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Primer

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Abstract

Microorganisms interacting with electrodes are at the centre of the evolving research field of microbial electrochemical technologies. The interdisciplinarity of the topic of microbial electrodes, including electrochemistry, microbiology and engineering, provides exciting opportunities and poses challenges. For further consolidation of the field, a solid methodology and approach as well as reporting are required. In this Primer, we provide an overview of the key parameters and main electrochemical methods, and the insights that can be obtained from microbial electrodes. These are exemplified and discussed for two case studies, one related to bioanodes and one related to biocathodes. The main applications of microbial electrodes, as well as challenges and directions for research, are summarized.

Sections

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Introduction

Microbial electrodes for sustainable technology

The rapid increase in the anthropogenic impact on the environment highlights the need for the development of new technologies capable of restoring Earth to its safe operating space[1]. Microbial electrochemical technologies (METs) are promising green technologies with the potential to contribute to meeting this challenge. Key component of METs are microbial electrodes, which combine electroactive microorganisms and electron-conducting materials (electrodes)[2]. Electroactive microorganisms are the biocatalysts that facilitate the conversion between electrical and chemical energy. These microorganisms can exchange electrons with the electrode via different direct and indirect mechanisms[3] (Box 1). Taking advantage of the strengths and opportunities of biofilms forms the basis for a wide variety of technologies and applications, such as wastewater treatment and soil remediation[4]. This Primer focuses on microbial biofilm electrodes (biofilm electrodes) that link the disciplines of electrochemistry and biotechnology, thus allowing the utilization of the best of both worlds.

The unique ability of METs to use and engineer the spatial decoupling of an oxidation reaction from a reduction reaction allows for a wide range of applications, such as sustainable energy, nutrient and metal recovery from wastewater, bioremediation, hydrogen production, water desalination, and the synthesis of added-value compounds[5–12]. As in all electrochemical systems, this spatial decoupling of redox reactions – being linked by electron flow via the external electrical circuit – is made possible by using two physically separated electrodes: anodes for oxidations and cathodes for reductions. These electrodes may serve both as electron acceptors (anodes) or electron donors (cathodes) for microbial conversions in a microbial electrochemical cell (Fig. 1).

Box 1 | Extracellular electron transfer mechanisms

The transfer of electrons between electroactive microorganisms and electrodes takes place via two different modes of extracellular electron transfer (EET): direct EET via immediate physical contact between the microorganisms and the electrodes, and mediated EET by means of soluble charge carriers[3,115–117]. Direct EET is typical for biofilm electrodes, but it can also occur when planktonic (suspended) microorganisms are in (intermittent) contact with the electrode. Direct EET can take place by contact between extracellular charge carriers on the membrane of the microorganisms, via nanowires or capacitive biofilm particles. In mediated EET, the metabolically derived electrons are transferred to redox compounds that, in turn, transfer them to the electrode (or vice versa). This means that microorganisms and electrodes do not necessarily need to be in proximity with each other.

Independently of the mechanism of electron transfer, the formal potential of the EET is the potential (energy level) at which the electrode receives the electron from, or donates the electron to, the microorganisms. It is not identical to the potential of maximum microbial energy gain at anodes or of minimum metabolic energy need at cathodes. The formal potential provides thermodynamic information for the reaction at the local conditions, rather than equilibrium or standard conditions. This is of importance as the conditions in living biofilms are neither at equilibrium nor standard.

One key feature of METs is that the electrode potentials can be set at given values, so that the driving force of the reactions can be steered. In general, electrochemical cells consist of an anodic half-cell and a cathodic half-cell. In each half-cell, a half-reaction takes place: an oxidation reaction occurs at the anode, and a reduction reaction occurs at the cathode. Each reaction is related to a corresponding change in Gibbs free energy, ΔG, which can be converted to a potential, E:

$$\Delta G = -z \times F \times E \quad (1)$$

(in which F is the Faraday constant and z is number of electrons per reaction).

The difference in potential between the anode and cathode is the cell potential, which represents the energy needed or released when coupling two electrochemical half-cells[2,6]. The difference between the potential of the cathodic reduction reaction (reduction of the electron acceptor) and the potential of the anodic oxidation reaction (oxidation of the electron donor) determines whether the reaction is spontaneous or not. In spontaneous reactions (positive cell potential), electrical energy can be harvested, whereas in a non-spontaneous reaction (negative cell potential), electrical energy is required for the reactions to take place. Decoupling these oxidation and reduction reactions thus introduces flexibility. For example, METs can be used to recover electrical energy from thermodynamically favourable, spontaneous reactions, or they can be fed with electrical energy to drive thermodynamically non-feasible, non-spontaneous reactions.

METs with microbial oxidation at anodes. The oxidation reaction occurs at the microbial anode, where a certain substrate is degraded when oxidized by electroactive microorganisms. This substrate can be an organic or inorganic electron donor, for example acetate, often used in laboratory studies as model component representing wastewater or complex organic matter found in real wastewater[13]. The electrons resulting from the oxidation of this substrate are transferred via extracellular electron transfer (EET) to the anode. From the anode, these electrons flow through an external electrical circuit to the cathode. At the cathode, a reduction reaction takes place. In METs with microbial anodes, two typical cathode reactions are the reduction of oxygen (O_2) to water[14] and the production of hydrogen from protons[15]. When O_2 is reduced to water, the cell potential is positive, resulting in a microbial fuel cell, and electrical energy can be harvested. By contrast, when hydrogen (H_2) evolution occurs at the cathode, the system is called a microbial electrolysis cell, and it needs an external input of electrical energy[5,6,16].

METs with microbial reduction at cathodes. Microbial cathodes can be used to drive reduction reactions[17]. When products such as organic fuels or chemicals or their precursors are generated, the resulting process is called microbial electrosynthesis. During microbial electrosynthesis at microbial cathodes, microorganisms take up electrons to convert a substance into a desired product[11]. One such conversion is the reduction of CO_2 to added-value compounds by pure and mixed cultures[18]. These compounds include soluble species like acetate and long-chain fatty acids (caproate and butyrate)[18–20], or gaseous compounds such as methane[21], but they also include fine chemicals like amino acids[22]. The formation of reduced compounds on the cathode can be attractive as a way to capture and fix CO_2, but also as a form of electrical energy storage. Microbial cathodes are often coupled to the oxygen evolution reaction at the anode, as also used in electrolysis cells, which causes challenges for strictly anaerobic biocatalysts[23].

The measured current is considered an electrochemical equivalent of the growth rate. Therefore, coupling the Monod equation, which describes microbial growth, with the Nernst equation, which describes electrochemical thermodynamics, and the Butler–Volmer equation, which describes electrochemical kinetics, creates an interconnection between the fields of biology and electrochemistry[24,25]. However, the growth yields — described as yield coefficients — based on carbon-source utilization differ when microorganisms grow in the presence of an electrode as compared with the case when no electrode is present. In addition, the yield based on current production is not rigorously linked to carbon turnover[26].

In this work, the choices for reactor setup, reactor operation and experimental conditions for basic study of biofilm electrodes are outlined. Electrochemical techniques that can be used to study different aspects of microbial electrodes are explained, and the reader is guided through the choice of techniques for answering research questions. In two examples, one for a bioanode and one for a biocathode, more detailed examples on choice of experimental setup, operation and conditions are outlined, and typical results and their interpretation are discussed. The opportunities for the use of microbial electrodes for the study of biofilms is further elaborated. This work focuses on basic electrochemical techniques and on the link between electrochemical conversions and chemical composition of the electrolytes. It encourages the reader to combine even more advanced electrochemical analyses with other insightful techniques, like molecular and optical techniques, that are not discussed in further detail in this Primer.

Experimentation

Studying microbial electrodes

Various techniques can be applied to microbial electrodes to better understand electroactive microorganisms, with electrochemical and chemical techniques being the most prevalent. For more advanced understanding, other techniques like molecular biology methods and optical techniques are useful. In this Primer, a streamlined methodology for setting up microbial electrochemical experiments is proposed, including guidelines for analysing the resulting data to draw relevant conclusions. Besides the minimal methodology, experimenters are urged to include additional relevant techniques to address more specific or detailed research questions. In this section, choices on reactor type, experimental conditions, and electrochemical techniques to grow and study biofilm electrodes are discussed.

