Techniques for the study and development of microbial fuel cells: an electrochemical perspective

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Microbial fuel cells (MFCs) represent a clean and renewable energy resource. To date, power generation in MFCs is severely limited. In order to improve performance, a wide range of techniques have been utilised for a fundamental scientific understanding of the components and processes and also to investigate MFC performance bottlenecks. This tutorial article reviews the electrochemical/electroanalytical techniques employed in recent MFC studies and discusses the principles, experimental implementation, data processing requirements, capabilities, and weaknesses of these techniques.

1. Introduction

Microbial fuel cells (MFCs) and enzymatic fuel cells (EFCs) are the two principal types of biological fuel cells (also called biofuel cells);[1,2] these are devices that transform chemical energy into electrical energy via electrochemical reactions involving microbes or enzymes either located in both anode and cathode compartments or in just one compartment; enzyme (protein) containing electrodes can also be employed in MFCs, thereby resulting in a hybrid of EFCs and MFCs.

MFCs generally consist of an anode, a cathode, an electrolyte (which can be in membrane form) and an electrical circuit; microbes located in one (or more) of the compartments are used to degrade/convert organic and/or inorganic substrates via their metabolisms. Electrons are produced at the anode and transferred through an external circuit to the cathode, whilst a charge balancing number of cations and/or anions are transferred between anode and cathode.

Utilising microbes or enzymes for electricity generation extends the range of fuels (substrates) that can be oxidised or oxidants that can be reduced; hence biological fuel cells can carry out specific tasks, and/or have niche applications, in addition to power generation. Two key issues that are required for a sustainable society are environmental protection and the generation of clean energy. MFCs are attracting worldwide attention, driven by the promise of clean and renewable energy from various wastes and wastewaters. Currently, MFCs can only produce low power outputs (< 6 W m^{-2}; ≤ 500 W m^{-3})[3] due to many factors related to the anode, the cathode, the chemical species present in the electrolyte, the ion–exchange or filtration membrane (if used), the microbial species present and their metabolisms, fuel cell configuration, and operational conditions.

Scaling up of these devices can be problematic, since what are considered as 'acceptable losses' at lab scale, become parasitic when scaled up to demonstration or commercial products.

A better understanding of all of the components is required, to allow the bottlenecks in MFC to be identified and to improve power outputs; the selection of the most appropriate techniques for MFC diagnosis and evaluation is vital in achieving this. To fully understand MFCs, knowledge of multiple technological disciplines is required, such as electrochemistry, microbiology, materials science and engineering, molecular biology and environmental engineering. It is a challenge for any research team to completely understand all of the theories and techniques used in the study of MFCs. An example is that some of the "electrochemistry" employed and/or described in some research papers can be described as ambiguous and/or inappropriate. In recent years a series of detailed review papers have discussed the MFC history, construction, reaction mechanisms, operational conditions and limitations.[2-9] However, review of the most appropriate electrochemical techniques required for MFC diagnosis has not been given the appropriate level of attention, with only a few such techniques being discussed.[10]

New mechanisms (microbial physiological, chemical and electron transport) are being gradually elucidated. Electrochemical/analytical techniques have an essential role in this continuing process; they are vitally important in analysing the limiting performances of each component, to optimise MFC operation, and to allow continued innovation. In this article, we review, from both electrochemistry and fuel cell materials chemistry perspectives, the techniques that are useful for the study and development of MFCs; the

aim is to complement previously published reviews, to highlight some of the capabilities and to expose weaknesses of these techniques.

2. Basic considerations

Generally speaking, the electrochemical techniques now being more routinely used in MFC studies have been used in the study of traditional electrochemical systems (*e.g.* chemical fuel cells and electrocatalytic systems) for many decades. The biological processes involved in electron transfer reactions in MFCs are more complex when compared with classical electrochemical systems that are, by comparison, relatively simple and clean. The main difference between chemical fuel cells [such as a hydrogen/oxygen (air) polymer electrolyte fuel cell (PEFC)] and MFCs is that the electricity generation in MFCs arises from the metabolic activity of microbes; the continuous and long term operation of MFCs mandates that systems must be run under conditions that are generally predefined by the requirements for optimal microbial growth and sustainability. Typical conditions include ambient temperatures and pressures, electrolytes with a low concentration of ions, and near neutral pH.

Maximum current densities of MFCs are typically < 3 mA cm^{-2}; these levels are significantly lower than those of PEFCs, which are of the order of > 1 A cm^{-2} (both normalised to geometrical electrode area). The performances and requirements of the electrode materials and catalysts in MFCs and chemical fuel cells differ substantially, even for the same reaction. One example is the oxygen reduction reaction (ORR)[11-14] where anions such as Cl$^-$, HSO$_4^-$, HPO$_4^{2-}$, and HS$^-$ are well known to be species that poison Pt catalysts and seriously interfere with the electrode reactions on Pt catalytic

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surfaces; however, the majority of MFC and related studies involve the presence of phosphate buffers, ionic strength adjusters (*e.g.* NaCl) and other nutrients. The low O_2 dissolution concentrations in electrolyte and the low H^+ concentration ($\approx 10^{-7}$ mol dm^{-3}) have a significant impact on the cathode ORR performance in a MFC operating at ambient temperature. It is essential that the nature of all the species (including spectator ions and microbes) are taken into account, especially when they can come into contact with the catalysts. This could be one explanation why non-Pt ORR catalysts show similar performance levels with MFCs containing platinum. MFC components (electrodes, catalysts, membrane etc.) must be tolerant to these species for long term operation.

The following sections briefly introduce MFCs from an electrochemical point of view, before discussing the suitability of the various techniques that can be used in studying these complicated systems.

2.1 Choice of electrode configurations

Two– and three– electrode configurations are commonly employed to determine MFC and electrode performances; a four–electrode configuration is sometimes used when the conductivity of an electrolyte (membrane) or electrode material is tested by electrochemical impedance spectroscopy (EIS).

In practice, a MFC only needs two electrodes for electricity generation, an anode and a cathode. A variety of materials have been used as electrodes: graphite foil, rods, granules, and fibre brush; carbon paper, cloth, felt, and foam; activated carbon cloth; reticulated vitreous carbon; electrodes modified with conductive polymers; and metals such as aluminium, nickel or stainless steel. The choice of materials used for the anode or

cathode generally depends upon the electrical potential window required. It is sometimes overlooked that the electrode materials and substances in the electrolyte are not electrochemically inert and may themselves generate currents in a potential range of the experiment in question. For example: (1) metal electrodes will generate currents due to chemical and/or microbial corrosion or passivation; (2) carbon materials will most likely not have pristine surfaces composed of 100% elemental carbon and will have heteroatom-containing surface groups such as quinones, amines, carboxylic acids, or other nitrogen containing functionalities, some of which can be electrocatalytically active; (3) electroactive binders are sometimes added for reasons of ease of electrode manufacture; (4) unwanted electroactive species (contaminants) are easily absorbed on the surface of electrodes prior to experiment and can also generate currents; (5) Pt electrodes will cause background currents by electrochemically oxidising a variety of substrate materials that may be present in MFCs *e.g.* sucrose or acetate; (6) electroactive chemicals exist naturally in various wastewaters. The background currents from the electrode materials and eletroactive chemicals present in the MFC should be evaluated in the absence of microbial species if meaningful results are to be collected. Those background/control experiments are necessary for the complete understanding of a system's electrochemistry, especially for complex devices such as MFCs.

The measurement of a cathode–anode two electrode configuration represents the overall MFC performance, which will be limited by many factors and is rarely completely reproducible due to changes in the growth, evolution, and metabolism of the consortium of microbes present. In order to avoid unwanted perturbations to the system and to simplify studies, a reference electrode (RE) and a counter electrode (CE,

sometimes called an auxiliary electrode) are often introduced into a MFC in order to analyse the behaviour of an individual working electrode (WE), which can either be the anode or cathode: this is then a three–electrode configuration. The potential of or the current generated at the WE can be precisely controlled in a three–electrode mode (this is not always possible with a two–electrode configuration).

