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Development of an Osmium Redox Polymer Mediated Bioanode and Examination of its Performance in *Gluconobacter oxydans* Based Microbial Fuel Cell

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Abstract: Gluconobacter oxydans (*G. oxydans*) cells together with an osmium redox polymer (ORP) [Osmium (2,2'-bipyridine)2(poly-vinylimidazole)10Cl]Cl were combined with a glassy carbon paste electrode (GCPE) to form a bioanode for a microbial fuel cell (MFC) based on *G. oxydans*. Although there are *G.oxydans*/ ORP combined bioanode in the literature, as far as it is known, this system is the first one where *G.oxydans*/ORP bioanode is combined with a cathode and a MFC is formed. After the optimization of experimental parameters, analytical characteristics of ORP/*G. oxydans*/GCPE bioanode were investigated. ORP/*G. oxydans*/GCPE showed two linear ranges for ethanol substrate as 1.0–30 mM ($R^2=0.902$) and 30–500 mM ($R^2=0.997$) and analytical range as 1.0–1000 mM. Limit of detection (3.0 s/m) and limit of quantification (10 s/m) values were calculated as 1.29 mM and 4.30 mM respectively where the RSD value was 1.16 % for n = 5. Combining the developed bioanode in the presence of 5.0 mM $K_3Fe(CN)_6$ mediator with a Pt wire cathode a double compartment MFC was obtained via a salt bridge. *G. oxydans*/GCPE bioanode based MFC had maximum power density of 0.133 μW cm^{-2} (at 33.5 mV), maximum current density as 8.73 μA cm^{-2} and OCP value of 156 mV. On the other hand, ORP/*G. oxydans*/GCPE based MFC showed maximum power density as 0.26 μW cm^{-2} (at 46.8 mV), maximum current density as 15.079 μA cm^{-2} and OCP value of 176 mV.

Keywords: microbial biofuel cell · osmium redox polymer · *Gluconobacter oxydans* · Glassy carbon paste electrodes

1 Introduction

Microbial fuel cells (MFCs) convert chemical energy into electricity by using microbial organisms as catalysts [1,2]. In biofuel cells including MFCs, there are some aspects that affect the performance of the developed systems. Anode materials [3], inoculum sources [4], substrates [5], separators [6], cathode type, cell design [7], operational parameters and modification including genetic engineering of bacteria are examples to influential factors that enhance the efficiency of electron transfer in MFCs [8]. For this reason, it is important to modify microorganism for increment of their catalytic properties, which may lead to more efficient MFC. Bacteria can use artificial redox mediators for extracellular electron transfer. Also, some types of microorganisms facilitate direct electron transfer with electrodes with the help of their cytochrome rich outer membranes, electrically conductive pili, nanowires [8, 9] or their own electron conducting mediators that are produced by themselves [8, 10–15]. The other important process that involves electron transfer which are widely used in bacteria based electrochemical systems is a "wiring" process that includes redox polymers like osmium redox polymers (ORP). With ORP it is possible to produce periplasmic membrane-bound PQQ dependent enzymes that can well oxidize a wide range of substrates. The efficient wiring was attributed to electron transfer between PQQ dehydrogenases and the ORP [8, 16]. Vostiar et al. pioneered this process by using a flexible polyvinylimidazole osmium functionalised polymer for electrical wiring of *Gluconobacter oxydans* (*G. oxydans*) cells [16]. Also Katrlik et al. developed two different *G. oxydans* based microbial biosensors based on either ferricyanide or a flexible polyvinylimidazole osmium functionalized polymer [17]. Electrochemical communication between *G. oxydans* cells and the electrode surface can also be accomplished by a series of hydrophobic mediators like ferrocene, 1,1′-dimethylferrocene, 2,5-dibromo-1,4-benzoquinone, and 2-methyl-1,4-benzoquinone [18].

In this work, a glassy carbon paste electrode (GCPE) was modified with *G. oxydans* cells and ORP to construct a bioanode. The amounts of ORP and *G. oxydans* cell

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were optimized and the analytical characteristics of the ORP/*G. oxydans*/GCPE bioanode were investigated. The effect of addition of a soluble low molecular weight mediator, $K_3Fe(CN)_6$, was also investigated. The ORP/*G. oxydans*/GCPE bioanode in pH 6.5 phosphate buffer anolyte was combined with a Pt wire cathode in pH 3.5 phosphoric acid solution via a salt bridge. As a result, a double compartment MFC was formed. Ethanol was used as substrate for the bioanode. Although there are several biosensor studies that stated the *G. oxydans* cell wiring and concluded as promising bioanodes [16–20], as far as we know this is the first study where a bioanode containing *G. oxydans* cells with ORP and $K_3Fe(CN)_6$, is used in a MFC system.

