In-Situ Electrochemical Behaviour of Biocathode Microbial Fuel Cell (MFC)

S. Venkata Ramana (ramanasudarsan@yahoo.com)

IBB–Institute for Bioengineering and Biosciences,Department of Bioengineering,Instituto Superior Técnico,Universidade de Lisboa, Av. Rovisco Pais,1049-001 Lisbon, Portugal;Food and Biotechnology Research Lab,South Ural State University(NRU),Chelyabinsk, Ru https://orcid.org/0000-0002-3206-9390

Cristina M. Cordas

LAQV, REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

Sara C. Matias

IBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

Luis Joaquim Pina da Fonseca

IBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

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Research

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Abstract

In the present work the electrochemical behaviour of microbial cells from a biocathode microbial fuel cell (MFC) functioning with wastewater was evaluated by cyclic voltammetry. In-situ electrochemical assays were performed and, under the tested experimental conditions, the biocathode medium was found to be the most efficient for the cathodic catalysed electrochemical reduction of oxygen. Different controls using sterile media and membranes covering the electrodes were performed and compared with the regular biocathode results. In the biocathode chamber, the presence of bacteria was associated with the enhanced active redox processes and with the higher electrochemical reduction of oxygen activity. The present study is a contribution to the understanding of the viability and advantages of the biocathodes use in MFC.

1. Introduction

Bacteria catalyse the anodic and cathodic half-reactions in microbial fuel cells (MFC) by metabolizing fuel and using the anode as electronic partner (Logan et al. 2006; Mohanakrishna et al. 2010; Kim et al. 2020). Research on MFCs has an enhanced interest since these have the ability to combine a wide range of biomass-derived fuels with long term durability of microbial consortia (Lovely 2006; Mekuto et al. 2020). Many different microorganisms have been utilized in MFCs, both as mixed and single strain cultures, such as *Geobacteracea* or *Desulfobulbus* families (Sukla et al. 2004; Lovely 2006; Du et al.2007; Tender et al.2008; Cao et al. 2019; Mekato et al..2020). Using cyclic voltammetry (CV), Bond et al. (2003) established the precedent that biofilms attached to anode and cathode electrodes exhibit electrochemical properties consistent with the hypothesis that these biofilms work as catalysts (Srikanth et al.2008; Venkata Mohan et al.2010; Velvizhi et al.2012; Massaglia et al.2019). In many systems the electrochemical features of the anode and the cathode are markedly different, reflecting possible fundamental differences in their extracellular electron transfer properties. Also, it was observed that the media also influences the electrochemical behaviour of the electrodes covered with biofilms, for instance sulphate reducing bacteria different mechanism in the presence of nitrate or sulphate. The potential, at which the electrodes are set, or the potential difference between them, has also been discussed as an important factor influencing the anode and cathode electrochemical response (Srikanth et al.2008; Marsili et al.2008; Cordas et al. 2008; Venkata Mohan et al.2010; Velvizhi et al.2012; Dall'Agnol et al. 2014; Massaglia et al.2019). Monitong the current as a function of potential and scan rate yields dependencies that can provide detailed mechanistic information about the electron transfer process from substrates (such as glucose, acetate etc.,) to the electrode surface. Changing the electrode potential will vary the driving force and may change the heterogeneous electron transfer step (across the catalyst/electrode interface). The present study focuses on the electrogenetic activity in the anodic and cathodic chambers of a MFC running with a biocathode and harvesting electricity from a suspension of bacteria cells in wastewater. In-situ electrochemical experiments were performed, including controls avoiding biofilms formation, to evaluate the biocathode MFC electrochemical behaviour.

2. Materials And Methods

2.1. MFC construction

Double chambered MFC was in-house constructed using acrylic glass material with equal volumes (working volume, 0.36L) of anode and cathode compartments, separated by a cationic exchange membrane (Nafion, Alfa Aesar, Germany). Graphite felts (GF, Alfa Aesar, Germany, 6 × 6 cm; 5 mm thick; surface area 36 cm^2) were used as electrodes. Copper wires were used for contact with electrodes. Appropriate sampling ports were designed. For the MFCs cathodes, a continuous air flow was provided through an air-pump to maintain constant the amount of dissolved oxygen (Fig. 1).

2.2. Biocatalyst

Aerobic mixed consortium from activated sludge was collected at the wastewater treatment plant of Chelas (Lisbon) and was used to inoculate the MFC anode and cathode compartments with simulated wastewater (1:3).

2.3. MFC operation

MFC was operated in aerated biocathode conditions (Fig. 1). MFC anodic and cathodic chambers were inoculated with aerobic mixed consortia and operated under respective microenvironment. Anode and cathode chambers were fed with artificial wastewater with composition, in g/L, comprising of sodium acetate (0.82) and sodium carbonate (0.31) as the sole carbon sources in the both chambers. For both chambers the remaining media composition was 50 mM phosphate buffer and nutrient solution: KH_2PO_4 (2.88 g/L), K_2HPO_4 (5.02 g/L), NH_4Cl (0.53 g/L), $C_{10}H_{16}N_2O_8$ (0.50 g/L), $MgSO_4$.$7H_2O$ (0.37 g/L), $MnCl_2.4H_2O$ (0.59 g/L), $COCl_2.6H_2O$ (0.08 g/L), $CaCl_2.2H_2O$ (0.11 g/L), $ZnCl_2$ (0.05 g/L), $CuSO_4.5H_2O$ (0.01 g/L), $AlK(SO_4)_2$ (0.01 g/L), H_3BO_3 (0.01 g/L), $Na_2MoO_4.2H_20$ (0.02 g/L), Na_2SeO_3 (0.001 g/L), $Na_2WO_4.2H_2O$ (0.01 g/L), $NiCl_2.6H_2O$ (0.02 g/L) and $FeCl_3.6H_2O$ (0.27 g/L) (Chen et al. 2008). Prior to feeding, the pH of the artificial wastewater was maintained at 7.0 in both chambers. Anodic chamber was spared with N_2 gas for 20 min to maintain the anaerobic microenvironment after the fed change and sampling; in the cathode chamber a continuous supply of air was maintained through an air-pump (ELITE-801, Holf C. Nagen, UK Ltd.) to keep constant the amount of the electron acceptor. The medium solution was changed when the voltage decreased to 50 mV and the suspended biomass was reserved, forming a complete fed-batch cycle. MFC was operated at room temperature (app. 25ºC) and electrodes were connected through a copper wire to a fixed load of external resistance of 1000Ω.