The type of RE used must be clearly reported as the potential difference between practical REs of different types and the standard hydrogen electrode differs; care is required when a RE is employed over long periods of time as there will inevitably be small potential changes *e.g.* from concentration changes inside the RE. Due to the fact that silver/silver chloride (Ag/AgCl) and saturated calomel electrodes (SCE) are cheap, practical, and easily prepared, and in addition have the ability to be 'revived' after long term operation, they are commonly the preferred option for REs.

Electrically conductive (e^-) and high surface area materials, such as Pt web or graphite foil, are usually employed as the CE, in order to prevent CE derived mass transport and activity limitations interfering with the measurement of the performance at the WE. The requirement of the CE is that it does not produce byproducts or contamination that would cause interferences to the anode or cathode reactions being studied (the tiniest trace of Pt on a WE can increase its activity towards a variety of species); it can, if required, be separated from the chamber/compartment by a glass frit or sinter to minimize CE–derived contamination.

2.2 Electrode reaction mechanisms and processes

Different catalysts for, and mechanisms of, MFC electrode reactions have been proposed. For the cathode,[9] catalysts such as carbon–supported platinum, metal tetramethyphenylporphyrins and metal phthalocyanine have been used for the ORR. As well as oxygen in air, electron accepting chemicals such as ferricyanide or Mn^{IV} species can be used. Biocathodes have also been reported.[15] For the anode, direct electron transfer (DET) from the microbes to the typically carbon–based anode via extracellular cytochromes[16] or conductive microbial pili (electrically conductive nanowires) have been observed.[17] Indirect (mediated) electron transfer (IET) to the anode is also possible via electroactive metabolites, such as hydrogen or sulphide,[18,19] or with the addition of non-natural (chemical) redox mediators.

For abiotic cathodes immersed in electrolyte solutions, the electrode reactions of MFCs are heterogeneous and take place in the interfacial region between the solid electrode and the solution. An example of a pathway for a simple oxidation or reduction process ($O + ne^- \leftrightarrow R$, where O is the oxidised species and R is the corresponding reduced form) on an electrode is given in Fig. 1. The distribution of charged species, which affects the electrode processes, differs in the electrode surface region and in the bulk solution and can take place in multiple stages as summarised below:[20]

1. Mass transport of the O'' species to the electrode surface where the reaction ($O + ne^- \leftrightarrow R$) occurs;

2. On the electrode surface, chemical reactions that might not involve an electron transfer processes [*e.g.* adsorption, desorption, protonation, decomposition,

electrodeposition steps ($O'' \leftrightarrow O'$ and $R'' \leftrightarrow R'$)] may precede or follow the electron transfer step (or steps);

3. Electron transfer ($O + ne^- \leftrightarrow R$) at the electrode surface;

4. Transport of the R' from the electrode surface to the bulk solution;

5. Transport of electrolyte including spectator ions and chemical species.

Electrode processes involving electron transfer can take place in several simultaneous or consecutive steps. The rate of an overall reaction is affected not only by the reaction processes at the electrode surface but also by the mass transport of species at the interface between the bulk solution and electrode surface region. Mass transfer can have contributions from: i) diffusion along concentration gradients; ii) migration when an electric field is present; and iii) convection in the presence of any form of agitation or forced motion. In traditional electrochemical systems, it is usual practice to design experiments where the conditions present facilitate the treatment of recorded data (*e.g.* leading to simplification of the electrochemical equations required for the calculation of kinetic parameters with the removal of unwanted factors). For example, an inert supporting electrolyte of ≥ 0.1 mol dm^{-3} concentration is often added to reduce migration effects and ohmic losses, and convection can either be avoided by preventing stirring and vibrations in the system being studied or precisely controlled using hydrodynamic techniques (see Section 3.11). However, the operational conditions typically found in MFCs [*e.g.* ≤ 50 mmol dm^{-3} (low concentrations) of stirred electrolyte] are predefined by the requirements of the microbes for optimum growth and metabolism and are not necessarily compatible with the objectives of an electrochemical study. Careful experimental design is essential in MFC studies when the aim of a study is to determine

fundamental kinetic parameters and the data must have some form of mass transport as well as ohmic corrections.

A potential pathway for DET between biofilm and electrode is shown in Fig. 2a. The region of biofilm formation on the electrode surface is critical to the operation of a MFC and both living and dead cells are present in the self–generating biofilm. The following steps can occur during the electrode reaction:

1. Transport of the substrate from the bulk solution to the biofilm region;

2. Transport of the substrate in biofilm region;

3. Metabolisms inside the different microorganisms that are present;

4. DET between the biofilm (*i.e.* the active sites of extracellular enzymes of the microorganisms) and the electrode;

5. Transport of the metabolites and the reaction products;

6. Transport of electrolyte including spectator ions and chemical species.

For anode reactions, living cells in the biofilm consume the substrate (electron donors) that is transferred from the bulk solution and the cellular metabolisms consist of a series of enzyme reactions, some of which yield the electrons. The intrinsic electron relay occurs in the microbes during the biological metabolism and a proportion of these electrons can be collected by the anode via extracellular cytochromes (when present).

The biofilm thickness is greater than the electrode surface region and the positions of extracellular enzymes have an important influence on the DET reactions (Fig. 2a):

a) DET can occur only when the active sites of extracellular enzymes are sufficiently close to the electrode surface;

b) When microbial cells in the biofilm are a certain distance from the electrode surface, it is impossible for DET generation to occur via extracellular cytochromes;

c) Some microbial cells contain electrically conductive pili, which can extend for tens of micrometers and may serve as electrical conduits for long range direct electron transfer.

Most redox proteins are known to undergo very slow heterogeneous electron transfer at electrode surfaces and exhibit irreversible electrochemical behaviour; one limitation of DET is that the active sites are typically buried deeply in the enzymes, which results in poor electrode kinetics. Fig. 2b illustrates the possible scenarios for indirect electron transfer (IET). Electroactive metabolites, such as phenazine, H_2 or HS^-, are produced by the living cells in the bulk solution and/or in the biofilm and can react at the electrode surface. There are two primary mechanisms by which this can take place:

a) When the cells are in contact with the electrode (*i.e.* the electroactive metabolites released from the cells are already on the electrode surface), the electron transfer reaction can occur with preceding and/or subsequent chemical reactions and/or surface reactions;

b) When the electroactive metabolites are released away from the electrode surface (*e.g.* by the suspended cells in the bulk solution), mass transport must occur in addition to the chemical reactions, electron transfer, and/or other surface reactions.

In some earlier studies,[2] artificial redox mediators (*e.g.* neutral red) were added to MFCs to enhance electron transfer processes. There are, however, often undesirable consequences when doing this (all of which make the use of such artificial mediators an

unsuitable choice for sustainable electricity generation in MFCs): these species tend to be toxic, they require regular addition and they often cause environmental problems. This review will not further discuss the functions of these artificial mediators.

In summary, there is typically a large variety of microbial species in commercially relevant MFCs, but only a part of these diverse consortia is involved in electrode reactions. The pathways for the electrode reactions, DET or IET, are interlinked and the processes involve a complex sequence of coupled steps. Microbial kinetics are directly linked to the reactions occurring at the electrode. Microbial growth is dependent on the substrate, metabolites and microbial concentrations, and an adaptation of the biofilm structure, and/or a change of the microbial community, will influence the electrochemical characteristics. The porosities and pore dimensions of the biofilm affect the transfer of metabolites to the electrode surface. Thick biofilms might enhance DET, but yield increased mass transfer losses (and associated impedances – see section 3). The transfer of protons, substrate and metabolites between electrode surface and bulk solution will also have an effect on the current generation.[21] For IET systems, higher power outputs are often obtained with the presence of higher concentrations of metabolites. The rate of the overall electrode reaction is determined by the rate of the slowest step in the sequence of coupled reactions. The metabolism of the microbes is generally the rate–limiting step and this limits the rate of current generation to that dictated by the rate of substrate oxidation by the active microbes; current cannot be generated at an electrode faster than the rate that the microbes can oxidise a substrate and transfer electrons to the electrode.

2.3 Microbes, configuration and operational conditions

Different MFC configurations have been designed for both batch and continuous operation modes: examples include two–chamber, single–chamber, upflow tubular and mini–MFC configurations. Many different separator types have been used in MFCs and these include:[22,23] cation or anion exchange membranes, bipolar membranes, nano–porous filters etc. A large variety of microbe–substrate combinations have been studied and new types are being continuously discovered.