2 Experimental

2.1 Chemical Reagents

Phosphate buffer solution was prepared from KH_2PO_4 (99.995 % pure, Merck). 1.0 M NaOH (97 % pure, Merck) and 1.0 M HCl were used for pH adjustments. MFC measurements were carried out in an aerobic double compartment system that contained an anolyte (100 mM phosphate buffer at pH 6.5), a catholyte (100 mM phosphoric acid solution at pH 3.5) and a salt bridge (10 mL of an aqueous solution containing 0.3 g of agar (Sigma) and 3.0 g of $KCl_{(s)}$ (Sigma) was filled into the salt bridge). Ethanol was used as substrate (99.9 % pure, Merck). Yeast extract granulated and D(+)glucose monohydrate (www.merckmillipore.com, Germany) were used at bacterial growth medium of *Gluconobacter oxydans* (*G. oxydans*). The [Osmium (2,2′-bipyridine)$_2$(poly-vinylimidazole)$_{10}$Cl]Cl (ORP) was synthesized by adapting previously published protocols [21, 22] and used for wiring of *G. oxydans* cells as a redox mediator.

$K_3Fe(CN)_6$ was also used as a mediator probe for electrochemical measurements and purchased from Sigma (www.sigmaaldrich.com, USA). Glassy carbon spherical powder (2.0-12 μm, Merck) and mineral oil (Sigma-Aldrich) were used for preparation of GCPE [23–26].

2.2 Instruments and Measurements

A μ-AUTOLAB TYPE III electrochemical analyzer was equipped with GPES/FRA and used for the electrochemical measurements (www.metrohm-autolab.com). A standard three-electrode cell containing a platinum wire as the auxiliary electrode, an Ag|AgCl (Ag|AgCl/KCl (1.0 M)) (filled with 1.0 M KCl, METROHM (www.metrohm.com)) as the reference electrode and either a *G. oxydans*/GCPE or an ORP/*G. oxydans*/GCPE was used as the working electrode. The electrodes were inserted into a conventional electrochemical cell. An IKA-CMAG HS 7 magnetic stirrer with heating was used to keep the temperature at 28 °C. Cyclic voltammetric measurements were recorded in a 10 mL cell filled with 100 mM phosphate buffer (pH 6.5) between −0.4 and 1.0 V with a scan rate of 50 mV s^{-1}. 5 mM $K_3Fe(CN)_6$ mediator and 10 mM ethanol substrate were added into this electrochemical cell during the measurements. Chronoamperometric measurements were recorded at 300 mV for 300 s duration time in the absence and presence of 5.0 mM $K_3Fe(CN)_6$ in the phosphate buffer (pH 6.5) at 28 °C under stirring conditions. An Autoranging Mini Multimeter (MN16A) was equipped for current and voltage measurements of the developed MFCs. A *G. oxydans*/GCPE or an ORP/*G. oxydans*/GCPE was used as the bioanode and a Pt wire was used as the cathode. Current (I) values of the MFC were measured while different external resistances (R) (from 1 Ω to 10 MΩ) were inserted to the circuit in order to calculate the cell voltage (Vcell) (Vcell = I R) and power (P) The current density (i) (i = I A^{-1}) and the power density (W) (W=P A^{-1}) values were obtained by dividing the current and the power values by the surface area of the bioanode (A = 0.126 cm^2). Open circuit potentials (OCP) were measured while there was no current flow in the system.