2.4. MFC Analysis

MFC was operating in a total of 28 fed-batch cycles, stabilizing at the 18 fed-batch cycle with high removal efficiency. The stabilized biocathode MFC was shown a maximum open circuit voltage (OCV) of 439 mV with a corresponding external resistor of 1000 Ω (operational parameters in Table 1). At this stage wastewater samples from MFC anodic and cathodic chambers were collected and its features were analysed by cyclic voltammetry (CV) to characterize the oxidation-reduction reactions of the suspension

bacteria cells containing media. Cyclic voltammetry analysis used a CHI 440B potentiostat from CHI Instruments, USA. The working electrode was a gold disk with ϕ = 2 mm (Bioanalytical systems, West Lafayette, model: 2014, USA). Ag/AgCl reference (RE-1B, BAS, Japan) and a platinum counter electrodes (Bioanalytical systems, West Lafayette, model: 4230, USA) were used in one compartment electrochemical cell. From each sample (control, supernatant, pellets and sonicated) 200 µl volume was taken, and placed on the cellulose membrane (14 kDa cut-off), that covered the working electrode, in a thin-layer configuration. The control was performed with artificial sterile medium placed on the cellulose membrane with the same procedure as the samples. Nitrogen gas was used to continuously flush the electrochemical cell during the measurements. CV was performed by applying a potential ramp to the working electrode at a scan rate of 10 mVs^{-1} after 5 min of equilibrium at open circuit potential. Potential window between + 0.6 and − 0.6V was used to exploit the oxidation/reduction processes characteristic of each sample. The working samples from biocathode MFC anodic and cathodic chambers' wastewaters, were separated into supernatant, pellets and sonicated, and prepared for the CV analysis by the following procedures: each 1.5 ml of wastewater samples were centrifuged at 12000 RPM for 5 minutes, and the upper layers (supernatant) were collected; the remaining pellets' samples were washed with 50 mM phosphate buffer (pH 7.0), centrifuged at 12000 RPM for 5 minutes, to the final remaining samples (pellets); the preparation of sonicated sample were as the previous pellets, but with the additional step of sonication in a water bath (NAHITA, Ultra Sonic 220–240 V) for 5 minutes.

Table 1
Biocathode MFC operating parameters.

Operation Parameters	Biocathode MFC
Batch mode operation time (days)	150
OCV (mV)[1]	439
Power density (mW/m^2)	54
COD removal efficiency (%)[2]	94
Coulombic efficiency (%)	33
Current density (mA/m^2)	122

Bio-electrochemical assays in situ were performed considering MFC reactor's anode and cathode as working and counter electrodes and vice versa, against Ag/AgCl reference electrode. In these assays, scan rate was 20 mVs^{-1} over the potential range + 0.8 to -0.8 V. Additional controls were measured in the MFC reactor using the same methodology, but with a cellulose membrane (14 kDa cut-off) covering the electrodes, as described elsewhere (Cordas et al.2008), to avoid any direct contact of bacteria cells with the electrodes.

3. Results And Discussion

3.1. Bio-electrochemical analysis of wastewaters samples from biocathode MFC

3.1.1. Anodic chamber

The samples were taken and prepared as described on Sect. 2.4 and analysed by cyclic voltammetry to determine its electrochemical activity. From the results shown in Fig. 2, it is possible to identify two redox processes associated with the presence of bacterial cells, namely an anodic wave around + 0.4 V and a cathodic one at 0 V, observed in all assays, more pronounced for the **pellets** samples. These seem to be the result of the presence of electron transfer proteins, such as cytochromes and small iron-sulfur proteins that possess metal centers (Nevin et al.2002; Lovely 2008; Wrighton et al. 2011; Mao and Verwoerd 2013; Massaglia et al.2019). A high cathodic current starts to develop at -0.5 V, also visible in the controls and in agreement with the high electrocatalytic activity of the gold electrode surface towards the oxygen reduction. The oxygen reduction, however, is slightly shifted to more negative potentials in the assays with the pellets sample, indicating the presence of biological material in the gold surface, probably making the diffusion of oxygen and its electrocatalytic reaction slower.

3.1.2. Cathodic chamber

The electrochemical characterization of the bacterial cells collected from the cathodic chamber of the MFC reactor is summarized in Fig. 3. The results of the biocathodic chamber do not show significant differences from the anodic. Again, it is possible to identify two redox processes associated with the presence of bacterial cells, namely an anodic wave around + 0.3 V, slightly shifted towards more negative values when compared with the anodic chamber results; and a cathodic one at + 0.1 V, observed in all assays, but more visible in the pellets' samples. Again, from the comparison with the control assays, one can speculate that these could be the result of the presence of proteins, possessing metal centers. The potentials at which the redox processes are visible are consistent with the potential values at which cytochromes and/or small iron sulfur proteins present redox activity (Nevin et al.2002; Lovely 2008; Wrighton et al.2011; Mao and Verwoerd 2013; Massaglia et al.2019). A high cathodic current starts to develop around − 0.5 V, also visible in the controls, that is slightly shifted to more negative potentials in the assays with the biological samples. As discussed before, this shift may be related with the presence of adsorbed material in the gold surface that hinders the electrode direct oxygen reduction process, as observed in the anodic samples. A small anodic wave develops around − 0.6 V, more visible for the supernatant samples that probably is due to the reverse reaction, namely, the oxygen regeneration from intermediate molecules adsorbed or retain at the surface. Comparing the results obtained between all the cathodic chamber assays (supernatant, pellets and sonicated) samples and corresponding controls, it is clear that the anodic and cathodic processes, with midpoint potential close to + 0.3 V, are visible in all samples, but not at the controls. The processes are more clearly observed in the pellet's assays and are the result of the biological material presence.