Components of an electrochemical cell. An electrochemical cell consists of reactor housing two electrodes (anode and cathode), and optional membrane and reference electrode(s). The minimum setup comprises two electrodes, with an electrical connection in between to form the external electrical circuit of the (microbial) electrochemical cell (as shown in Fig. 1). The connection between electrodes can be established through a conductive wire, for example, a metal wire, which, through connection to a load, forms the external electrical circuit[2,6]. Such a system is classified as a two-electrode configuration. A cell voltage between both electrodes can be measured or applied to determine the resulting current. This configuration is useful when one is interested in assessing a full electrochemical cell, given that no information on the individual half-cell performance of the anode and cathode can be extracted. For example, from a change in cell voltage as a function of time it cannot be concluded if the change in voltage is caused by changes at the anode, cathode or both.

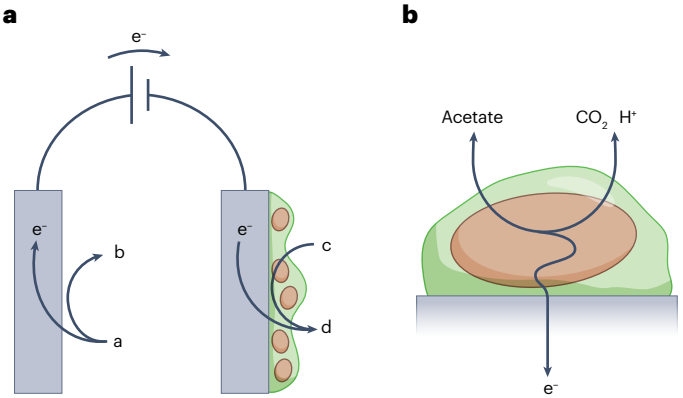

Fig. 1 | An electrochemical cell consisting of a microbial electrode coupled to a second (counter) electrode. a, Oxidation reactions take place at the anode, in which the oxidation of compound a to compound b results in the release of electrons. Reduction reactions, here catalysed by microorganisms, take place at the cathode, where electrons from the electrical circuit will reduce compound c to compound d. The combination of oxidation and reduction reactions determines if the reactions occur spontaneously (electric power is generated) or if the reactions require an energy input (electric power) to proceed. **b**, Example of an electroactive biofilm on an electrode, with the reaction of acetate producing CO_2, H^+ and electrons. This reaction can occur in both directions. When the biofilm oxidizes acetate, the electrons are released to the electrode, and the assembly of electroactive biofilm and anode is called a bioanode. For the reverse reaction, when the biofilm reduces CO_2 to produce acetate using electrons from the electrode, the assembly of electroactive biofilm and cathode is called a biocathode. Many different anodic oxidation and cathodic reduction reactions can be catalysed by microorganisms.

For a deeper understanding of the performance of either electrode, it is advisable to include a reference electrode, resulting in a three-electrode configuration requiring the use of a potentiostat (see next section). The reference electrode should be placed in close vicinity to the electrode of interest (working electrode). The working electrode can be the anode or cathode, the other electrode is termed counter-electrode[27]. With this setup, the potential of the working electrode relative to the reference electrode can be determined by measuring or controlling the cell voltage (the difference between the potential of the counter-electrode and that of the working electrode). Alternatively, while controlling the potential of the working electrode relative to the reference electrode, the cell voltage can be measured. As the reference electrode forms a fixed point on the potential scale, information on the performance of the anodic or cathodic half-cell can be extracted. If there is a change in cell voltage, it can be determined if this is caused by changes of the working electrode potential (in relation to reference electrode) or other processes occurring in the reactor.

Reactor setup and considerations. The simplest reactor configuration involves studying the biofilm electrode inside one bottle filled with a suitable solution as growth medium and containing another electrode. Both electrodes are electrically connected with a wire via a resistor forming the external circuit. To grow a biofilm on one electrode, the first step is to inoculate the reactor with a source of electroactive bacteria. This inoculum can be taken from different environments, like wastewater treatment plants, anaerobic digesters and sediments[28,29]. In a single bottle, the reactants and products move freely

in the solution. Therefore, when only using two electrodes connected by an external circuit, it is challenging to determine (and control) which electrode acts as anode and which as cathode.

Most researchers use reactors with two compartments (or chambers) separated by a membrane. Given that METs rely on the spatial separation of anodic oxidations and cathodic reductions, the use of a two-compartment reactor allows for the separate analysis of anodic and cathodic half-cells, which is not possible in single-compartment reactors. When using a two-compartment reactor, the reactants and products are mostly retained in one compartment by the membrane, allowing for the assessment of selectivity and efficiency of the half reactions. Several types of membranes can be used to separate the anolyte (electrolyte solution in the anode compartment) from the catholyte (electrolyte solution in the cathode compartment). For instance, anion exchange membranes can be utilized to preferentially transport anions from catholyte to anolyte, and cation exchange membranes can be utilized to preferentially transport cations from anolyte to catholyte. Instead of ion exchange membranes[30,31], other separators such as porous membranes or cloths are used[32].

Electrochemical modes of operation. When electrodes are connected via a load, only thermodynamically spontaneous reactions that generate electrical energy can occur. This means that cell voltages at different loads can be measured and coulombic efficiency and energy efficiency calculated to determine the fuel cell performance (Box 2). When including a reference electrode in this setup, and measuring electrode potentials relative to it, the cell voltage and half-cell potential at a given load and current is determined[2]. However, when a load is applied, the potential of the electrode can only be measured and not maintained, limiting this approach to thermodynamically favourable reactions.

Alternatively, a potentiostat can be used to connect both electrodes. A potentiostat is a more advanced electronic device that can measure and control cell voltage and electrode potentials. Use of a potentiostat provides a more advanced understanding of electrochemical half-cells. Potentiostats can be used to apply a wide range of electrode potentials or currents, including direct and alternating current[27]. The use of a potentiostat allows, for instance, the application of a constant potential relative to the reference electrode to a working electrode. Afterwards, the current at the working electrode can be monitored over time to study biofilm growth. As the potential of the working electrode is permanently re-adjusted by the potentiostat to the set value, a defined electrochemical environment is provided to the working electrode, regardless of other reactions that may be occurring in the system and at the counter-electrode.

Reactor design considerations. Reactors have many different shapes and sizes. For an overview of typical reactor configurations and designs, the reader is referred to relevant review papers[33–35].

Several factors should be considered for the design and size of the reactor and electrodes. The first consideration is the ratio of electrode area to electrolyte solution volume. For example, when targeting a more fundamental understanding of processes occurring inside the electroactive biofilm or on the level of microbial cells and its components, small electrodes in larger volumes without high specific surface area are adequate. When targeting more applied research in which high rates and efficiencies per reactor volume become important, high surface area to volume ratios should be used. Furthermore, high surface area to volume ratios will lead to higher changes in concentrations and thus likely a more precise measurement of the amounts of reactants and/or products. This will then allow for a more precise calculation of, for example, the coulombic efficiency. The absolute reactor volumes are also important. In low-volume reactors, the introduction of additional sensors, such as pH sensors, is challenging, and sampling leads to a high relative loss of electrolyte solution volume.

Given that different electrode designs and sizes are used, it is common to normalize parameters, such as current, to an area or volume leading to a current density (j). This ensures that reported results are comparable across experiments. Normalization can be done using the working electrode area $A_{electrode}$ as the available area for the biofilm to grow for normalization. When working with 2D electrodes, capacitive

Box 2 | Calculation of performance parameters in microbial electrochemical technologies

The energy efficiency (EE) can be determined by multiplying the coulombic efficiency (CE) (also called cathodic efficiency for cathodes) by the voltage efficiency (VE):

$$EE = CE \times VE$$

The equations for calculating coulombic efficiency for both microbial anodes and cathodes are given below; in these equations, i is the current (A = C s^{-1}) integrated over the time (t), $S_{consumed}$ (mol) is the amount of substrate (S) consumed at time t, P_{formed} (mol) is the amount of desired product (P) formed at time t, z is the number of electrons per mol of S or P (mol e^- mol^{-1}), and F is the Faraday constant (96,485 C mol^{-1}):

$$CE_{anode} = \frac{\int_0^t i\,dt}{S_{consumed} \times z \times F} \times 100\%$$

$$CE_{cathode} = \frac{P_{formed} \times z \times F}{\int_0^t i\,dt} \times 100\%$$

Note that, for a continuously fed system, the substrate or product concentration is multiplied by the inflow rate (in L s^{-1}) and $z \times F$, and $\int_0^t i\,dt$ is replaced by current (in A).

