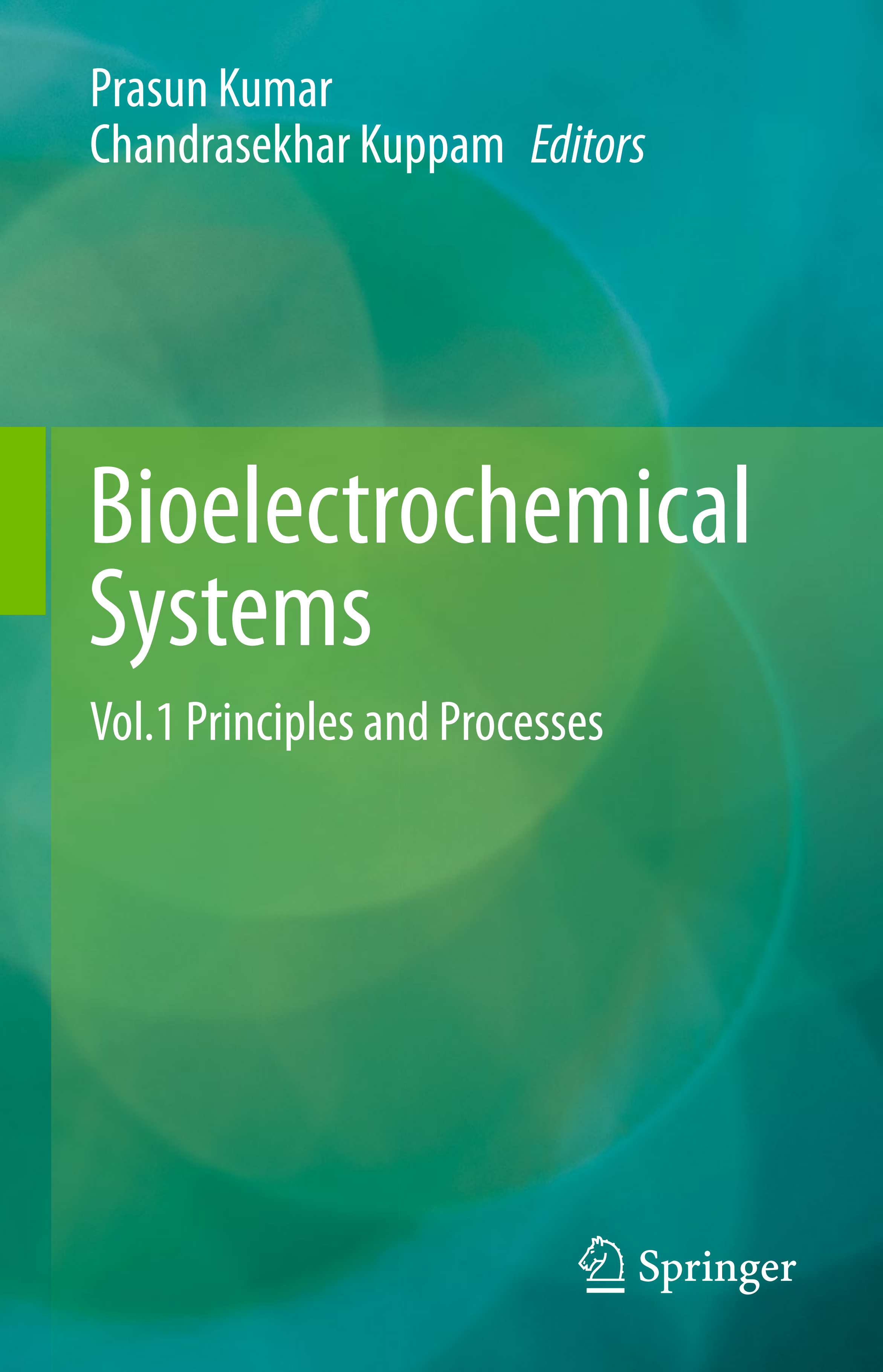

Prasun Kumar
Chandrasekhar Kuppam *Editors*

Bioelectrochemical Systems

Vol.1 Principles and Processes

Springer

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Prasun Kumar • Chandrasekhar Kuppam
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Dedicated to my Parents

Preface

Microbes are ubiquitous and versatile in nature showing strong interactions among themselves and others living in the vicinity. These interactions with living beings have gained renewed interest and value. Microbial activities are finding wider applications ranging from bioremediation, bioenergy, and biomedicine to agriculture and industry. During the past decade, there has been a transition from chemical processes to biological methods, largely because the latter are eco-friendly and expected to be sustainable. Exploiting green technologies to realize the circular economy is the new trend amid the increasing demand for energy. In recent times, the necessity of wastewater treatment as well as its management has been considered an important research area. Integrative technologies have paved the way for the value addition of the whole process. Among these, bioelectrochemical systems (BESs) are gaining popularity in the realm of increasing energy demand, pollution, and concerns for global warming. The increasing dimension and diversification of BES hold the promise for maximal extraction and value addition to the existing tools being exploited for renewable and sustainable energy across the globe. The widespread application and/or integration of such technology will certainly be helpful for developed and developing countries to eliminate and overcome the problems associated with waste management, clean energy, water, remote power generation, etc. Electricity, hydrogen, and methane are some of the major outputs of BES with a concomitant removal and/or treatment of wastewater. Among these, electricity can be classified as "super low-carbon fuel" that can be utilized for decentralized power production leading to revenue addition in the form of renewable energy credit and other greenhouse gas emission credits. The rapidly developing tools to improve the BES and its applicability have instigated us to bring out a comprehensive reference book. This book is an attempt to cover most of the information related to BES and its variants. This book is a two-volume set devoted to bioelectrochemical systems (BESs) and the opportunities that they may offer in providing a green solution to growing energy demands worldwide. In this first volume, established research professionals explain the underlying principles and processes of BESs, the roles of various catalysts, and the mechanism of microbial electrosynthesis. This volume forms a sound foundation for understanding the potential industrial applications of this technology, which include in particular the generation of high-value chemicals and energy using organic wastes. The second volume focuses on the applications of BES in diverse fields and how such systems can be realized in the real-life scenario. The implication of BES in metal recovery, pollutant removal, and energy production has been particularly emphasized. Readers will also find up-to-date information on microbial biofilm- and algae-based bioelectrochemical systems for bioremediation and co-generation of valuable chemicals. Usage of MFC in rice fields and the challenges associated with the pilot-scale operations are among the few unique topics covered in this book. A thorough review of the performance of this technology and its possible industrial applications is presented. The book is designed for a broad audience, including undergraduates, postgraduates, energy researchers/scientists, policymakers, and anyone else interested in the latest developments in BES. In this book, the learned scientific community has put their best efforts to share their expertise, which they have gained through their immense experience targeted toward understanding bioelectrochemical systems. This book is a true reflection of the sincerity of the scientific community, who promptly agreed to contribute their creation for the young minds, who are likely to benefit and take this world a step further into the future. I am truly humbled by the help rendered by all the contributing authors. I am running short of words to adequately acknowledge the worthiness of their efforts.

My true inspiration to write this piece of work stems from the faith in me and the constant support of Mrs. Usha Banbari and Mr. R.L. Banbari (parents), Aparna (Sister), and my wife (Stally). I must also acknowledge the support of my teachers, especially Dr. Vipin C. Kalia. He played his role to perfection as a leader, as a torchbearer who refined my skills and stimulated the researcher in me. The joy and enthusiasm he has for research were contagious and motivational for me. I also acknowledge the direct and indirect support provided by my seniors—Sanjay K.S. Patel, Mamtesh Singh, Jyoti Kushwah, Ashish Bhusan, Lalit Singh, and Preeti Bansal—and my friends—Awdhesh, Sanjeet, Subhasree, Madan, Pavan, Praba, and Ezhaveni.

Cheongju, Republic of Korea Prasun Kumar

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About the Editors

Prasun Kumar is presently working as an Assistant Professor at the Department of Chemical Engineering, Yeungnam University, Republic of Korea. He holds a Ph.D. in Biotechnology from CSIR-Institute of Genomics and Integrative Biology, Delhi, India. His main areas of research are biopolymers, microbial biodiversity, bioenergy, microbial biofilms, quorum sensing, quorum quenching, and genomics. He has over seven years of experience in applied microbiological research including over 2 years postdoctoral research experience as BK-21 plus fellow at Chungbuk National University, Republic of Korea. He made a significant contribution while working on valorization of lignocellulosic biowastes or cheap raw materials into high-value products such as H_2, Polyhydroxyalkanoates, lipids and Carotenoids. To his credits, there are over 29 articles in various peer-reviewed SCI journals including Trends in Microbiology, Biotechnology Advances, Bioresource Technology, etc. To date, his work has fetched decent citations with an h index of 25 and an i10 index of 32. In addition, he has edited two books: (i) Microbial Applications Vol. I: Bioremediation and Bioenergy (2017) and (ii) Soil Microenvironment for Bioremediation and Polymer Production (2019) and also authored 10 book chapters. He has been contributing to scientific society by actively reviewing articles for more than 32 SCI journals and was awarded the peer review award by Publons in the year 2018. He serves as the editorial board member of few international journals and also worked as the guest editor for the journals, namely 'Polymers' and 'Frontiers in Bioengineering and Biotechnology'. He is a life member of the following scientific societies: Association of Microbiologists of India, Indian Science Congress Association, and Biotech Research Society of India (BRSI). He is also a member of the American Society for Microbiology.

Chandrasekhar Kuppam is a Ph.D. in Biological Science from the Academy of Scientific and Innovative Research (AcSIR), Indian Institute of Chemical Technology (CSIR-IICT), Hyderabad, Telangana, India. He has worked as a senior research fellow at CSIR-IICT. He also worked as a postdoctoral researcher at the School of Applied Bioscience, Kyungpook National University, Republic of Korea. He is presently working as a Postdoctoral Researcher at the School of Civil and Environmental Engineering, Yonsei University, Republic of Korea. He made a significant contribution while working on bioelectrochemical systems, bioenergy from waste biomass, biohydrogen production, Electrofermentation technology, Biofuels and biochemical production from waste biomass, valorizing lignocellulosic biowastes, and waste remediation technologies including bioelectrochemical remediation. He has published more than 30 articles in various scientific journals, including Bioresource Technology, International Journal of Hydrogen Energy, Renewable and Sustainable Energy Reviews, Waste Management, Journal of Biotechnology, International Journal of Molecular Sciences, Journal of Hazardous Materials, Energies, Chemosphere, Environmental Technology and Innovation, Biomass Conversion, and Biorefinery, Alexandria Engineering Journal, Energy Conversion and Management, and Critical Reviews in Biotechnology. He has authored over 15 book chapters. His work has been cited 1740 times with an h index of 22 and an i10 index of 28. He is a life member of the following scientific societies: (1) life member in The Biotech Research Society of India (BRSI), (2) senior membership in Asia-Pacific Chemical, Biological and Environmental Engineering Society (APCBEES), (3) senior member in Institute of Research Engineers and Doctors, and (4) Frontiers member. He is serving as a review editor in bioenergy and biofuels, then as a guest faculty, Department of Engineering, University of Naples "Parthenope," Naples, Italy. He has also presented his work at several national and international conferences.

Chapter 1
Bioelectrochemical Systems: Principles and Applications

Divya Naradasu, Xizi Long, Akihiro Okamoto, and Waheed Miran

Abstract Bioelectrochemical systems (BESs) are emerging environmental biotechnologies that involve microbial interfacial electron transfer and electrochemical transformations for achieving sustainable energy and carbon neutrality. BESs provide an excellent strategy for the processes based on microbial metabolic oxidation and reduction in comparison to conventional chemical and environmental processes. Thus, a plethora of applications including electricity production via oxidation of the waste biodegradable substrates in the anode compartment and the use of this electricity (along with additional required energy) for production of chemicals and energy carriers (such as H_2) in the cathode compartment has sparked a great interest in BESs. In this chapter, a brief introduction to BESs is provided along with reviews about microbial, technological, and thermodynamic fundamentals of the BES

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technology, and different applications and the latest progress in the field of BESs are briefly discussed.

Keywords Bioelectrochemical system · Electron transfer mechanism · Extracellular electron transfer · Environmental biotechnology · Microbial fuel cell · Microbial electrolysis cell

1.1 Introduction

1.1.1 Fundamentals of BESs

Bioelectrochemical systems (BESs) are generally described as systems that imply electrode reactions by using microorganisms as biocatalyst. Microbial fuel cells (MFCs) and microbial electrolysis cells (MECs) are prominent examples of BESs as the fast-growing biotechnologies. They merge microbial and electrochemical processes to produce bioelectricity, H_2 or other valuable chemicals (Logan et al. 2019). A classic BES system comprised of two electrodes, an anode and a cathode linked to each other by using an external conductive wire for completing an electric circuit. MFCs are employed for electricity generation, while MECs use electricity to drive chemical reactions such as the production of H_2 and/or other valuable chemicals in the cathode compartment (Fig. 1.1). For achieving this, wastewater can be used as a source of bacteria and energy for both MFCs and MECs, and during the process, organics from the wastewater can be removed, thus treating the wastewater (Nevin et al. 2010; Logan 2009; Rozendal et al. 2009).