3.2. In-situ bio-electrochemical analysis of biocathode MFC

3.2.1. Anodic chamber

Cyclic voltammetry assays were carried out in the anodic chamber using regular inoculated media and also sterile media for comparison of the in situ electrodes behaviour. Electrochemical features of control assays using a cellulose membrane (14 kDa cut-off) covering the anode and cathode electrodes, were evaluated to determine if the observed electrochemical activity is due to the formed biofilms or redox compounds released into the solution resulting from the bacteria metabolism. The resulting voltammograms (Fig. 4) for the anode containing biofilm assays present considerable differences from the controls (sterile and membranes' electrodes). Voltammograms of the anode biofilm show three anodic processes, approximately at + 0.2, -0.1 and − 0.35 V, and one cathodic peak around 0 V. Also, a broad cathodic wave, associated to the reduction of oxygen in solution starts to develop around − 0.3 V. Using the sterile media, a different pattern is observed, and two broad processes can be observed, an anodic wave presenting the maximum intensity around + 0.15 V, and a cathodic wave with maximum current intensity around − 0.30 V. The membranes' control has shown much less intense currents, where small broad anodic and cathodic processes around + 0.2 V and − 0.35 V, respectively, are visible in a similar pattern to the sterile medium (Table 2; Fig. 4.). Based on the results, in the regular anodic media with biofilm formation, redox compounds (electron shuttles) resultant from the biological material presence seem to be responsible for the enhanced electron transfer activity to the anode and catalytic activity and, consistently, producing an operative MFC.

Table 2
Potential values of the redox processes in sterile media, non-sterile (biocathode) and control (membrane) conditions.

Medium	Anodic chamber		Cathodic chamber	
	E_{pa}/V	E_{pc}/V	E_{pa}/V	E_{pc}/V
Control (membrane)	+ 0.20	-0.35	+ 0.26	-0.25
Sterile media	+ 0.15	-0.30	+ 0.05; +0.3	-0.2; -0.53
Non-sterile (biocathode)	+ 0.2; -0.1; -0.35	0; -0.3 to -0.75	-0.1; +0.15	-0.05; -0.5
E_{pa}, anodic peak potential; E_{pc}, cathodic peak potential				

3.2.2. Biocathodic chamber

The cyclic voltammetry analysis of the aerobic biocathode chamber was performed as described for the anodic chamber in the previous section (3.2.1). As shown in Fig. 5, no obvious redox processes were observed from the control biocathode chamber, covered with the cellulose membrane. The voltammograms using the biocathode show two anodic peaks, namely a main intense peak at -0.1 V and a smaller wave at + 0.15 V and two cathodic processes approximately at -0.05 and − 0.5 V. Using sterile media two anodic waves develop around + 0.3 and + 0.05 V and two cathodic processes are also visible, a broad wave around − 0.2 V and a smaller wave at -0.53 V. The membranes' control shows less intense

currents, and a small anodic process around + 0.26 V and a cathodic approximately at -0.25 V is visible (Table 2; Fig. 5). Based on the results, the biocathode containing attached biofilms reveals more electron transfer activity when compared to both the sterile media and the membranes' control. The results point to a decisive role of the biocathode biofilm and metabolic released redox biological compounds (electron shuttles) in the power density production in the biocathode MFC.

4. Conclusions

The electrochemical assays of the wastewater samples retrieved from the MFC running with a biocathode clearly show the presence of anodic and cathodic redox processes. These are better observed in the assays using pellet samples and were indexed to the biological material presence. The possible occurrence of extracellular bacterial proteins with electron transfer properties must be taken into consideration. The in-situ MFC assays using the chambers' electrodes, which are covered by biofilms, have shown interesting redox features evidencing the biofilms role in the production and conduction of electrons. Under the tested experimental conditions, the biocathode operating conditions seem the most favourable for cathodic electrochemical reduction of oxygen. The attained results confirm that the biocathode is a viable alternative to the use of conventional catalysts in MFC's.

Abbreviations

CV, cyclic voltammetry; **MFC,** microbial fuel cell; **OCV**, open circuit voltage

Declarations

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Authors' contributions

S.Venkata Ramana,Cristina M.Cordas, Sara C.Matias: experimental procedures, results discussion, and data treatment; Luis P.Fonseca : research supervisor/coordinator

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Availability of data and materials

The datasets generated for this study are available on request to the corresponding

author.

Ethics approval and consent to participate

Not applicable.

Consent for publication

All authors agreed with this publication.

Competing interests

There are not competing interests.

Author details

[a]IBB – Institute for Bioengineering and Biosciences, Department of Bioengineering, Instituto Superior Técnico, Universidade de Lisboa, Av. Rovisco Pais, 1049-001 Lisbon, Portugal

[b]LAQV, REQUIMTE, Faculdade de Ciências e Tecnologia, Universidade Nova de Lisboa, 2829-516, Caparica, Portugal

[c] Food and Biotechnology Research Lab, South Ural State University/National Research University, 454080, Chelyabinsk, Russian Federation