2.3 Preparation of *G. oxydans* Culture

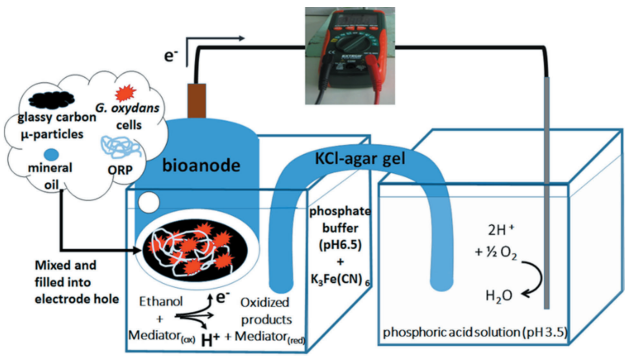
The strain of *G. oxydans* DSM 2343 was obtained from DSMZ (German Collection of Microorganisms and Cell Cultures, Braunschweig, Germany, www. dsmz.de). *G. oxydans* was maintained on agar containing (g L^{-1}): D-glucose, 100; yeast extract, 10; calcium carbonate, 20; agar, 20 [27]. The cell biomass was prepared by aerobic cultivation at 28 °C on a rotary shaker in 250 mL flasks filled with 50 mL of medium. The growth medium contained glucose, 0.5 % and yeast extract, 0.5 %. The culture, inoculated from the slant agar, was incubated for 17 h at 28 °C in a shaking incubator to reach the late exponential phase. The cell growth was followed spectrophotometrically via measuring the optical density at 600 nm to obtain a value that was used to be able to take the same amount each time on the electrode surface [28]. Strain stocks were stored at −18 °C with 25 % (v v^{-1}) glycerol at early phase. Then, for the electrochemical measurements, the cells from one of the cultivation flasks were collected by centrifugation (10 min, 4000 × g), re-suspended in sterile 0.9 % NaCl solution and centrifuged again. After removal of the washing solution completely from the cells, the bacterial pellet was dissolved in the required amount of phosphate buffer at pH 6.5. These solutions were stored at −18 °C when not in use.

2.4 Preparation of Electrodes and Fabrication of MFCs

A GCPE electrode was prepared by mixing proper amounts of glassy carbon micro particle, mineral oil (the ratio of glassy carbon micro particle: mineral oil was 80:20 % ww^{-1}). Then the resulting paste mixture was filled into the hole (2.0 mm radius, 3.0 mm deep) on a Delrin body, where a copper wire provides the electrochemical connection. The surface of the electrode was polished on a plain paper before every measurement. The

G. oxydans/GCPE microbial bioanode was prepared by dropping the required amount (μL) of cells (1.693×10^9 cell titer μL^{-1}) onto the electrode surface. The ORP/*G. oxydans*/GCPE was also prepared in the same way but additionally an amount of ORP (10 mg mL^{-1}) solution was dropped onto the electrode surface after coating with *G. oxydans* cells.

In order to construct the MFC, a *G. oxydans*/GCPE or an ORP/*G. oxydans*/GCPE microbial bioanode and a Pt wire cathode were connected to a multimeter and dipped into 10 mL of anolyte and catholyte solutions that were combined with a KCl(sat.) salt bridge that facilitates the charge transfer between the cells. The anolyte chamber contained 100 mM phosphate buffer (pH 6.5), 5.0 mM $K_3Fe(CN)_6$ as mediator and ethanol as substrate and the temperature was kept at 28 °C. The catholyte chamber contained just a phosphoric acid solution at pH 3.5 prepared by adjustment of the pH of 100 mM phosphate buffer (pH 6.5) by addition of 1.0 M $HCl_{(aq)}$ into this solution. The double compartment MFC system and preperation of the electrodes are given in Scheme 1.



Scheme 1. Illustration of the preparation of the composite microbial bioanode and the construction of the MFC.

3 Results and Discussion

3.1 Electrochemical Bioconversion Mechanism of Ethanol on ORP Modified Bacterial Bioanode

It has been known that in *Gluconobacter* species, ethanol can be oxidized by two different metabolic pathways: oxidation by cytosolic NAD-dependent aldehyde and alcohol dehydrogenases (ADH), where transportation occurs through the cytoplasmatic membrane or the direct oxidation in the periplasmic space by membrane-bound PQQ-dependent dehydrogenases [29–31]. On the other hand combination of ORP with *G. oxydans*/GCPE enhances the electron transfer because conductive nature of ORP provides better communication between the electrode surface and enzymes in bacteria. Also, since the ORP is positively charged, because of the electrostatic interactions, more stable contact is provided with negatively charged bacterial surface structures including charged protein molecules [16, 32–34].

In the developed system another mediator, $K_3Fe(CN)_6$ was also used. In order to obtain the effect of this mediator to the developed system and examine the difference between glucose and ethanol substrates, chronoamperometric measurements were conducted in the presence and absence of $K_3Fe(CN)_6$ (Figure 1A a and b and Figure 1B). Also for the control experiments, the responses of only ORP modified GCPE in the presence and absence of $K_3Fe(CN)_6$ were also monitored (Figure 1 A) c and d). For the conduction of these experiments, 10 mM ethanol (for whole Figure 1A and Figure 1 Bb) and 10 mM glucose (for Figure 1 Ba) were added into the working cell at 100.s

As can clearly be seen from the chronoamperometric results, *G. oxydans* cells were well adapted to the electrode surface and the highest catalytic current was obtained for 10 mM ethanol conversion in the presence of $K_3Fe(CN)_6$ in the phosphate buffer .