There are many factors that affect MFC performance and even one small, seemingly insignificant change can lead to a significant substantial deterioration in performance. The correct choice of experimental technique(s) for any specific research objective is very important. Testing of identical MFCs in parallel must be conducted to evaluate reproducibility and repeatability; data from only one test may lead to misunderstandings due to the complex nature MFC systems.

3. Electrochemical/electroanalytical techniques

The power generated by a MFC is quantified in terms of power output, $P = V_{cell}$ (V) $\times I$ (A). The open circuit voltage (OCV) is the voltage of a MFC under a no–load condition (no current generation) and can be measured with a high impedance voltmeter or potentiometer. The open circuit potential (OCP) is the potential of an electrode (cathode or anode) measured against a suitable RE, again using a high impedance–measuring device. An overpotential, a measure of the irreversibility of the processes occurring, is the difference between the equilibrium (reversible) potential of an electrode with zero net

current and its operating potential with a net current flow; it broadly represents the extra energy ("kick") needed to force the electrode reactions to proceed at the required rate.[24] The cell voltage of a MFC can be expressed in terms of the overpotentials associated with different fundamental phenomena as shown in the following equations:[25]

$$E_{cell} = E_c - \eta_{act,c} - \eta_{conc,c} - E_a - \eta_{act,a} - \eta_{conc,a} - \eta_{ohm}$$ Equation (1)

$$E_{cell} = E_c - IR_{act,c} - IR_{conc,c} - E_a - IR_{act,a} - IR_{conc,a} - IR_{ohm}$$ Equation (2)

where E_c and E_a are the reversible OCPs for the cathodic and anodic reactions respectively; η_{act} are the charge transfer derived overpotentials of the anode or cathode (alternatively called charge transfer losses or activation losses – the subscripts a = anode and c = cathode); η_{conc} are the concentration overpotentials of the anode or cathode (also called mass transport losses); η_{ohm} are the ohmic overpotentials of MFCs (alternatively called ohmic losses – Ohms Law $V = I \times R$). Each of the overpotentials for various steps can be represented with an associated resistance: R_{act}, R_{conc}, and R_{ohm}, (Equation 2); R_{act} and R_{conc} are not real electrical elements and are better described as impedances,[20] but they can be useful for characterizing MFCs and their constituent electrode reactions *e.g.* R_{act} is mathematically related to the much more important exchange current density, a parameter discussed later in Section 3.3. Caution is required in using the term of "internal resistance" as the chemical fuel cell community considers this to refer to R_{ohm} only, whereas many MFC studies use the term to mean the sum of all of the R_{act}, R_{ohm} and R_{conc} that are present in the system. A small impedance (resistance) represents a low overpotential and a rapid process; a key challenge for improving MFC power outputs is to reduce these impedances.

3.1 MFC polarization techniques

The term "polarization" (which is old fashioned and sometimes misleading) is the change of electrode potential (or MFC voltage) from its equilibrium state due to a flow of current. Polarization curves are plots of electrode potential (or MFC voltage) as a function of current or current density. Such plots contain a wealth of information and can easily be obtained with a suitable potentiostat (capable of measuring power inputs – *i.e.* power is supplied to the instrument from the power source being studied) or a variable external resistance load; for highly reproducible, precise measurements it is essential that an electronic load with the appropriate current ranges is used.

There are four options for the measurement of MFC polarization discharge curves: (a) constant resistance discharge measured by connecting different resistors to the MFC and measuring the resulting currents and voltages; (b) potentiodynamic polarization, *i.e.* linear sweep voltammetry (LSV) where current is measured with slow voltage scan rate (*e.g.* 1 mV s^{-1}); (c) galvanostatic discharge, where the current is controlled and the resulting voltages measured; and (d) potentiostatic discharge, where the voltage is controlled and the resulting currents measured.

For the above options, constant potential (potentiostatic) discharge measurements may be the most suitable for investigations that are probing the fundamental science that is involved in biofuel cells, especially when the electrochemistry of enzymes is involved: i) electroactive biological species (enzymes) can rapidly switch their activities on or off depending on the potentials present; hydrogenase fuel cell catalysts are a classic example as they can suddenly switch off (deactivate) when the potentials are raised beyond a certain point, this being due to the oxidation of the metal centres to non–active oxidation

states.[26] This is in contrast to chemical fuel cell studies where constant current (galvanostatic) discharges are preferred as constant reaction rates (electron generation/consumption rates) are occurring at the electrodes. ii) for constant resistance discharge techniques, both the current outputs and cell voltages will be varying with time (two variables, however small their variation). In comparison, potentiostatic experiments involve controlled and constant cell voltages and require only measurement of the current (the only major electronic variable). Constant potential and current data are useful when MFCs are designed as power supplies for practical systems; constant resistance values do not yield much useful information for MFC studies, especially when MFCs of different configurations and dimensions are being compared. Even when chemical fuel cells have been tested for real world scenarios, the variable load has been via a controlled and time varying current profile (and not by a external resistance profile). The use of electronic loads has clearly benefited the development of chemical fuel cells. Constant resistance measurements are not necessary when accurate electronic loads or battery/fuel cell test equipments are available. iii) finally, it is often difficult to determine when using LSV measurements (even with a slow scan rate) if a system is achieving a steady state for each data point collected. The factors affecting the steady state operation for MFCs are much more complicated than for traditional electrochemical systems.

The time that should be left for equilibration before recording data points at each resistance/current/potential is still being debated (seconds, minutes, hours). Unlike with a chemical fuel cell (times frames in the range of several seconds to several minutes are typically used when recording galvanostatic data, after which steady potentials are normally achieved), it is difficult to record a credible steady state polarization curve with

a MFC for the following principlal reasons: (a) the nature of the microbial species/communities is evolving with time and electrode potential, with concurrent changes in the biofilm (for chemical fuel cells, the composition and structure of catalysts change only very slowly with time via various degradation reactions – < 100 μV h^{-1} in well the best systems); (b) the concentrations of electroactive metabolites and/or substrate may also be constantly changing with time and potential (chemical fuel cells supplied with gases can be tested or operated with very precise and constant fuel/oxidant supplies); finally (c) the mechanisms of electrode reactions and operational conditions may be different for each different type of MFC, each requiring different sampling rates. MFCs can exhibit stable behaviour in simple time–related voltage and current experiments using fixed resistance load, but long–term polarization testing requires caution as there is a continual risk of the presence of the above changes.[27,10] In contemporary MFC studies, pseudo–steady state conditions are generally present during testing and a minimum of several minutes per data point is necessary. The optimum time for data point recording needs further discussion by MFC researchers, as it will inevitably be a function of the system being studied and can affect numerous parameters.

An ideal polarization curve for a power generation device (MFC or chemical fuel cell) includes three characteristic regions located at different current ranges (either well segregated, as is typical with chemical fuel cells, or with varying and significant levels of overlap, as is more typical with MFCs) as shown in Fig. 3a and summarised below:

a) The region of charge transfer overpotentials ($\eta_{act,a} + \eta_{act,c}$) is located at low currents and derives from the slowness (irreversibility) of the reactions taking place on the surface of the electrodes. The charge transfer overpotentials depend

on the nature of the electrode materials, catalysts, reactant activities, electrolyte including any spectator species that are present, electrochemical mediators, biofilm, electrode microstructure, microbial species and their metabolisms, and operational conditions such as temperature;

b) Ohmic overpotentials typically manifest themselves at intermediate currents in the polarization curve and are caused by ionic resistances in the electrolyte (especially for MFCs, due to low ionic concentrations), membrane, and biofilm, and by electronic resistances in the electrodes, current collectors, interconnects, and the electronic components between the measuring instrument and the power generation device under study;

c) Mass transport overpotentials result from the changes in concentration of the reactants (or products) at the interface between electrode surface region and bulk electrolyte; this overpotential is prevalent at relatively high current densities if the reactants cannot be supplied to the electrode reaction zones at the rate required to sustain the generation of current. The presence of high concentrations of product species can also lead to the reduction of transport of reactants to where they are needed. Mass transfer derived overpotentials are affected by the geometry and the structure of electrodes and biofilm, the nature of the electrolyte, the metabolites, and the products that are present.