The voltage efficiency is the ratio between the practical (measured or applied) cell voltage E_{cell} and the equilibrium cell voltage E_{eq}, that is the theoretical (thermodynamic) cell voltage based on Gibbs free energy change. For spontaneous reactions, the voltage efficiency is described as:

$$VE = \frac{E_{cell}}{E_{eq}}$$

For non-spontaneous reactions, the voltage efficiency is described as:

$$VE = \frac{E_{eq}}{E_{cell}}$$

For further details on energy efficiency, efficiency and voltage efficiency, see also ref. 2.

Table 1 | Selected electrochemical techniques

Technique	Information	Pros	Cons
Chronoamperometry	Measurement of current as a function of time, when applying a constant potential to the working electrode	Basic electrochemical parameters like coulombic efficiency and *j* current (density) can be determined	Limited information on mechanisms and kinetics
Chronopotentiometry	Measurement of cell voltage or half-cell potential as a function of time, when applying a constant current to the working electrode	Basic electrochemical parameters like steady-state potential at a given current can be determined (note: the measured potential is a mixed potential)	If biological activity (conversion rate) does not match the set current, other electrode reactions like oxygen or hydrogen evolution may occur that may affect the biofilm
Linear sweep voltammetry	Current is measured as a function of the applied potential to the working electrode while changing the potential in one direction	Basic information on formal (measured) potentials of oxidation or reduction including extracellular electron transfer can obtained	Limited to only oxidation or only reduction reactions
Cyclic voltammetry	Current is measured as a function of applied potential to the working electrode while changing the potential in a potential window forward and backward	Identification of formal potentials as well as kinetics of oxidations and reductions including extracellular electron transfer	Parameter choice (especially scan rate and potential window) is of importance; often overinterpreted in literature
Electrochemical impedance spectroscopy	Response of voltage (full cell) or potential (half-cell) to a sinusoidal change in potential or current at different frequencies	Allows distinguishing between different resistances, capacitances and diffusion in the system	Valid and meaningful equivalent circuit needs to be used and verified; often interpretation is done without validation, leading to misinterpretation

This table lists selected electrochemical techniques typically used for research on biofilm electrodes in a three-electrode configuration, including the information that can be gained with these techniques, and their pros and cons[2].

processes are more important when electrodes with high specific surface area are used:

$$j = \frac{i}{A_{\text{electrode}}}$$

Alternatively, a normalization relative to the volume of 3D electrodes or the reactor can be suitable to gain volumetric current densities (mA cm^{-3} or A m^{-3}). However, the normalization process is a topic of debate and thus researchers must report how the area or volume is determined.

Bioelectrochemical reactors can be operated in different feeding modes: batch, fed-batch or continuous mode. A batch mode is characterized by a single pulse of electron donor (for anode) or electron acceptor (for cathode). Fed-batch mode requires regular re-addition, whereas in a continuous mode there is a continuous flow of electron donors or acceptors in the influent. The choice of flow conditions will affect the type of insights and results obtained.

Electrochemical techniques and considerations. The current related to redox reactions (Faradaic current) is of most interest when studying microbial electrodes. In addition to Faradaic current, capacitive current may have a role. There is also pseudo capacitive current that we will not discuss further here[36]. The capacitive current originates from physical-chemical processes taking place at the electrode surface, and it is observed when changing the electrode potential. Capacitive processes play a more important role when electrodes with high specific surface area are used. For example, activated carbon, with high specific surface area of the order of 500 $m^2 g^{-1}$, has a very high capacitance[37,38], whereas electrodes with low specific surface area, like glassy carbon, have a very low capacitance[39]. The capacitive current may not be of interest, and it is often unwanted, see ref. 2 for further details. Table 1 gives an overview of the most used electrochemical techniques, including the information that can be gained with each technique and its pros and cons.

The most basic techniques used to study microbial electrodes are chronoamperometry and chronopotentiometry. Chronoamperometry measures the current as a function of time when applying a fixed potential at the working electrode relative to the reference electrode. Chronopotentiometry measures the working electrode potential and/or cell potential as a function of time when a current is applied. These techniques can be used for setting the potential or current at one fixed value, or they can be programmed to apply different potentials or currents for specific time periods. When the steady-state of current and potential is reached at different conditions, the current–potential behaviour of the electrode under study can be analysed.

Linear sweep voltammetry (LSV) involves the application of a change in potential in one direction from a start potential to an end potential over time, while measuring the resulting current. For microbial electrodes, LSV can provide first information on the formal potentials of EET and on the limiting current (maximum conversion rate) of microbial electrochemical reactions[40]. This technique is advantageous for studying reactions that occur in one direction (either oxidation or reduction reaction)[27]. When studying the oxidation and reduction behaviour of the microbial electrode, cyclic voltammetry (CV) can provide more detailed insights[27,41,42]. CV applies a change in potentials over time from a start potential to a reverse potential and back to a start potential. Therefore, the working electrode potential is changed within a range in two directions (in a cycle), while the current response is measured. CV allows determining the measured formal potentials of both oxidation and reduction processes and hence the identification of the formal potentials of the EET. A formal potential can be determined from the CV scan as the arithmetic mean of the peak potential for the oxidation and the peak potential for the reduction. When used at different scan rates, additional information can be gained, for example kinetics or the capacitance of the electrode[43]. The above-mentioned techniques measure or apply direct current, whereas electrochemical impedance spectroscopy (EIS) uses alternating current[2,27,44,45]. When using EIS, either a sinusoidal perturbation is applied to the potential (for half-cells) or voltage (for full cells) of a system at different

frequencies, or a sinusoidal perturbation is applied to the current at different frequencies[45]. Based on the recorded effect of this perturbation on the current (or potential/voltage) of the system under study as a function of frequencies, the impedance can be analysed. In simple words, impedance reflects different resistances, so, based on impedance data, electrochemical characteristics such as capacitances, and electric and charge transfer resistances can be derived.

Data collection and analysis

Experimental runs can vary in duration, spanning from short periods (hours or days), for example when observing the initial growth phase of the biofilm on the electrode, to longer periods, often months or longer. This extended runtime is needed when studying the long-term operation of MET reactors and tracking changes in biological conversion rates and biofilm communities over time. The length of the experiment and the research question determine the sampling frequency. There is no strict rule for the sampling frequency, but it is important that trends and answers to the research questions can be extracted from the dataset. For example, when monitoring the current profiles of a microbial anode running for months, recording hourly measurements provides a comprehensive overview of trends in the current, whereas when studying biofilm changes over hours or days, a higher frequency of data collection may be useful. Dynamic techniques, such as LSV and CV, often collect data at second or even sub-second intervals. However, when studying capacitive processes, millisecond data may be of importance. Importantly, electrochemical data are usually recorded non-invasively, incurring in minimal operating costs. Moreover, when using a potentiostat, data collection is facilitated by user-friendly software. Sampling may interfere with the operation of liquid and gaseous samples. Interference may arise from, for example, opening the reactor lid when no permanent sampling port is present, liquid sampling outside an anaerobic chamber, or stopping the electrochemical experiment to move the reactor. Furthermore, analysis of these samples comes with costs and consumption of resources, and the chemical analysis data are not in the same format as the electrochemical data. Therefore, all data need to be brought manually together for analysis, which can be done with basic software like MS Excel or R.

Safety considerations

Owing to the low voltages and the generally non-harmful nature of microorganisms (classified as safety level 1 in the European Union), typically only standard safety measures for chemical and biological laboratories need to be considered. However, experiments conducted under real conditions require increased safety measures, owing to the risks associated with the presence of pathogens when utilizing complex inocula from real wastewater streams or other sources[46]. Here, safety concerns arise from various factors, such as using genetically modified organisms, research focusing on degradation of harmful or toxic components (for example, hydrogen sulfide, organosulfur compounds and BTEX (benzene, toluene, ethylbenzene and total xylenes)), and the use of specific reactants, for example formaldehyde, in biofilm-staining procedures. Furthermore, experiments performed on a large scale pose safety risks related to increasing pressures resulting from the production of a gaseous product, such as cathodic H_2, at high conversion rates.

Results

In this section, two practical examples with specific research questions are introduced to explain the generic approach to set up experiments and to choose methodologies. Supplementary Table 1 presents key information on measurement techniques and performance indicators for biofilm electrodes and METs, to support decisions regarding experimental setup and method selection.