Consequtively the electrocatalytical behaviour of ORP/*G. oxydans*/GCPE microbial bioanode was examined for glucose and ethanol substrates by introducing 10 mM glucose (Figure 1 Ba) or 10 mM ethanol (Figure 1 Bb) into the 5 mM $K_3Fe(CN)_6$ including working medium. The current values were obtained as 0.30 μA after 10 mM glucose addition and 1.4 μA after 10 mM ethanol addition into the phosphate buffer. Although the bacteria were grown in the glucose medium as a carbon source, it is obvious that the usage of ethanol as a substrate yields better current values. There are similar works in the literature that ethanol was used as a substrate where glucose was used as carbon source however there is not exact explanation for that [35–37]. In our opinion, with the usage of glucose as a carbon source, alccohol oxidase could be activated more than ADH.

On the other hand, in the presence of 10 mM ethanol and with ORP/*G. oxydans*/GCPE, the addition of 5 mM $K_3Fe(CN)_6$ changed the obtained current value drastically from around 0.49 μA to almost 1.4 μA (Figure 1A a and b respectively). Lo Gorton's group explained this phenomenon. According to them, freely diffusing $K_3Fe(CN)_6$, might reach respiratory enzyme molecules that did not have any contact with ORP [16] and by this way conduction of electrodes between G. oxydans cells and the electrode surface may become easier due to the electron shuttle behavior of $K_3Fe(CN)_6$. Overall as a result, with the combination of these two mediator effects, higher current values were obtained. On the other hand the redox behavior of ORP was examined in the presence and absence of bacterial cells by CV. Cyclic voltammograms of ORP modified GCPE and ORP/*G. oxydans*/GCPE electrodes were recorded in 100 mM PBS at pH 6.5 between -0.5 and 1 V (Figure 1C). In order to define formal potential (E°) value of the ORP, the mean value of the anodic and cathodic peak potentials of ORP/GCPE voltammogram (Figure 1 Ca) were used. Eventually it was found that **ΔE** was equal to +217 mV vs. Ag|AgCl reference electrode. The value was in the interval of previously reported potentials (between +15 and +489