In BESs, unique type of microorganisms are the crucial players that can transport electrons from their intracellular electron transport chain to an external solid electron acceptor (electrode) or vice versa. This electron exchange reaction with extracellular solids is called "extracellular electron transfer" (EET). Typically, in MFCs, these electrically active bacteria oxidize organics available in wastewater in the anode compartment in the absence of oxygen, i.e., bacteria anaerobically release electrons, protons, and CO_2 while breaking down organics. Anode collects the electrons via different possible pathways from bacteria, which reaches the cathode via an external circuit. Cation exchange membranes are usually placed for separating the anode and cathode sections to allow the protons transport. The electricity is ultimately harvested from the exterior circuit across an external resistor. At the cathode, reaction between electrons and protons along with oxygen (usually from the air) takes place. As a result, water is formed and hence circuit is completed. Whereas MEC is the amended form of MFC in which oxygen is generally not present in the cathodic chamber, and hence a reaction of electrons and protons (H^+) take place at the cathode to produce hydrogen (H_2) (Kadier et al. 2016). As it is not a spontaneous reaction, a small amount of additional energy of approximately +0.2 V or more (other than that produced by the bacteria) is required to operate this system. MECs can also be modified for other processes so that more valuable products can be

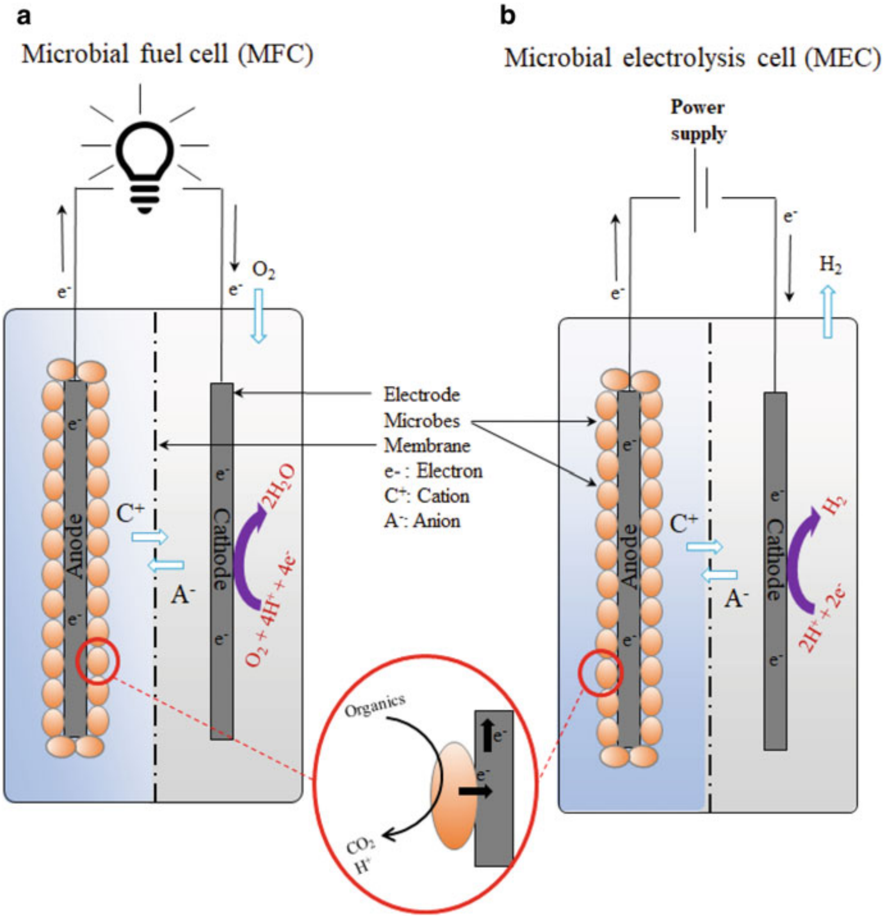


Fig. 1.1 Typical schematic diagram of the two most commonly employed bioelectrochemical systems: (**a**) MFC for current generation (**b**) MEC for H_2 production. Adopted from (Yasri et al. 2019)

produced. In addition to MFCs and MECs, BES technology has been modified and given different names based on different functions. This includes microbial remediation cell (MRC), microbial desalination cell (MDC), microbial solar cell (MSC), microbial chemical cell (MCC), microbial electrosynthesis (MES), etc., details of which can be seen in the literature (Harnisch and Schroder 2010; Kumar et al. 2018; Bajracharya et al. 2016).

1.1.2 Extracellular Electron Transfer Mechanisms

EET is the fundamental process involved in BESs that couples microorganisms and insoluble electron donors/acceptors, based on which various applications can be deployed. Over the years of fundamental research, two distinct modes of EET mechanisms are proposed: direct extracellular electron transfer (DET) linked to electrode attached microbes and an indirect extracellular electron transfer (IET) from electrode non-attached microbes via electron shuttles.

1. Direct extracellular electron transfer (DET)

   DET between the microbes and electrodes has been proposed to happen in two major ways:

   a. Electron transfer from the directly attached microbe's outer membrane proteins and the electrode's surface. The outer membrane protein complex spanning across the membrane of microbes enables the transport of electrons directly from the cell interior to the external electrode surface.
   b. The second mode of DET occurs between the microorganism and the electrode when electrons are transferred through cell extensions termed as pili or nanowires. These are the extensions from the microbial outer membrane and attached to the surface of the electrode. As nanowires can range up to tens of microns, cells can make contact with the distant electrode surface to transfer electrons.

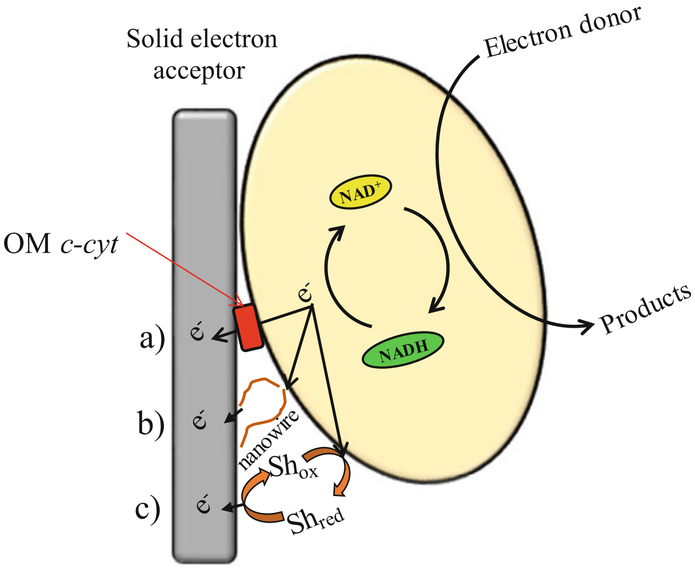
2. Indirect or mediated transfer of electrons from the microorganism to the electrode surface occurs via electrons shuttling compounds that are naturally present in the niche or may be secreted by the microorganism such as flavins.

These mediators can help in transport of electrons to and from the electrode as they can display cyclic redox properties. This mechanism is helpful in a long-range electron transfer which can result in sustainable and efficient current generation. These mechanisms of EET also help microorganisms to build up multi-layered structures called biofilm.

Many studies have been reported on microorganisms which can utilize metals as electron acceptors and reduce them (Fredrickson and Zachara 2008; Lovley 2011; Richardson et al. 2012). Some known bacterial strains are *Shewanella oneidensis* (Myers and Nealson 1988), *Geobacter metallireducens* (Lovley 1993), *Desulfuromonas acetoxidans* (Roden and Lovley 1993), *Pelobacter carbinolicus* (Lovley et al. 1995), members of the genus *Desulfuromusa* (Fredrickson and Gorby 1996), *Ferrimonas balearica* (Lovley and Coates 2000), *Geovibrio ferrireducens* (Caccavo et al. 1996), and *Geothrix fermentans* (Coates et al. 1999). Extensive studies have been reported for both *Shewanella* and *Geobacter* spp. with reference to the importance of outer-membrane multiheme cytochromes (OMCs) and electrically conductive pili (e-pili) or nanowires which enables DET (Gorby et al. 2006; Reguera et al. 2005; Esteve-Nunez et al. 2008; Lovley 2008; Shi et al. 2009; Richter et al. 2012).

1.1.2.1 Outward Electrons Transfer (Microbes to Anode)

The transfer of electrons outwardly from intracellular electron transport chain to extracellular electron acceptors is a unique phenomenon by which electroactive microbes perform respiration (Kumar et al. 2017a). In BESs, the advantage of microbial electron transfer capability to minerals is being used to collect the electrons by placing a solid electrode as an insoluble solid terminal electron acceptor (Logan 2009), thus extracting electrons as the current. In a BES system, electrode in the anode compartment assists the growth of bacteria to form biofilm. The key mechanisms that have been identified how microbial electron transfer occur to the electrodes can be seen in Fig. 1.2. The electrons produced as a result of bacterial metabolism make their way to the electrodes through (a) DET via membrane protein structures such as cytochromes (Okamoto et al. 2012; Busalmen et al. 2008; Kim et al. 2002) or (b) via e-pili or nanowires (Reguera et al. 2005; El-Naggar et al. 2010), or (c) via the involvement of redox mediators which can be secreted by bacteria itself or added from external source (Pham et al. 2008; Marsili et al. 2008; Rabaey et al. 2005; Park and Zeikus 2000; Tang et al. 2010; Feng et al. 2010). Electron transfer by DET mechanism is the most evident in case of *Geobacter sulfurreducens* (Inoue et al. 2010; Yi et al. 2009; Malvankar and Lovley 2012)



a) DET through outer membrane (OM) proteins
b) ET through nanowires
c) ET through redox shuttles Sh_{ox}: Shuttle oxidized, Sh_{red}: Shuttle reduced

Fig. 1.2 Schematic of EET mechanisms depicting the possible modes of electron transfer from microbes to the electrode in a typical BES. Adopted from Patil et al. (2012a) and *Shewanella oneidensis MR-1* (Gorby et al. 2006; Meitl et al. 2009; Bouhenni et al. 2010).

Moreover multi-copper proteins (OmpB and OmpC) are other vital redox proteins involved in EET for iron reduction apart from OMCs (Holmes et al. 2008). Many other bacteria like *Thermincola ferriacetica* (Marshall and May 2009), *Desulfobulbus propionicus* (Holmes et al. 2004), *klebsiella pneumoniae* (Zhang et al. 2008), *Hansenula anomala* (Prasad et al. 2007), and *Enterobacter aerogenes* (Zhuang et al. 2011) have either reported or proposed to carry out DET. Also Gram-positive microbes such as *Thermincola potens* have also been proposed to carry out DET on the basis of their physiological, genomic, and electroanalytical analysis (Wrighton et al. 2011; Carlson et al. 2012).

Apart from DET, other major electron transport pathway involved the mediated electron transfer (MET) where redox mediators or shuttling molecules transfer electrons between cells and electron acceptors (Lovley et al. 1998; Pierson and Pierson 2010). This indirect ET to anodes involved self-secreted or artificial mediators. Among the self-secreted mediators the most common examples include flavins (riboflavin (vitamin B2; RF) and flavin mononucleotide (FMN)) which are secreted by *Shewanella* (Marsili et al. 2008). It is important to mention that few studies have further suggested that secreted flavins can bound to OMCs, enabling EET in *Shewanella* while acts as cofactors (Okamoto et al. 2013, 2014). Phenazine derivatives (mainly pyocyanin (PYO) and phenazine-1-carboxamide) secreted by *Pseudomonas aeruginosa* (Rabaey et al. 2004) are other less commonly reported mediators. Among artificial EET mediators, methyl viologen, methylene blue, resazurin, anthraquinone 2,6-disulfonate (AQDS), etc. are well-known mediators involved in MET (Watanabe et al. 2009; Allen and Bennetto 1993; Philips et al. 2016).

Further, indirect electron transfer mechanism is found at anodes by reduced energy carriers such as H_2 which are produced during the bacterial metabolism (Rabaey et al. 2007). This mechanism is largely known in MFCs that is used by microbes such as *yeast* which are capable of fermentative reactions (Niessen et al. 2005; Karube et al. 1977). Owing to fermentation reactions and their products, such systems give lower coulombic efficiency and hence show less efficiency for the electricity production. More than one mode or overlapping electron transfer pathways are also well reported (Fredrickson et al. 2008; Lies et al. 2005).

In latest developments about EET mechanisms, new mechanistic basis of EETs are found. A recent study uncovers a new ET chain that helps in the microbial growth involving insoluble electron acceptors in case of *Listeria monocytogenes*, a Gram-positive bacterium and food-borne pathogen resides in the human gut (Light et al. 2018). This newly discovered EET approach lacks the well-known multi-heme assembly (as found in the case of Gram-negative EET bacteria *Geobacter* and *Shewanella*). Eight-gene locus is found to be responsible for EET in this bacterium. The identified locus is encoded by a special NADH dehydrogenase which is responsible for transferring the electrons to a distinctive membrane localized quinone pool. Further, number of proteins facilitates the assembling of extracellular flavoprotein, which involves free flavins and helps in mediating the electron transfer to extracellular acceptors. Orthologues of this flavin based protein conduit exists in many other Gram positive strains from Firmicutes phylum which includes many pathogenic and commensal strains of the gut bacteria (Light et al. 2018).

Addition to this, more pathogens have also been identified to carry out EET but without in-depth findings about mechanism involved in these strains (Pankratova et al. 2018; Naradasu et al. 2018). These results advocate a large predominance of EET-capable microorganisms across the diverse environments other than the environmental sources (such as host-associated bacterial communities and infectious disease).