3.2 Electrode polarization techniques

A MFC polarization curve yields the overall fuel cell performance under specific operating conditions, but fails to give information on the performances of the individual

electrodes (anode or cathode), hence it is difficult to elucidate the limiting factor of MFC performance. However, a RE can be easily introduced in one or more of the MFC chambers so that it is possible to record the individual potentials of anode or cathode (as well as the overall cell voltage of the MFC – modern electrochemical testers can measure the impedance spectra of all of these simultaneously, see Section 3.5); single electrode polarization curves can, therefore, be easily obtained as shown in Fig. 3b. The overpotentials of a MFC are the sum of the (non–linear) overpotentials of the anode and cathode and the (linear) internal ohmic overpotentials of the MFC. By analysing the potential changes of the anode and the cathode at varying currents, the performance limiting factor can often be revealed. For example, a previous study showed that a biocathode showed inferior performance compared to the anode,[28] and that increasing the efficiency or surface area of the cathode should be targeted to enhance power generation. As it will become apparent in Section 3.5, to simplify analyses and to reveal more information, polarization plots can be corrected for IR_{ohm} losses *i.e. IR*–corrected voltages (= $V_{cell} + I \times R_{int}$) can be plotted against $\log_{10} I$, where R_{ohm} is the internal ohmic resistances between the relevant electrodes; an example of this is found Figure 4 of reference 29.

In a three–electrode configuration, it is possible to obtain the performance of an individual anode or cathode using a variety of techniques including galvanodynamic or potentiodynamic measurements (other relevant techniques are detailed in the sections below). Galvanodynamic measurements[12] have been used to investigate the cathode performance of cobalt– and iron–based ORR catalysts in different concentrations of aqueous electrolyte. It was demonstrated that limited buffering capacities of the

electrolyte will lead to undesirable decreases in the cathode performance due to pH changes at the cathode. Rosenbaum *et al.*[30] used galvanodynamic electrochemical methods to evaluate the anode performance and electrocatalytic activity with microbial fermentation products; hydrogen, formate, and lactate can be oxidised by tungsten carbide catalysts in a neutral pH electrolyte, whilst no activity was observed for ethanol electro–oxidation. Potentiodynamic methods were used by Manohar *et al.*[31] to compare the performances of anodes in buffer, buffer–substrate, and buffer–substrate–microbe systems; the analysis of their results allowed the determination of the exchange current densities and Tafel slopes, detailed below.

3.3 Butler–Volmer equation and tafel curves

Charge transfer overpotentials are controlled by the rate of heterogeneous electron transfer, and the kinetics of this process are described by the Butler–Volmer equation[20] when the reactants are abundant and the current is small enough that the ohmic and concentration overpotentials are negligible:

$$I = Ai_o \left\{ e^{\left(-\frac{\alpha nF \eta_{act,c}}{RT} \right)} - e^{\left(\frac{(1-\alpha)nF \eta_{act,a}}{RT} \right)} \right\}$$
Equation (3)

where I is the current, A is the electrode active surface area, i_o is the exchange current density, α is the charge transfer barrier (symmetry coefficient), n is number of electrons involved in the electrode reaction, and $\eta_{act.}$ is the charge transfer overpotential. Typical electrode reactions occur in more than one elementary step, and there is an overpotential associated with each step. The i_o is a fundamental parameter in the rate of electrooxidation or electroreduction of a chemical specie at an electrode at equilibrium; a

large i_o indicates a fast reaction (approaching reversible, as found with the hydrogen oxidation reaction at a Pt electrode), whereas a small i_o indicates a slow reaction (often an irreversible electrode reaction, such as the reduction of oxygen (often irreversible even when employing Pt electrodes).

The Butler–Volmer equation can be simplified to equation (4) in the high overpotential region (> 118/n mV, where n is the number of electrons exchanged), yielding the Tafel equation:[20,25]

$$\eta_{act.} = b \log_{10}\left(\frac{i}{i_o}\right)$$
<div align="right">Equation (4)</div>

where i is the current density and b is Tafel slope (mV dec^{-1}), which is an important experimental parameter commonly used to probe the mechanism of an electrode reaction. Plots of overpotential against $\log_{10} i$ are known as Tafel Plots (see Fig. 4); i_o and b are obtained by extrapolation of the linear region of the curve to $\eta_{act.} = 0$.

Exchange currents or exchange current densities in MFCs have previously been calculated from Tafel plots.[12,31,32] Several methods have been proposed with the aim of reducing charge transfer overpotentials and therefore increasing i_o:

1. Improved catalysts or biocatalysts, which can considerably decrease activation barriers, have been used to improve the electrode performance;

2. Modification of the electrodes can significantly alter the biofilm composition of electrode surface, which can facilitate an electrode process such as the direct electron transfer between a protein and the electrode, or can produce selectivity toward a particular process (especially in EFC studies);[32]

3. Utilisation of electrode materials with higher specific surface area (*e.g.* with a highly porous structure) has the potential to provide more available reaction sites

for electrode reactions and increase the geometric current density. Microbes may not be able to access the surface areas inside smaller sized pores, however, ignoring the specific surface area of the electrode materials (especially with carbon based electrodes, where these surface areas are easily obtained using gas absorption/mercury porosimetry measurements) may lead to a loss of vital information. An example is that a electrode with higher specific surface area substantially improved performances for sulphide oxidation;[19]

4. The rates of chemical reactions involving electroactive metabolites are generally faster than for reactions involving biological metabolisms; employing optimised operational conditions (*e.g.* raising the temperature to levels optimal for microbial metabolism and for maximum enzyme reaction activity and/or increasing the concentrations of reactants)[33] may be effective;

5. The evolution of microbial species can be adapted to allow enhanced electrode performance; analysis of gene expression and identification of beneficial mutations can allow improvements via genetic engineering and the identification of more of the electroactive species and more effective biofilm structures.

3.4 Current interruption (CI)

CI techniques have been used extensively to measure the internal ohmic resistance of chemical fuel cells and more recently of MFCs.[27] As shown in Fig. 5, the basic principle of the CI is to interrupt the current flow and to observe the resulting voltage transients. On current interruption, the ohmic overpotential is separated from other overpotentials as the former is a near instantaneous process, whereas the relaxation times for other voltage

loss phenomena are significantly longer. CI measurements can be conducted using low cost electronic equipment, and resulting data are easily interpreted. The primary disadvantage is that very short duration (< 10 μsec) measurements of the perturbation to the system are required for precise and accurate determinations, and it is possible to overestimate the voltage changes if data collection is not rapid enough (sample rate is too low). Another disadvantage of using CI is that it is difficult to distinguish between charge transfer and mass transfer impedances.

3.5 Electrochemical impedance spectroscopy (EIS)

EIS takes advantage of the large spectrum of time scales over which different processes occur in the system being studied. It is a powerful tool for examining chemical and physical processes in solutions, at solid–liquid interfaces and at solid–solid interfaces, as it allows the separation of the different voltage loss phenomena.[20,29,34,35] There are two common graphical representations used in EIS: Nyquist plots and Bode plots (Fig.6). The main shortcoming of Nyquist plots is that it does not show the frequency represented by each data point (each point being a representation of the impedance vector in the complex plane at a particular frequency); Bode plots show this frequency information as they are plots of the magnitude and phase angle of the impedance vector versus frequency.

Recently, MFC studies using EIS have been reported.[31,36,37] A small amplitude (*e.g.* 10 mV rms – rms = root mean squared) alternating current (a.c. – sine wave) perturbation signal is applied to the MFC being tested; small perturbations are used to ensure non–linear harmonic effects are not interfering with the data collection and also to prevent damage to the biofilm attached to the electrode surface (systems are often perturbed far

from equilibrium in the cyclic voltammetry or potential step methods detailed in Section 3.6). To avoid non–linear responses when measuring EIS spectra at different currents or potentials, it is vital that the d.c. polarization curves are inspected first so that the correct settings can be deduced. The amplitude of either the voltage or current perturbations (when conducting experiments with potential or current control respectively) must be selected such that responses will be linear at steady or pseudo–steady state conditions (Fig. 7) and within the current/voltage ranges of the electronic equipment used in the test *e.g.* do not use a voltage perturbation signal that will result in a current amplitude (*e.g.* 10 nA) that is smaller than the current measuring resolution (*e.g.* 0.1 μA) of the instrument. In general, it is best to use the smallest perturbation signal that still produces noise free data; perturbations of large amplitude will result in distortions of the EIS spectrum due to non-linear responses.