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Figures

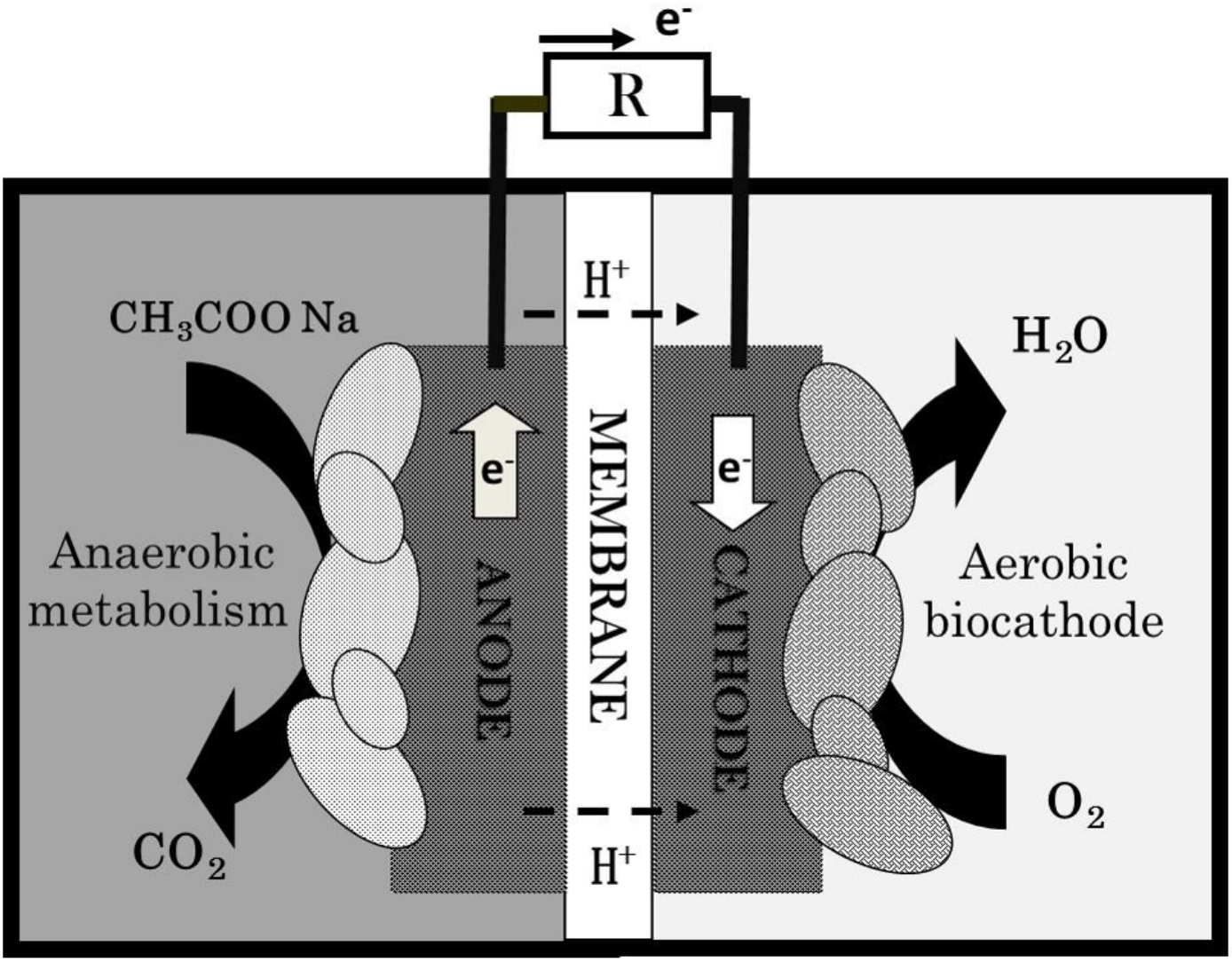
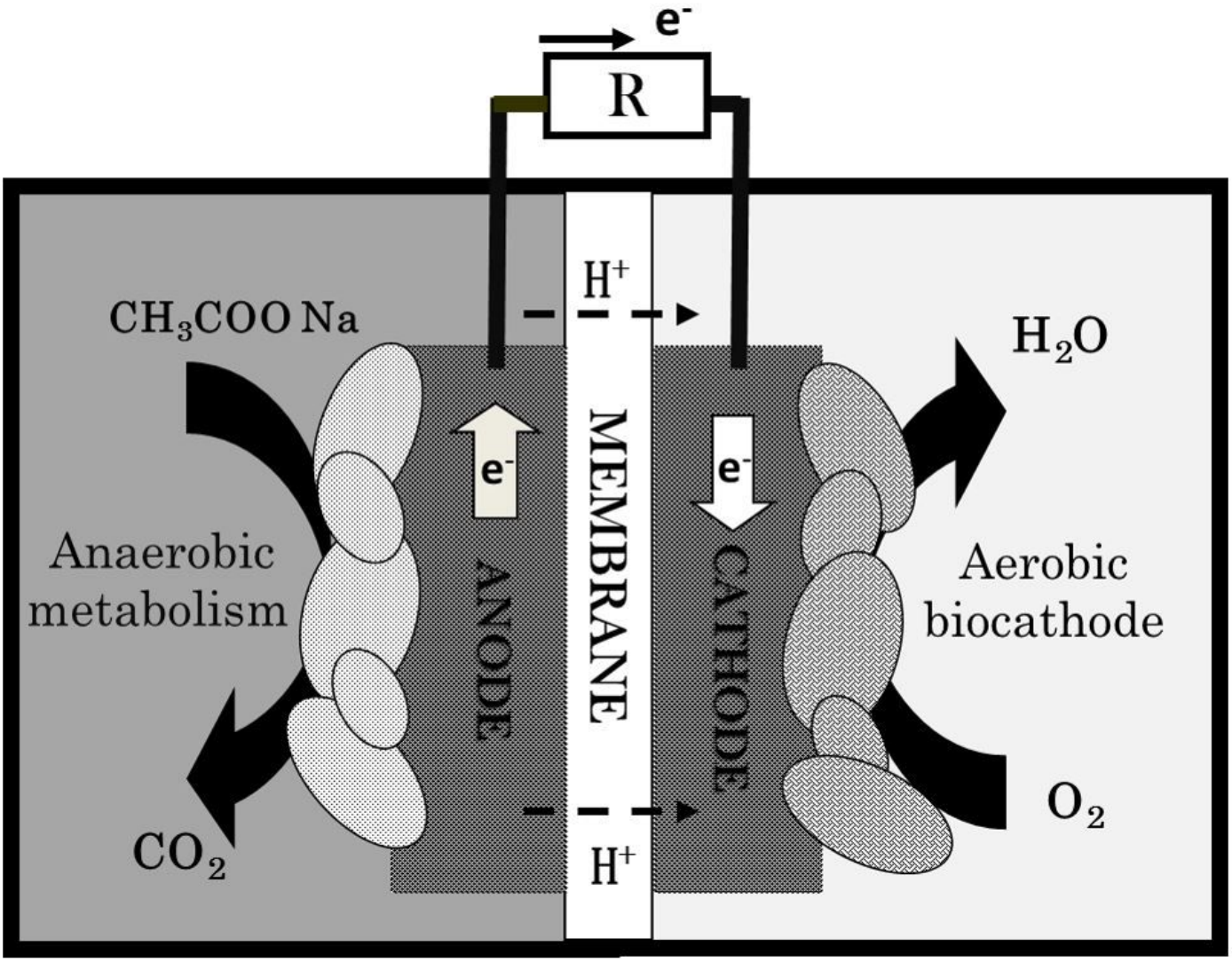


Figure 1

Schematic diagram of biocathode MFC.



