Energy & Environmental Science

Accepted Manuscript

ROYAL SOCIETY OF CHEMISTRY

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Microbial Electrochemistry and Technology: Terminology and Classification

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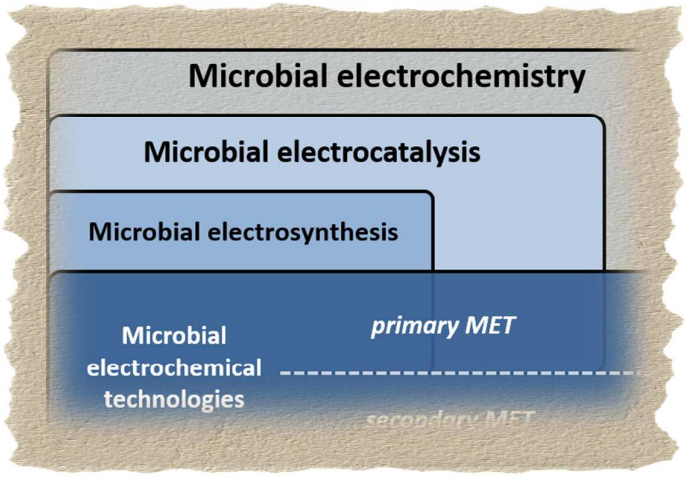


TOC

Abstract

Microbial electrochemistry is the study and application of interactions between living microbial cells and electrodes (*i.e.* electron conductors, capacitive materials). For a long time this subfield of bioelectrochemistry has been the interest of mainly fundamental researchers. This has considerably changed during the last decade and microbial electrochemistry gained interest from applied researchers and engineers. These researchers took the microbial fuel cell (MFC), which is a system that converts the chemical energy of organic material in wastewater into electric power, from a concept to a technology. In addition, a plethora of derivative technologies, such as microbial

electrolysis cells (MECs), microbial desalination cells (MDCs), photomicrobial fuel cells (photoMFCs), microbial electrosynthesis (MES), and biocomputing have been developed. The growing number of systems is often referred to in literature under the termini bioelectrochemical system (BES), microbial electrochemical technology (MET), or electrobiotechnology. Within this article we introduce a classification of technologies based on interfacing microbiology and electrochemistry. We argue that BESs comprise all systems based on bioelectrochemistry, with a further layer of termini through the use of METs. Primary METs are based on extracellular electron transfer (direct or mediated), whereas secondary METs include systems in which electrochemistry is connected – at least through ionic contact – with a microbial process *via* the electrochemical control or adaptation of environmental parameters, such as pH or metabolite concentration level.

Key words: Bioelectrochemical system, BES, microbial electrochemical technology, MET, microbial electrochemistry, bioelectrochemistry, electromicrobiology, electrobiotechnology, microbial fuel cell, microbial electrosynthesis

Broader Context

The interface of microbiology and electrochemistry is a fascinating playground for fundamental research and strives to become a vital field of technology. Thereby, its interdisciplinary research community has not only developed a plethora of concepts and applications, but also numerous and often doubling or overlapping termini. For continuing success of the field, however, the communication and language among the researchers, as well as with stakeholders and the public must be unambiguous. Therefore, a clear terminology and classification of concepts and technologies is needed, for which this article provides a first scaffold.

1. Introduction

A quick literature search with the search phrase “microbial fuel cell” shows an almost exponential increase in research publications over the last three decades, with more than 700 publications in 2013 (Scopus, September 2014). The steep increase over the last decade reflects the growing research activity as well as the growing research community all over the world. This community – which recently has organized itself in the *International Society for Microbial Electrochemistry and Technology* (ISMET) - stems from very different areas, including environmental engineering and

technology, biochemistry, electrochemistry, physics, mathematical modelling, and microbiology. The rapid growth in the development and the interdisciplinary nature of the field has lead to an ever-growing number of concepts, termini, and applications, making it increasingly difficult to classify technology. Here, we propose a classification of technologies based on interfacing microbiology and electrochemistry. Thereby, definitions of the most important key termini are provided. These definitions and classifications shall provide a scaffold for the further development of and form a basis for a unification of scientific language in this field.