Detailed information on the ohmic internal resistances, and on the charge and mass transfer impedances, can be obtained on the analysis of correctly collected EIS spectra. The Nyquist plot and the Bode plots of a single chamber MFC with an air cathode developed for wastewater treatment are shown in Fig. 6. The ohmic internal resistance is often determined by the high frequency intercept of the curve with the real impedance axis (Z_{re} – the *x*–axis).[20] The internal ohmic resistances recorded using EIS data are much more accurate than when using simple single frequency resistance measurements (1 kHz measurements are commonly employed in cheap ohmmeters). EIS can, like CI, overestimate internal ohmic resistances, especially at high d.c. discharge current densities; however, this overestimation tends to occur at current densities of > 0.5 A cm^{-2}

(geometric) achievable in H_2/O_2 chemical fuel cells, which are unlikely to be achieved with MFCs.

With EIS technique the conductivity of electrode materials and membranes can be easily measured. The conductivity of cation–exchange membranes (CEM), such as Nafion, can be as high as 0.1 S cm^{-1}, but only when the CEM is in the proton–exchange form (H$^+$ conduction). Conductivities of these membranes are lower when in the Na$^+$ / K$^+$ / Ca^{2+} forms since H$^+$ ions have higher mobilities than metal cations, mainly because proton conduction involves the Grotthuss mechanism;[38] CEMs are likely be present in MFCs in a form where a variety of metal cations or other chemical species are present. Careful selection of materials, MFC configurations, electrode spacing, and electrolyte concentration will allow reductions in ohmic losses. Salt-bridge-containing or high internal resistance (*ca.* 3.9 MΩ) systems[39] have limited power generation because most of the electricity being generated is dissipated as heat (not a desirable outcome: MFCs operating at ambient temperatures will never be applicable as co–generation systems). The conductivity of the electrode material has a practical importance. It is rarely a serious issue with lab scale MFCs, but low conductivity will lead to major problems (uneconomic performances) at the pilot scale or with full scale devices. The thickness and conductivity (an intrinsic property) of the electrolyte membrane also affect the internal ohmic resistance of the energy generation device. Thin membranes reduce ionic resistance but tend to have a higher rate of oxygen crossover to anode compartment, which undesirably reduces coulombic efficiency and/or power production; thin membrane electrolytes might also lead to an increase in the crossover of other species to the cathode, which leads to performance limitations *i.e.* additional mass transport and/or

activation derived losses (catalyst poisoning or blocking of surface sites for ORR). A radical modification is achieved through the removal of the electrolyte membrane – the membrane–less system. Cheng *et al.*[40] reported the maximum power production of a membrane–less MFC achieved with a 2 cm dimensional spacing between the anode and cathode; the power decreased with reduced space due to oxygen crossover from the cathode side to the anode chamber; the studies suggest that the anode and cathode spacing should be less than 2 cm for high power generation when a separator is present in MFCs.

Charge transfer processes occur with smaller time constants (= $R_{act} \times C_{dl}$ if modelled by parallel *RC* circuits in EIS, where R_{act} is the charge transfer impedance and C_{dl} is the capacitance of the electrode's double layer) when compared to mass transport (diffusion/migration) processes. Processes occurring with different time constants can often be identified using EIS measurements. For example, the difference between the charge transfer parallel *RC* impedance (kinetic control – semicircle at medium–high frequencies) can be clearly distinguished from the mass transfer derived impedances (sloped line at low frequencies in Fig. 6a). Charge and mass transport related impedances are common non–ohmic phenomena and require overpotentials to enable a current flow; the diameter of the semicircular charge transfer *RC*-related impedance response in Nyquist plots will change exponentially with overpotential (or electrode potential). The capacitance of the interface between electrode and solution changes when a biofilm attaches to the surface of electrode. Manohar *et al.*[31] found, using EIS methods, that in the presence of the species MR–1 the OCP of an anode became more negative and the capacitance increased; both of these affected the MFC power output.

To reduce mass transfer impedances in MFCs, the electrode structure and configuration should be designed to facilitate the rapid transport of the reactants, products, supporting electrolyte and substrate in order to avoid excessive concentration-derived polarizations. The accumulation of metabolites in the biofilm can hinder microbial activity, whilst mass transport between the anode and cathode chambers tends to alter the pH in each and results in further performance decreases. Stirring and forced convection enhances mass transport and have been shown to improve the power output of EFCs;[41] MFCs exhibit analogous behaviour *e.g.* the anode reaction in a sulphur–pollutant–containing wastewater treatment system was controlled by a diffusion process.[18] Appropriate stirring or forced convection may lead to denser biofilms in systems operating with a direct electron transfer mechanism, leading to enhanced anode performances. For systems operating with indirect electron transfer, the concentration of electroactive metabolites has an important effect on current outputs;[19,42] higher concentrations of electroactive metabolites may reduce the mass transport and charge transfer impedances, as well as internal ohmic resistances by solution conductivity increase. A MFC combined with a reservoir that is designed for the accumulation of electroactive metabolites is a technology that promises high current outputs.

In comparison with CI, EIS is a very sensitive technique yielding a wealth of information such as kinetic parameters, determination of the reaction mechanisms, electrolyte and electrode conductivities, and biofilm behaviours. However, MFC studies, due to their complexity, need to be undertaken carefully in terms of how they approach EIS data collection and analysis, especially when equivalent circuit modelling is used to interpret results and the reaction mechanism of electrode is not known. Each EIS

equivalent circuit element introduced (resistor, capacitance or inductor) must represent something in the real system: the tendency to unnecessarily increase the number of circuit elements, or to replace capacitors with constant phase elements without justification, in order to gain better fits to the collected data must be avoided.

3.6 Cyclic voltammetry (CV)

The determination of the mechanisms of electrode reactions underlying oxidation or reduction reactions can be achieved using a variety of electrochemical techniques. The most common and straightforward technique is CV, which requires a three-electrode configuration to obtain accurate results. Fig. 8 shows the important parameters that can be obtained from CV of a reversible redox couple ($Fe(CN)_6^{3-} + e^- \rightarrow Fe(CN)_6^{4-}$). It is relatively easy to show using CV whether a chemical system under study is reversible or irreversible: for a fully electrokinetically reversible couple, the ratio of anodic to cathodic peak currents = 1 and the potential separations between the peak potentials = 59.2 mV/n at room temperature, where n is the number of electrons transferred in the electrode reaction. Compared to reversible systems, quasi- or irreversible phenomena will exhibit larger separations between peak potentials and/or one or more peaks that are reduced in size (the electron transfer process takes more time to respond to the applied potential in these non-reversible systems). MFC studies employing CV generally use forward and backward voltage sweeps with rates in the range of $1 - 100$ mV s^{-1}. Multiple peaks in the cyclic voltammograms of bio–electrochemical system may be observed due to multi–step parallel or consecutive (series) mechanisms, or to the presence of several different redox species.

In protein film voltammetry, the analysis of the first derivative of CV allows the estimation of the potential at which the rate of increase of the catalytic wave reaches a maximum; this has been used to reveal that the oxidative and reductive potential sweep possess two (or more) inflection points in some systems.[26,43]

In MFC studies, CV experiments have been used extensively to: (i) investigate the mechanisms of electrode reactions involving both direct and indirect electron transfer between the biofilm and the electrode; (ii) determine the redox potentials of the chemical or biological species involved at the anode or cathode (for a reversible redox couple, the average of the cathodic and anodic peak gives the reversible potential for that couple referenced against the RE being employed); and (iii) to evaluate the performance of the catalysts being studied.

For anode reactions, only microbial extracellular cytochromes have been proven, to date, to be active for direct electron transfer mechanisms. However, some CV–based MFC studies shows characteristics of inter-conversion between active–inactive states, *i.e.* inactivation is observed during the positive potential sweep and reactivation is observed on the return sweep; this is not an expected behaviour of cytochromes but is similar with that of enzymes (e.g. hydrogenase) that have been studied in EFCs.[26] The shape of these voltammograms is an initial indication that "turnover" enzymes or multi-enzyme systems might be contributing to the anodic current.