Examples: bioanode and biocathode experiment

The microbial biofilm anode example considers growing a microbial biofilm anode that oxidizes acetate to HCO_3^- using a three-electrode configuration. To study the performance of this bioanode and to analyse the mechanisms of EET, several parameters need to be monitored. The coulombic efficiency of the degradation of the substrate should be determined as well as the formal potentials E_f of the redox-active compounds at the electrode, and which of these compounds are involved in the EET.

The biocathode examples consider a microbial cathode that reduces HCO_3^- to acetate that is being coupled to a water-splitting anode in a MET reactor. To study the performance of this biocathode over a longer time period, several parameters need to be monitored. The product selectivity for acetate should be determined in combination with the energy efficiency, as well as how these factors change over the operation throughout 8 weeks.

Choosing the reactor and electrode material. For the bioanode, focus is on using CV to characterize the EET mechanisms and their formal potential (see Supplementary Table 1). For CV experiments, the electrode material ideally has low capacitance, and thus, typically, a 2D electrode like a graphite rod (low capacitance) or glassy carbon (minimal capacitance) is used. A reference electrode, being most often an Ag/AgCl (sat. KCl)-electrode, should be placed at closest proximity to the working electrode, so that minimal voltage gradients occur between the anode and the reference electrode, and the anode potential can be precisely controlled. In terms of reactor, the most practical approach is to use a two-chamber system to make sure there is minimal crossover of hydrogen formed at the cathode towards the anode[47]. When studying a pure culture, for example *Geobacter*, reactor materials that can be sterilized should be selected. Furthermore, depending on the microorganism under study, not only sterility but also anaerobicity need to be assured during reactor assembly and operation. For a constant biofilm environment, it is beneficial to have a defined concentration of substrate (acetate), so continuous feeding is advised. An advantage is that sufficient pH buffer capacity can be provided, to avoid biofilm activity changes, owing to acidification as well as community stratification for electrode microbiomes[48].

For the biocathode experiment that considers the full cell, a reactor should be selected that offers a high electrode surface area to cathode volume ratio to achieve high volumetric conversion rate. Additionally, a reactor configuration with high energy efficiency requires a minimized distance between electrodes. One option would be to use a flow reactor with flow channels containing 3D cathodes that are well-connected to the current collector[49]. A current collector should be placed along the length of the electrode, ensuring good homogeneous contact through pressure. A reference electrode should be included and positioned near the current collector. For constant current operation, performance can be analysed by measuring the cathode potential. For constant potential operation (chronopotentiometry), performance can be evaluated by measuring current (chronoamperometry). For longer-term stable operation, it is ideal to have continuous feeding of CO_2, so that continuous conversion of HCO_3^- to acetate can be achieved.

Analysing the performance of microbial electrodes. The performance of microbial electrodes is often assessed using coulombic efficiency. This metric indicates the proportion of electrons gained via substrate that are converted to electric current for the anode or the proportion of supplied electric current that is converted to the product for the cathode. To assess coulombic efficiency, it is necessary to measure the amount of electrons (the charge), typically recorded as current over time, based on the total amount of substrate consumed or product generated. For a single or a defined number of organic or inorganic compound(s), concentration changes are measured using methods such as liquid chromatography, for example HPLC (high-performance liquid chromatography), or gas chromatography for volatile fatty acids, alcohols and gaseous products like hydrogen or methane. For complex mixtures of substances like wastewater, a sum parameter like the chemical oxygen demand (COD) is a suitable measure to estimate the concentration of oxidizable components. COD can be determined, for instance, using spectrophotometric kits. The use of (micro)sensors in the electrolyte solution allows for real-time monitoring of (local) concentrations of substrate or products[50,51].

Measurement of concentration of substrate(s) and product(s) is crucial to assess the coulombic efficiency and the energy efficiency of METs[2,52] (see Box 2).

Making experimental decisions using key equations for data analysis. The first step in the choice of the control strategy and process conditions is to analyse the thermodynamic equilibrium potential(s) for both systems. For a detailed description of how to set up reaction equations and calculate the corresponding Gibbs free energy change and electrode potential at actual conditions, we refer readers to refs. 2,6.

For the bioanode in which acetate is oxidized to HCO_3^-, the following reaction takes place:

$$\text{Anode}: C_2H_3O_2^- + 4H_2O \rightarrow 2HCO_3^- + 9H^+ + 8e^-$$

Assuming that the actual conditions during operation are $[C_2H_3O_2^-] = 8$ mM, $[HCO_3^-] = 5$ mM and pH = 7, the standard potential of this reaction can be converted to the potential at actual conditions using the Nernst equation:

$$E_{eq} = E^{\theta} - \frac{RT^{\theta}}{z_r F}\ln(K_{eq})$$

in which E_{eq} is the equilibrium potential at actual conditions (V), E^{θ} is the equilibrium potential at standard conditions (V), R is the gas constant (J mol^{-1} K^{-1}), T^{θ} is the standard temperature (K), z_r is the mol of electrons per mol of reaction, F is the Faraday constant (C mol^{-1}) and K_{eq} is the equilibrium constant defined as:

$$K_{eq} = \frac{P_1^{\upsilon_1} \times P_2^{\upsilon_2}}{R_1^{\upsilon_3} \times R_2^{\upsilon_4}}$$

where P_1 is the concentration of product 1 with υ_1 the corresponding stoichiometric factor for this product, P_2 the same for product 2, R_1 is the concentration of reactant 1 with υ_3 the corresponding stoichiometric factor, and R_2 the same for reactant 2. The equilibrium potential of the bioanode at actual conditions is then −0.298 V versus NHE (normal hydrogen electrode), equivalent to −0.503 V versus Ag/AgCl (assuming that the reference electrode is a Ag/AgCl (3 M KCl) electrode with a potential of +0.205 V versus NHE).

The cathode reaction is the hydrogen evolution reaction:

$$\text{Cathode}: 2H^+ + 2e^- \rightarrow H_2$$

In this example, only the anodic half-cell is of interest, and a potentiostatic operation is implemented. Therefore, the cathodic half-cell can be neglected. One point of attention is that the hydrogen formed at the cathode should not enter the anode as it may serve as electron donor. This can be avoided by flushing the catholyte with nitrogen gas.

For the biocathode in which HCO_3^- is converted to acetate, a reaction coupled to water oxidation at the anode, the full reaction equation is needed:

$$\text{Anode}: 2H_2O \rightarrow 4H^+ + O_2 + 4e^- \quad \text{(multiply by 2)}$$

$$\text{Cathode}: 2HCO_3^- + 9H^+ + 8e^- \rightarrow C_2H_3O_2^- + 4H_2O$$

$$\text{Overall}: 2HCO_3^- + H^+ \rightarrow C_2H_3O_2^- + 2O_2$$

The actual conditions during operation are $[C_2H_3O_2^-] = 20$ mM, $[HCO_3^-] = 20$ mM, pH = 6 and $pO_2 = 0.2$ bar. Using the same approach as in the bioanode example and using $E_{cell} = E_{cathode} - E_{anode}$, the equilibrium cell voltage at standard conditions can be calculated: −1.04 V versus NHE. The equilibrium cell voltage at actual conditions is then, using the Nernst equation, $E_{eq} = -1.09$ V versus NHE.

The performance data in both examples can then be analysed as detailed in Box 2.

Bioanode. The growth and development in activity of the biofilm is measured through the changes in current as a function of time, at constant anode potential. The anode potential should be more positive than the equilibrium potential (here, −0.503 V versus Ag/AgCl) to provide the electroactive biofilm with sufficient energy to grow. At the same time, the chosen potential should not be too positive to allow for a reasonable voltage and energy efficiency. For this example, an anode potential of −0.050 V versus Ag/AgCl is chosen. Given that the current is related to the presence of active redox compounds in the biofilm, generally, higher currents are associated to higher biomass concentrations and/or higher concentration of redox compounds in the biofilm. Using CV during non-turnover and turnover conditions can allow the determination of which redox-active compounds are present in the biofilm and which of those are involved in EET.

Biocathode. For an established biocathode, the acetate production rate in the biofilm is expected to correlate with the applied current. By sampling both the catholyte and cathode headspace, and measuring the acetate concentrations, potential intermediates and alternative products (other volatile fatty acids, H_2 and CH_4), the coulombic efficiency for each product can be calculated. To determine the energy efficiency, this coulombic efficiency towards the desired product is multiplied by the voltage efficiency.