2. Terminology and definitions

To distinguish processes, materials, and applications at the interface of biology and electrochemistry from conventional electrochemistry, generally the prefix *bio* is used to indicate that a biological moiety (*e.g.* a cell, enzyme, or organelle) plays a key role. Examples are *bio*fuel cells, *bio*sensors, *bio*electrocatalysis, and, of course, *bio*electrochemistry. When looking at a specific biological system and its use in electrochemistry the prefix *bio* is usually waived, resulting in, for example, enzyme electrochemistry, protein electrochemistry, and microbial electrochemistry (Figure 1). The same applies to the deriving bioelectrochemical systems (BESs), which are consequently denominated as microbial fuel cells or enzymatic fuel cells for the example of *bio*fuel cells. However, quite often, a doubling in the terminology is used with the attribute and the prefix *bio*: microbial *bio*fuel cell, microbial *bio*electrochemistry. Here, we advise on a clear terminology, and therefore strongly suggest to waive the prefix *bio* when referring to a specific biological system.

The development of a classification requires the establishment and widely use of respective definitions and terminologies. In the following paragraphs the key termini microbial electrochemistry and microbial electrocatalysis are defined and the derived technologies as well as their interrelations are introduced. These technologies are illustrated on selected examples in section 3.

Microbial electrochemistry is the study and application of interactions between microorganisms and electron conductors. These electron conductors comprise physical electrodes (*e.g.* made from graphite or metals, being for instance solid or granular) and naturally occurring conductive materials such as metal oxides.[1] In the latter case, microbial electrochemistry can help explaining processes observed in e.g., geomicrobiology. In the following sections, the different electron conductors will be subsumed under the electrochemical term *electrode*.[2]

Microbial electrochemistry is a subfield and belongs into the field of bioelectrochemistry with its sister subfields enzyme electrochemistry, protein electrochemistry, or DNA electrochemistry

(Figure 1). In this definition, microbial electrochemistry may be considered synonymous with **electromicrobiology**,[3] although the latter term may put the emphasis stronger on the biological aspect of electrode-microbe interactions.

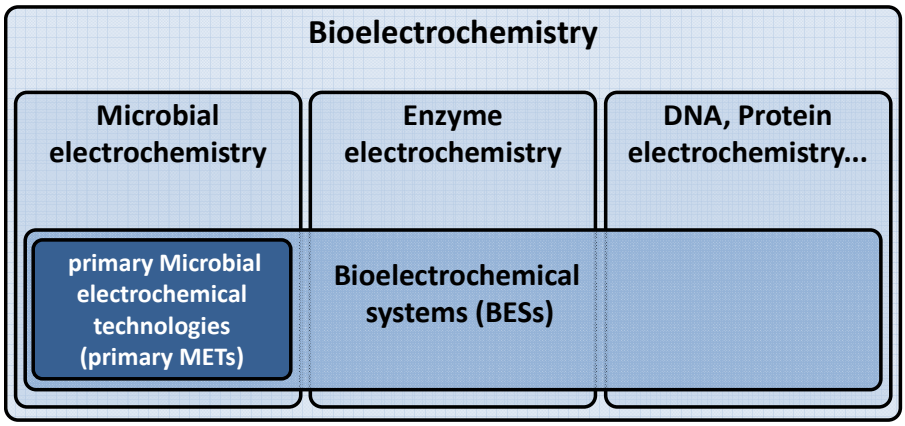


Figure 1: Venn-diagram illustrating the sub-disciplines of bioelectrochemistry and the interrelations of bioelectrochemical systems (BESs) and microbial electrochemical technologies (METs).

Interactions between microbial cells and electron conductors can be of either capacitive or Faraday nature (Figure 2). Capacitive interactions describe the change of the double layer capacity of an electrode, for example, as a result of cell attachment or detachment. The attachment of microbial cells at an electrode (illustrated in Figure 2A by means of the lipid layer of the cell membrane) leads to a displacement of water molecules and ions from the double layer, and thus to a diminishing electrochemical (double layer) capacity – leading to the flow of a charge balancing (capacitive) electric current. Microbial Faraday processes describe all kind of oxidation and reduction reactions of microbial cells and of any molecular species involved in microbial extracellular electron transfer (see section 3.1). Prominent examples for such microbial Faraday reactions are pseudo-capacitive processes (Figure 2B) in which microbial cells and microbial biofilms are oxidatively or reductively charged or uncharged (*i.e.* acting as a microbial supercapacitor)[4] as well as microbial electrocatalysis (Figure 2C).

The interactions between microorganisms and electrodes that are included in microbial electrochemistry should take place at physiologically important potentials. That means that the applied potential should not be too positive for anodic activities or too negative for cathodic activities, since too positive and negative potentials would harm the microorganism through the degradation of physiologically important biomolecules. It will take a scientific debate to decide on the maximum potentials, and certainly the physiological potential window depends specifically on the used microorganism and the natural redox conditions it is adapted to. Thus, aerobic

microorganisms are adapted to redox potentials governed by oxygen, whereas anaerobes may not tolerate such high potentials. Here, cytochrome c proteins on the outside of the microorganisms are damaged already at an anodic potential of +0.6V vs. SHE.[5] At the cathode side, the hydrogen evolution reaction may serve as a general limit of the physiological range, although several biochemical processes exist that take place at potentials more negative than the $H_2/2H^+$ couple.