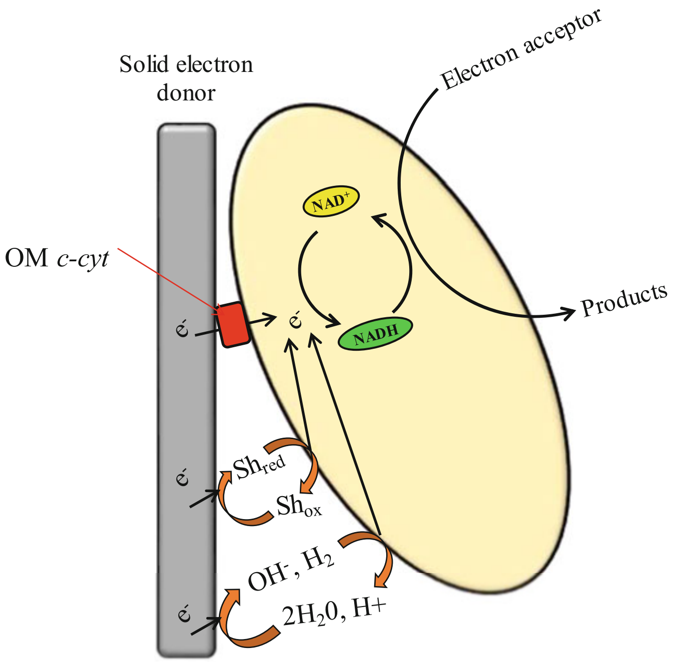
Overall, combining the mediated and the DET capabilities of EET microbes for enhancing BESs performance by improving ET rates can be a highly vital strategy (Patil et al. 2012b). Further understanding about ET mechanism across Gram positive microbes is also a key task, which can help in developing new designed electrodes and enhancing BESs performance. Further, understanding of EET mechanisms in human pathogens and potential application in medical field will open a new era of exciting research.

1.1.2.2 Inward Electrons Transfer (Cathode to Microbes)

Abundant mechanistic information is available about EET from bacteria to the anodes, while the details for opposite direction EET process, i.e., electrons transfer from the cathode to microbes are relatively unclear. Electroactive microbes living in the environment with abundant soluble electron acceptors and inadequate soluble electron donors are well placed for electrons uptake from solid electron donors (Kumar et al. 2017a).

Inward EET-capable microbes are classified among the category of electrotrophic bacteria that can attach to the electrode and uptake electrons for performing microbial metabolic reduction. This reversing of electron flow for bacterial reduction metabolism gave an excellent possibility for synthesis of value-added fuels and chemicals such as in MEC systems (Patil et al. 2012a). Several electron uptake mechanisms from the electrodes have been proposed (Fig. 1.3). These include either (a) direct electron uptake from the electrode via OMCs or (b) an indirect electron uptake through soluble redox shuttles, or (c) via oxidizing the hydrogen by several pathways. These mechanisms depend on the bacterial inoculum, the operating conditions, and the target reactions.

DET mechanism has been proposed for *Geobacter* spp. biofilms based on findings that electrons were accepted from a solid graphite electrode for respiration (Gregory et al. 2004). Also *Geobacter* spp. (Gregory et al. 2004) and *Shewanella oneidensis* (Ross et al. 2011) pure strains have shown direct electron uptake from electrodes, although thin films were formed for these strains in comparison to bio-anodic counterparts. The bidirectional flow in *Shewanella oneidensis* is mediated by the same OmcA–MtrABC respiration pathways (Ross et al. 2011), while genome probe shows that a periplasmic monohaem cytochrome, PccH, is critical in inward electrons transfer by *G. sulfurreducens* (Strycharz et al. 2011). The direct



a) DET through outer membrane (OM) proteins
b) ET through redox shuttles Sh_{ox} : Shuttle oxidized, Sh_{red} : Shuttle reduced
c) ET through oxidation of hydrogen by microorganisms

Fig. 1.3 Schematic of the EET mechanisms from electrode to microorganisms in BESs. Adopted from (Patil et al. 2012a)

inward electron uptake from electrode to bacteria is also found in bacterial communities like *betaproteobacteria* (Chen et al. 2008) and *firmicutes* (Croese et al. 2011). Further, *Clostridium pasteurianum* showed increase in butanol yield by cathodic ET without of any external mediators (Choi et al. 2014). Although in these cases exact ET channels for uptake of extracellular electrons are not yet well determined; the membrane redox enzymes are likely responsible for the reduction reaction.

Reduction of CO_2 to organic compounds by accepting electrons from the electrode has been reported in many acetogenic bacteria, such as *Sporomusa ovata* (Nevin et al. 2010), *Sporomusa sphaeroides*, *Sporomusa silvacetica*, *Clostridium aceticum*, *Clostridium ljungdahlii*, and *Moorella thermoacetica* (Nevin et al. 2011). These studies excluded the presence of H_2 as a carrier of electrons from the electrode to the microbes, revealing the possibility of DET mechanism. Sulfate reducing bacteria such as *Desulfovibrio ferrophilus IS5* has also found to carry out inward ET by OMCs (Deng et al. 2018).

Utilization of self-secreted mediators has been shown to involve in ET from the cathode to bacteria, e.g., pyrroloquinoline quinone (PQQ) by *Acinetobacter calcoaceticus* (Freguia et al. 2010) and unknown mediator by hydrogenophilic dechlorinating bacteria (Aulenta et al. 2010). The electron uptake by microbe through H_2 is also discovered for denitrification in wastewater treatment. Villano et al. showed electro-reductions of carbon dioxide to methane involving DET and indirect ET from the cathode to bacteria by a hydrogenophilic methanogenic bacteria (Villano et al. 2010).

Overall, it is cost-effective and green approach to use bacterial cathodes such that lower value pollutants get reduced and value-added or benign products are produced (Tremblay and Zhang n.d.). ET mechanisms involved in reduction reactions at electrodes for production of high value chemicals are less explored, and hence there is a need of extensive research in this area to have impactful contribution in development of sustainable societies.

1.1.3 Fundamentals of Thermodynamics in BESs

The performance efficiency of BESs is largely decided by the reactions' energy losses, usually stated as overpotential, and the extent electrons generated would result in wanted products, stated as coulombic efficiency (Hamelers et al. 2010). Microbial cells can harvest the energy (i.e., Gibbs free energy, ΔG) by oxidizing substrates and transfer part of this energy for growth and their maintenance. The transferred energy that acts as a driving force for cells' growth mainly consists of heat (i.e., enthalpy, ΔH) and final products (i.e., entropy, ΔS) (Heijnen 2010). The driving forces of enthalpy and entropy for microbial growth are related to Gibbs free energy as:

$$\Delta G = \Delta H - T\Delta S$$

The microbes get energy for their growth and maintenance by breaking the energy source and measured by employing the Gibbs free energy difference between reactants and products at standard conditions.

$$\Delta \mathrm{G}^0 = \sum \mathrm{v}_\mathrm{i} \Delta \mathrm{Gi}^0, \mathrm{Products} \ - \sum \mathrm{v}_\mathrm{i} \Delta \mathrm{Gi}^0, \mathrm{Reactants}$$

where v_i is a coefficient of stoichiometry for the component i in the reaction.

However, energy harvesting is not always under standard conditions and thermodynamic activity and temperature conditions may vary. Therefore, ΔG^0 can be corrected for non-standard conditions (ΔG) by the below relation.

$$\Delta G = \Delta G^0 + RT\ln\left(\frac{\Sigma a, Products}{\Sigma a, Reactants}\right)$$

Other than Gibbs energy, redox potential can also describe driving forces and it is well suitable for oxidation-reduction reactions where the transfer of electrons takes place. The redox potential determines whether a substrate will get oxidized after donating electrons or reduced after accepting electrons. The compounds having negative potential donate electrons, while with comparatively positive redox potential accept electrons. Redox potentials state a thermodynamic potential and therefore required a reference states for comparison which can be achieved by using a standard potential (E^0) for the definite redox species to the electrochemical reference half-cells (e.g., SHE) for the standard condition. The standard potential of reactions and Gibbs free energy can be correlated as:

$$E^0 = -\frac{\Delta G^0}{zF}$$

Here $F = 96{,}485$ C/mol and represents Faraday's constant, while z represents the number of electrons transferred.

In BESs, many reactions occur at their own specific potential. The thermodynamic favorability (energy produced or required) for reactions can be determined by combining oxidation and reduction reactions. In electroactive microbes in BESs, maximum energy available is limited to merely a part of the energy available based on redox potential difference in electron donors and terminal electron acceptors. Moreover, there is a common misconception that electrode potential co-determines the thermodynamic frame of electroactive microbes. The comparison of model strain *Geobacter* spp. and microbes using soluble terminal electron acceptors for respiration has shown that a substantial share of the ET chain of electroactive microbes capable of direct EET do not contribute in building of the proton-motive force and therefore, electrodes potential do not co-determine the thermodynamic frames (Korth and Harnisch 2019). It was also showed that the effect of electrode potentials for harvesting of energy is only a kinetic effect. Under less electrode potential, NADH accumulates as a result of slower direct EET rates and hence leads to a limited use of the thermodynamic frame, but for anode potentials $\geq$0.2 V (vs. SHE), the EET kinetics, NAD^+/NADH ratio, and the thermodynamic frame's exploitation are maximum, and a potential increase above that does not result in improvement of energy harvesting (Korth and Harnisch 2019).

For better understanding of the thermodynamic framework for microbial activity, take an example of glucose (Fig. 1.4), where during catabolic reactions, electrons are transferred from glucose (having negative redox potential) through many intracellular redox components to a final electron acceptor, oxygen (having positive redox potential). During the process, a chain of consecutive electron transfer steps is involved in which energy is obtained from electrons is stored in reduced energy equivalents for bacterial survival. Also, potentials of the intracellular redox components are getting further positive in a stepwise manner (e.g., electron transfer from NADH ($E0' \approx -300$ mV, Fig. 1.4) to menaquinone ($E0' \approx -50$ mV, Fig. 1.4) and finally to terminal electron acceptor (e.g., O_2, $E0' \approx 820$ mV, Fig. 1.4)). This sequence of steps for the electrons transfer need to be maintained for energy harvesting (Korth 2016).

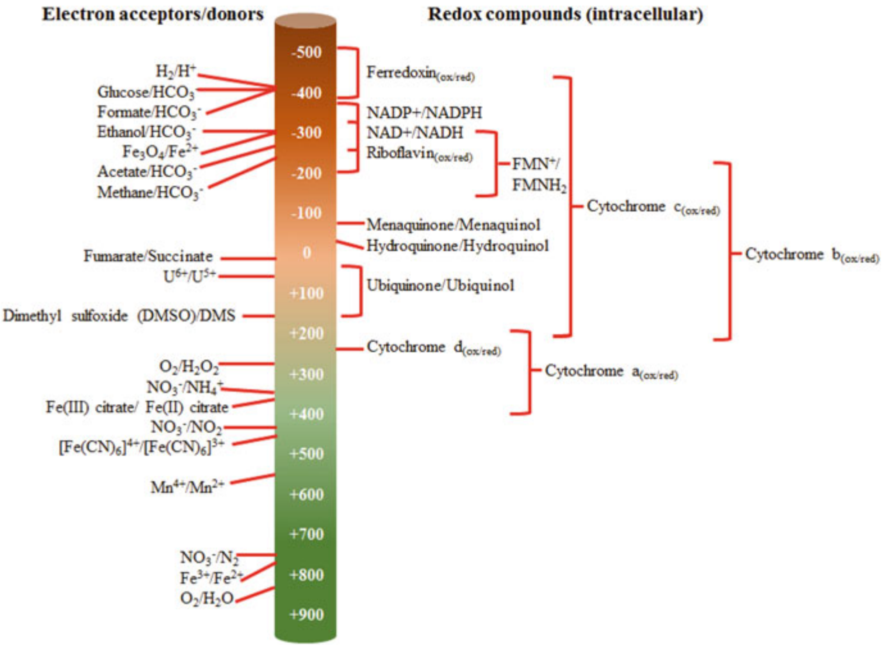


Fig. 1.4 Representation of redox potentials (in mV vs. SHE) of electron donors, acceptors, and intracellular redox compounds at biochemical standard conditions (298.15 K, 101.325 kPa, pH = 7, thermodynamic activity = 1). Compounds are symbolized with their oxidized/ reduced form. Adopted from (Rabaey and Rozendal 2010; Kracke et al. 2015; Korth 2016)

For aerobic microbes, oxygen is the main final electron acceptor that enables the fast growth of microbes as its reduction potential is very positive. This leads to higher harvested energy. Nevertheless, an adaptive capacity of microorganisms in different environments results in a different forms of energy harvest, for example, energy harvesting by using insoluble terminal electron acceptors, which set the basis for BESs.

1.2 BESs Performance Evaluation

1.2.1 Electrode Potential and Circuit Voltage

In BESs, the potential of electrodes (anode or cathode) can be measured by determining the voltage against the known potential of an electrode which is called as reference electrode (Logan et al. 2006). The potential of BES usually reflects the enrichment status of microbial microorganisms and the ability of microorganisms for electric energy output. The electrode potential is also a crucial parameter to describe the electrode reaction substrates, polarization, and reversibility. In BES experiments, Ag/AgCl reference electrode is the commonly used reference electrode, because it is simple, stable, and nontoxic. In saturated KCL solution, Ag/AgCl has a potential of 0.197 V vs SHE at 25 °C, and calomel electrode has 0.242 V vs SHE at 25 °C (Bard et al. 1980).