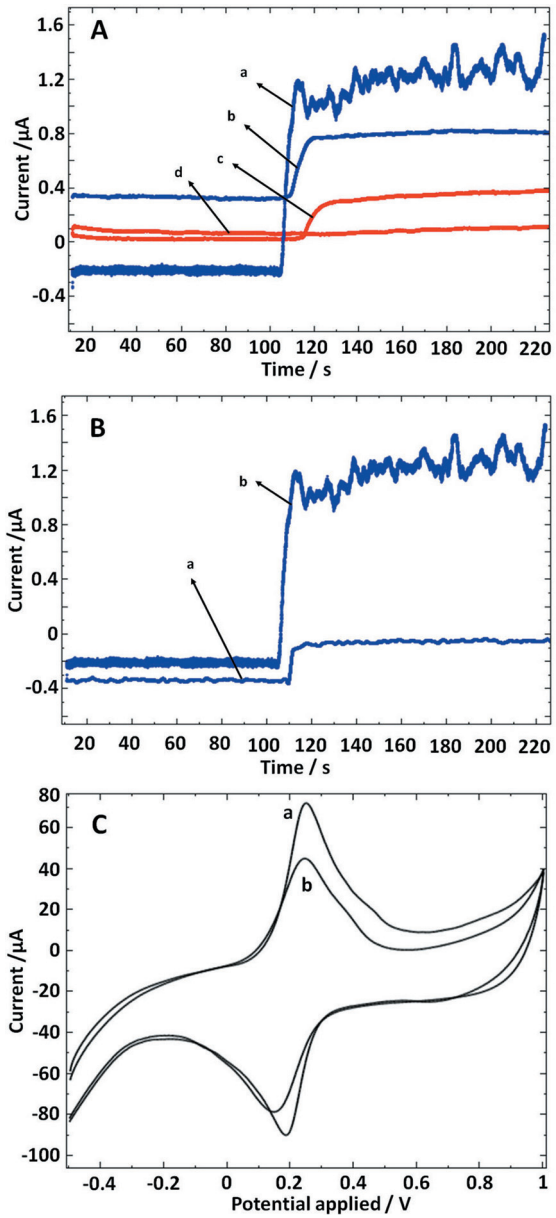


Fig. 1. A) Obtained chronoamperometric results of (a) ORP/*G. oxydans*/GCPE microbial bioanode in the presence of 10 mM ethanol and 5.0 mM $K_3Fe(CN)_6$ (b) ORP/*G. oxydans*/GCPE microbial bioanode in the presence of 10 mM ethanol (c) ORP/GCPE measured in the presence of 10 mM ethanol and 5.0 mM $K_3Fe(CN)_6$ (d) ORP/GCPE measured in the presence of 10 mM ethanol; B) Chronoamperometric measurements of ORP/*G. oxydans*/GCPE microbial bioanode after addition of a) 10 mM glucose substrate, b) 10 mM ethanol substrate into the 5.0 mM $K_3Fe(CN)_6$. All of the experiments were conducted into 100 mM phosphate buffer (pH 6.5), with 5µL (1.693 x 10^9 cell titer μL^{-1}) *G. oxydans* bacteria and 5 µL (10 mg mL^{-1}) ORP at 300 mV; C) Cyclic voltammograms of (a) ORP/GCPE and (b) ORP/*G. oxydans*/GCPE microbial bioanode in 100 mM phosphate buffer (pH 6.5) between -0.5 and 1.0 V, scan rate; 50 mV s^{-1}. 5.0 µL (1.693 x 10^9 cell titer μL^{-1}) of *G. oxydans* and 5 µL (10 mg mL^{-1}) ORP were used on GCPE modification.

mV versus NHE [42, 43]) and also in accordance with other related ORP studies [8]. From the Figure 1C, it is obvious that the peak current of the ORP/GCPE decreases clearly after the introduction *G. oxydans* cells into the electrode structure. This is attributed to the interaction between positively charged Os center of ORP and the negatively charged parts of cell wall of the bacterial cells. These viable cells are obstructing the electron transferring system in the polymer matrix by blocking redox centers [8].

3.2 Optimization of Experimental Parameters

3.2.1 Optimization of ORP Amount

For the optimization of ORP amount, various amout of ORP included ORP/*G. oxydans*/GCPE was immersed into 100 mM phosphate buffer (pH 6.5) solution which contained 2.0 mM $K_3Fe(CN)_6$ and 10 mM ethanol. The cyclic volammograms were recorded between −0.4 and 1.0 V potential range with a scan rate of 50 mV s^{-1} and the results were presented in Figure 2A. In order to prepare ORP/*G. oxydans*/GCPE electrodes 0, 1.0, 3.0, 5.0, 7.0 and 10 µL of ORP solutions (10 mg mL^{-1})were dropped onto the 5.0 µL (1.6) of *G. oxydans* cells containing GCPEs. As can be seen from Figure 2A inset, the maximum current value is reached after the addition of 5.0 µL of ORP and then for the next additions, the current values remain almost constant. For this reason, 5 µL of ORP was chosen as the optimum ORP value and used for further studies.

3.2.2 Optimization of $K_3Fe(CN)_6$ Amount

After the optimization of ORP amount, the effect of $K_3Fe(CN)_6$ mediator on the developed microbial bioanode's performance was examined. The working conditions were: 10 mM ethanol substrate in 100 mM phosphate buffer (pH 6.5) with 5.0 µL *G. oxydans* and 5.0 µL ORP modified GCPE bioanode electrode. In order to define the optimum amount 0, 0.5, 1.0, 2.0, 3.0, 5.0 and 7.0 mM of $K_3Fe(CN)_6$ mediator solutions were added into the working cell. The best current value was observed after the addition of 5.0 mM $K_3Fe(CN)_6$ solution (Figure 2B). After the addition of 7.0mM, the current value almost stays constant and as a result, 5.0 mM $K_3Fe(CN)_6$ solution was used for further studies as the optimum $K_3Fe(CN)_6$ amount. On the other hand, as can be seen from the Figure 2B, without any $K_3Fe(CN)_6$ but in the presence of optimum ORP amount in the medium, smallest current value was obtained. Again this situation could be explained due to the reachment of freely diffusing $K_3Fe(CN)_6$ mediator to the enzymes in the bacteria which ORP did not have any contact.