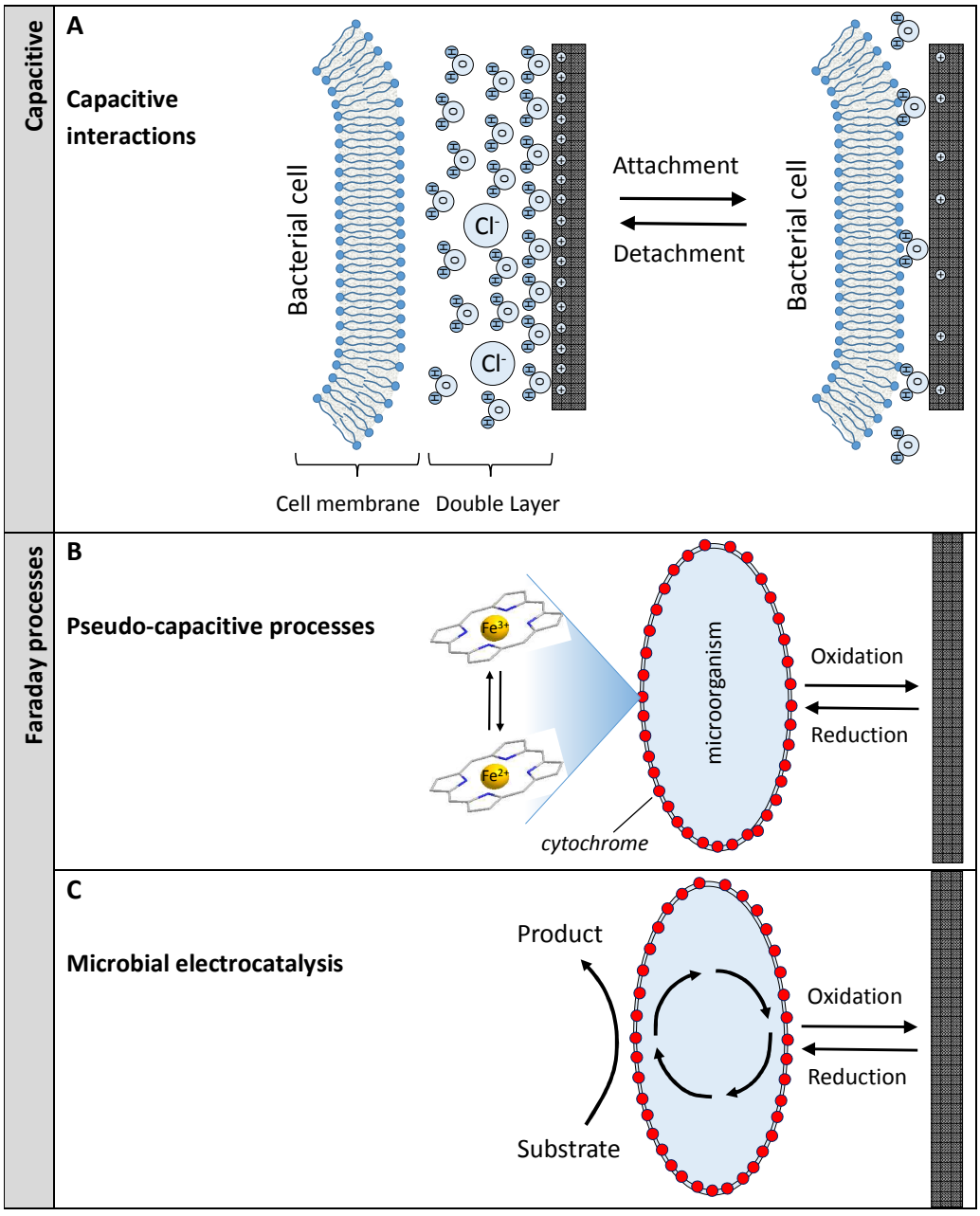


Figure 2: Major electrochemical interactions between microorganisms and electrodes.

Microbial Electrocatalysis describes the acceleration/facilitation of an electrochemical reaction by microorganisms based on extracellular electron transfer (see section 3.1). Microbial electrocatalysis can also be defined as the reduction of the overpotential (that is necessary to achieve a certain reaction rate) or the increase of the rate constant and thus current flow (at a given potential) of an electrochemical reaction[2] by the action of microbes. Strictly speaking, the term catalysis should be not applicable to a microbial system, since living cells usually actively participate by deriving a share of energy (*e.g.* in terms of reduction equivalents) from the reaction.[6] Yet, from the practical viewpoint of the reaction facilitation, this terminological inaccuracy may be tolerable.