The performance of a BES can be directly evaluated measuring the circuit voltage (V). The current (I) passing through an external resistance (R_{ext}) can be determined by using a current meter, or can be calculated by Ohm's law:

$$I = \frac{V}{R_{\mathrm{ext}}}$$

1.2.2 Coulombic Efficiency

The coulombic efficiency (CE) is defined as the ratio of total coulombs transferred to the electrode from the substrate in actual, to theoretically possible coulombs that can be transferred if all the substrate removal results in current production (Logan et al. 2006).

The total output of the MFC is obtained by integrating the current over time:

$$C = \int_0^t i dt$$

The usually used CE formulas are listed:

$$\mathrm{CE} = \frac{C \times 32 \times 1000}{4 \times F \times V_{\mathrm{anode}} \times \mathrm{COD}} = \frac{C}{Fnv\Delta C}$$

where C is the amount of electrons (Q), t is the measurement time (s), i is the current (A), COD is the chemical oxygen demand (mg/L), F is Faraday's constant (96,485 C/mol), n is the number of electrons, v is the volume of anolyte (L), and ΔC is the concentration difference of the substrate (mol/L) during t time.

1.2.3 Power Density

The power density is an important indicator of BES performance. In most cases, a power density curve is determined by using a changing external resistance method usually from 10^5 to 10 Ω. The power density (P) from a BES has an inverse relation with the square of total system resistance according to:

$$P = \frac{V^2 R_{\mathrm{ext}}}{\left(R_{\mathrm{ext}} + R_{\mathrm{in}}\right)^2}$$

where R_{ext} is external resistor, R_{in} is internal resistor. The time interval of about 5–30 min is used when changing the R (depending on a stable value of the potential) (Zhang et al. 2009). In some other studies, the linear sweep voltammetry (LSV) plot is also utilized to get the power density (Reddy et al. 2010).

1.2.4 Energy Efficiency

Energy efficiency (EE) describes the ratio of the energy available to a device to its input energy. The available energy for the output may be electrical energy, heat, or mechanical work. In a BES reactor, the EE is defined as the ratio of electrical energy and the combusting energy of substrate organics:

$$\eta = \frac{Pt}{\varDelta C Q \varDelta c_H}$$

where Δc_H is combusting heat (J/mol). P is power (W). Q represents the feed flow rate (L/s), and $\varDelta C$ represents the consumed substrate (mol/L).

1.3 Applications of BESs

1.3.1 Wastewater Treatment and Electricity Production

The development of sustainable wastewater treatment infrastructure is an excellent strategy that has a potential to tackle several challenges of energy shortage, resource depletion, and environmental pollution (Zhuang et al. 2012). The greatest potential for the practical application of BESs is so far considered in wastewater treatment. Many types of bacteria which are being used in BESs have the ability to remove the organics and toxic reagents present in wastewater (Harnisch and Schröder 2010; Huang et al. 2015), and organics in wastewaters can be used as a source of fuel for BESs (Khan et al. 2015; Xu et al. 2014). Therefore, two important functions can be performed simultaneously, i.e., (a) Wastewater treatment, (b) Electricity generation. The integration of MFCs with the currently available treatment technologies for wastewater treatment is a more realistic, low-cost, and feasible approach. Many integration strategies have already been proposed as shown in Fig. 1.5.

Many types of industrial wastes have been reported to treat and simultaneously produce electricity in MFCs ranging from readily degradable to more complex wastes, which includes breweries, food processing, paper recycling, agricultural,

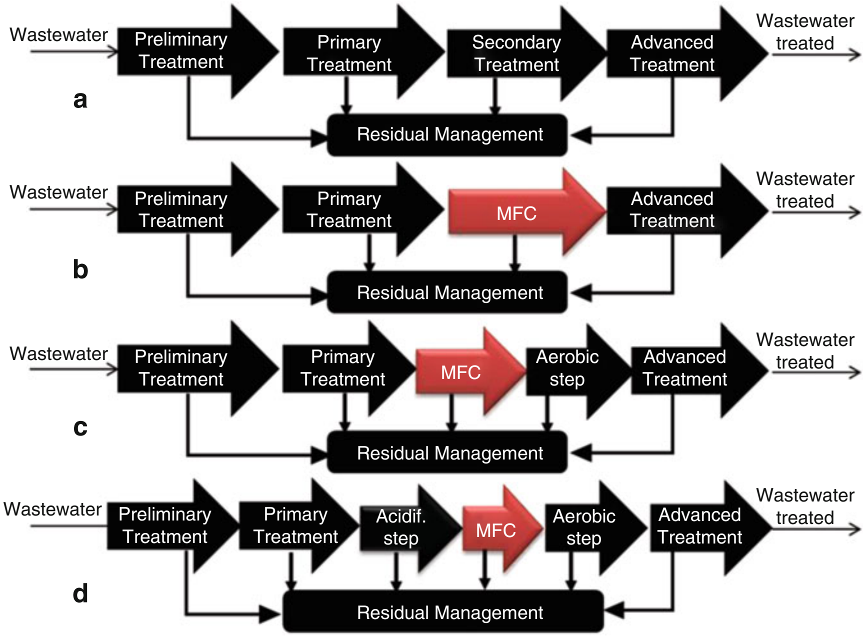


Fig. 1.5 (**a**) Typical wastewater treatment plant with no MFC. (**b**) MFC replaces biological reactor in the secondary treatment. (**c**) Polishing step is introduced after MFC treatment unit. (**d**) Acidification step before and polishing step after MFC treatment unit. Adopted from Escapa et al. (2014)

municipal, refineries, and textile wastewater (Pant et al. 2010; Chandrasekhar and Ahn 2017; Deval et al. 2017; Mohan and Chandrasekhar 2011b).

The power production largely depends on the ease of biodegradation of organic source, loading rate, and conversion efficiency. More power is generally achieved from easily degradable organics, whereas much low electricity can be produced by complex and recalcitrant wastes. Among recalcitrant wastes, phenols which are toxic because of conjugated structure of benzene have been treated in MFCs and energy generation was simultaneously achieved (Luo et al. 2009; Huang et al. 2012). Moreover, the chlorinated phenols' dechlorination can also be achieved by MFC. The accumulated SRB and other strains such as *Arcobacter*, *Aeromonas*, *Pseudomonas*, *Acinetobacter*, *Cloacibacterium*, and *Shewanella* spp. in the anode chamber show the ability of their degradation (Miran et al. 2017b; Hassan et al. 2018). Also, pharmaceutical wastewater is another kind of recalcitrant pollution which has been treated in MFC (Song et al. 2013; Wen et al. 2011). Further, azo dyes which are extensively applied colorant for various fibers, leather, wood, paper, and plastic (Yildiz and Boztepe 2002; Khan et al. 2015) have a complex molecular structure which constrained the penetration across the membrane cells and the degradation by intracellular enzymes (Liu et al. 2017). It was found that anodic microorganisms can efficiently decolorize dye wastewater by employing MFCs (Sun et al. 2009; Miran et al. 2018).

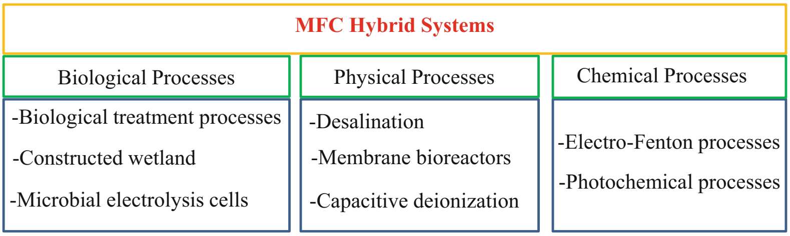


Fig. 1.6 Classification of MFC hybrid systems

MFC has also been readily coupled with other technologies for water treatment and other applications. MFC technologies are targeted from the initial improvement of current output to pollutant removal, desalination, synthesis, fermentation, etc. A number of coupling methods have been derived. The well-known MFC coupling technologies are represented below (Fig 1.6) and details can be found in literature (Zhang et al. 2019).

1.3.2 Biosensors

The rapid sensing and analysis of toxins is highly challenging due to the complex nature of such compounds. Traditionally, the techniques used for the monitoring of toxins involved analytical techniques such as ultraviolet (UV) spectrometry and high performance liquid chromatography (HPLC) (Abrevaya et al. 2015; Zhou et al. 2017). Nonetheless, these techniques are laborious and not fully compatible in the in situ detection. The development of biosensors have led to rapid and selective detection of various compounds (Su et al. 2011); however, the recognition components in biosensors such as fluorescence based compounds, enzymes, immobilized microorganisms, piezoelectric, magnetic, or micromechanical elements are expensive and require time-consuming procedures (Mehrotra 2016; Luong et al. 2008). Additionally, the large-scale applications are restricted by low sensitivity and specificity. Therefore, the development of fast and low-cost biosensor for toxins monitoring is of great interest worldwide. BES-based biosensors have recently emerged as notable tools for the environmental pollutant monitoring. They have the advantage of being instant and convenient technology with the potential of permanent and long-term monitoring (Pasternak et al. 2017; Velasquez-Orta et al. 2017). A schematic of the BES-based biosensor and its principle of operation is shown in Fig. 1.7. The versatility in construction and application modes has resulted in the development of different BES-based biosensors which are being discussed in this section.

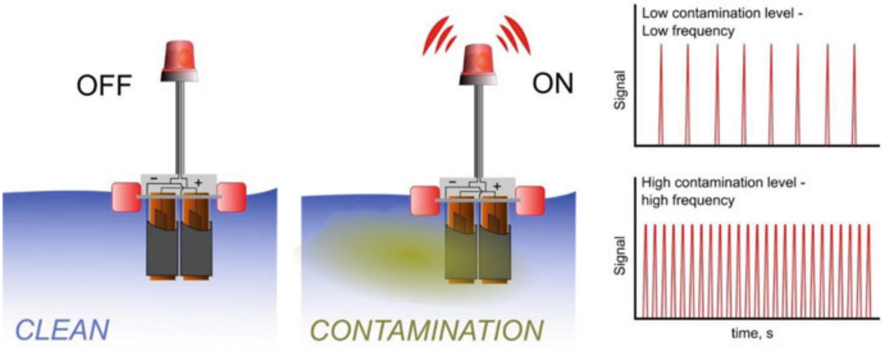


Fig. 1.7 A schematic of the BES-based biosensor and its principle of operation. Adopted from Pasternak et al. (2017)

1.3.2.1 Biological Oxygen Demand Biosensors

Among many biosensor applications, analysis of the biological oxygen demand (BOD) is one of the highly prevalent applications of BES-based biosensors in wastewater monitoring (Kumlanghan et al. 2008; Nakamura et al. 2007). The conventionally applied procedures for BOD measurements generally take 5–7 days for samples to be incubated in the dark (Jouanneau et al. 2014) along with expertise of a technician who have skills to accomplish repeated results, whereas the analysis time for BES-based BOD biosensors is significantly reduced (minutes to hours). Moreover, this methodology possesses stability in the long term along with a high reusability rate (Kharkwal et al. 2017). This shows the high suitability of BES-based BOD biosensors for field online monitoring applications. However, the disadvantage associated with the BES-based BOD sensors in a majority of the cases is its upper limit of amperometric linear calibrations, which is 250–340 mg/l BOD_5, assigned to the substrate saturation at anode biofilm. Active research is in progress to extend the detection range, e.g., recently, linearity of response was extended to 720 mg/L BOD_5 (1175 mg/L COD) with R^2 of 97%, when a combination of three BESs was linked hydraulically in the series (Spurr et al. 2018). Further improvement in designs of these sensors for the increase in sensing range will help in commercialization of such type of biosensors for high BOD wastewaters.

1.3.2.2 Heavy Metal Biosensors

Numerous types of heavy metals are very vital for humans as well as plants and animals when available in trace amounts; though, in large quantity they cause severe toxicity and may lead to damages of organs and tissues. Heavy metals monitoring by BESs are a new strategy developed owing to its easy operation. Heavy metals like Hg^{2+}, Cr^{6+}, Pb^{2+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Zn^{2+}, and Ni^{2+} have been tested in BESs and their inhibition rate on current output was monitored (Xu et al. 2015b; Tran et al. 2015; Wang et al. 2016; Wu et al. 2018a). Also capability of monitoring the toxicity of multiple heavy metals can be a more practical approach. For instance, a dual BES system for monitoring the twelve types of metal (Ba^{2+}, Be^{2+}, Co^{3+}, Cu^{2+}, Fe^{2+}, Mn^{2+}, Hg^{2+}, Pb^{2+}, Se^{4+}, Tl^{3+}, V^{5+}, and Zn^{2+}) has been developed with a minimum detection limit of each metal below 1.0 mg/L (Jae Sun et al. 2015). Also in another study six heavy metals (Cu^{2+}, Zn^{2+}, Pb^{2+}, Hg^{2+},Cd^{2+}, and Cr^{3+}) having the limit of detection of 2 mg/L were tested in a two-chamber BES with real-time monitoring (Yu et al. 2017). Heavy metals can inhibit the respiration of microbes which is the basis for developing BES-based heavy metals biosensor as the current output of BESs associated with metals concentration (Kim et al. 2007). Further improvement towards selectivity of heavy metals will help in deploying theses BES-based environmental biosensors in the field.