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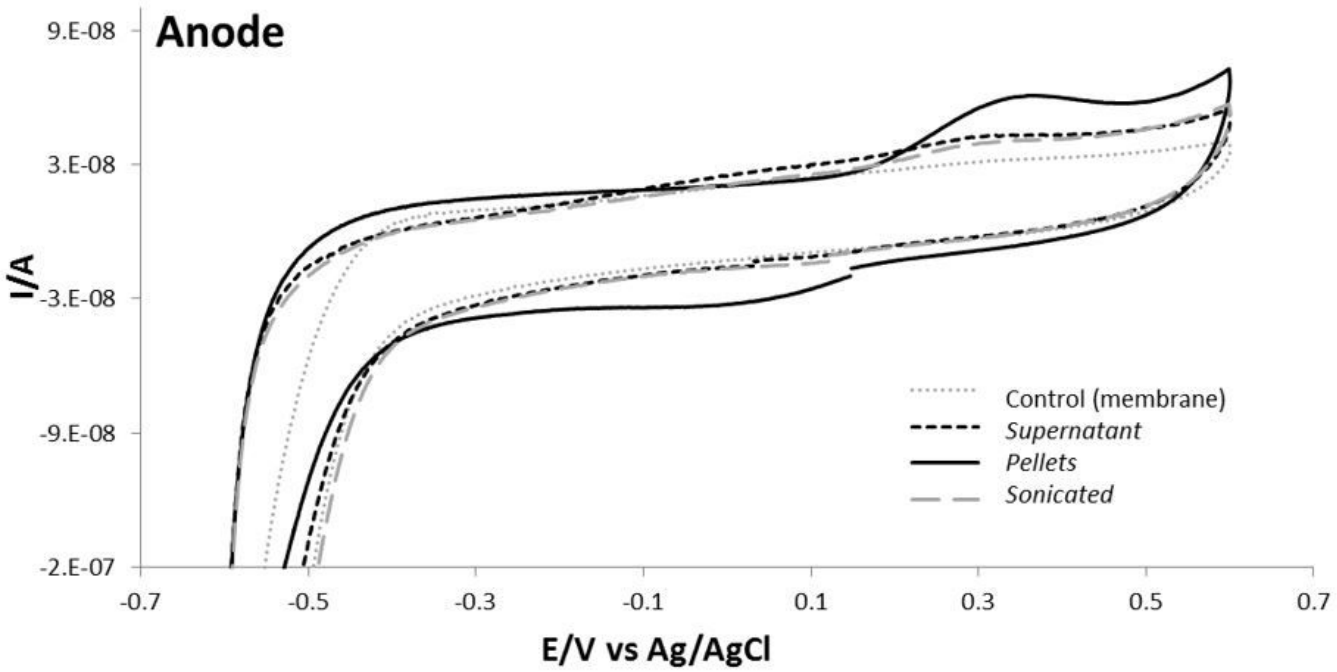
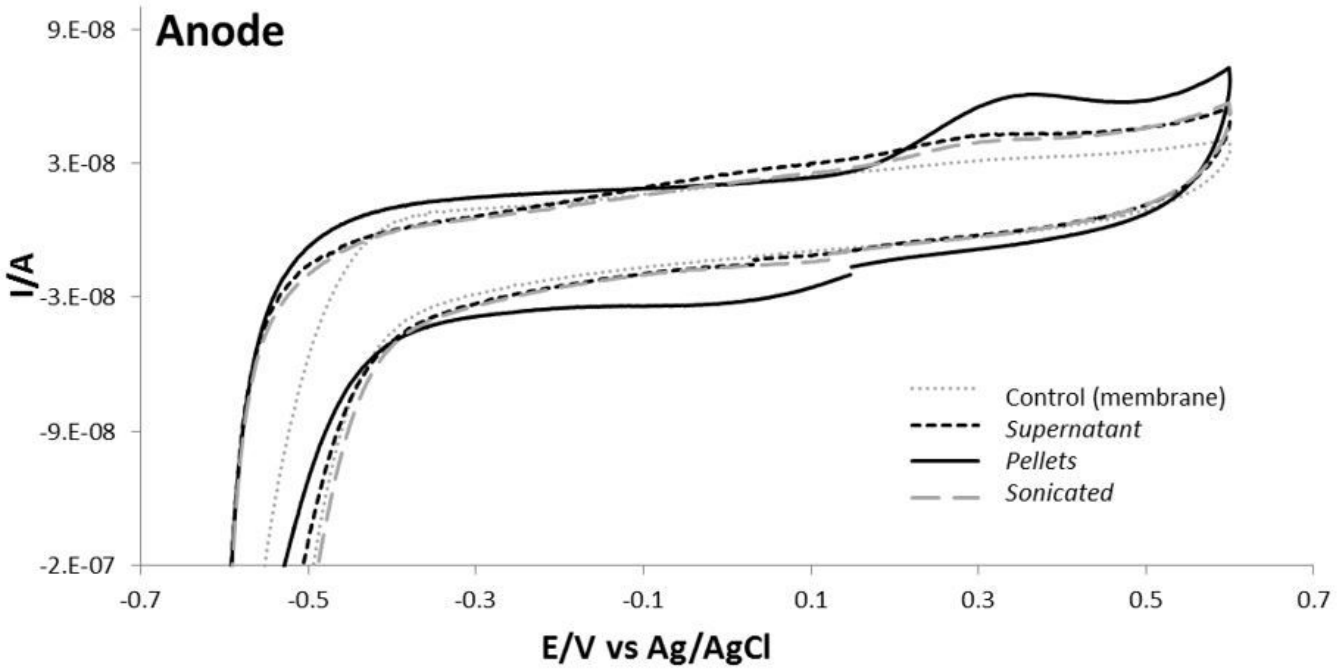


Figure 2

Ex-situ cyclic voltammetric features of anodic chamber wastewater sample with bacteria cells in suspension; scan rate 20mV/s.