Microbial electrocatalysis can either be used for the purpose of substrate degradation – emphasis of environmental applications, such as microbial fuel cells (with the focus of wastewater treatment and energy recovery), or for the purpose of synthesis – opening the scope of BESs for biotechnological applications.

Microbial Electrosynthesis has been described by different authors as the microbial electrochemical conversion of carbon dioxide into organic compounds[7] or, more general, the production of (complex) organic matter using microbial electrocatalysis.[8] Based on definitions for chemical synthesis[9], we propose to define microbial electrosynthesis (MES) in a broader sense: MES is the execution of microbially catalysed electrochemical reactions to transform a substance into a desired product. Thereby, microbial electrosynthesis reactions comprise anodic (oxidative) and cathodic (reductive) processes. The generation of carbon dioxide in a microbial fuel cell is not a microbial electrosynthesis, since it is not the purpose of the MFC operation to produce CO_2 but to degrade organic matter (in wastewater) and to produce electric energy.

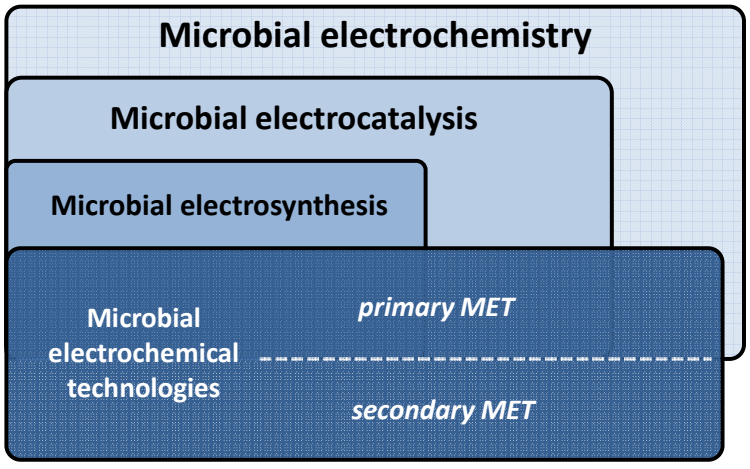


Figure 3: Venn-diagram illustrating the interrelations of the subfields of microbial electrochemistry and microbial electrochemical technology.

Microbial Electrochemical Technologies (METs) can now be derived *as technologies or applications that utilize the electrochemical interaction of microbes and electrodes.* Based on the nature and the degree of interaction, we can distinguish between **primary** and **secondary METs** (Figure 3). A **primary MET** uses processes that fall within microbial electrochemistry (*i.e*. the above described Faraday or capacitive interactions of microorganisms and electrodes at physiologically important electrode potentials). In the great majority these interactions involve extracellular electron transfer (see section 3). On a system level, when looking at the actual bioelectrochemical device (*e.g*. a MFC, MEC), primary METs are often denominated as **bioelectrochemical system (BES)** (see discussion below)**.**

A **secondary MET** utilizes more indirect interactions, which do not fall within microbial electrochemistry. These interactions comprise, for example, the control or adjustment of the microbial reaction environment (such as pH, oxygen partial pressure, and metabolite [*e.g.* substrate or product] concentration) by means of electrochemical processes. It is important to note that an ionic connection between the electrochemical system and microbial system should exist to make this control or adjustment possible. Thus, electrochemical systems and microbial systems must be in close vicinity of each other and cannot be separated spatially.

For a differentiation between primary METs and secondary METs, also the applied electrode potentials may serve as an indicator: whereas in primary METs, the used electrode potentials would thermodynamically lie within the physiological range of the used microorganisms, the use of high voltages (strongly negative or positive electrode potentials) often indicates a secondary MET.

In addition, for a differentiation between METs and systems that are not METs, the existence or nonexistence of a functional connection in the form of at least an ionic connection/interaction between the electrode(s) and the microorganisms would be an indicator. Since any electrochemical cell consists of at least two electrodes – cathode (at which the reduction takes place) and anode (at which the oxidation takes place) – it has to be stated that in a MET at least one of the two electrodes has to be functionally connected with a respective microbial process.

Bioelectrochemical systems (BESs) have recently become a synonym for primary METs. However, BESs comprise systems, which derive from all subfields of bioelectrochemistry, including enzyme, microbial, protein, DNA- or neuro-electrochemistry.[10, 11] Thus, primary METs are only a sub-section of BESs. Yet, when describing or discussing an actual microbial electrochemical device, the term BES may be more applicable than MET, since *system* describes physical devices, whereas *technology* is more abstract and comprises the entity of technical tools, machineries, and procedures.