1.3.2.3 Volatile Fatty Acid (VFA) Biosensors

The current methods of VFA detection, such as HPLC, gas chromatography (GC), calorimetry techniques, and titration are not only complicated but also involve many steps (Raposo et al. 2013; Siedlecka et al. 2008). Therefore, the development of a convenient VFA detecting tool is vital especially for anaerobic digestion process monitoring. BES-based biosensors have been employed as a novel device for VFA monitoring. VFAs such acetate, butyrates, and propionates have been categorized by applying coulombic efficiency (CE) and cyclic voltammetry (CV) methods. Even though the CE method requires long sampling times, a better linearity can be obtained for charge vs individual VFAs concentration ranging from 5 to 40 mg/L (Kaur et al. 2013). In comparison to the traditional anaerobic digestion, BES can improve the biodegradation rate of propionate and butyrate, representing an effective methodology for sensing of VFAs and organics removal. Therefore, BES-based VFA sensors have wide usage prospects for the monitoring of AD processes with a greater sensitivity and a fair wider range. Nevertheless, some problems need to be resolved in the future such as influences of fermentation metabolites and other variations by the different kind of inhibitors. Overall for BES-based biosensors, robustness, further improvement in range, and selectivity for target products should be enhanced and times for measurement should be decreased. Furthermore, building small and inexpensive BES-based VFA sensors are important that can sustain the low-cost use of these systems.

1.3.3 Environmental Remediation

Microorganisms are known for having various strategies for depolluting and detoxifying their environment and led to the transformation of toxic environmental contaminants into safe end products. However, bioremediation processes have many challenges such as contaminant's concentration, microbial activity of mixed communities over time, and the energy consumption. Numerous environmental toxins released into the environment persist for very long periods and wield harmful effects on the biosphere. Current systems are not adequate for rapid removal of persistent environmental pollutants from the natural environment (Fernando et al. 2019). BESs are known for both the oxidation and reduction based processes for remediation of underground contaminants. In comparison to conventional biological treatment or chemical processes, BESs employ single or multiple electrodes which are not closed reactors for pollutants' remediation. Solid electrodes in this system work as non-exhaustible electron acceptors/donors for stimulating as well as enhancing the biodegradation of toxins and, during the enhanced bio-oxidation, bioelectricity can be produced (Mohan and Chandrasekhar 2011a). Additional benefit associated with BES for bioremediation is no involvement of any chemicals or energy input, and generated current can be used for running of wireless sensors which can be employed as instantaneous bioremediation indicators.

The complex petroleum organics, such as BTEX complexes (benzene, toluene, xylenes, ethylbenzenes, etc.) have been reported to bioremediate using BES systems. Morris et al reported that diesel (C8–C25) degradation was improved by 164% in comparison to OCV condition. The electrode was used such that it was linked to the underground hydrocarbon which donate electrons and O_2 at the ground surface which accepts electrons (Morris et al. 2009). Investigations on biodiesel, phenol, total petroleum hydrocarbons (TPH), polycyclic aromatic hydrocarbons (PAHs), 1,2-dichloroethane, pyridine, etc. have been also stated, validating BES can be used as a practical technology for degrading petroleum hydrocarbon with simultaneous current generation (Lovley and Nevin 2011; Chandrasekhar and Venkata Mohan 2012). In contrary, oxidative toxins are treated by employing electrode as the electron donor. In comparison to anodic oxidation process which is largely depend on microbes; the removal of toxins can be attained with or without microbe at cathode. Chlorinated solvents like trichloroethene (TCE) and perchloroethene (PCE), known for high toxicity or carcinogenic nature, have been reported to degrade by using negatively polarized solid-state electrode which donate electrons with and without electron shuttles (Aulenta et al. 2008). In other studies, reduction of perchlorate (Butler et al. 2010), Cr(VI) (Wang et al. 2008), Cu(II), and radioactive uranium (Gregory and Lovley 2005) have also been achieved in BESs with cathode as electron donors. The major benefit associated with the use of a solid electrodes as an electron donor instead of soluble electron donor is the reduction of contaminant (e.g., U(VI) to U(IV)) which is a stable precipitate at the electrode. After pulling out electrodes from the contaminated site, deposited U(IV) can be recovered from the surface, and ultimately reuse after recovery. Once the cost-effective material for BES constructions is available and problems of scalability are resolved, BES will be developed as a very effective strategy to solve the issues of xenobiotic's pollutants removal in the environment friendly manner.

1.3.4 Value-Added Chemical Production

The large focus of research after the birth of BES field has been developing and improving of MFCs primarily with bioanode, but it is now rapidly expanding in the fields of biocathodes due to many interesting developments. MES, a type of BES, can employ electricity for driving the synthesis of fuels and high value chemicals by employing microbes as catalysts which also results in the treatment of waste streams (Fig 1.8) (Rabaey and Rozendal 2010). In comparison to high cost chemical catalyst, electroactive bacteria that attached to the electrodes are low-cost and self-regenerating catalysts which efficiently accomplish oxido-reduction reactions. The dual benefits associated with this system are carbon sequestration and value-added chemicals' production (Irfan et al. 2019). The synthesis of various products in MES largely governed by biocathodes, conditions for electron uptake, microbes involved in the oxido-reduction reactions, reduction potentials in the process, redox mediators' activity, etc. (Kumar et al. 2017a, 2017b). A wide range of valuable compounds have been produced by MES such as H_2, acetate, CH_4, ethanol, butanol, H_2O_2, etc. (Miran et al. 2017b; Marshall et al. 2013; van Eerten-Jansen et al. 2015; Bajracharya et al. 2015). The chemical compounds obtained from MES can be used as precursors for the production of downstream industrial products such as polymeric products, diesel or kerosene resembling products, plasticizers, and as lubricating agents in many industries (van Eerten-Jansen et al. 2015). Moreover, formic acid which can be produced by MES (Zhao et al. 2012) is considered as an effective carrier of energy for its large application in pulp and paper industries.

Many organic compounds, viz. acetate, butyrate, and lactate, largely exist in effluents of wastewater plants and fermentation units. These organics are valued

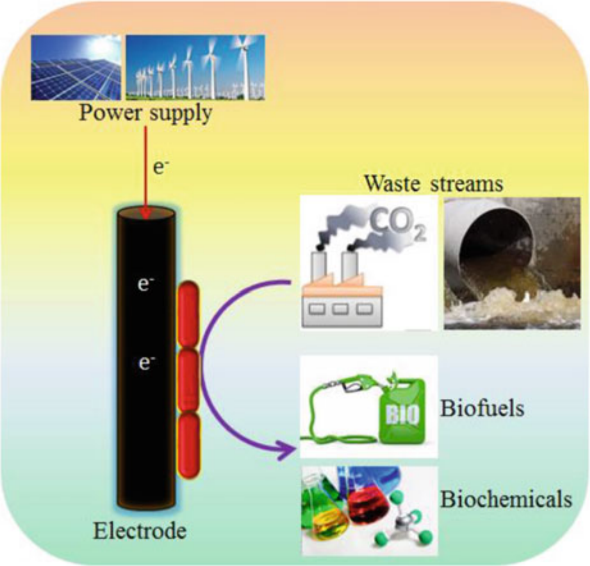


Fig. 1.8 Schematics of MES showing treatment of waste streams and formation of high value products

products but due to their low concentrations, extraction is not a cost-effective option. Therefore, MES has been employed for the conversion of these low chain organics to a long chain and other useful products (Steinbusch et al. 2010; Raes et al. 2017). Although higher value compounds can be obtained from low resource cost feeds, studies are required to compare if controlling the redox potential and supplying current to cathodes is economically feasible in comparison to current technologies. Nevertheless, further improvements in this technology platform can help in overcoming many of the fundamental challenges of a future bioeconomy.

1.3.5 Nutrient Recovery

Nitrogen and phosphorous are considered as major pollutants in the wastewater whose removal and recovery are well required for sustainable treatment systems. Nitrogen is conventionally removed by biological nitrification and denitrification processes which involved a very high energy and cost in wastewater treatment. New technologies are being developed for removal of nitrogen which targets the reduction in the requirement of energy and recovery of ammonium nitrogen. BESs have a good potential for the recovery of ammonium nitrogen with good profits from waste streams rich in nitrogen such as urine, swine liquor, digester liquor and landfill leachate, etc. (Nancharaiah et al. 2016). In comparison to traditional denitrification which involves heterotrophic denitrifying bacteria, denitrification by BES involves autotrophic denitrifying bacteria which have the electrons uptake ability from the electrodes. Consequently, biocathode in the BES was developed for denitrification which results in an efficient reduction in nitrate/nitrite and a concurrent electricity generation (Clauwaert et al. 2007; Zhao et al. 2016). Also, phosphorus from the wastewaters is being rid of traditionally by the polyphosphate accumulating organisms largely as polyphosphate granules. Moreover, chemical precipitation is also used for the recovery of phosphorous as Fe-P or struvite. Struvite precipitation is also used for phosphorus recovery in BES, as BES has the potential to develop high pH conditions in the cathodic chamber and provide raw materials for the process. Cusick et al. achieved struvite production in a BES by employing single-chamber MEC, where soluble phosphate up to 40% was recovered by struvite precipitation at a rate of 0.3–0.9 $g/m^2/h$ (Cusick and Logan 2012). In comparison to the MFC, a MEC maintained high pH condition owing to hydrogen production in the cathodic compartment, inferring that a MEC-based BES may be more suitable for struvite formation. Other strategy for phosphorus recovery in BES involved exchange of hydroxide ions generated by the cathode reaction with phosphate ions from wastewater which resulted in removing 52.4 ± 9.8% of phosphate (Zhang et al. 2014). Further enhancement in the capabilities of BES for recovering nitrogen and phosphorous will help in the development of BES-based nutrient recovery biotechnologies.

1.3.6 *Heavy Metals Recovery*

Heavy metal-containing wastewaters are discharged from industrial processes such as mining, metallurgy, machinery manufacturing, chemical, plating, and tannery (Wang and Ren 2014). Human health is threatened by the accumulation and persistence of heavy metals in food chains (Li et al. 2014). At present, heavy metal wastewaters are being commonly treated by physical and chemical approaches like precipitation, electrolysis, and ion exchange (Kadirvelu et al. 2001; Mier et al. 2001). However, these processes have the shortcomings of high cost and secondary pollution. Therefore, researchers are more committed to explore a green, cheap, and efficient processing technology. In addition, heavy metals are valuable industrial materials. With the increase of mining volume year by year and the decrease of reserves, the recovery and utilization of heavy metals have become a top priority. BES being cost-effective, efficient, without secondary pollutants, and new treatment technology that can simultaneously remove and recover heavy metals along with electricity generation is of great advantage (Tao et al. 2014).

The driving force (potential) for metal recovery comes from the oxidation of organics. Both direct and indirect reductions are utilized for the heavy metals recovery process. The main mechanisms reported for heavy metals removal (Fig. 1.9) includes, (1) Sorption or formation of coprecipitation with sulfide produced by SRBs in the anode. In BESs, SRBs oxidized organics and reduced sulfates to sulfide while simultaneously generating current. Then, sulfide reacts with the dissolved heavy metal cation and precipitates, thus achieving the accumulation of heavy metals and further extracting for recovery. (2) Heavy metals reduction at the cathode. This strategy is more prevailing in BES as the toxicity of heavy metals does

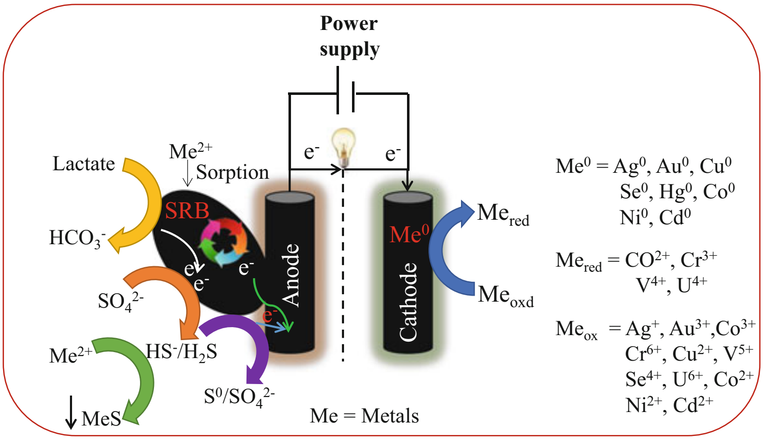


Fig. 1.9 The mechanisms of heavy metals removal in BESs. Adopted from Nancharaiah et al. (2015) and Miran et al. (2017a)

not interfere with the metabolism as in the anode microorganisms, which is favorable for the recovery of heavy metals in BES.

Many reports showed that metal ions like Ag^+, Au^{3+}, Co^{3+}, Cr^{6+}, Cu^{2+}, Hg^{2+}, Se^{4+}, or V^{5+} can be reduced at the cathode for removal and recovery (Huang et al. 2014; Tao et al. 2011; Heijne et al. 2010). When heavy metals are removed by the coprecipitation or biocathode-assisted method, the concentration seriously affects the production performance and recovery efficiency of BES. Many research works have reported that low concentrations of heavy metals induce heavy metal resistance and can promote biological metabolism. However, the toxic effect of heavy metals on bacteria increased significantly along with enzyme inactivation and degeneration with the increase in concentration (Alexandrino et al. 2011; Kamika and Momba 2012). It was reported that the tolerance of bacterial species to V^{5+} is 110–230 mg/L (Kamika and Momba 2012). Over this concentration range, the number of colonies decreased with increasing concentration. Abourached et al. found that because heavy metals are highly toxic, it inhibits the growth of microorganisms and reduces cell density at higher concentrations. Also the voltage was restored after replacing the heavy metal solution with medium without any heavy metals (Abourached et al. 2014). Wu et al. exhibited the tolerance strength of Cu^{2+} in a single-chamber BES up to 12.5 mg/L (Wu et al. 2018b). By a gradual increase in strength of Cu^{2+} from 0 to 12.5 mg/L, a decreasing voltage trend from 0.57 V to 0.43 V was observed. Also by increasing metals initial concentration to 15.0 mg/L, there was a decrease by ~79% in removal efficiency (Hao et al. 2016). Analogous pattern was also observed in the cathode. As the concentration of heavy metals increased, the cathode reduction rate constantly decreased, but the total removal increased (Xu et al. 2015a). These reduced products adhere to the surface of the cathode, which increases the internal fouling of the electrode and causes the electrode to scale, preventing the heavy metal from continuing to reduce, thereby reducing removal rate and efficiency (Tao et al. 2011). Nancharaiah et al.'s review article can be seen for further details about heavy metals recovery in BESs (Nancharaiah et al. 2015).