CV is a simple technique and results are obtained in a relatively short time. However, background experiments (with blank electrolyte) are mandatory for high quality mechanistic studies. When microbial communities are present in MFC chambers, the peak current and the peak potential coming from the electrode interface reactions might

involve direct electron transfer and/or indirect electron transfer. For complex systems, where there are many unknowns (especially relevant to wastewaters), the following CV testing protocols are recommended, as a minimum, to aid understanding:

a) Effects of electrode and electrolyte: Employ a biofilm–less electrode in un–inoculated or abiotic electrolyte;

b) Effect of the biofilm: Employ a biofilm-coated electrode in the un–inoculated or abiotic electrolyte;

c) Effect of the substrate: Employ a biofilm–less and a biofilm-coated electrode in the un–inoculated or abiotic electrolyte with the addition of fresh substrate;

d) Effects of the suspended cells: Employ a biofilm–less electrode in an inoculated electrolyte;

e) Effect of the metabolites: Employ a biofilm–less electrode in the inoculated electrolyte with consumed substrate(s).

By comparing the results of these CV experiments (*i.e.* the peak currents and the peak potentials at same scan rate using a same size electrode) the electron transfer mechanisms of the electrode should be clarified to an extent. The collection of reproducible CV scans is required for rigorous understanding, especially in systems with low concentrations of electroactive metabolites.

The characteristics of CV depend on several factors, such as the electrode surface pretreatment, the rate of the electron transfer reactions, the chemical and biological species present and their thermodynamic properties, the concentration of electroactive species and their rates of diffusion and the sweep rate. It should be noted that many electrode materials used in MFCs cannot produce reversible electrochemical reactions

even for the classic reversible redox couple $Fe(CN)_6^{3-}/Fe(CN)_6^{4-}$. The main reason for this is that the heterogeneous processes of electrode reactions can be significantly affected by the microstructure, roughness and function groups present on the electrode surface. One example is that a simple electrode will often produce different electrochemical reaction rates before and after polishing, and will show different CV profiles. When the accurate determination of kinetic parameters is investigated, it is essential that the electrodes and the operational conditions are appropriate.

3.7 Differential pulse voltammetry (DPV)

DPV is a voltammetric technique with an improved sensitivity compared to CV and LSV methods. An important limitation of both CV and LSV is the substantial background levels from capacitive, non–Faradaic currents. Enhanced discrimination of Faradaic currents (electron transfer to and from an electrode) can be obtained using DPV, where the potential perturbation consists of small pulses is superimposed upon a staircase waveform. DPV studies can also provide improved selectivity for observing different redox processes compared with CV and LSV.

In DPV measurements using sterile carbon electrodes in a growth medium and with carbon electrodes colonised by *Shewanella*, Marsili *et al.*[44] found that a layer of flavins was adsorbed on the electrodes and these acted as external electron transfer acceptors (especially in older biofilms), lower concentrations of secondary compounds were present in the electrolyte.

3.8 Chronoamperometry (CA)

CA is an electrochemical technique where the potential of anode or cathode is controlled (or stepped) and then held constant with the resulting currents being monitored as a function of time. Bond *et al.*[45] inoculated a MFC chamber containing *Geobacter sulfurreducens*, acetate as the electron donor, and a graphite electrode held at an oxidising potential (+200 mV versus Ag/AgCl). A DET process was indicated for the MFC as the biofilm electrode continued, unaffected, to generate an acetate–dependent current when the medium was replaced with an anaerobic buffer lacking nutrients. It is important to appreciate that the anode potentials influence and regulate biofilm activity as well as the microbial cell growth rate. The study of three comparable reactors, containing microbial communities that were continuously fed with acetate, with anode potentials poised at 0, −200 and −400 mV versus Ag/AgCl respectively, led to the conclusion that the optimal anode was at −200 mV under the conditions tested.[27] In another experiment, a tungsten carbide modified anode was immersed in a glucose substrate solution that had been freshly inoculated with heat-treated soil, and data on microbial growth and oxidation of metabolites as function of time were recorded.[30] A final example is where both carbon fibre veil and activated carbon cloth anodes were compared during a study of the oxidation current for microbially produced sulphide at anode potentials of +200 mV versus Ag/AgCl; the highly porous activated carbon cloth electrode gave substantially better performances.[18]

3.9 Chronopotentiometry (CP)

CP involves the study of potential as a function a time at an electrode operating with a constant current (the opposite of CA above). A recent study using this technique investigated air cathodes coated with polytetrafluoroethylene containing layers.[46] The electrode potentials of the layered carbon cloth electrodes were measured whilst applying a constant current. The resulting potentials were then plotted as a function of current densities to evaluate the electrode performances. A maximum performance was achieved with coulombic efficiencies of up to 42% when a four-layer-coated electrode was employed.

3.10 Other simple time–related voltage, potential and current measurements

There are other basic techniques that can be employed to investigate both MFCs and the individual performances of electrodes as a function of time without precise control of potential or current. Information such as voltage versus time profiles, acclimation times, the effects of substrate addition and/or depletion, electrolyte effects, and the changes in current on bacterial evolution and enrichment can all be obtained.[47,48]

The potential of various carbon based anodes as a function of time in wastewater containing sulphur pollutants was recently investigated.[19] The results indicated that the anodic potential was primarily controlled by the concentration of microbially produced sulphide in the solution. Another study, that analysed potential/time curves, showed that biologically produced H_2 also had an effect on the behavior of modified Pt anodes.[49] These studies demonstrate that the electroactive species (not only the biofilm) should be

considered when probing electrode mechanisms, such species are commonly found in wastewaters and significantly affect the electrode performance.

3.11 Rotating–disk electrode (RDE) and rotating ring disk electrode (RRDE)

Hydrodynamic techniques, such as RDE and RRDE, are electrochemical experiments where mass transfer is carefully controlled and are essential for studying the precise kinetic parameters of electron transfer and for detailed probing of electrochemical reaction mechanisms. These techniques have been used in the evaluation of catalyst or modified electrode performances and for quantifying the number of electrons involved in ORR.[20] The desired cathode process is the full (4e⁻) reduction of oxygen to water, but partial (2e⁻) ORR will occur on carbon-based electrodes and results in the production of significant quantities of highly reactive hydrogen peroxide (H_2O_2), which may affect microbial metabolism. RRDE studies involve a RDE with an additional ring electrode (poised at a separately controlled potential – hence the need for a bipotentiostat) to identify the products (including peroxides) of the electrochemical reactions occurring on the central disk electrode. These techniques cannot, however, always be used to probe the electrochemical behaviour of biofilms on electrode surfaces since biofilm can be fragile and likely to be destroyed under conditions of high speed rotation.

Another possibility that will allow experiments with rapid mass transfer is to design experiments, which can study the electrochemistry of a single microbe on some form of electrochemically inactive but electronically conductive electrode surface. The hydrogen oxidation reaction has been studied on electrode tips containing single Pt nanoparticles of defined size.[50]

3.12 Denaturing gradient gel electrophoresis (DGGE)

DGGE is one of the most sensitive electrophoretic techniques available and has become a routine and essential technique in the field of environmental microbiology for characterisation of microbial population structures and ecology. In many MFC studies, the development (evolution) of microbial communities over time have been monitored using DGGE involving polymerase chain reaction (PCR)–amplified 16S rRNA gene fragments.[51] *Shewanella* and *Geobacter* species have been proven to be electroactive microbes for direct electron transfer, but analysis of the communities in the anode biofilm revealed a diversity of microbes much greater than these simple iron reducing bacteria alone; many novel microbes appear to be enriched in the MFCs. The types of microbes that are enriched in a MFC depend strongly on the substrate and environmental conditions; no typical consortium has been detected from any clone library to date.[52,53]

PCR-DGGE is dependent on intact nucleic acid rather than viable or non-viable cells, and does not distinguish between living and dead cells in MFCs. As a consequence, it may result in misleading results with the complex communities in wastewater treatment systems. Much remains unknown about the role and the interactions of microbes within both the electrode biofilm and the suspended communities, and the contribution towards electricity generation of microbial cells at different electrode surface regions. Understanding how microbial populations and biofilm structure evolve with time is essential if superior MFC power levels are to be achieved.