3. Microbial Electrochemical Technologies

3.1 Primary METs

Although microbial electrochemistry involves capacitive and Faraday processes (Fig. 2), current primary METs rely exclusively on Faraday processes – mostly on microbial electrocatalysis. Microbial electrocatalysis requires that electrons (or reduction equivalents) are exchanged between the intracellular electron transfer chains and an electrode, across the cell membrane. This electron exchange is called extracellular[a] electron transfer (EET). The two most important mechanisms, which are the functional basis of all so far reported primary METs, are direct extracellular electron transfer and mediated extracellular electron transfer in anodes, whereas for cathodes only very few mechanisms have been reported.[12]

Direct Electron Transfer Mechanisms: the prime example for an inherent functional connection between a bacterial cell and an electrode is direct electron transfer by electro-active bacteria (often referred to as electricigens,[13] anode respiring bacteria,[14] or exoelectrogenic bacteria[15] in anodes or electrotrophs in cathodes[16] using transmembrane redox-proteins such as cytochromes[17-19] or nanowires.[20] Direct electron transfer involves that the microorganism is in the vicinity of the electrode, which usually means that the microorganisms are permanently attached to an electrode in the form of a microbial biofilm (Figure 4A).

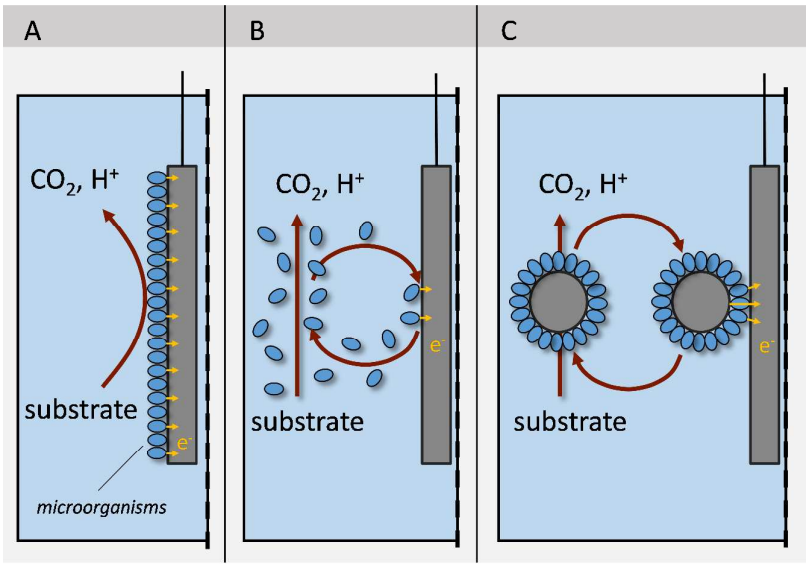


Figure 4: Direct electron transfer in exemplary anodic BES half-cells A) Biofilm electrode, B) via suspended cells, C) via capacitive biofilm particles

[a] The term extracellular is equal to exocellular (Extra (Latin) = Exo (Greek) = outer)

Although detachment of the cells results in termination of the electron transfer, the charge storage capacity of metal reducing bacteria, such as *Shewanella*,[21] allows for an intermittent contact and electron transfer based on passive or active (electrokinesis) cell movement towards an electrode[22] (Figure 4B) or the use of capacitive biofilm anode particles in a fluidized reactor (Figure 4C).[23] In both cases the fermenter can be separated from the actual electrochemical cell (as in the case of Figure 4B), which, however, requires the pumping of the microbial broth from the fermenter through the electrochemical cell for electrochemical oxidation/ reduction.

Mediated Electron Transfer Mechanism: many electroactive bacteria exploit mediated extracellular electron transfer based on molecular redox compounds. In nature (in soil and in sediments) this involves humic compounds as electron carrier.[24] In technical systems, the discovery of endogenous (microbial) redox mediators, such as flavins[25] or phenazines[26, 27] (secondary microbial metabolites), made the use of exogenous (artificial) redox mediators in microbial fuel cells obsolete.[6] For other applications such as microbial electrosynthesis the use of exogenous mediators is still a suitable, and often necessary approach. [28]