Statistical analysis and data corrections. In MET literature, basic statistical analyses are frequently used to report results, with the exception of scaled reactors or pilot studies, in which often only one or two reactors can be operated. However, also for lab-scale experiments, the availability of replicates in an experimental set is often limited, with n being 2 or 3, and typically fewer replicates are used for long-term experiments than for short-term experiments. Although the literature often presents averages and standard deviations, it is not always clear whether the data conform to a normal distribution or whether this representation offers more valuable information when compared with showing all the data points. Without comprehensive statistical analysis, presenting and analysing results with all the data points is required to

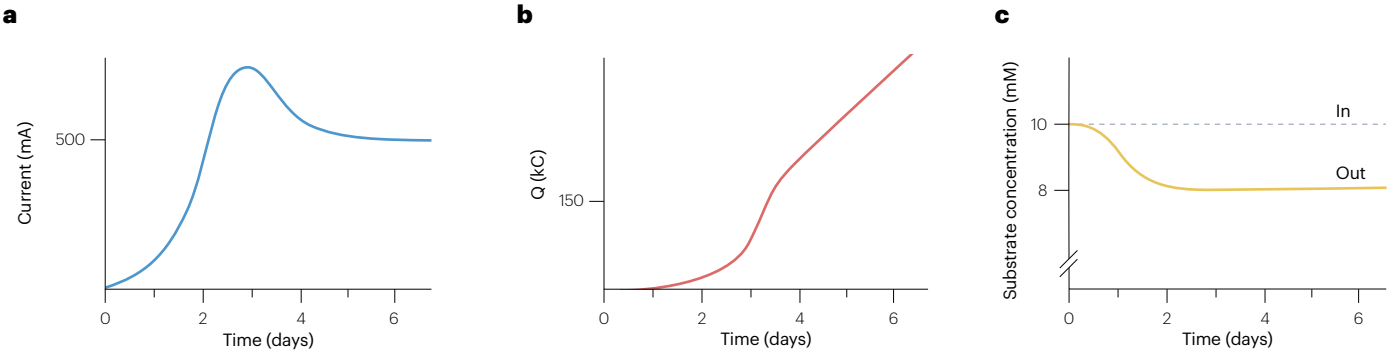

Fig. 2 | Example results for the bioanode experiment using continuous flow reactors. a, Current profile over time; in the first few days, there is a steady increase in current that leads to a maximum current on day 3; afterwards, a decrease to a stable current of 500 mA is reached. **b**, Cumulative charge (Q) over time resulting from the current flow described in part **a**. **c**, Substrate concentration in the anolyte over time; continuous feeding of 10 mM results in a stable (outflow) concentration of 8 mM.

elucidate trends among replicates. This will allow readers to interpret differences between the different conditions tested in each study. Even though more advanced software such as R, Python and Sigmaplot, can be used, Origin or MS Excel is often a robust and sufficient choice to process (bio)electrochemical data. Besides calculations, corrections of the raw data can be used to make graphs readable. For example, moving averages of datasets can help to better visualize the trends over longer time periods.

Working example: bioanode

First, the experiment will be started and operated to allow the bioanode to develop at constant anode potential of −0.050 V versus Ag/AgCl using chronoamperometry and with continuous feeding of acetate. This will typically take a few days or up to 2 weeks. In Fig. 2, a typical current profile and the acetate concentration in the anolyte are shown as a function of time. The current (Fig. 2a) is typically representative of the substrate conversion on the electrode. In the first few days, the slow current increase can be linked to the colonization of the electrode surface by the first microorganisms and the formation of microbial clusters, which leads to an exponential current increase (exponential growth phase). After this initial phase, the current flattens out and remains constant over time. Here only little or no growth takes place in addition to metabolic maintenance, as the biofilm has reached a steady state (for the given conditions). The current is stable during the steady state, but microbial growth can still occur, and part of the biomass may leave the system as planktonic cells[53]. During continuous feeding, the acetate concentration in the anolyte decreases as the biofilm starts to grow on the electrode, and, afterwards, it typically remains constant over time because of the constant current. Figure 2b shows the cumulative charge resulting from the measured current with time. The evolvement of acetate concentration is shown in Fig. 2c.

In this example, the bioanode is fed with 10 $mmol_{acetate}$ l^{-1} at a flowrate of 1 ml s^{-1}; when reaching steady-state, the resulting stable acetate concentration is 8 mmol l^{-1}. The coulombic efficiency of the anode, CE_{anode}, can be calculated over the whole experiment, or over a defined period of time. The coulombic efficiency will initially be low, because carbon and energy (from the substrate) are used for biofilm growth. When there is a mature biofilm in steady state on the anode surface, coulombic efficiency is high. In this example, the coulombic efficiency is calculated after day 5, when a stable current of 500 mA and stable acetate concentration of 8 mmol l^{-1} is reached (see Box 2).

$$CE_{anode} = \frac{500 \times 10^{-3}\frac{C}{s}}{(10-8) \times 10^{-3}\frac{mol_{acetate}}{l}\, 0.001\frac{l}{s} \times 8\frac{mol_{e^-}}{mol_{acetate}} \times 96,485\frac{C}{mol_{e^-}}} \times 100\%$$
$$= 32\%$$

The coulombic efficiency of the bioanode when operated in continuous mode showing constant 500 mA, that is 500 mC s^{-1}, on day 5 was 32%, meaning that, of the electrons released as a result of acetate conversion, 32% ended up as electric current, and the other 68% were diverted to other sinks, for example biofilm growth and/or formation of other products, like methane.

CV can be used to study the bioanode at different growth stages. For this analysis, different scan rates—the speed at which the potential changes with time—can be used. The suitability of the scan rates depends strongly on the electrode under study as well as the research questions to be addressed. Here, the simplest case is discussed, in which the steady state should be reached during the CV experiment, meaning that consecutive CV scans have to closely overlap with each other. For this example of a bioanode, a scan rate of 5 mV s^{-1} is used. When applying the scan rate to a blank electrode without biofilm (before inoculation), a background cyclic voltammogram is recorded, both in the presence and absence of acetate (see Fig. 3, blue lines). The current resulting from the change in potential is only capacitive, given that no electrochemical reaction takes place. When conducting the same measurement on a biofilm electrode in the absence of acetate, a different CV signal is obtained. Two pairs of oxidation and reduction peaks can be identified (Fig. 3b), and the arithmetic mean between oxidation and reduction peak is the measured formal potential of the underlying redox processes. In the case of our bioanode, two formal potentials of possible sites of EET, E_{f1} at −0.375 V versus Ag/AgCl and E_{f2} at −0.100 V versus Ag/AgCl, can be identified. These are formal potentials of possible EET sites, but which is related to EET can only be determined when performing the same measurement in the presence of the electron donor. Therefore, when performing CV with acetate (Fig. 3a), a current increase is observed for more positive anode potentials that accelerates above a certain anode potential. The steepest point of the increase (being mathematically the maximum of the first derivative; see also Supplementary Table 1) is the formal potential of the actual EET site.

Therefore, the cyclic voltammograms reveal that the bioanode performs EET with a site possessing E_{f2} at −0.100 V versus Ag/AgCl. It can now be speculated what the related molecular moiety is. For instance, based on literature data, a cytochrome may be that moiety, but more advanced methods are needed to really identify it. At the same time, the obtained information is already useful, for instance for energetic considerations or modelling.

Working example: biocathode

The biocathode should be grown over a longer period of time, until it reaches a steady state in terms of productivity. Subsequently, chronopotentiometry is used to assess the polarization behaviour of the biocathode (Fig. 4a). This is done by applying increasing as well as decreasing currents, and recording the potential over time. There is an initial negative peak in cathode potential, after which the cathode potential increases towards a rather stable value. This pattern is repeated for each potential step. As the current increased, higher cathode overpotentials (more negative cathode potentials) were measured. Based on these measurements, a cathode polarization curve can be constructed from the chronopotentiometric experiments by plotting the cathode potential (*y* axis) as a function of the current density (*x* axis) (Fig. 4b). Now, it becomes clear how the cathode potential changes between −0.4 V versus Ag/AgCl at 0 mA down to −1.7 V versus Ag/AgCl at a current value of −30 mA, at which the cathode potential seems to stabilize. Finally, the selectivity of reactions is shown in Fig. 4c, in which the coulombic efficiency towards the different products (hydrogen, acetate, butyrate and others) is shown.