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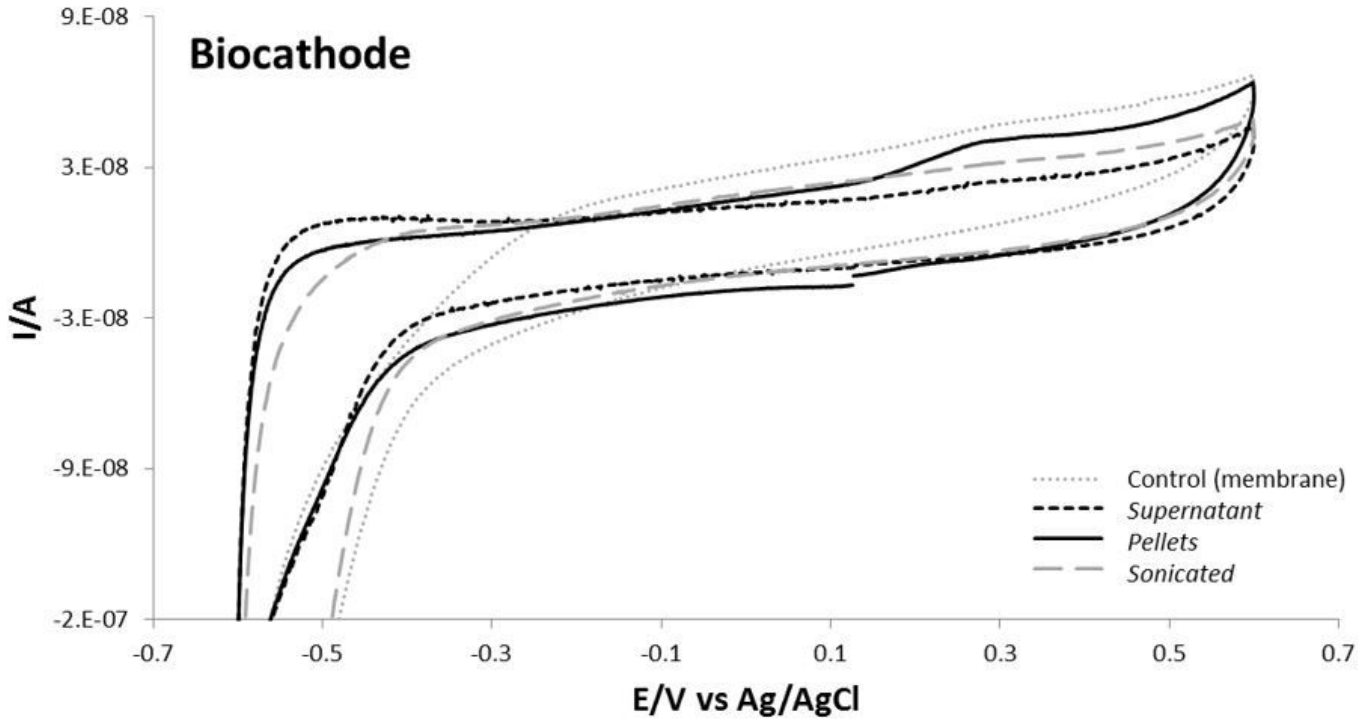
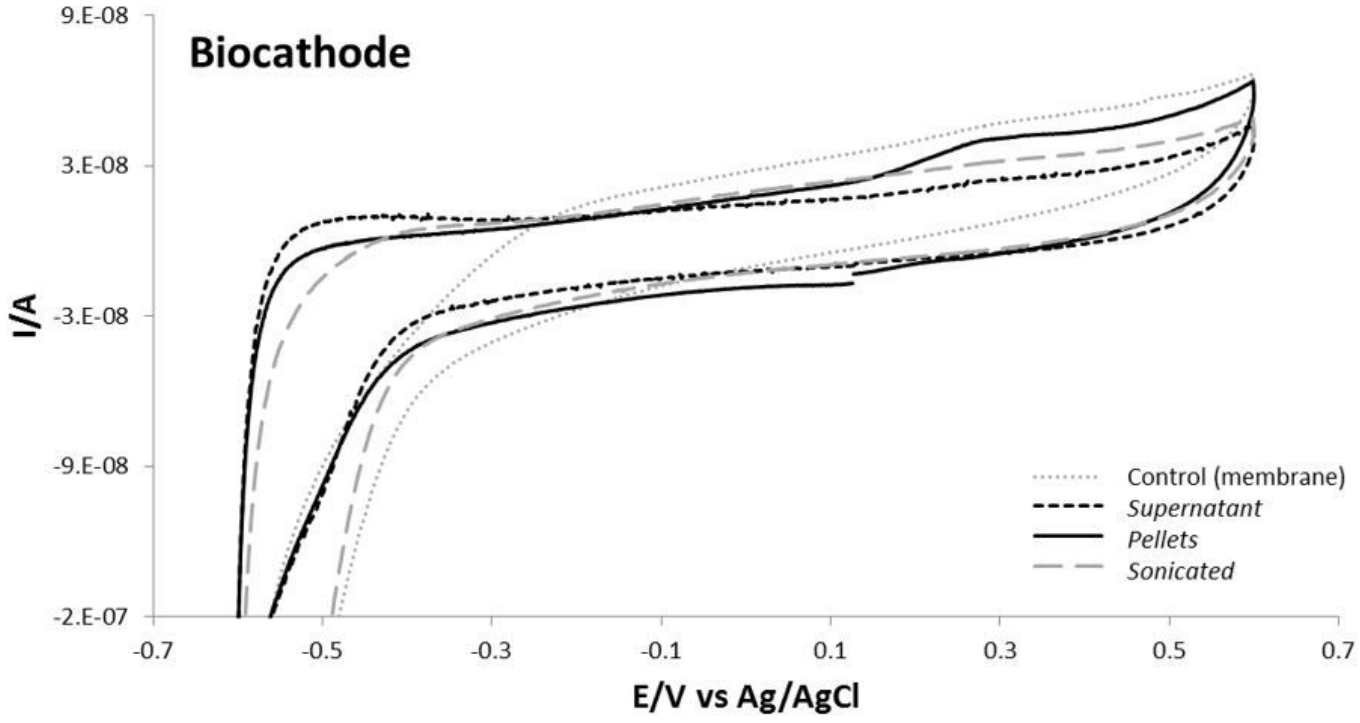


Figure 3

Ex-situ cyclic voltammograms of cathodic chamber wastewater samples with bacteria cells in suspension; scan rate 20mV/s.