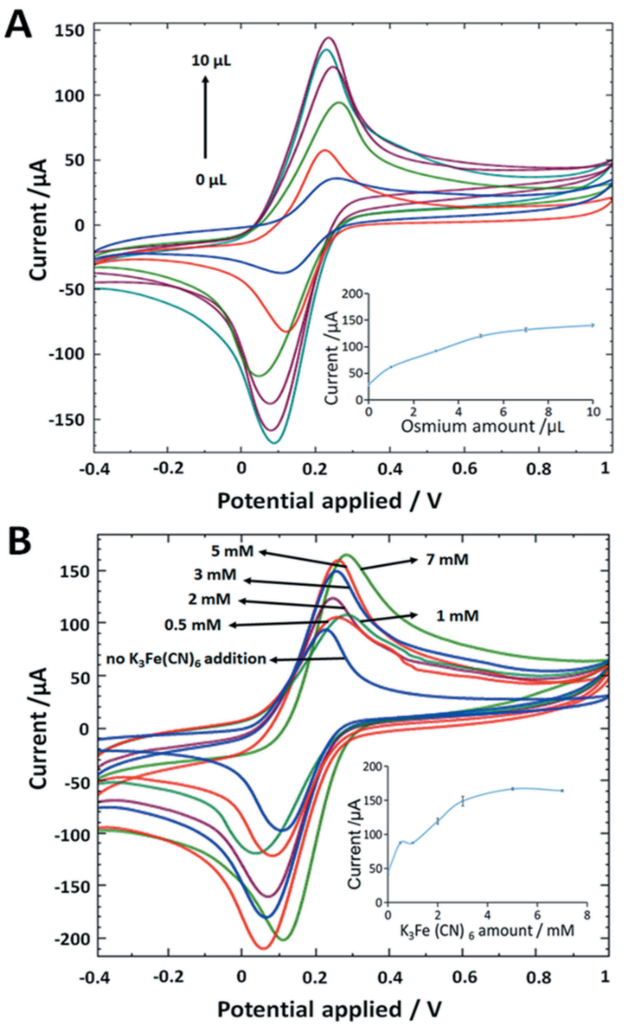


Fig. 2. A) Cyclic voltammograms and graph (inset) of ORP amount optimization, that is used for wiring of the 5.0 µL (1.693 x 10^9 cell titer μL^{-1}) of *G. oxydans* on GCPE. (2.0 mM $K_3Fe(CN)_6$ and 10 mM ethanol in 100 mM phosphate buffer (pH 6.5), between −0.4 and 1.0 V, scan rate; 50 mV s^{-1}); B) Cyclic voltammograms and optimization graph (inset) for $K_3Fe(CN)_6$ amount. 5.0 µL (1.693 x 10^9 cell titer μL^{-1}) of *G. oxydans* and 5 µL (10 mg mL^{-1}) ORP were used on GCPE. $K_3Fe(CN)_6$ solutions were added into 10 mM ethanol containing 100 mM phosphate buffer (pH 6.5), measurements were recorded between −0.4 and 1.0 V, at scan rate of 50 mV s^{-1}.

3.2.3 Optimization of G. Oxydans Cell Amount

Effect of *G. oxydans* cell amount onto the biosensor response was also examined (Figure 3). For this purpose, 5.0 mM $K_3Fe(CN)_6$ mediator and 10 mM ethanol together with 100 mM phosphate buffer (pH 6.5) were used. Then the cyclic voltammograms were recorded between −0.4 and 1.0 V potential range with a scan rate of 50 mV s^{-1}. In order to prepare *G. oxydans*/GCPE electrodes 1.0, 3.0, 5.0, 7.0 and 10 µL (1.693 x 10^9 cell titer μL^{-1}) of *G. oxydans* cells were dropped onto the GCPE surface and dried at room temperature. After the cyclic voltammetric measurements of each electrode, it was observed that 5.0

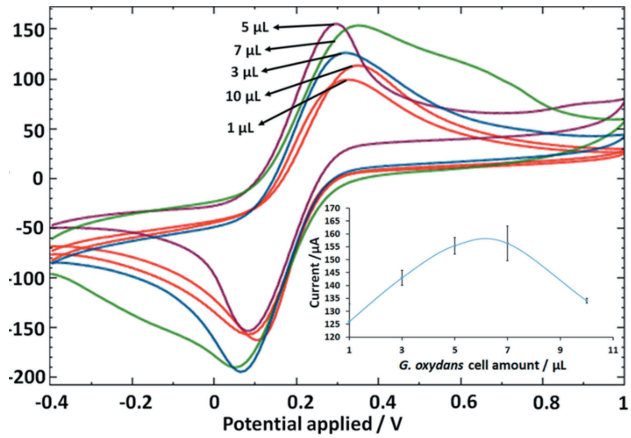


Fig. 3. Cyclic voltammograms and optimization graph (inset) of *G. oxydans* cell amount. 5 µL (10 mg mL^{-1}) ORP was used on GCPE. (5.0 mM $K_3Fe(CN)_6$ and 10 mM ethanol in 100 mM phosphate buffer (pH 6.5) between −0.4 and 1.0 V, scan rate; 50 mV s^{-1}).

