* sp. and *Pseudomonas* sp. [7,15], the response was not pronounced for *R. capsulatus* in the presence of substrate, which might be due to the mass transfer and/or kinetic limitations. However, at a constant applied potential the wild type *R. capsulatus* with the Os polymer modified graphite electrode showed steady state current generation at different substrate concentrations (Figs. 3A & 4A). The increased (although lower than previously observed with other bacteria) current response in presence of substrate (as can be seen in CV in Fig. 2D) in comparison to the CV obtained in absence of substrate (Fig. 2C) and steady state current generation at constant potential (Fig. 3A inset) indicates the bioelectrocatalytic activity and thus the electrochemical communication between viable *R. capsulatus* with the Os polymer modified graphite electrode.

The electrical wiring of *R. capsulatus* with electrodes is due to the strong electrostatic interactions between the negatively charged bacterial cells and the positively charged adsorbed Os polymer matrix. Therefore, the electron transfer can be attributed to the sequential electrical conduit starting from intracellular redox proteins to external Os redox centers at the polymer matrix and finally towards the electrode surface (as illustrated in "Scheme 2"). *R. capsulatus* lacking the capsule also exhibited similar electrochemical behavior with CV measurements (data not shown). Control experiments with an electrode only modified with wild type *R. capsulatus* cells in a solution containing succinate did not reveal any current generation (data not shown) indicating the necessity of Os-polymer for successful bioelectrocatalysis.

The quantitative evaluation of the electron transfer efficiency and thus, bioelectrocatalytic performance of *R. capsulatus* was investigated with amperometric measurements by applying a constant potential. For the Os polymer modified electrodes, a +300 mV vs. Ag|AgCl (0.1 M KCl) potential was applied (a potential value sufficiently more positive than the E°′ value of the polymer, to guarantee a potential independent response). The viability and stability of the cells trapped at the Os polymer modified electrode surface is a critical issue. Therefore, the operational stability of the Os polymer modified graphite electrodes with both *R. capsulatus* strains in the flow cells was investigated by monitoring the current response of the system with 2 mM succinate. After initial adjustment of the flow system for a stable baseline (for 30–45 min) with only buffer, the system was continuously fed with succinate. We observed a stable current generation (~4.25 μA/cm^2) for about 4 h with wild type *R. capsulatus* (Fig. 3A inset). Afterwards, a continuous decrease in current response was observed with time and reached approximately 35% of the initial response after 18 h (Fig. 3). The decrease in current response can be attributed to the lowering of the microbial substrate oxidation. This is due to a gradual decrease in the number of active cells on the electrode surface. As we provide only carbon source but no other nutrients in the flow buffer, the cells do not multiply as they do naturally in presence of complete growth medium. Therefore the cell number continues decreasing with time. Also there is detachment or washout of the cells from the electrode, in particular with mutant cells. In order to check the role of the continuous supply of succinate on the generated current the pump was stopped after approximately 18 h, which resulted in a drastic decrease in the current response. It is well known that the lipopolysaccharide capsule layer helps many organisms to adhere to the substratum very firmly and lead to biofilm formation [51]. Thus, the stability of wild type *R. capsulatus*