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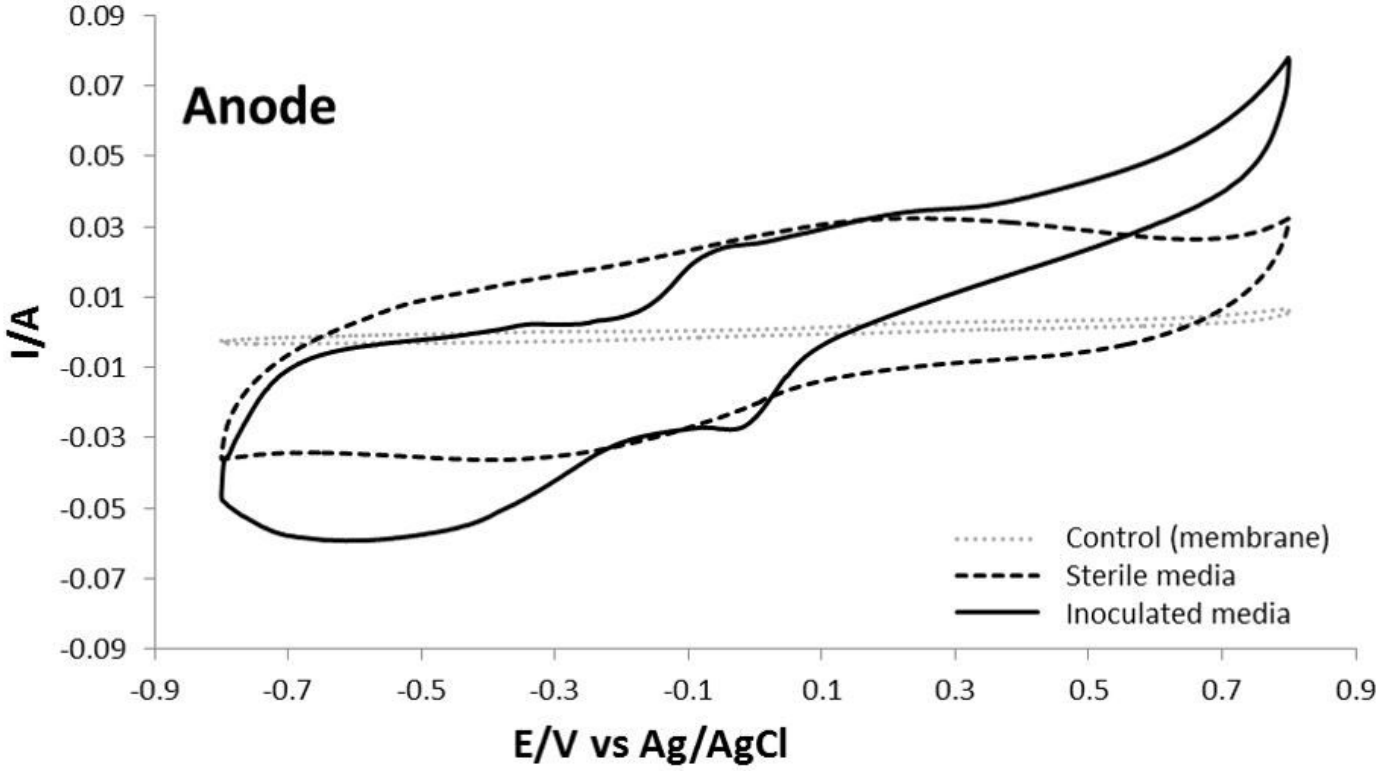
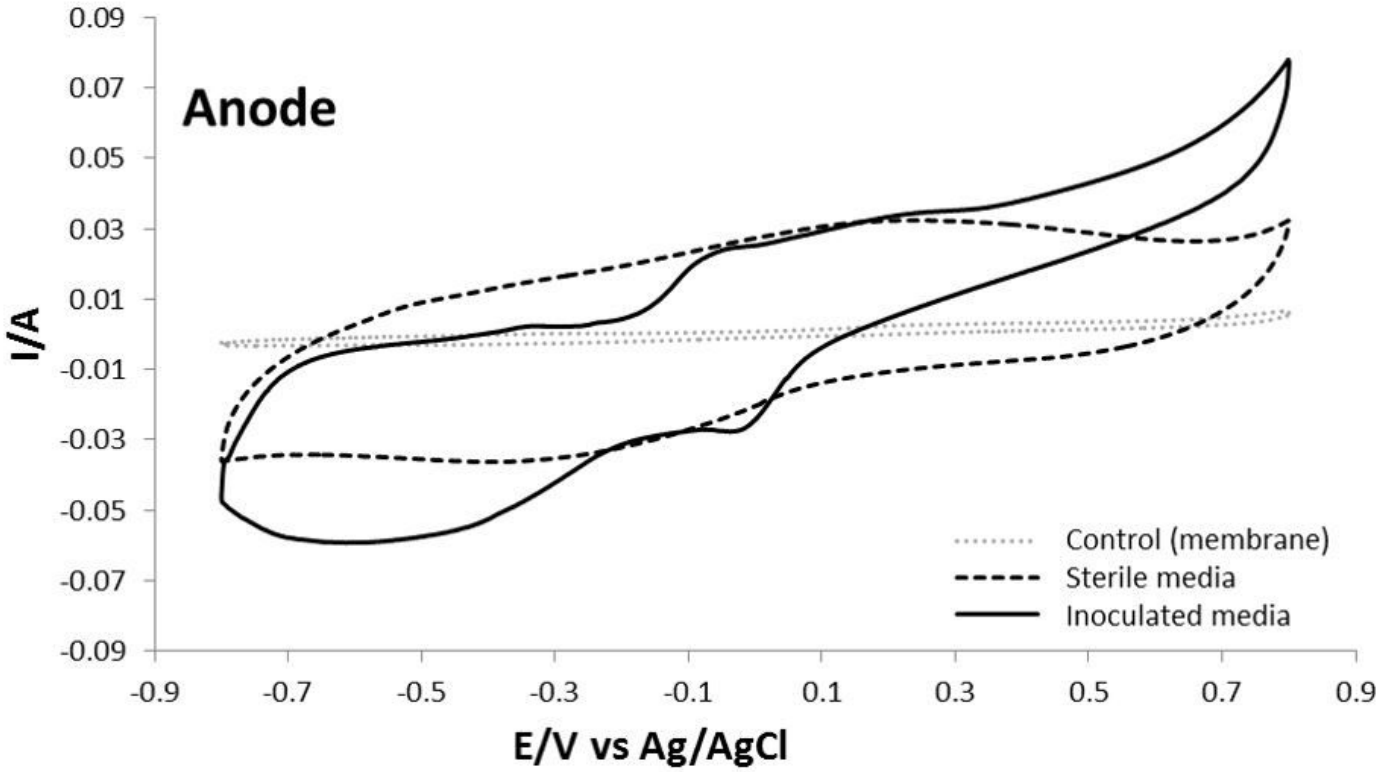


Figure 4

In-situ cyclic voltammograms of the MFC anodic chamber; scan rate 20mV/s.