µL *G. oxydans* cells exhibits the maximum current value. For the addition of 7.0 µL of the cells, almost the same current value is obtained and after 10 µL cell addition, the current value decreases (Figure 3). As a result, 5.0 µL *G. oxydans* cells was chosen as the optimum amount.

3.3 Analytical Characteristics

After the determination of optimum experimental conditions, linear and analytical ranges of developed bioanode was examined for varying ethanol concentrations like 1.0, 3.0, 5.0, 10, 20, 30, 50, 100, 250, 500 and 1000 mM. ORP/*G. oxydans*/GCPE bioanode showed two linear ranges as 1.0–30 mM with the equation of $y = 2.4278x + 120.19$ ($R^2 = 0.902$) and 30–500 mM with equation of $y = 0.1283x + 187.37$ ($R^2 = 0.997$) (Figure 4B). The analytical range of ORP/*G. oxydans*/GCPE was obtained as 1.0–1000 mM (Figure 4C). LOD (3.0 s/m) and LOQ (10 s/m) values were calculated as 1.29 mM and 4.30 mM respectively where RSD value was 1.16% for n = 5. Measurements were recorded under optimum conditions in 5.0 mM $K_3Fe(CN)_6$ containing 100 mM pH 6.5 phosphate buffer between −0.4 and 1.0 V potential range with 50 mV s^{-1} scan rate.

3.4 Polarization and Power Measurements of the Constructed MFC

G. oxydans/GCPE and ORP/*G. oxydans*/GCPE bioanodes were combined with a Pt cathode in a double compartment MFC and current-voltage measurements were done by using a multimeter. Polarization and power measurements were performed and calculated as explained in the experimental section. The bioanode was immersed into 100 mM phosphate buffer (pH 6.5), 5.0 mM $K_3Fe(CN)_6$ mediator and 250 mM ethanol substrate containing anolyte and Pt wire was dipped into catholyte

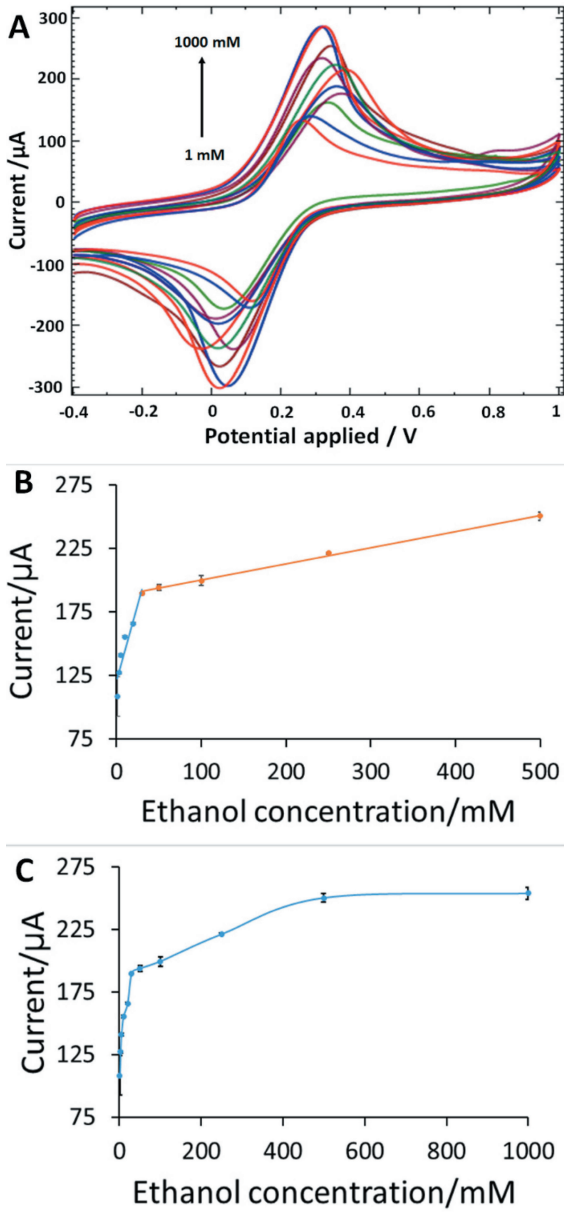


Fig. 4. A) Cyclic voltammograms B) calibration graph and C) analytical range graph of 5.0 µL (10 mg mL^{-1}) ORP and 5.0 µL (1.693 x 10^9 cell titer $µL^{-1}$) *G. oxydans* modified ORP/*G. oxydans*/GCPE bioanode in the presence of 1.0; 3.0; 5.0; 10; 20; 30; 50; 100; 250; 500 and 1000 mM ethanol substrate and 5.0 mM $K_3Fe(CN)_6$ containing 100 mM phosphate buffer (pH 6.5), measurements were recorded between −0.4 and 1.0 V, at the scan rate of; 50 mV s^{-1}.