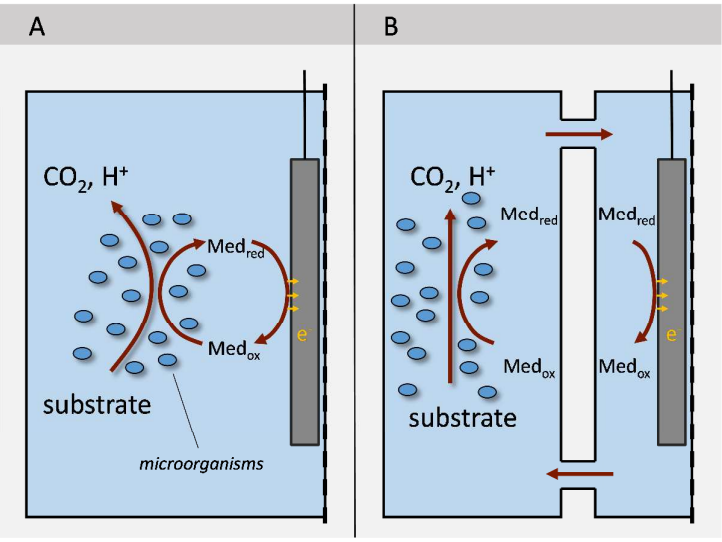
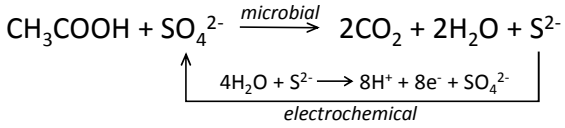


Figure 5: Mediated electron transfer in exemplary anodic BES half-cells A) Integrated ("conventional") BES, B) BES consisting of separate fermenter and electrochemical cell.

Generally, the concentration level of mediators is orders of magnitudes lower than that of the substrate or product. Mediator recycling between bacterial cells and electrode and *vice versa* is, therefore, essential for a significant electrochemical substrate conversion, respectively product formation. Thus, there are strong, immediate functional connections between the electrode and the microorganisms, although mediator recycling does not have to take place in the close vicinity of the microbial cells and the latter can grow also as planktonic (free swimming) cells away from the

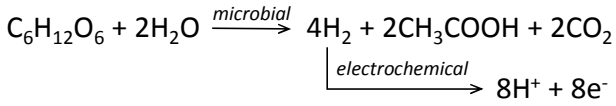
electrode (Figure 5 A). This spatial disconnection allows to perform the electrochemical mediator recycling in an electrochemical cell separate from the biological reactor (Figure 5B). [28]

Apart from secondary metabolites also primary metabolites or primary substrates can facilitate electron transfer. One example is the use of sulphur-species as electron shuttles. Sulphate reduction is a process in which bacteria, such as *Desulfovibrio*, use natural, dissolved sulphate as terminal electron acceptor.[29] In a bioelectrochemical system, an abiotic electrochemical re-oxidation can sustain the reaction at low sulphate level and could, for instance, be exploited to extract electric energy in a benthic microbial fuel cell[30] (Scheme 1).



Scheme 1: Simplified equation of the microbial oxidation of acetate via sulphate reduction and its electrochemical re-cycling.

This reaction requires the use of electrocatalytic anodes to facilitate the complete oxidation of sulphide to sulphate.[31] A second example of using primary metabolites for mediated electron transfer is the use of hydrogen, which is a product from fermentative and photo-heterotrophic processes, as electron carrier (Scheme 2).



Scheme 2: Simplified equation of the mixed acid fermentation of glucose, e.g. by *Clostridia*; with subsequent abiotic electrochemical hydrogen oxidation.

Here, two situations can be distinguished. For case 1, the hydrogen is directly (*in situ*) oxidized in the microbial solution.[32-35] Electrode and microorganisms are functionally connected, as it has been demonstrated that *in-situ* hydrogen depletion enhances microbial hydrogen generation (Figure 6A).[36] For case 2, the evolved hydrogen is separated *via* the gas phase and is burned in a separate hydrogen fuel cell. Whereas case 1 is a typical (primary) MET, case 2 is not a MET or a BES, since the electrochemical process is functionally disconnected to the microbial process. Case 2 (Figure 6B) is different to Figure 5B, since in the latter case no separation of the electron carrier from the microbial solution takes place and the functional connection of electrochemical and microbial step is given (please note that here the ionic contact between the microbial and electrochemical process is maintained).