For assessing the coulombic efficiency of the biocathode in Fig. 4c, the fraction of electrons towards acetate, butyrate and H_2 are shown as a function of time, for a well-established, that is steady-state, biocathode. Especially at constant current control, it is often observed that most of the electrodes produce H_2 at the start of the experiment, as also shown in this example. As the biofilm develops on the cathode, the H_2 is consumed, and the product spectrum changes from mostly H_2 to acetate and, in a later stage, also butyrate. The reason that some balances are not closed (meaning obtaining 100% after summing all the fractions) relates to the occurrence of other processes and products that are not quantified, for example, biofilm growth or reduction of oxygen (crossover from anode, or diffusion from surroundings). Based on the measured concentrations of acetate over time, the acetate production rates can be calculated, as an important measure to analyse the (volumetric) biological conversion rate. Note that the conversion rate can also provide information on the consumption of compounds (intermediates) to produce other products. For example, a negative acetate production rate suggests that overall, acetate was consumed, which could be linked to the production of butyrate.

To obtain the energy efficiency, the voltage efficiency needs to be calculated. For this purpose, the applied voltage between anode and cathode needs to be measured. In this example, it is assumed that this cell voltage was stable at −2.5 V at −30 mA (data not shown). The (calculated) equilibrium voltage was −1.09 V. This results in a voltage efficiency of $VE = \frac{-1.09\,\text{V}}{-2.5\,\text{V}} \times 100\% = 44\%$. Multiplying the voltage efficiency by the coulombic efficiency (towards the desired product) yields the energy efficiency with, for example in week 7, an *EE* of 44% × 70% = 31%. Therefore, an energy efficiency of 31% reflects the fact that for each kWh of electrical energy supplied to the microbial electrochemical cell, 0.31 kWh of energy are stored as chemical energy in the form of acetate. The energy efficiency is a strong function of the current (conversion rate), as also voltage efficiency and coulombic efficiency change with current. Therefore, depending on the goal of the conversion (high conversion rate or high energy efficiency), the desired operating conditions can be identified. In addition, the voltage losses can be further analysed in detail as described in refs. 2,54 to determine the extent to which the components in the full cell (overpotentials, ohmic losses and transport losses) contribute to the voltage losses. These results will allow deriving guidelines on further improving and engineering these systems towards higher efficiencies.

Applications

Research on microbial electrodes and METs has spanned over two decades. These subjects are studied both for acquiring fundamental understanding and for developing practical applications. This section discusses different examples of how microbial electrodes and METs can be applied.

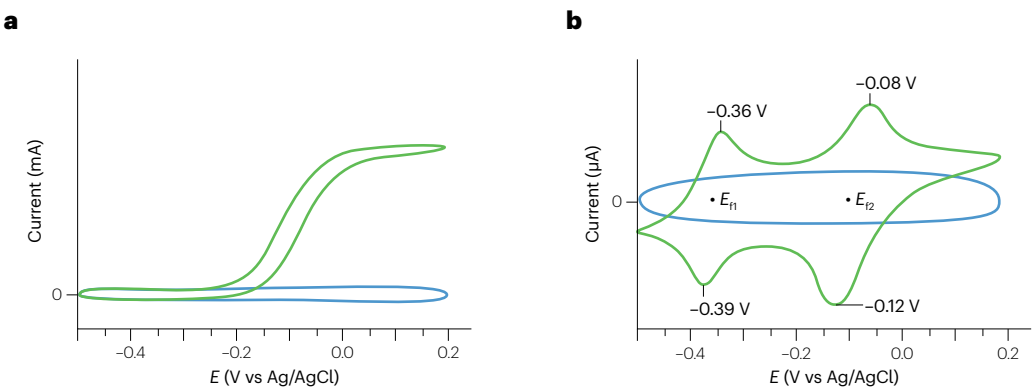

Fig. 3 | Cyclic voltammograms of an electrode with and without biofilm. **a**, Example of a cyclic voltammogram for an electrode that is blank, meaning without biofilm (blue line), and for electrode with biofilm, under turnover conditions (in the presence of acetate; green line). The voltammogram for the bioanode shows a typical increase in oxidation current with increasing anode potential, reaching a maximum current at anode potentials more positive than 0.0 V versus Ag/AgCl. From the point of steepest slope of the curve (the inflection point), the formal potential of the extracellular electron transfer can be determined. **b**, Under non-turnover conditions (in the absence of acetate), the blank electrode shows similar behaviour to under turnover conditions, with a small capacitive current in oxidative as well as reductive directions. The bioanode shows two oxidation and two reduction peaks – for which the midpoint potentials being formal potentials E_{f1} and E_{f2} can be determined – which indicates that two redox components are present in the biofilm.

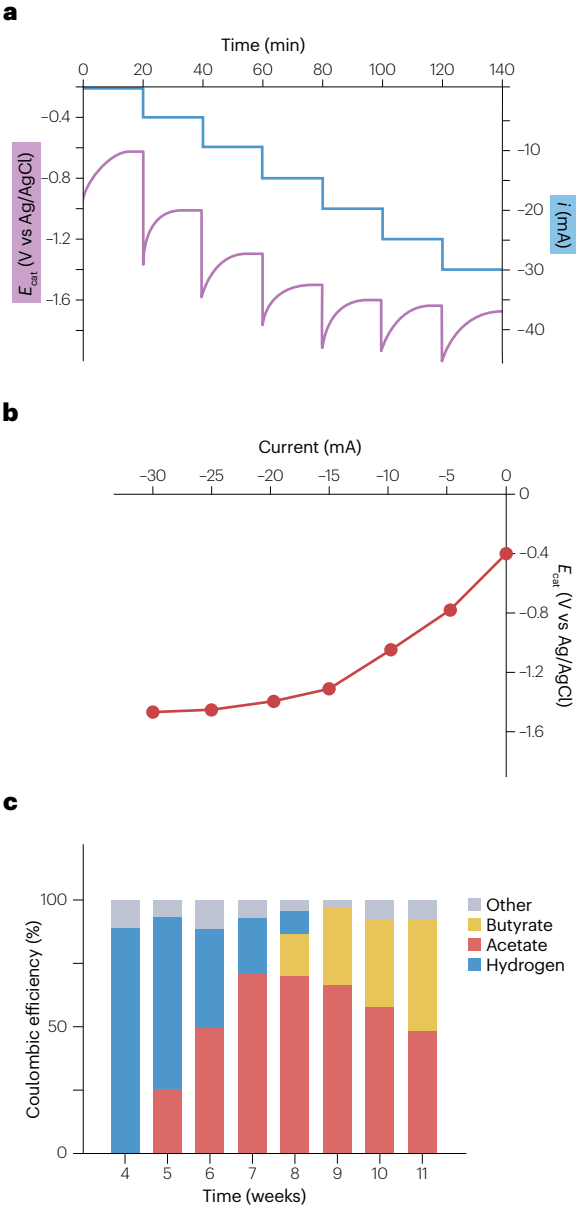

Fig. 4 | Analysis of performance through polarization curves and efficiency. Example of a chronopotentiometric measurement conducted at a biocathode, in which the current value is changed stepwise with time, and the measured cathode potential is shown (part **a**); the translation of these chronopotentiometric measurements into a polarization curve that shows the change in cathode potential as function of current (part **b**); and the coulombic efficiency towards the different products, hydrogen, acetate, butyrate and others (part **c**). The product distribution changes as a function of time.

Fundamental understanding

Studying the ecology of biofilm microbiomes. The use of electrodes can help to understand mechanisms of electron transfer between microorganisms and electrodes, but also between microorganisms. Electrodes offer the unique opportunity to control the potential at which electrons are released or taken up, and at the same time can be used to study how this affects microbial biofilm ecology and changes in microbial communities, for example when combined with next-generation sequencing[55].

Studying EET mechanisms. Many microorganisms can exchange electrons with an electrode, either directly or via (self-produced and excreted, or externally added) redox-active components. Electrochemical techniques, such as CV, are unique tools to identify the formal potential of electron transfer between microorganisms and electrodes, and with that they can give insights into the electron transport mechanisms. Not only may these findings be useful for biofilm electrodes, but they can also shed light on EET in biocorrosion as well as microbiomes[56–58].

Studying structures for biological conductance. Many studies have addressed the conductance of electroactive biofilms and the presence of conductive structures, for example pili, that have a role in electron transport through the biofilm to the electrode. Conductance has also been studied in cable bacteria[59]. These biological conductive structures have a wide reported range of conductivity and may offer new possibilities to produce conductive materials with unique properties, which are of interest for biocomputing[60,61] or catalysis[62].