chamber that just contained pH 3.5 phosphoric acid solution. The two compartments were connected with each other via KCl $_{(sat.)}$ filled salt bridge. *G. oxydans*/GCPE bioanode based MFC showed the maximum power density of 0.133 µW cm^{-2} (at 33.5 mV), the maximum current density of 8.73 µA cm^{-2} and OCP value of 156 mV (Figure 5 A). ORP/*G. oxydans*/GCPE based MFC showed the maximum power density of 0.26 µW cm^{-2} (at 46.8

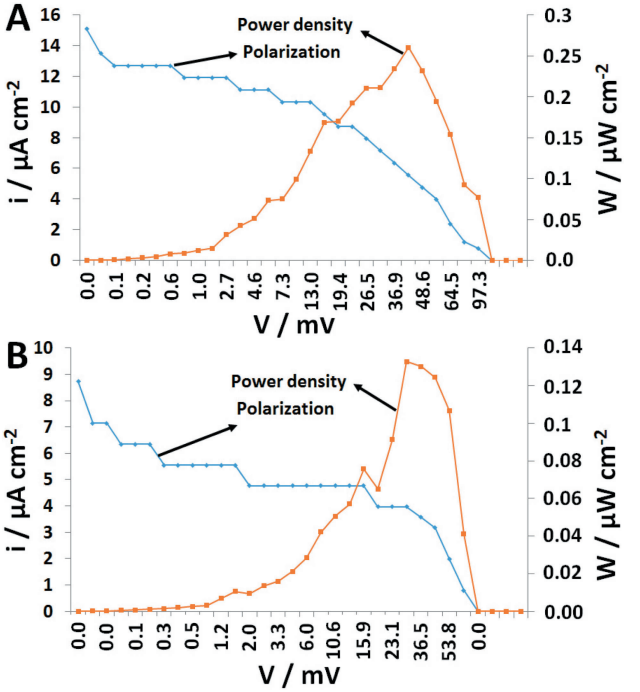


Fig. 5. Polarization and power density curves of A) *G. oxydans*/GCPE bioanode, B) ORP/*G. oxydans*/GCPE bioanode and Pt wire cathode based double compartment MFCs. All the conditions as in Figure 3.

mV), the maximum current density of 15.079 µA cm^{-2} and OCP value of 176 mV (Figure 5B).

There are similar previously reported *G. oxydans* whole cell based bioanode systems that were used in the MFC structure. Alferov et al. immobilized *G. oxydans subsp. industrius* RKM V1280 into a synthetic matrix based on polyvinyl alcohol modified with N-vinylpyrrolidone and used as bioanode for the microbial fuel cells. Compared to our system, they managed to obtain higher power density like 7 mW/m^2 [38]. On the other hand, in their other studies, they reported lower power output like 7.23 nW [39] and lower OCP value as 55 mV [40] than *G. oxydans* and ORP combined MFC system. Considering ORP based MFC systems, Hasan et. al. used *R. capsulatus* cells on ORP modified electrode and obtained the current density output like 4.25 µA cm^{-2} [8]. On the orher hand, in another study where *G. oxydans* were combined with carbon nanotubes for ethanol oxidation, a maximum current density of 261 ± 4 µA cm^{-2} was obtained which is higher than the presented bioanode system [41].

4 Conclusion

A bioanode containing *G. oxydans* cells with ORP and $K_3Fe(CN)_6$, was developed and combined with a Pt cathode to form a MFC for the first time. When the obtained results were compared with similar MFC studies, it is clear that the present system has promising output values as a MFC system. Also because of the composite nature of

GCPE, it can be said more practical and economical MFC has been developed. Having said that, for the future studies, in order to increase the efficiency of the developed MFC system, different types of electrodes (nanomaterial modified etc.) together with a membrane instead of the salt bridge could be used. Also, various cell designs could be tried to increase the output values of ORP/G.oxydans based MFC system.

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