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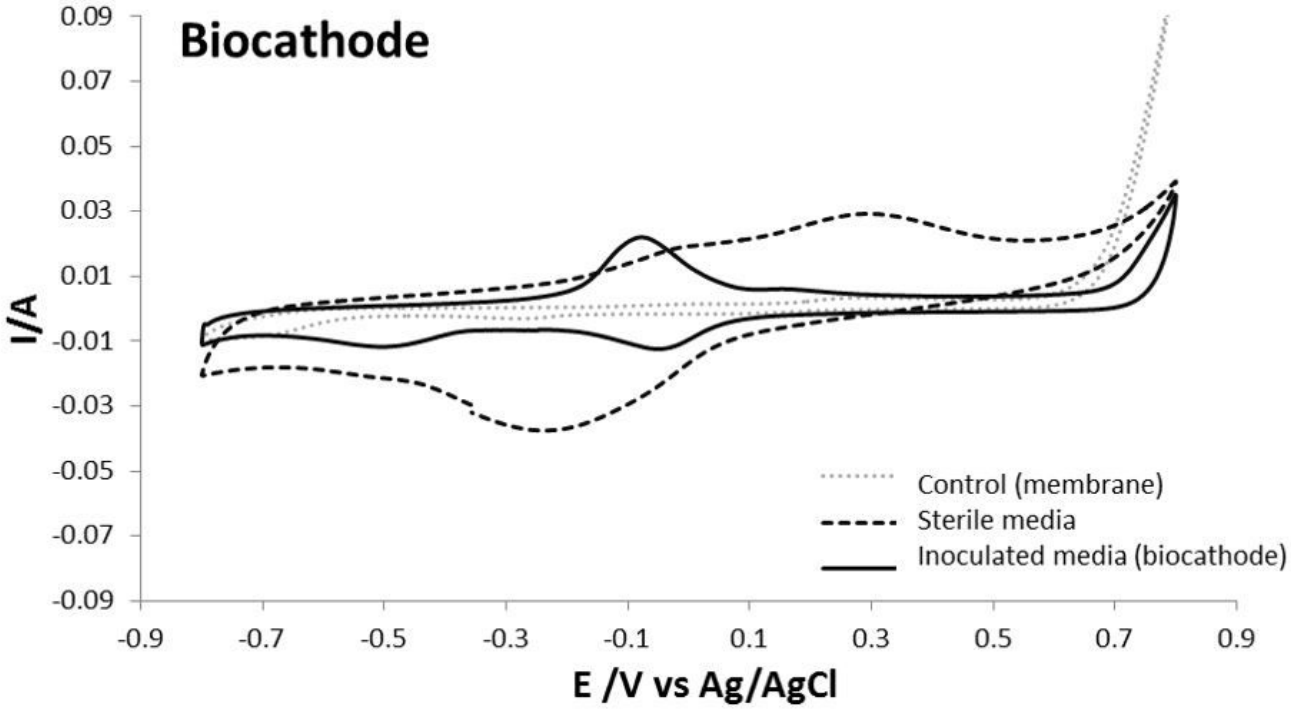

Figure 5

In-situ cyclic voltammograms of the MFC biocathodic chamber; scan rate 20mV/s.

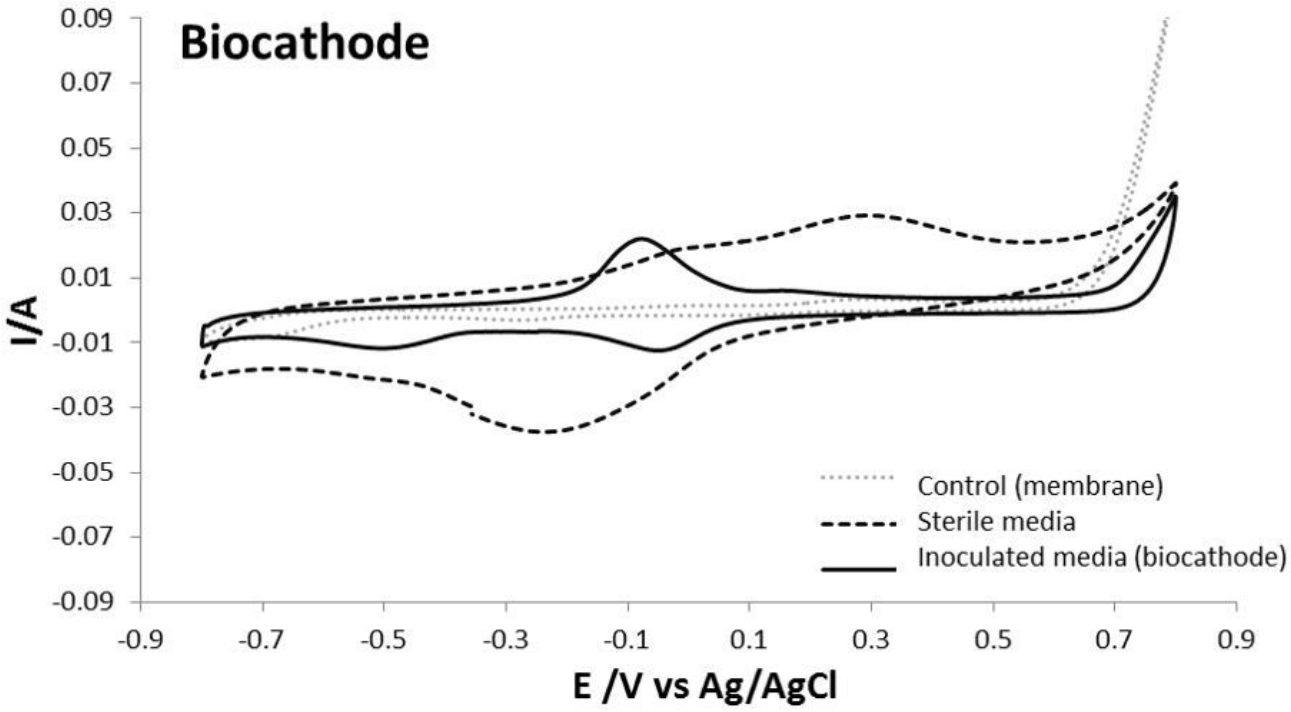

Figure 5

In-situ cyclic voltammograms of the MFC biocathodic chamber; scan rate 20mV/s.

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