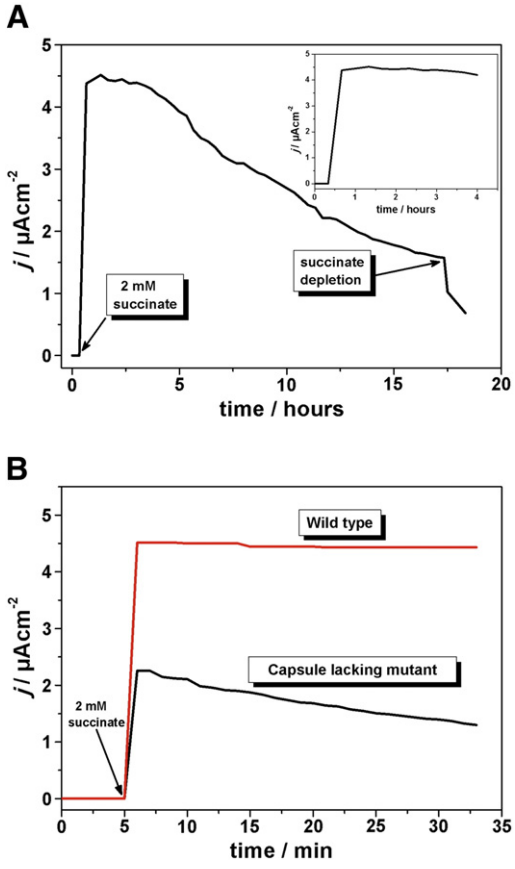

Fig. 3. (A) Stability characteristics (current density vs. time) for wild type *R. capsulatus* cells on osmium redox polymer modified electrode. Measurements were performed in flow mode (0.5 ml/min). Inset figure shows the stable current generation for approximately 4 h. (B) Comparison between current density responses of intact wild type and capsule lacking *R. capsulatus* cells mediated by osmium redox polymer (E_{app} = +300 mV vs. Ag|AgCl, 0.1 M KCl) adsorbed on graphite electrode. Measurements were performed in flow analysis mode after removing oxygen from the solution.

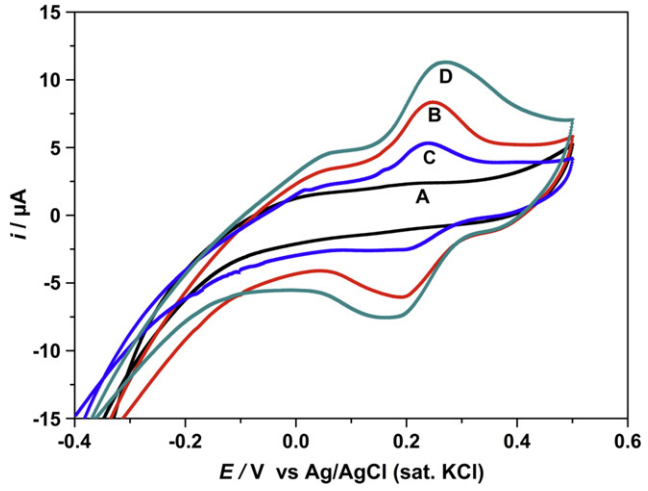

Fig. 2. Cyclic voltammograms of the (A) bare graphite (B) Os polymer modified graphite (C) Os polymer modified graphite in the presence of cells in only 20 mM MOPS buffer (0.1 M KCl) at pH 7.4 and (D) Os polymer modified graphite in the presence of cells with 2 mM succinate in 20 mM MOPS buffer. The scan rate was 2 mV s^{-1}. The measurements were conducted with wild type *R. capsulatus*.

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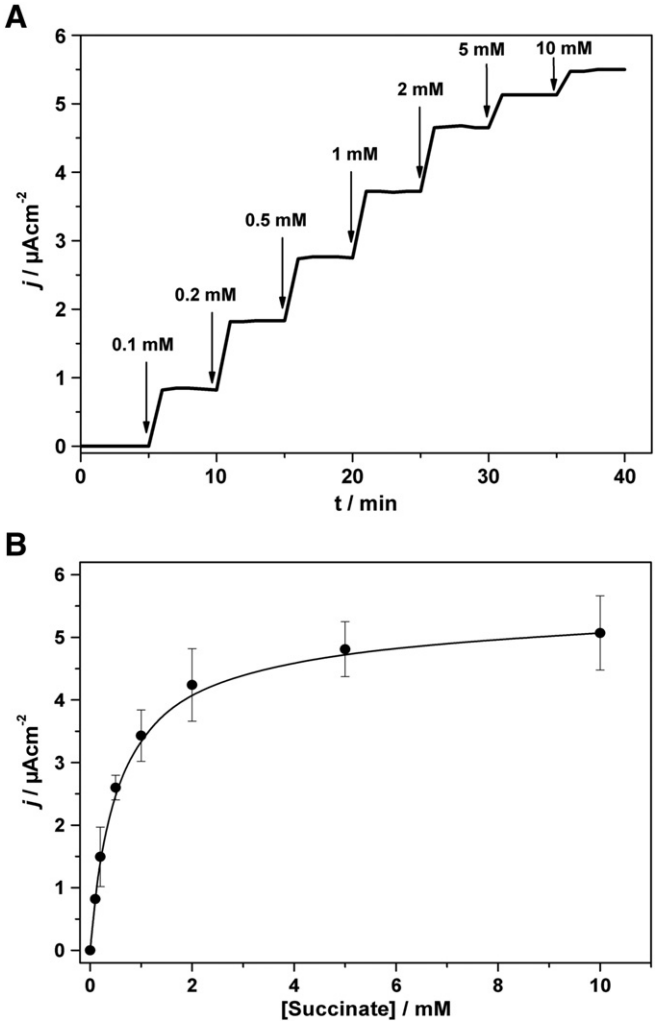
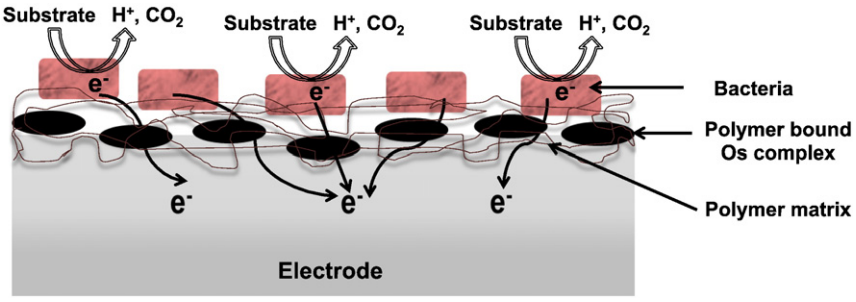