In summary, the electrons produced by MFC can be used for heavy metal removal/reduction. This method is environmentally friendly and further improvements in BES systems will help in the development of BES-based heavy metal-containing waste/wastewater technologies.

1.4 Conclusions

More detailed mechanistic studies about outward and inward EET of physiologically and phylogenetically diverse microorganisms are helping in advancing BES technology applications. However, mechanistic understandings are still limited to few microorganisms in addition to model strains and need to be further explored in detail. The major goal associated with BES technology is electricity recovery from wastewater due to its potential of decreasing overall treatment costs. The integration of BESs with the currently available treatment systems for wastewater treatment is a more realistic, low-cost, and feasible approach. Despite the exponential improvement in BESs performance in the last decade and a half, the upscaling is still the big issue; and therefore, BESs can also be used for various portable applications. The large focus of research after the birth of the BES field has been developing and improving MFCs primarily with bioanode, but it is now rapidly expanding in the fields of biocathodes. Further, BES is increasingly getting attention as a more versatile technology for a number of applications, which includes chemical production, environmental remediation, nutrient recovery, water desalination, and sensors. Applications in the medical field will open a new era of exciting research in BESs. Overall, BESs still require scaled-up by many orders of magnitude for achieving practical implementation for different applications, and reactor designs need to be integrated with currently available infrastructures.

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Chapter 2
Bioelectrochemically Assisted Anaerobic Digestion: Principles and Perspectives

Jhansi L. Varanasi and Amrit Lal

Abstract Anaerobic digestion (AD) constitutes the microbial conversion of organic matter into biogas under anaerobic conditions. This technology has gained significant attention over the past few decades owing to the ever-increasing concerns over the fossil fuels and the growing need for renewable energy production. Different varieties of wastes and residues can be used for biogas production via AD. Biogas typically comprises methane and carbon dioxide. One of the disadvantages of the conventional AD process is the need for biogas upgradation to improve the purity of methane. This in turn leads to requirement of high level of investments when carried out at commercial scales. Apart from AD, another process that has provided a new horizon to the methane generation process is electromethanogenesis. In this process, CO_2 is bioelectrochemically converted to methane with the help of electrotrophic methanogens. It has an added advantage of CO_2 fixation along with wastewater treatment and energy generation. In spite of numerous advantages, several technical and economic barriers hinder the practical implementation of this technology at commercial scales. Combining AD and electromethanogenesis can be a possible means to overcome the drawbacks of each individual technology. This chapter briefly summarizes the working principles, bioenergetics, the interplay of microorganisms, the critical factors affecting the process, etc. along with a perspective of future research needs of the bioelectrochemically assisted anaerobic digestion technology.

Keywords Biogas · Biomethane · Anaerobic digestion · Electromethanogenesis · Biocathode

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2.1 Introduction

The rising concerns over global warming and negative impact of fossil fuels have promoted researchers to focus upon alternate clean, renewable, and energy-efficient fuel sources. Biomass (including energy crops and organic wastes) is the most abundant renewable energy resource in the world and it has an added benefit of maintaining a balanced carbon cycle with respect to global carbon dioxide levels (Kralova and Sjöblom 2010). Moreover, using biomass resources such as municipal, industrial, and agricultural organic wastes aids in mitigating their treatment and disposal issues. Various biochemical and thermochemical strategies have been developed over the years to convert biomass into usable form of energy such as ethanol, butanol, methane, hydrogen, etc. (Aro 2016; Nigam and Singh 2011). The selection of the ideal biofuel depends upon several factors such as the conversion efficiency, economies of scale, environmental impact, product use, etc. Biogas production via anaerobic digestion (AD) process is an efficient means for renewable energy production from wet and dry biomass (Abbasi et al. 2012). It is a well-established technology and generates less environmental pollutants as compared to other conversion technologies. Methane accounts for almost 50–65% of the total biogas released during AD process (Bouallagui et al. 2005; Mata-Alvarez et al. 2000). It can be used as both domestic fuel and transport fuel. Currently, various small scale and large scale commercial AD plants are being operated in different parts of the world.

AD mainly comprises the anaerobic breakdown of organic compounds to produce biogas or biomethane. The advantage of this technology is that it can utilize a wide range of feedstock, unlike ethanol fermentation process which relies on specific energy crops and extensive pretreatment strategies. The major steps involved in AD are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Fig. 2.1). During hydrolysis, complex organic matter is enzymatically degraded into simple molecules. These simple molecules are then assimilated by the acidogenic bacteria and further converted to short-chain carboxylic acids during acidogenesis. During acetogenesis, the short-chain carboxylic acids are converted to acetate, CO_2, and H_2 in the presence of fermentative bacteria. Finally, in methanogenesis, the byproducts of acetogenesis, i.e., acetate, CO_2, and H_2 are converted to biogas and/or methane by the action of methanogens

AD technology is cheaper and much simpler. It is proved to be a promising alternative to the conventional activated sludge process as it has an added advantage of energy generation along with wastewater treatment (Kumar et al. 2018). Biogas can be a potential substitute for LPG and has several advantages such as its recovery is simpler as the product (gas) automatically separates from the substrates, it generates rich organic manure that can be used instead of harmful chemical fertilizers, it can be used as a power source, and it can provide rural employment opportunities. However, there are certain environmental and economic barriers like which need to be addressed to make this process an efficient alternative to the existing fossil-based fuels. The major challenge is the low productivity of this

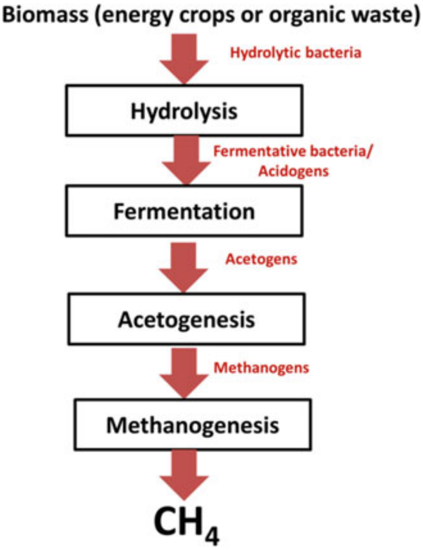

Fig. 2.1 Anaerobic digestion pathway

process since processes such as fermentation and methanogenesis can proceed from hours to days. Moreover, although numerous studies have been conducted on evaluating the energetics of biomethane production till date, the complete understanding of the microbial consortium involved and the complex microbial interactions is still lacking. Another limiting factor is the energy inputs required for upgrading the biogas quality as certain applications (such as fuel cells) require pure form of methane for operation. The presence of hydrogen sulfide, ammonia, carbon monoxide, etc. in the biogas lowers its calorific value and thus constraints its usability in internal combustion engines (Mao et al. 2015). Therefore, it is essential to upgrade biogas in an economical way so as to obtain high fuel quality methane.

2.2 Bioelectrochemically Assisted Anaerobic Digestion

Bioelectrochemical systems (BES) are novel technologies which are used to convert wastes into electricity, fuels, or other value-added chemicals (Shemfe et al. 2018). On the basis of source of electrons, BES can be categorized as electron producing systems (such as microbial fuel cells (MFCs)) and electron consuming systems (such as microbial electrolysis cells (MECs)). MFCs derive energy from the oxidation of organic substrates and generate electricity during the process (Deval et al. 2017). On the other hand, the cathodic reduced end products of MECs such as hydrogen, methane, or other value-added compounds are not thermodynamically feasible and require additional energy inputs (from an external DC voltage supply) to drive the reaction (Kumar et al. 2018). In recent years, with the discovery of electrotrophs i.e. bacteria that can uptake electrons from the solid electrode surfaces, new horizons for application scope of BESs have emerged (Seviour and Hinks 2018). One of the most widely studied applications of biocathodes is electromethanogenesis which involves ability of certain methanogens to uptake electrons from the solid metal surfaces and produce methane using CO_2 as the sole carbon source. The basic principles behind cathodic CH_4 generation are similar to H_2 production in MEC (Kadier et al. 2018). However, a biocathode is essential for electrocatalytic CH_4 production. In this process, the cathode acts as electron donor and the methanogenic bacteria uptake electrons from the solid surface (cathode) to convert CO_2 to CH_4 (Cheng et al. 2009). The process is seldom used for upgrading the biogas obtained from the anaerobic digestion process (Cerrillo et al. 2018). Typically the biogas obtained from the conventional AD process comprises large amounts of CO_2 (about 30–40%). This CO_2 can be further converted to CH_4 using the MEC process and improves the overall energy efficiency of the process. Various studies have reported improvement in CH_4 production using coupled AD-MEC process (Table 2.1)

It was reported that by using the combined AD-MEC process, the CO_2 content in the biogas below 10% was achieved (Sangeetha et al. 2017). Cerrillo et al. observed that MEC system helped in recovery of ammonium produced during AD and aided in the final effluent polishing step (Cerrillo et al. 2017). Thus, BES can be looked upon as a complementary technology to AD rather than competing technology, which can be integrated together to overcome the inherent limitations of each technology. Thus, coupling of anaerobic digestion and MEC can be a convenient means of upgrading methane production from organic wastes. Various syntrophic interactions occur between the different microorganisms present in the AD-MECs, which ultimately result in enhancing methane formation (Fig. 2.2).

The three major pathways for CH_4 production in AD-MECs viz. (a) Conversion of carbon dioxide and hydrogen into methane, (b) Conversion of acetate into methane, and (c) Direct CO_2 conversion to methane. The most prevalent mechanism is the acetate conversion to methane by acetoclastic methanogens given as (Eq. (2.1))

$$CH_3COOH \rightarrow CO_2 + CH_4 \tag{2.1}$$

In the presence of electrotrophic methanogens, the CO_2 can be directly reduced to methane (Cheng et al. 2009) (Eq. (2.2))

$$CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O \tag{2.2}$$

Fermentative (Eq. (2.3)) and abiotic (Eq. (2.4)) H_2 production, and its conversion to CH_4 by hydrogenotrophic methanogens (Batlle-Vilanova et al. 2015)

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 4H_2 + 2CO_2 \tag{2.3}$$

$$2H^+ + 2e^- \overset{\text{catalyst}}{\rightarrow} 2H_2 \tag{2.4}$$

Table 2.1 Reported studies on hybrid AD-MECs

Inoculum/source	Substrate	Applied potential (V)	COD removal (%)	Cumulative methane yield (L g^{-1} COD)	References
Anaerobic digester sludge	Glucose	0.9	65.3	0.25	Wang et al. (2018)
Anaerobic digester sludge	Food waste leachate	0.3	–	0.34	Lee et al. (2017)
Sludge fermentation liquid	Glucose	0.8	60	0.12	Cai et al. (2016)
Activated sludge	Acetate	0.8	92.1	0.27	Sangeetha et al. (2017)
Cow dung	Sewage sludge + food waste	0.04	–	0.15	Prajapati and Singh (2018)
Anaerobic digester sludge	Food waste	0.3	76.1	0.34	Park et al. (2018)
Anaerobic digester sludge	Dried ground corn silage	0.2	–	0.22	Koch et al. (2015)
Municipal wastewater treatment plant sludge	Waste activated sludge	0.6	–	0.14	Chen et al. (2016)
Anaerobic digester sludge	Synthetic wastewater	0.4	74.6	–	Tian et al. (2018)
Municipal wastewater treatment plant sludge	Thermal-alkaline-pretreated sludge	1.8	51.3	0.2[a]	Xiao et al. (2018)
Anaerobic digestion effluent	Glucose	1.0	86.6	0.41	Choi and Lee (2019)
Anaerobic digester sludge	Glucose	0.3	93	0.32	Gajaraj et al. (2017)
Digested pig slurry	Synthetic wastewater	-0.8 (cathode)	42	–	Cerrillo et al. (2018)
Anaerobic digester sludge	Food waste	1.2	–	0.32	Choi and Lee (2019)

[a] Reported in L g^{-1} VSS

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \tag{2.5}$$

Apart from the aforementioned pathways, another indirect route for methane production in AD-MECs involves abiotic H_2 production (Eq. (2.4)) and its conversion to acetate by acetogenic bacteria (Eq. (2.6)) (Schuchmann and Müller 2016)

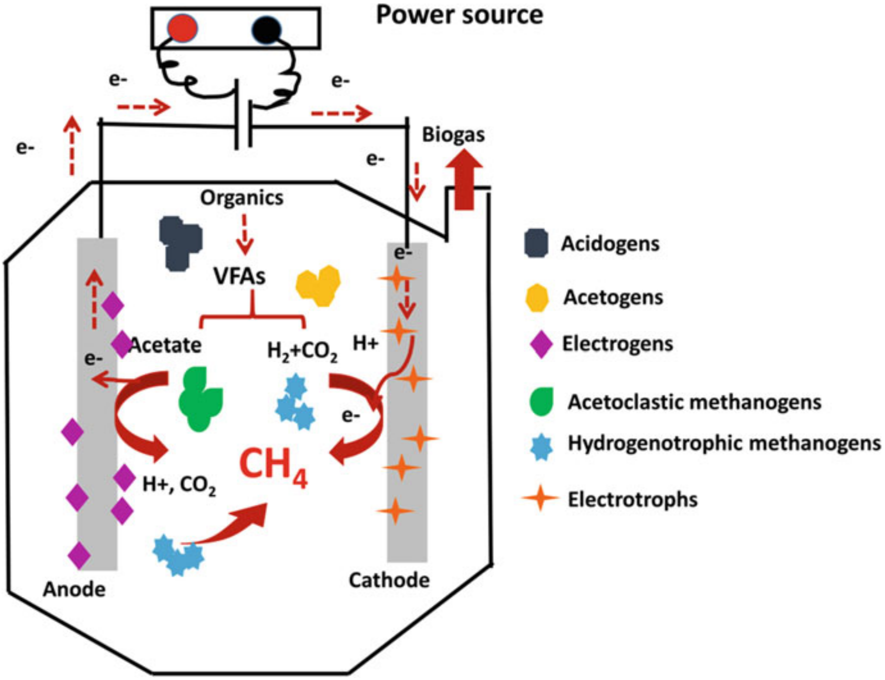

Fig. 2.2 Schematic representation of CH_4 upgradation in hybrid AD-MEC via syntrophic interactions of different microorganisms

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \tag{2.6}$$

The acetate formed by Eq. (2.6) can be converted back to methane by Eq. (2.1). A more detailed description of the bioenergetics involved during methane production using different biochemical pathways is given below.