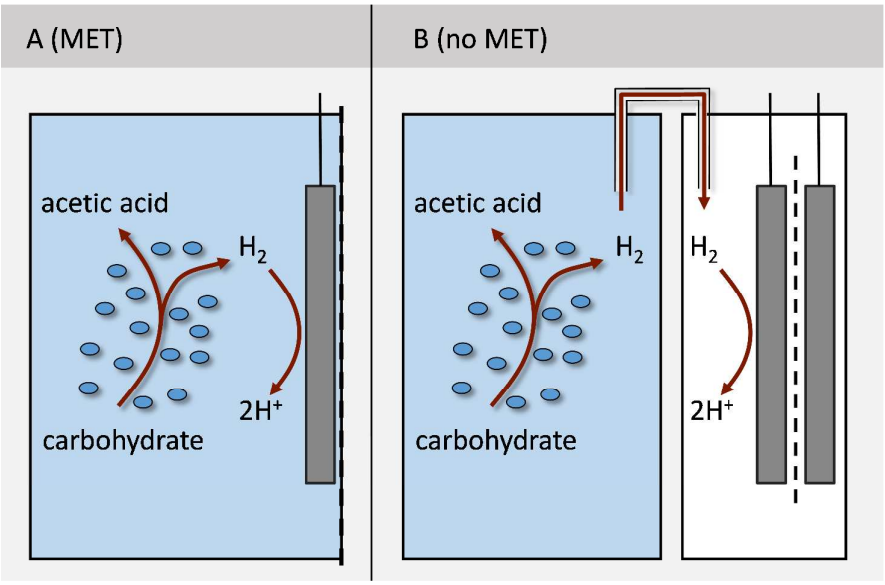


Figure 6: A) Anodic half-cell of a hydrogen-mediated BES B) Hybrid system consisting of a fermenter and a separate hydrogen fuel cell.

The provided examples are typical for the anode reactions in microbial fuel cells and microbial electrolysis cells. They can, however, be generalized and applied for cathode reactions and for other types of METs, such as microbial electrosynthesis, biocomputing[37, 38] and the electrochemical redox balancing of fermentation processes.[39, 40]

3.2 Secondary METs

Compared to primary METs, secondary METs are based on a more indirect functional connection of electrochemistry and microbiology – for example, on the adaptation and control of environmental parameters such as pH, oxygen partial pressure, metabolite concentration by means of electrochemical processes. A prime example for illustration is microbial fermentation that is ionically coupled with membrane electrolysis extraction (Figure 7).[41,42] In this process the fermentation product (*e.g.* a carboxylate) is extracted from the bacterial broth by mean of an (abiotic) electrolysis process, while pH control occurs simultaneously – both extraction and pH control are advantageous for the microorganisms.

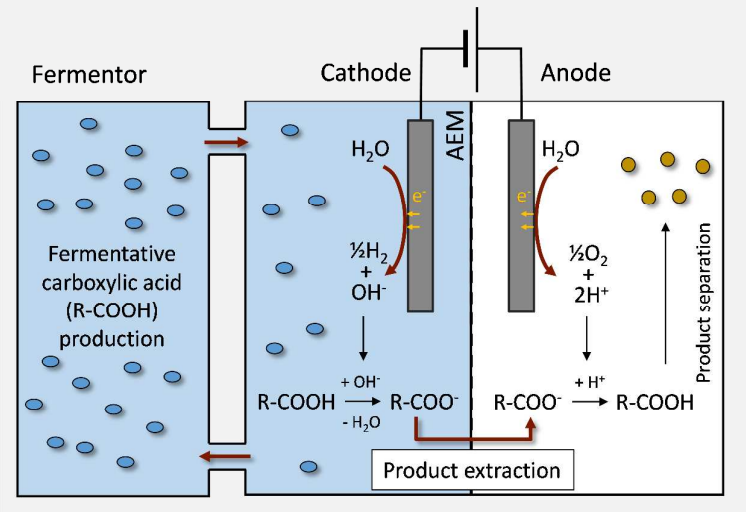


Figure 7: Membrane electrolysis extraction as an example for a secondary MET. The electrochemical reaction (water electrolysis) serves as the driving force to extract an anionic product from the fermentor with microorganisms (blue ovals). The carboxylate is extracted from the broth and separated as phase-separated oil droplets (gold circles) for *n*-caproic acid with a relatively low maximum solubility. Hydroxyl formation at the cathode result in lower requirements for sodium hydroxide.[42]

The purpose of the electrolysis process is to create an anion flux from the fermenter solution *via* an anion exchange membrane into a product chamber. The negatively charged carboxylate ions can, thus, be extracted and separated from the fermentor, and the product yield can be enhanced, since product inhibition can be prevented at the constantly low product concentration in the bacterial broth. The ionic connection generates an additional advantage due to the electrochemical production of hydroxyl ions at the cathode, reducing the procurement need of sodium hydroxide to maintain high enough pH levels in the fermentor due to carboxylic acid formation (Figure 7).[42] The electrochemical production of protons at the anode generates acidic conditions that induces phase separation of *n*-caproic acid (Figure 7). The benefit for fermentation is lost when the electrochemical cell is separated from the biological process. A similar approach is the integrated product removal by electrochemically induced crystallization.[43]