Fig. 4. (A) Current density response of intact wild type *R. capsulatus* cells mediated by osmium redox polymer ($E_{app} = +300$ mV vs. Ag|AgCl, 0.1 M KCl) on graphite electrode at different concentrations of succinate (indicated by arrows) in 20 mM MOPS buffer. Measurements were performed in flow analysis mode. (B) Dependence of the bioelectrocatalytic current densities on the succinate concentration. The data are derived by depicting the steady state current from experiments analogue to those presented in panel A.

can be attributed to the outer capsule layer, which helps the biofilm to remain intact and thus stable for a long time at the Os polymer matrix adsorbed on the electrode. In comparison, with the capsule lacking *R. capsulatus* the current response was stable for less than 1 h and reached approximately 10% of the initial response after 3 h (data not shown). This indicates the wash out/detachment of physically adsorbed capsule lacking bacterial cells from the graphite electrode during continuous flow operation. As a consequence, with regard to the stability observations all flow mode experiments were planned and executed within 1 h and 4 h (after a stable baseline was obtained) for capsule lacking and wild type strains respectively. Furthermore, as can be seen from Fig. 3B, the wild type strain (~4.25 µA/cm^2) exhibited almost a 3.5 times higher bioelectrocatalytic current generation than the capsule lacking strain (~1.55 µA/cm^2) with 2 mM succinate in flow mode measurements. The superior current response with wild type compared to capsule lacking *R. capsulatus* can be attributed to their stability and conductive capsule — polymer matrix. Although Os polymers exhibit excellent communication with the electrode, these findings raise the concerns about their stability as mediators and their applicability in BFCs. We anticipate that the use of some non-toxic cross-linking agents may increase the long term stability of the Os polymer electrodes.

Fig. 4A shows the dependence of the bioelectrocatalytic current generation on the substrate concentration. After adjustment of the flow cell to continuous flow conditions with only buffer, the system was exposed to a stepwise increase in the succinate concentration after every 5 min interval. It can be clearly seen that the current density increases with increasing substrate concentration up to 10 mM. The subsequent pumping of ≥10 mM succinate in the system resulted in a slight decrease in the current response (data not shown). The decrease in the current signal at high substrate concentrations could be attributed either to product inhibition or pH changes inside the cell. The maximum bioelectrocatalytic activity (~5.5 µA/cm^2) was achieved at 10 mM succinate. Fig. 4B summarizes the results of experiments similar to those shown in Fig. 4A by depicting the succinate concentration dependence of the steady state current densities. Furthermore, one can also see the linear dependence of the current response on the succinate concentration at low range (up to 2 mM). A similar kind of a linear dependence of the current response on the succinate concentration in the µM (5–200) range was observed for capsule lacking *R. capsulatus* (data not shown) and also have been reported by Coman et al. for *Bacillus subtilis* [18].