2.2.1 *Conversion of Carbon Dioxide and Hydrogen into Methane: Bioenergetics of Hydrogenotrophic Methanogens*

Similar to the conventional AD process, volatile fatty acids, ethanol, carbon dioxide, and hydrogen are also produced in AD-MEC during the acidogenesis process. A considerable amount of dissolved carbon dioxide and hydrogen remains in the spent media which can act as energy sources for hydrogenotrophic methanogens (Eq. (2.5)). It is reported that hydrogenotrophic methanogens contain mainly two types of [Ni-Fe] hydrogenases: Coenzyme F420-reducing hydrogenase; and coenzyme F420-nonreducing hydrogenase (Shima et al. 2002). The latter is also known as methyl viologen-reducing hydrogenase. A third hydrogenase, called as methylene tetrahydromethanopterin dehydrogenase, is also found in methanogens. This contains nickel and/or iron-sulfur clusters.

2.2.2 Conversion of Acetate into Methane: Bioenergetics of Acetoclastic Methanogens

Lee et al. highlighted that the acetoclastic methanogens are favored in the combined process in contrast to hydrogenotrophic methanogens which in turn improve the substrate removal rate and biogas production (Lee et al. 2017). Pathway for methane generation from acetate includes three major following steps:

1. Acetate to acetyl-CoA activation
2. Decarbonylation
3. Methyl transfer to methyl-S-CoM and reductive demethylation of methyl-S-CoM to methane

The major enzymes involved during CH_4 formation from acetate include CO dehydrogenase (for decarbonylation), methyltransferases (for methyl transfer to methyl-S-CoM), and methyl-coenzyme M reductase (catalyzes terminal step of coenzyme M reduction to methane).

2.2.3 Direct Conversion of CO_2 to Methane: Bioenergetics of Electrotrophic Methanogens

Methanogenic electrotrophs are capable of deriving their energy from the cathode and convert CO_2 into methane. However, the electron transfer mechanisms of these organisms are less understood in contrast to acetoclastic and hydrogenotrophic methanogens. The first report on methane-producing biocathode was given by Cheng et al. (2009). One of the possible electron transfer mechanism involves direct reduction of an electron shuttle methanophenazine (MP), which is present in the cytoplasmic membrane of methanogens (Biegel et al. 2011). The oxidation of this reduced MP at the heterodisulfide reductase enzyme results in buildup of proton motive force and reduction of heterodisulfide leading to methane generation. On the contrary, certain studies have reported the ability of methanogens for direct CO_2 reduction by an electrode (Bajracharya et al. 2017; Gajaraj et al. 2017).

2.3 Reactor Designs for AD-MEC

Various configurations of AD-MECs have been reported, most of which are modifications of the conventional AD system. Zhang and Angelidaki (2012) described a submersible MEC system connected to an MFC, which was used as a power source to drive the methane production (Zhang and Angelidaki 2012). Sangeetha et al. (2017) developed single-chambered tubular upflow AD-MECs with carbon brush anodes and nickel mesh cathodes. They concluded that the optimization of appropriate electrode position and placement in the reactors is crucial for their performance and development (Sangeetha et al. 2017). Gajraj et al. designed single-chambered cylindrical AD-MEC with reticulated vitreous carbon as electrodes (Gajaraj et al. 2017). Apart from the hybrid AD-MECs, few studies have reported two-stage AD-MECs such that the biogas produced from AD can be used in the cathode of MEC for upgrading the methane content of AD (Cerrillo et al. 2018; Zhen et al. 2017). Typically biogas from AD comprises 60–70% methane and 30–40% CO_2. This leftover CO_2 can be channeled toward methane production in two-stage AD-MEC. A two-stage AD-MEC system was operated by Villano et al. (2010) who observed high purity of methane when biogas comprising CO_2 was sparged to the cathode of MEC (Villano et al. 2010). The above findings suggest that AD-MEC is a promising system for upgrading of biogas produced by AD, with simultaneous treatment of organic waste. Further advances in the reactor configrations are needed so as to recover the inhibitory products of AD such as ammonium which would further improve the quality of the effluent and enhance the overall stability of the process. Additionally, it is essential to evaluate and optimize AD-MEC systems under different operational regimes to ensure performance stability.

2.4 Methanogenic Assemblages

Methanogens are the major populations that are responsible for methane production in AD-MECs. They mainly belong to the domain Archaea, which are distinguished from the domain Eubacteria by a number of contrasting characteristics, such as the absence of a muramic acid-based peptidoglycan in the cell wall, presence of isoprenoid-rich membrane lipids, which is linked with glycerol, and the and distinct ribosomal RNA (Balch et al. 1979). Methanogens can be taxonomically classified into five orders *Methanobacteriales*, *Methanococcales*, *Methanomicrobiales*, *Methanosarcinales*, and *Methanopyrales* (Primrose and Twyman 2006).

2.4.1 Methanobacteriales

The order *Methanobacteriales* mainly consists of rod-shaped methanogens which use CO_2 as the energy source except for *Methanosphaera* sp., which are cocci in shape and use hydrogen as the energy source. The order *Methanobacteriales* comprises two families, *Methanobacteriaceae* and *Methanothermaceae. Methanobacteriaceae* is a diverse family, which includes the genera *Methanobacterium*, *Methanobrevibacter*, and *Methanosphaera*. *Methanothermaceae* contains a single genus, *Methanothermus*, of extremely thermophilic methanogens (Lauerer et al. 1986).

2.4.2 Methanococcales

They consist of halophilic, chemolithotrophic microorganisms that produce methane by reducing CO_2. They use hydrogen or formate as the energy source. These are coccoid-shaped, marine methanogens that include three thermophilic species (*Methanocaldococcus*, *Methanothermococcus*, and *Methanoignis*) and one mesophilic species (*Methanococcus*). *Methanococcus* also includes some hyperthermophilic marine cocci that grow rapidly at 85° like *Methanococcus jannaschii*. It is the first methanogenic archaea whose complete genome was sequenced.

2.4.3 Methanomicrobiales

This order also includes mesophilic and thermophilic methane producers that are slightly halophilic. They are osmotically sensitive due to the presence of the S-layer, a protein that is present in the cell wall. They have different shapes viz., helical/spiral (*Methanospirillum hungatei*), cocci (*Methanocorpusculum* sp.). It mainly consists of three families, viz., *Methanospirillaceae*, *Methanomicrobiaceae* and *Methanocorpusculaceae*. They require mainly acetate as a carbon source, e.g., *Methanomicrobiales* sp.

2.4.4 Methanosarcinales

This genera include *Methanosarcina*, *Methanosaeta* (*Methanothrix*), *Methanolobus*, *Methanococcoides*, *Methanohalophilus*, and *Methanohalobium*. This group is also known as methylotrophic methanogens because they mainly use methyl group-containing compounds such as methanol, methylamines, or methyl sulfides as a substrate for methane production. Another unique characteristic of this group is that none of them can use formate as a catabolic substrate. *Methanosarcina mazei* belongs to genus *Methanosarcina* which is found in semi-aquatic environments such as sewage receptacles and anoxygenic, moist soils.

2.4.5 Methanopyrales ord. nov.

The genus *Methanopyrus* contains a single species, *Methanopyrus kandleri.* It is a gram-positive bacilli, and grows at a very high temperature. Their cell wall consists of a unique type of pseudomurein, which contains ornithine in addition to lysine. N-acetyl glucosamine is absent in its cell wall. They are hydrogenotrophic methanogens and reduce CO_2 to methane.

2.5 Social Interaction Among Microorganisms

2.5.1 Microbial Syntrophy

2.5.1.1 Acidogens, Electrogens, and Methanogens

The doubling time for fermenters is much less as compared to electrogens. Therefore, when complex carbohydrates are used as substrates, they are first fermented by the mixed anodic communities. During fermentation, acidogens break down the complex carbohydrates into simpler monomers and further into short-chain volatile fatty acids like acetate butyrate, propionate, and ethanol and produce hydrogen and carbon dioxide in the reducing environment. These end products of acidogens are substrates for anodophilic respirers (Chabert et al. 2015). On the other hand, methanogens can utilize acetate, CO_2, and H_2 to produce methane. Thus, the electricity and methane generation in AD-MECs is almost entirely a syntrophic process.

2.5.1.2 Interspecies Hydrogen/Electron Transfer

The catabolic reactions of homoacetogenic bacteria (bacteria that catalyze the formation of acetate) are in most cases thermodynamically not feasible and become feasible when reducing equivalents (such as H_2 or formate) are effectively scavenged by their syntrophic partners—the methanogens (Boone et al. 1989). This finding was a breakthrough in understanding the syntrophic interaction between a hydrogen-producing and a hydrogen-oxidizing organism that coexist by breaking a single substrate. Another example of microbial syntrophy is the electric syntrophy between electrogenic and electrotrophic microorganisms described by Kato et al. (2012), which can be a key process for sustainable energy production in AD-MECs (Kato et al. 2012).

2.5.2 *Microbial Competition*

The methanogenic consortium developed from the sewage sludge or cow dung contains metal-reducing bacteria and sulfate-reducing bacteria as co-contaminants. Sulfate-reducing bacteria can utilize organic acids, alcohols, amino acids, and aromatic compounds as potential electron donors. Metal-reducing bacteria such as Fe^{3+} reducers can also outcompete other organisms if potential electron acceptors are present in the system. In a habitat where the organic substrate (electron donor) is limiting, a hierarchy for competition for electron donor is observed. Metal-reducing bacteria such as Fe^{3+} reducers can outcompete other organisms if potential electron acceptors are present in the system. This is followed by a succession of sulfate-reducing bacteria, methanogens, and acetogens.

2.5.2.1 Competition for Hydrogen

A considerable amount of hydrogen remains in a dissolved state after the first stage of hydrogen production which can be consumed by methanogens in the second stage. The hydrogen utilization rate is limited by the hydrogen transfer rate between gaseous and liquid phases. The competition for hydrogen under anaerobic conditions could be examined by observing the apparent K_m value for hydrogen utilization. The higher K_m value indicates limitation of the uptake hydrogenases for utilizing hydrogen at lower partial pressures. The methanogens have apparent K_m values of 4–8 μM hydrogen (550–1100 Pa) which is higher than the sulfate-reducing bacteria (about 2 μM).

2.5.2.2 Competition for Acetate

In a strict anaerobic environment with low acetate concentration, both electrogens and methanogens can compete for the same substrate. In BES conditions, methanogens (if present) have an advantage over electrogens as they are not dependent upon the contact with the electrodes and can thrive in planktonic environment (Zhang et al. 2019). However, such a problem arises only during long term operation of the system and is dependent upon the anodic potential.

2.6 Performance Assessment

The efficiency of the CH_4 recovered during MEC process can be calculated in terms of measured current (r_i) and based on substrate conversion (r_{CH4}) such that

$$r_i = \frac{n_{CE}}{n_{th}} \tag{2.7}$$

$$r_{CH_4} = \frac{n_{CH_4}}{n_{th}} \tag{2.8}$$

where, n_{th} is the theoretical maximum moles of CH_4 based on acetate consumed, n_{CE} is the maximum moles of CH_4 from the measured current, and n_{CH4} is the no. of moles of methane measured.

n_{th} can be calculated as (Eq. (2.9))

$$n_{th} = \frac{S_i - S_f}{M_s} \tag{2.9}$$

where S_i is the initial acetate concentration, S_f is the final acetate concentration, M_s is the molecular weight of acetate (thermodynamic limit is 1 mole CH_4 per mole of acetate). n_{CE} can be calculated as (Eq. (2.10))

$$n_{CE} = \frac{\int_0^t It}{8F} \tag{2.10}$$

where, $\int_0^t It$ is the total charge obtained after time t, F is Faraday's constant, and 8 is the thermodynamic limit (8 moles of electrons transferred per mole of methane).