Enrichment of electroactive microbiomes. Electrodes offer a new way to enrich microbial strains or communities that are challenging to enrich through conventional methods[63]. Not only do they offer a surface to attach to, but they also provide an additional way to accept or donate electrons via EET, besides supplying those donors/acceptors as chemical species in solution. Therefore, microorganisms capable of electron exchange with electrodes become selectively enriched at the electrode surface. Subsequent transfers (dilutions) of electrode material with biofilm into fresh solutions offer a novel method for enriching electroactive microorganisms[64,65]. This also includes weak electroactive microorganisms[66].

Practical application

Energy recovery from wastewater. The first foreseen application of METs was the direct conversion of organic matter in wastewater to electricity using microbial fuel cells. The uniqueness of this process lies in the fact that this conversion occurs in a single step at the bioanode[67]. In addition to electricity generation, energy recovery can occur in the form of hydrogen production at the cathode when electricity is supplied. Currently, microbial fuel cells and microbial electrolysis cells are not (yet) competitive with existing wastewater treatment systems, but they hold promise for the future[68,69]. Their initial applications were primarily observed in the treatment of high-strength, defined and homogeneous wastewaters, such as those found in the food industry.

Sensors for organic matter in (waste)water. Biofilm electrodes can be used to measure substrate conversion[70,71]. The current and total charge produced at the bioanode directly reflects the conversion of electron donors, such as acetate or other organic/inorganic matter, assuming that all substrate at the anode is converted to electric current. Microbial electrodes offer an alternative to chemical analyses for assessing the conversion of components at the anode through electrochemical measurements. The main limitation, but also a strength in this application, is its limited selectivity. Only sum parameters like COD can be assessed, or there should be a single substrate available for the biofilm, with minimal biofilm growth, ensuring a coulombic efficiency of 100%.

Sensors for compounds in water. Electroactive microorganisms can respond to specific compounds in water, allowing for real-time monitoring[72]. For example, when in contact with biodegradable substrates, electroactive microorganisms generate an electric current, serving as a sensor for the presence of biodegradable substrates in water[73]. Alternatively, contact with toxic substances reduces the current generated by electroactive biofilms, and thus a drop in current can indicate the presence of toxic components. Interestingly, currents can be detected at very low values, enabling the detection of compounds at low concentrations. Electroactive biofilms can act as a sensor for compounds in water by monitoring changes in the current signal, but they do not directly identify the specific components present.

Power-to-X processes, especially microbial electrosynthesis from CO_2. Biocathodes can use electricity to convert CO_2/HCO_3^- to more reduced organic components, such as methane, acetate, fatty acids and alcohols, or other chemical building blocks or even proteins[18,21,52,74]. This reaction is normally combined with water oxidation (oxygen evolution) at the anode, providing a new way for power-to-X processes. Major challenges include achieving high rate, selectivity and energy efficiency towards the desired product while maintaining an anoxic cathode. Furthermore, downstream processing can be a major hurdle as often only a limited concentration (low titre) can be achieved.

Directing fermentations. In fermentation processes, the availability of reducing equivalents will affect the selectivity and rate. Electrodes can supply (or take away) reducing equivalents at a controlled energy level (potential) and may create local gradients that favour certain processes. This allows electrodes to be used to enhance fermentation selectivity or even produce new products[75–79]. However, a challenge is the requirement of two electrodes; moreover, a counter-reaction (possible oxygen evolution) may harm the process.

Nutrient and metal recovery. The potential gradient and movement of electrons from anode to cathode can be used to transport charged ions between the compartments. For example, the removal and recovery of NH_4^+ can be achieved[80–82]. For a concentrated stream containing both organic matter and NH_4^+, like urine[83–85], the bioanode can even supply a substantial part of the energy required for NH_4^+ transport. Other nutrients, such as sulfur and phosphate, can also be recovered using METs[86]. Additionally, metals can be selectively removed from wastewater, generally through their reduction at cathodes[9,87]. During this reduction, the dissolved metal ion will be converted to its solid form. Control of cathode potential can be used to selectively remove and recover metals. A challenge in this process is maintaining a neutral pH at the bioanode, while maintaining the low pH of the metal-containing solutions at the cathode.

Pollutant degradation. METs can also find their application in the degradation of different pollutants at bioanodes and/or biocathodes[8]. Examples are the removal and degradation of benzene[88], uranium[89], organic and inorganic sulfur compounds[90,91], and micropollutants like pesticides and pharmaceuticals[92,93].

Reproducibility and data deposition

Factors affecting the reproducibility of METs

Reproducibility in research on MET reactors, but also biofilm electrodes, is currently based on duplicates or triplicates and can, in some rare exceptions, go up to quintuplicates or more[94]. However, reproducibility is still very challenging, especially when longer-term biological experiments are performed. One approach to increase the number of replicates for certain studies, such as when screening materials, requires special potentiostats that can implement multiple working electrodes in the same reactor compartment to study the current (or potential). This increase in the amount of electrochemical data cannot always be linked to biological conversions, given that the change in concentrations of compounds is determined by all electrodes together and cannot be assigned specifically to one electrode. It is expected that, in the future, high-throughput platforms will gain attention as a tool to maximize data generation and thus enrich the knowledge and allow more statistically supported data on METs, microbial electrodes as well as electromicrobiology.

Data reporting standards

The interdisciplinary nature of METs has the advantage of scientists contributing insights from their respective fields of expertise. However, this presents challenges concerning the use of methodologies and interpretation of data, which may not have the same standards. Therefore, it is important to define a minimal set of data and environmental variables to be reported for MET in general and also for studies on biofilm electrodes. For example, scientific studies should include a minimal number of replicate experiments, a minimal time duration of the experiment, the use of reference microorganisms and reference media and conditions, the use of at least one reference electrode, a minimal set of operational conditions (potential and current) and methods (to be chosen, for example, based on Table 1), and a minimal set of environmental variables (pH, temperature and conductivity).

This minimal set of data and variables, however, is not so easily defined, given that the field of METs ranges from fundamentals to applications. The type of research focus will determine which type of measurements and what duration of experiments is required. For example, for fundamental studies at small scale that are shorter (days or weeks), it will be easier to include several replicates than for engineering studies that last longer (months or even a year). For fundamental studies that require anaerobic conditions, it will be more challenging to measure and report all operational and environmental conditions, whereas for engineering studies, those measurements can be more easily integrated in the reactor setup.

There is currently no specific data depositing system for METs, given the recent development of this field. This also holds true for electrochemical data in general, but it is different for molecular biology data for which, for example, sequence repositories like NCBI (National Center for Biotechnology Information) are available. Over time, data from MET research will undoubtedly move towards big data, allowing machine learning-based and artificial intelligence-based data mining and predictions, as already seen in recent studies[95]. The interdisciplinarity of this field presents a unique challenge to establish interlinked datasets across biology, electrochemistry and engineering.

In all instances, it is essential to establish a solid foundation for research. Therefore, it is recommended to perform at least independent triplicates when studying biofilm electrodes and to report all used materials and calculations. Special attention should be paid to assumptions and approximations, particularly relating to electrode surface areas. Detailed information can be included as supplementary information.