These examples clearly illustrate the functional connection between the microbial and the electrochemical process that goes beyond microbial electrode interactions. Other examples are more difficult to classify, such as the electrolysis enhanced anaerobic digester (eAD), in which abiotic electrodes are immersed in a fermenter such as an anaerobic digester (AD), to enhance methane formation,[44] or aid in metabolite degradation to carbon dioxide. Three mechanisms have been proposed for the electrochemical enhancement of the anaerobic digestion, they all lead to different process classifications: It has been proposed that oxygen is released anodically during water

electrolysis in the AD broth (mechanism one). This leads to a microaerophilic environment, which in turn enhances substrate hydrolysis. The hydrolysed substrate can, thus, be more efficiently digested.[44] This can be considered as a secondary MET, since the electrochemical process is connected to the microbial process *via* the change of the environmental variable oxygen pressure. In mechanism two, hydrogen, being formed at the cathode of the electrolyser, is used as a co-substrate for the fermentation with enhanced methane formation by hydrogenotrophic methanogenesis. Since the electrochemically formed reduction equivalents are directly and microbially utilized for product formation, this could be denominated as a primary MET. It is important to note, that hydrogen can be generated at physiologically important electrode potentials. A third, more profane role of the electrode in the AD reactor (mechanism three) is the simple enhancement of the biomass retention on the electrodes in the AD reactor.[45] In this case no electrochemical reaction is necessary and the technology is not a MET at all, but simply anaerobic digestion with biomass retention.

A recent example of placement of electrodes in a fermentor to generate formic acid from carbon dioxide, with a direct consumption of the formic acid by microorganisms [46] may appear like a primary MET. However, the potential necessary to produce formic acid from CO_2 is strongly negative and lies clearly outside the physiologically important potential range. It should therefore be denominated as secondary MET.

Another secondary MET may be seen in electrobioremediation.[42] Here, electrodes are inserted into soil and a voltage is applied to degrade pollutants from the soil. The main mechanisms involve the change in the environment similar to the above example of eAD (*vide supra*), abiotic reactions steps of the pollutants as well as the enhancement of the bioavailability of the pollutants and nutrients by electroosmosis and electromigration.[47] Microbial electroremediation cells at physiologically important potential differences, which exploit the activity of electrochemically active bacteria, on the other hand, should to be counted as primary MET.[48]

4. Summary & Outlook

The here presented approach shall not be understood as comprehensive or rigid. The fields of microbial electrochemistry and microbial electrochemical technologies are rapidly growing, and growing technologies need the possibility to develop their terminology. Our goal was to take a first step to start a discussion on a common language and terminology. Therefore, we hope that this article will serve as a scaffold for a continuing and lively discussion that contributes to the shaping and maturation of research and development at the interface of microbiology and electrochemistry.

Acknowledgements

We thank two anonymous referees for their very critical and constructive comments that helped to shape the discussion and article. F.H. acknowledges support by the BMBF (Research Award "Next generation biotechnological processes - Biotechnology 2020+") and the Helmholtz-Association (Young Investigators Group); L.T.A. acknowledges support by the U. S. Army Research Laboratory and the U. S. Army Research Office under contract/grant number W911NF-12-1-0555. This work was supported by the Helmholtz-Association within the Research Programme Renewable Energies.

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Microbial Electrochemistry and Technology: Terminology and Classification

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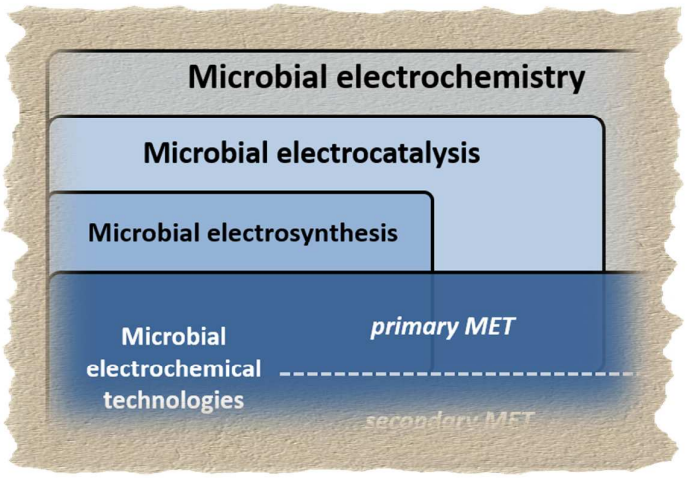
Table of Contents



This paper provides a scaffold for the development of a clear and consistent terminology and classification of microbial electrochemistry and microbial electrochemical technologies.