3.13 Combined electrochemistry–spectroscopy techniques

Spectroelectrochemical techniques facilitate an in vivo understanding of molecular structures, electron transfer mechanisms, and the interactions between microbes and electrodes. Using direct current surface enhanced infrared (IR) absorption spectroscopy and subtractive interfacial Fourier transform IR spectroscopy, a recent study investigated the interface between *Geobacter sulfurreduces* and a gold electrode.[54] The analysis of the IR spectral bands linking the increasing presence of protein at the interface with time to the increase in current, thereby demonstrating that the extracellular cytochromes were responsible for the electron transfer to the gold electrode.

3.14 Scanning tunnelling microscopy (STM) and atomic force microscopy (AFM)

STM and AFM are both high–resolution and powerful types of microscopy. These are not direct electrochemical techniques but merit inclusion as they can provide complementary information on electrode materials. STM or AFM can be used to image conducting substrates and microbial pili in MFCs studies. For example, the pili on *Geobacter* species were recently recognised using these techniques;[17] the presence of such pili raises the possibility of long range DET from one microbe to the electrode.

The understanding of the function of these nanowires is in its infancy and the mechanism of how the proteins of these microbial pili interact with extracellular electron acceptors is poorly understood. This is an important area for future study, in which *in situ* electrochemical (and associated) techniques will play a vital part. Techniques combining AFM with electrochemical measurements have recently been developed for the study of

the ionically conductive channels in proton–exchange membrane,[55] and these may have uses in MFC studies.

3.15 Other techniques worth consideration

Alternative electroanalytical techniques that aid in the investigation of MFCs in the future and to further improve the fundamental understanding of the processes occurring are: i) square wave voltammetry, which has detection limits as low as 10^{-8} mol dm^{-3} when used as an electroanalytical technique to determine the nature and concentrations of electroactive metabolites at trace levels;[20] ii) electrochemical quartz crystal microbalance measurements can be used as a non–destructive on–line monitoring tool with regards to the formation of biofilms, and has been used to obtain information on the biofilm–electrode interface;[56] iii) a.c. voltammetry is receiving increasing interest in the electrochemical community; iv) microelectrode techniques may be useful for in situ investigations of the conductive pili; and v) electrochemistry combined with other techniques, such as microarrays, microscopy, or fluorescent *in situ* hybridization experiments, may provide a great deal of insight about the behaviour of the DNA, microbes, and other electroactive species that are present in the systems being studied.

4. A summary of the key points

To date, the commercial viability of MFCs will rely on side benefits and not purely on the generation of electrical power. An ever expanding range of cross–disciplinary techniques are now being employed to study MFCs with the aim of enhancing both fundamental understanding and innovation in the field. Each of the

electrochemical/electoanalytical techniques described in this review provides a range of valuable information that is useful in the study of MFCs. More details about these techniques can be obtained from the classical electrochemistry literature *e.g.* reference 20. The following summarises some of the key points from the main text.

1. There are various biological and chemical reactions occurring in MFCs, but only a few steps are directly involved in electron transfer processes of the electrode reaction. Background experiments should be conducted to identify and clarify the electrochemical reaction mechanisms, and the effects of the electrode materials, biofilm, substrate and metabolites. The spectator ions/chemicals present in MFC systems cannot be ignored and will fundamentally affect any electrocatalysts that are present. This is especially true of species such as HS^-, Cl^-, HPO_4^{2-}, and HCO_3^-/CO_3^{2-}.

2. Experiments that measure reproducibility and repeatability (such as running identical MFCs in parallel and at different times) should be conducted.

3. The inclusion of a reference electrode will allow the routine (and often simultaneous) collections of anode, cathode and whole cell potentials at different MFC current outputs. Modern equipment will also allow the simultaneous collection of the impedance spectra at steady or pseudo–steady state conditions for each of the above.

4. With the availability of low cost electronic loads or battery test systems, a constant potential (potentiostatic) measurement may be preferable when studying MFC discharge performances.

5. Each study must define the use of term "internal resistance". This should refer only to internal ohmic resistances. Ideally, the collection of IR_{ohm} corrected voltage data should be routine.

6. Keep the analysis of impedance spectra as simple as possible, and ensure that each element (*e.g.* capacitor) that is introduced in equivalent circuit modelling is fully justified by the system under study, *i.e.* each circuit element should represent an identified parameter.

7. Carbon materials from different suppliers/sources will not be the same. The surface chemistry (specific surface area, surface functionalities and morphology) of the electrode materials should be evaluated because they have an effect on biofilm and/or on other species.

8. Oxygen reduction on carbon–based electrodes will produce peroxide species under neutral pH conditions, with $2e^-$ oxygen reduction being predominant on carbon surfaces. The effect of peroxide on the system performance (and durability) should be taken into account or even studied.

Acknowledgments

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Figure captions

Fig. 1 Schematic of the pathways involved in solution phase electrochemical reactions composing of a series of simultaneous or consecutive steps: mass transfer, chemical reactions or other surface reactions, electron transfer. The different species (O, O′, O″, R, R′ and R″) represent the corresponding forms before and after the processes such as adsorption, desorption, protonation and electron transfer. (Adapted from reference 20).

Fig. 2 Schematic of the pathways involved in solution during electrochemical reactions with the presence of a solid biofilm electrode: a) direct electron transfer between the biofilm and the electrode; b) indirect electron transfer via the electroactive metabolites.

Fig. 3 a) The ideal current–voltage polarization curve of biological fuel cell, and b) the separate current–potential polarization curves of cathode and anode where a reference electrode has been introduced into the system.

Fig. 4 A typical Tafel plot for an electrode reaction, from which exchange current density and Tafel slope can be determined.

Fig. 5 An ideal voltage transient in a MFC after current interruption. The MFC is firstly operated at a fixed current, and then current is interrupted at t_o.

Fig. 6 a) A Nyquist plot and b) a Bode plot of the impedance spectrum of a single chamber MFC measured with two–electrode configuration under open circuit voltage.

The frequency range was $10^6 - 0.1$ Hz with a potential perturbation signal of 10 mV rms. R_{ohm} was the ohmic internal resistance, $R_{act,}$ was the charge transfer "resistance".

Fig.7 Schematic diagram illustrating the need for choosing the amplitude of the stimulus signal correctly. The diagram shows the measurement of impedance spectra at different MFC d.c. discharge currents with potentiostatic control, a.c. potential perturbations and the measurement of the resulting a.c. current responses: a) nonlinear response (distortion in impedance spectra); b) ideal linear response; and c) linear response where the current amplitude is too small to be accurately measured by the instrument (very noisy impedance spectrum).