The efficiency of the Os polymer was also compared to that of a freely diffusing mediator, hexacyanoferrate (III). In this case, the system was polarized at a potential of +300 mV for the Os polymer modified electrode and the results were compared (Fig. 5). Once a stable baseline was obtained with only buffer, a 2 mM succinate containing buffer was continuously pumped into the system. Immediately after a stable current response with 2 mM succinate for approximately 10 min was obtained, a flow buffer containing 2 mM hexacyanoferrate (III) in addition to 2 mM succinate was pumped to the system. As expected, the low molecular weight and freely diffusing monomeric hexacyanoferrate (III) proved to be a more efficient mediator compared with the Os polymer. As can be clearly deduced from Fig. 5, approximately a five times increased current was observed with hexacyanoferrate (III), when using 2 mM succinate as substrate with capsule lacking *R. capsulatus* cells. A similar hexacyanoferrate (III) effect was observed with wild type *R. capsulatus* (data not shown). Furthermore, the observations are well in agreement to the previously obtained results with *Gluconobacter oxydans* [7] and with engineered *Bacillus subtilis* [18].



Scheme 2. Proposed extracellular electron transfer from the viable bacteria to the electrode mediated by an osmium redox polymer.

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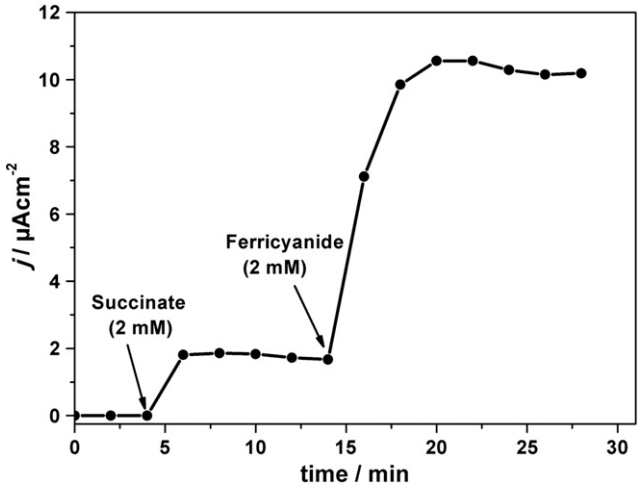

Fig. 5. The effect of ferricyanide on the current density generated by the osmium polymer mediated capsule lacking *R. capsulatus* cells modified graphite electrode. The applied potential was +300 mV vs. Ag|AgCl (0.1 M KCl) and the rest of the conditions were similar as mentioned before.

Here it has to be stressed that the *R. capsulatus* cells used within this study were directly harvested from the MPYE medium without pre-adjustment of the cells to succinate as substrate. Any prior supplementation of substrate into the MPYE during inoculum preparation might considerably increase the response of the bacteria with respect to that substrate. The immobilization of the bacteria/mediator on the electrode and ensuring an efficient electron transfer from the enzyme/bacteria to the electrode via the mediator are decisive features of mediated electrochemical biosensors. Based on these results and our previous research, we anticipate that the use of Os polymers as electron shuttles in whole cell biosensors and MFCs can enable the possibility of exploration of wide range of microbial catalysts for both anode and biocathode applications. With further investigations, we also assume that the use of Os polymer modified electrodes may simplify the design and the use of electrochemical microbial biosensors, particularly for on-site applications and for flow systems.

4. Conclusion

In this study we have demonstrated the possibility to "wire" heterotrophically grown *R. capsulatus* to both gold and graphite electrodes with the aid of an Os redox polymer. Wild type *R. capsulatus* cells embedded in the Os polymer matrix demonstrated their ability to generate a significant current with succinate as substrate. The study further showed the linear dependence of the current response on substrate concentration (in the μM range). Although stability is a main concern, the results of this study are encouraging for the possible exploitation of non-exoelectrogens as biocatalysts in microbial biosensors and BFCs with Os polymers. Further work is in progress to exploit photoheterotrophically grown *R. capsulatus* at anoxic conditions for phototrophic MFCs.

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