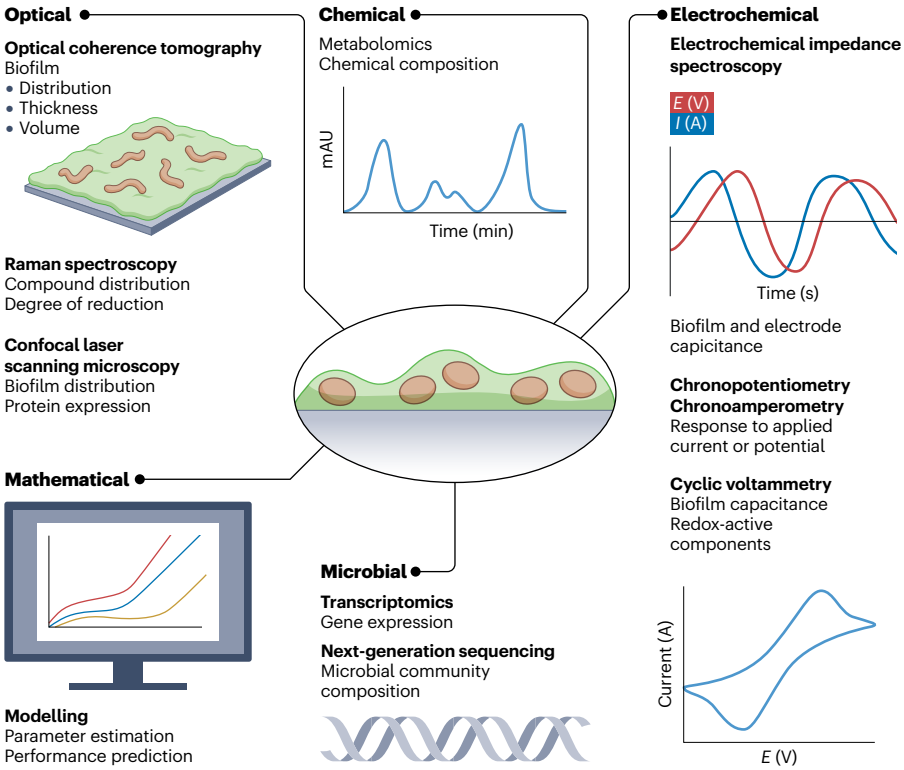

Fig. 5 | Methods to determine microbial electrode performance. Using chemical and electrochemical techniques together provides basic information on the performance of microbial electrodes. In addition to those, many different optical, microbial and mathematical methods can be used for a more in-depth understanding.

Limitations and optimizations

Methods in which basic electrochemical measurements are combined with the chemical analysis of substrates and products can provide a robust dataset to answer several research questions. In some cases, more in-depth answers on different scales are required, for which the basic data form the foundation. Therefore, for more in-depth studies, further analysis techniques are required.

For example, when interested in quantifying the amount of biomass on the electrode surface or visualizing the location of specific redox-active components in the cells, optical techniques can be used for real-time measurements[96]. Examples of optical techniques suitable for in situ and real-time application, particularly on the living microbial electrode, include optical coherence tomography[97,98], confocal laser scanning microscopy[99,100] and magnetic resonance imaging[101,102]. In addition to optical methods, alternative chemical measurements for biomass quantification are available, but they are often destructive. Examples include measuring COD and/or total nitrogen on the electrode post-experiment[37,97] or measuring the consumption of ammonium during the experiment[103,104].

The use of novel cutting-edge methods can expand the current toolbox of techniques and enhance the depth of insight. For instance, the fast-developing methods in molecular biology have enabled the analysis of genes, transcription, metabolites and proteins[105], as well as the untapped biofilm matrix[106,107]. These methods realize their full potential when combined with basic chemical and electrochemical characterization. This also holds true when more advanced electrochemical techniques like EIS are applied.

Figure 5 shows how (a combination of) different optical, electrochemical, molecular biology, chemical and mathematical methods can be used to achieve in-depth understanding of microbial electrodes.

Combination of techniques for characterization

Using only electrochemical or chemical techniques, or their combination, does not afford a complete insight into microbial electrodes and METs. Therefore, for more in-depth insights into biofilm properties and performance, particularly EET mechanisms, it is crucial to combine electrochemical techniques with optical techniques[108], molecular tools and mathematical modelling[25,109–111].

Through the determination of practical biofilm densities, achieved through correlations between chemical and optical quantification of biofilms[97], or by using theoretical biofilm densities based on thermodynamic models[109,112], optical techniques can be used to determine the different electron sinks. In addition to quantifying biofilm growth in situ, it is possible to measure biofilm composition, including intracellular and extracellular electron storage compounds[98]. Molecular biology techniques, such as next-generation sequencing for DNA analysis of the microbial community within the biofilm, as well as RNA, proteome and metabolome analysis, can further complement electrochemical and optical characterization. These techniques allow, among other things, mapping and tracking how the community changes over time, which proteins are involved in EET and how protein expression is affected by the operating conditions. Other methods can be used to further investigate METs and to cover and integrate more disciplines.

Outlook

METs represent an interdisciplinary research field with many fundamental and applied research questions. Despite progress, there are still challenges in advancing this field and applying laboratory-scale systems to pilot and full-scale applications.

Scientific methods and approaches are developing rapidly, and many of these developments are also relevant for biofilm electrodes.

Glossary

Chronoamperometry
An electrochemical method in which a potential is applied to the working electrode (versus the reference electrode), and the resulting current is measured as function of time.

Chronopotentiometry
An electrochemical method in which a current is applied between anode and cathode, and the resulting potential (of the anode or cathode versus the reference electrode) is measured as function of time.

Coulombic efficiency
The part of electrons from a converted substrate that ends up as electric charge (for anodes), or the part of electrons from electric charge that is converted to the (desired) product(s) (for cathodes).

Cyclic voltammetry
(CV). Electrochemical method in which the potential of the working electrode is changed (versus the reference electrode) from an initial value to an end value, and back to the initial value. Such a cycle is typically repeated several times. The resulting current is measured, and information can be gained on the formal potential of electron transfer processes.

Electroactive microorganisms
Microorganisms that can exchange electrons with an electrode. They can either donate electrons to an anode or accept electrons from a cathode.

Energy efficiency
Ratio between the energy harvested in the form of electricity and the energy supplied in the form of substrate (for fuel cell mode), or ratio between the energy in the desired product and the energy supplied in the form of electricity (for electrolysis cell mode).

Extracellular electron transfer
(EET). Electron transfer to solid surfaces that are located outside the microbial cell. EET typically occurs through extracellular charge carriers (for example, cytochromes and flavins) located on the outer cell membrane or via soluble compounds (such as redox shuttles) like phenazines or flavins.

Microbial electrochemical technologies
(METs). Technologies based on the interfacing of microbial and electrochemical conversions. Primary METs are based on the principle that microorganisms perform extracellular electron transfer for which they form biofilms at the anode or cathode.

Microbial electrolysis cell
A type of microbial electrochemical technology that involves coupling a bioanode, which converts organic or inorganic matter, with a thermodynamically unfavourable cathodic reaction, requiring electric power.

Microbial electrosynthesis
The synthesis of a desired product by a microbial electrochemical technology (MET). In a narrower sense, a type of MET in which the reaction of the cathode is the conversion of CO_2 to organic products, for example formate or acetate, catalysed by microorganisms performing extracellular electron transfer.

Microbial fuel cell
A type of microbial electrochemical technology that involves coupling a bioanode, which converts organic or inorganic matter, with a thermodynamically favourable cathodic reaction, resulting in the harvesting of electric power.

Polarization curve
A graph in which the potential of the working electrode (versus the reference electrode) is shown as a function of the current. Information can be gained on the losses (overpotentials) that occur at the electrode and how these depend on the rate (current).

Voltage efficiency
Ratio between actual cell voltage and theoretical (equilibrium) cell voltage (for fuel-cell mode), or ratio between theoretical (equilibrium) cell voltage and applied cell voltage (for electrolysis cell mode).

For example, the field of molecular biology, not only to identify (active) microbial communities but also to analyse gene expression, is rapidly evolving. It is expected that further identification of markers for electroactive microorganisms and extracellular electron transfer will lead to further insights into microbial communities and electroactive microorganisms. This may even lead to microbial resource mining of electroactive species and microbiomes in high throughput, which was impossible until now. Artificial intelligence and big data may provide promising new strategies to control, analyse and optimize METs. Integrated modelling and experiments will further enhance our mechanistic understanding and will offer opportunities for the (re) design of METs. The integration of techniques from different fields and disciplines (Fig. 5) will lead to further and in-depth understanding of all aspects of METs and of biofilm electrodes.

Over the next 5–10 years, there are many opportunities for research and application. From an application point of few, some examples of METs are already on the market, such as sensors (SENTRY). There are several sister technologies (Aquacycl and Electrochaea) demonstrating their MET technology on a larger scale. The successful operation of METs in wastewater treatment and in power-to-X application will need to be further demonstrated. In this context, there is a need for the further exploration of strategies for scaling up to determine whether a modular or size-based approach is more suitable, depending on the application scenario[113]. Early-stage life cycle and technoeconomic assessments may help to guide and pave the way towards implementation.

The field would benefit from the standardization of methods and approaches. Initial steps have been taken towards this, for example in a cross-laboratory study with identical reactors and variation in inocula only[114]. METs have potential applications beyond the current field. This includes application in health, for example sensor development and biocomputing, as well as bio-inspired material development and understanding the role of microorganisms in corrosion processes.

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