Fig. 8 A classic cyclic voltammogram of a fully reversible redox couple showing the important parameters: i) peak potentials; ii) peak currents and iii) the potential difference ΔE between the reduction and oxidation peaks.
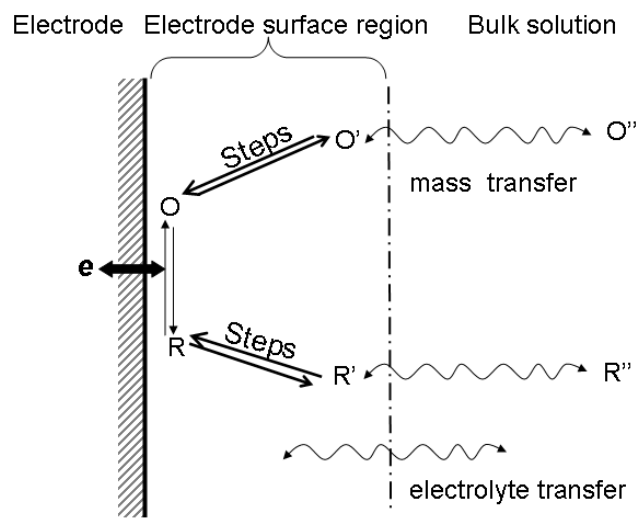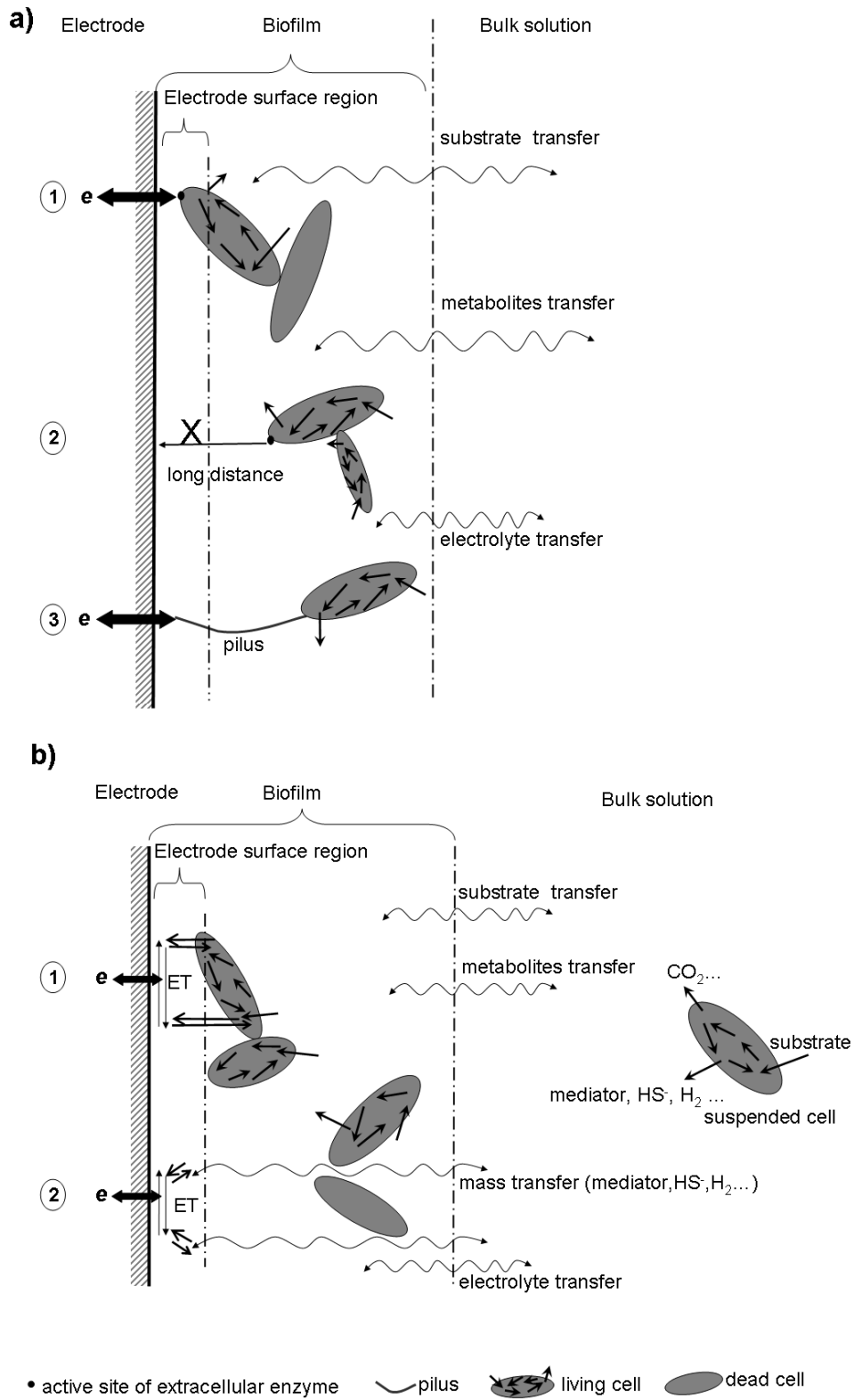
Fig. 1

a)

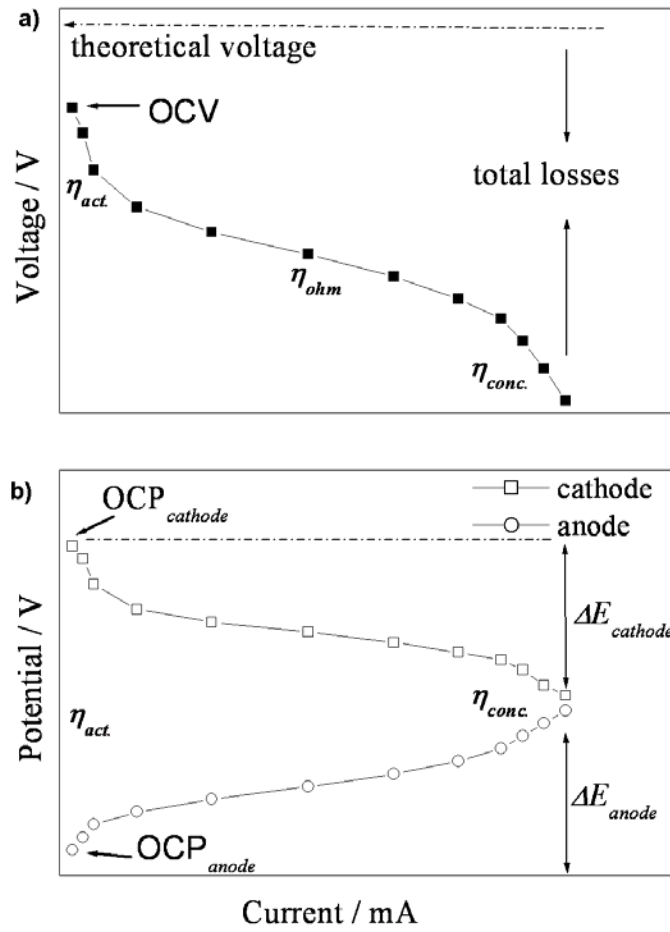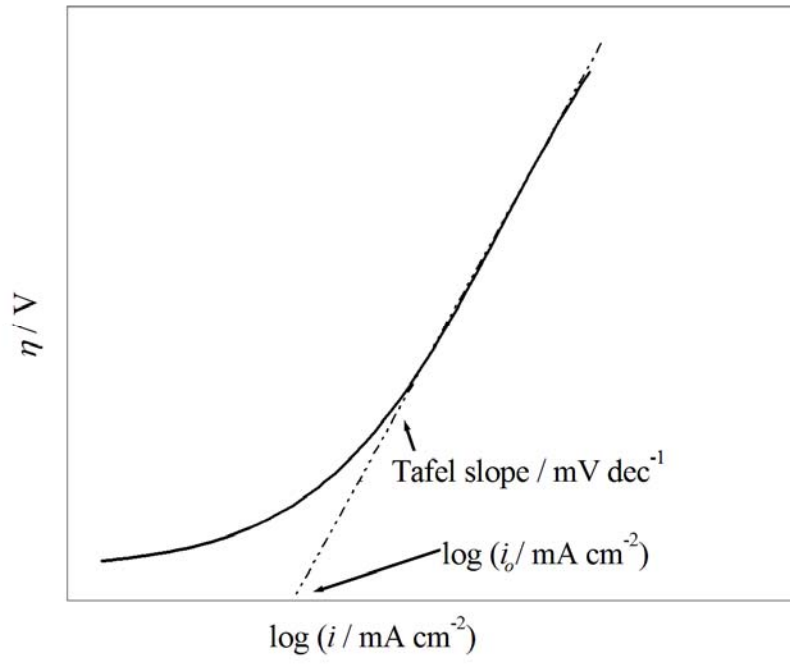Electrode   Biofilm   Bulk solution

Electrode surface region

substrate transfer

① e

metabolites transfer

② X
long distance

electrolyte transfer

③ e
pilus

b)

Electrode   Biofilm   Bulk solution

Electrode surface region

substrate transfer

metabolites transfer   CO₂...

① e ET

substrate

mediator, HS⁻, H₂ ...
suspended cell

② e ET

mass transfer (mediator,HS⁻,H₂...)

electrolyte transfer

• active site of extracellular enzyme   ⌣ pilus   living cell   dead cell

Fig. 2

Fig. 3

Fig. 4

a)

b)

Fig. 5

a)

b)

Fig. 6

Fig. 7

Fig. 8