2.7 Critical Factors Affecting Bioelectrochemically Assisted Anaerobic Digestion

2.7.1 Temperature and pH

Temperature and pH of the media are the two most influencing parameters associated with any microbial process. Both pH and temperature govern metabolism by mediating the enzymatic reactions. Several enzymes are required in the metabolic pathway of the AD viz., glycolysis, fermentation, methanogenesis, etc., and efficiency of the enzymatic machinery is very much controlled by pH. It also plays a crucial role in oxidation-reduction potential of the cells. Since all the enzymes have an optimum pH for their maximum activity, it becomes imperative to study the role of pH in methane production (Weld and Singh 2011). Due to accumulation of metabolite like volatile fatty acids during dark fermentation process, the pH drops with time. This decrease in pH (3.8–4.2) results in hydrogen production cease. Accumulation of volatile fatty acids can also disrupt the cell membrane's integrity which leads to the disturbance of internal pH. A metabolic shift from acidogenesis to solventogenesis takes place at low pH. Hydrogen production and substrate conversion improve when reaction pH is maintained at optimum value. Similarly, extreme temperatures can lead to denaturation of enzymes required for the growth and maintenance of the microorganism. Most of the studies have reported an optimum temperature range of 30–37 °C.

2.7.2 Applied Potentials

The applied potentials are crucial for the conversion of CO_2 to CH_4 in hybrid AD-MECs. Thermodynamically, a theoretic potential of -0.224 V (vs. NHE) is needed to drive the electroreduction of CO_2 to CH_4 at the cathode. However, due to the electrochemical losses, much higher applied voltages are used to overcome the energy barrier. Applied potentials in the range of 0.2 V–2.0 V have been reported for producing CH_4 in MECs (Choi et al. 2017; Park et al. 2018). It has been demonstrated that CH_4 production increased with increase in the applied potentials of the system (Chen et al. 2016). However, extremely high applied potentials can negatively affect the methanogenic consortia, electron transfer routes, electron transfer rates, etc. and can lead to loss of microbial viability (Wang et al. 2017). Therefore, an optimal potential needs to be selected so as to achieve the scalability of the process. Usually, the optimal set potentials vary in different studies due to the discrepancies in the cell configuration, cathode materials, type of inoculum, etc. The applied potentials can also lead to generation of H_2 at the cathode, which can be used as electron donor by the methanogens for methane productions.

2.7.3 Hydraulic Retention Time

Hydraulic retention time (HRT) also known as hydraulic residence time is a measure of the average period of time for which a compound remains in a reactor. Hydraulic retention time is a ratio of reactor volume and flow rate of feed. In a continuous operation of bioreactors, HRT for cell biomass becomes a crucial parameter because a very low HRT may lead to the washout condition. Thus, an optimum HRT must be studied to obtain the highest rate of methane production. By manipulating the HRT, microbial profile in a reactor may shift. Acidogens have higher specific growth rate as compared to the methanogens. Thus, lower HRT would lead to enrichment of acetogenic hydrogen producers inside the reactor and the methanogens would get washed out (Dong 2009; Pant et al. 2013). The HRT also influences end metabolite formation which is related to the change in the microbial profile. The Organic Loading Rate (OLR) is a function of HRT. Thus OLR is also considered as one of the influencing parameters in a continuous mode of AD-MEC operation.

2.8 Perspectives and Outlook

Biogas based electricity generation is gaining popularity in recent years owing to the major shift toward renewables. However, the high capital costs and longer payback periods make the process economically unattractive. Integrating AD with MEC can be an attractive strategy to overcome the limitations of the conventional AD process. AD-MEC process has the potential to generate high-quality methane due to the electroreduction of CO_2 to CH_4 which in turn reduces the biogas upgrading costs. A preliminary concept of treatment of waste sludge with biogas upgrading using integrated AD-MEC is shown in Fig. 2.3.

At present, the AD-MECs are still limited to bench-scale studies and further advances in fundamental understanding as well as process development is needed to achieve scalable AD-MEC designs for practical consideration. Significant efforts

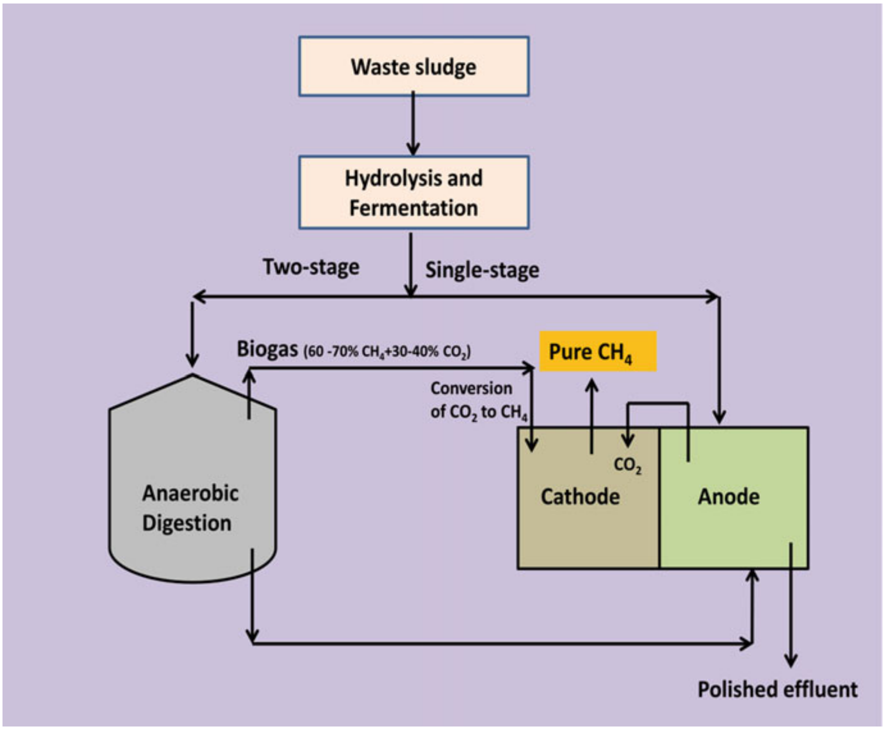

Fig. 2.3 Treatment of waste sludge with biogas upgrading using integrated AD-MEC

have to be made with respect to electrode materials, surface area, spacing, applied voltages, functional microbiome, etc. Reducing the startup time and increasing the productivity of methane production are essential to prove the reliability of these systems. The combined influence of various factors affecting AD-MEC performance has to be assessed systematically. Techno-economic assessment of AD-MEC is crucial to evaluate the industrial viability of the process and assert its advantage over AD or MEC process alone. Additionally, a more comprehensive life cycle analysis based on pilot-scale AD-MECs is warranted for elucidating the environmental impact of the process.

2.9 Conclusion

Bioelectrochemically assisted the anaerobic digestion process is an attractive means to alleviate the global energy crisis. AD-MECs generate better quality final effluent, enrich CH_4, and reduce the overall CO_2 emissions which make them quite appealing. Further elucidation of the hybrid AD-MEC process is needed to decipher the electron transfer mechanisms and their impact on the microbiome. Also, extensive bench-scale and pilot-scale studies are needed to ensure the longevity and stability of the process. Although the technology is still in its nascent phase of research, the recent advances in process designs and adequate understanding of the syntrophic interactions among the microbial assemblages in AD-MEC provide promising aspects for its commercialization.

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Chapter 3
An Insight into Biological Photovoltaic Cell Based Electrochemical System

Gini Rani, Vijay Jaswal, Rajesh Banu, and K. N. Yogalakshmi

Abstract Biological photovoltaic cells can be called as living solar cells. They use oxygenic photoautotrophs such as cyanobacteria and algae, instead of silicon, to capture light energy for photolysis. The organisms such as cyanobacteria and algae capture light energy during the process of photosynthesis and perform charge separation of water molecules (photolysis), producing protons, electrons, and oxygen molecules. The electrons thus produced are transferred to the anode and through external circuit they move to cathode to get reduced to water, producing electric current. Biophotovoltaic (BPV) are different from traditional silicon based solar photovoltaics (SPV) cells in a number of ways. Unlike SPV, the presence of water is imperative in BPV for the algae/cyanobacteria to perform photolysis. The BPV are self-renewing in nature and do not require any external carbon source for growth. The technology of BPV can be incorporated in bioelectrochemical systems (BES) to generate green energy. BPV based electrochemical technology can be used as solar bio-battery or bio-solar panel. It can also be utilized in low powered devices such as alarm clocks. Despite the multiple advantages of BPV, still they are in the threshold of its development due to its energy conversion efficiency. The chapter would comprehensively explain the principle, working, and application of biological photovoltaic systems.

Keywords Biophotovoltaic cell · Oxygenic photoautotrophs · Semiconductor device · Electrochemical system · Microbial fuel cell

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3.1 Introduction

In the entire solar system, sun is the largest object having massive definitive source of energy for earth's entire life form. The nuclear fusion reactions occurring in the sun produce 386 billion MGW of power (World Energy Resources 2016). Every second, around 3.86e33 ergs (5 million tons) of energy is released in the form of gamma radiations (Solar Energy Research Institute 1982). As these rays move towards the surface of the earth, they get absorbed and reemitted constantly, causing dilution of the radiations. A part of radiation is reflected back, while some get scattered by the atmosphere. The atmosphere attenuates a major portion of the spectrum (~50% or more). However, the earth's atmosphere gets a steady supply of 1 kW/m^2 of solar energy which is carbon neutral, renewable, and abundantly available throughout the world (Silvi 2004).

Photosynthesis is a natural process of harnessing sun's energy. For the production of biomass, photosynthetic organisms utilize only 0.25% of the total sunlight falling on them (Driver and Bombelli 2011). Nevertheless they are able to harness more than 10 times the annual human energy consumption. Therefore, man-made photosynthetic techniques (imitating natural photosynthesis) such as photovoltaic cells (PVC) with potential to convert sun's energy with the efficiency of nearly 10–15% have been devised. High cost and requirement of semiconducting materials in its extremely pure form are the biggest shortcomings of this technique. Therefore, a novel technology, the biological solar cells with high conversion efficiency and low cost was devised. The biological solar cells are a kind of natural technology which generates clean energy by making use of oxygenic photoautotrophs. The photosynthetic fractions harvest sunlight and convert it directly to electrical energy. The oxygenic photosynthesis material can either be algae or cyanobacteria with potential to produce electrons and protons by oxidizing water molecule using sun as a source of energy. The charge separation mechanism occurs in algae/cyanobacteria during the process of photosynthesis, i.e. a chain of reactions occur when sunlight falls on them producing H^+ ions (by water splitting), oxygen, and electrons. These components are crucial in converting CO_2 and other inorganic substances into proteins and carbohydrates, which help algae to grow and thrive. This algal photosynthesis is utilized to produce electrical energy through biological photovoltaic (BPV) devices. An extensive detail about BPV devices, its principle, configuration, and applications is mentioned in the forthcoming sections of this chapter.

3.2 Principle of Photovoltaic Systems

3.2.1 History

Edmund Becquerel, a French physicist observed for the first time in 1839 the phenomenon of photovoltaic effect (PV) using an electrolytic cell consisting of a pair of metal electrodes dipped in weak conducting solution. Becquerel noticed generation of mild electric current when certain metals were exposed to sunlight. PV effect is an indispensable process in which solar devices convert sunlight to electric current. The PV effect was then studied in solid substance (selenium) in the 1870s. A decade later (1880s), PV cells based on selenium were devised. These devices were able to convert 1–2% of sunlight into electricity. However these devices did not manage to gain much popularity owing to its high cost and negligible output. During the 1940s, remarkable properties of photovoltaic process were shown by the accidental formation of p-n junctions (Green 2001). It served as basic building block in designing semiconductor devices. Since then it began to be commonly used as rectifier in electronic materials. It further triggered development of point contact diodes, transistors, junctions, fixators, and then finally development of integrated circuits. A major breakthrough came during the year 1940s and early 1950s with the production of extremely pure crystalline silicon through the method named Czochralski method. Czochralski method is used for bulk production of single crystals, in which pure silicon ingot is put in the crucible along with silicon doped boron pieces and melted using radiofrequency heaters or resistance. A seed crystal is dipped into it and then gradually withdrawn. Since then, most of the solar cells developed are fabricated using either monocrystalline or polycrystalline (large grained) silicon material. A detailed timeline of the historical events in the development of solar cells is summarized in Table 3.1. The development of photovoltaic devices initially started with discovery of the phenomenon of photovoltaic effect. This effected into the subsequent development of the material selenium. Similarly more and more materials were discovered that possessed the property of photoconductivity. The availability of the material proved to be major limiting factor in the commercialization of this technique. Therefore materials having similar properties while also being cheap were studied extensively. Crystalline silicon showed better results in conversion efficiency. Its structure was altered to achieve enhanced efficiency. Meanwhile other composite materials were also discovered, which have been mentioned earlier.

3.2.2 Ideal Materials for Photovoltaic Devices

Solar cells are kind of photovoltaic tool which creates voltage on exposure to sunlight because the material used in PVC is a semiconductor. Solar cells are fundamental units of PV devices, modules, and panels. Majority of the solar cells are developed in a pattern to capture sunlight before reaching the surface of the earth while some are designed for working in space. Semiconductor materials are capable of absorbing light and transferring part of absorbed energy to electrons and holes, which produced DC current. The preferred materials for PVC are selected on the basis of the cost of production and the extent to which absorption properties compliment the solar spectrum. Solar cells are categorized as first, second, or third generation cells. The first generation cells are traditional wafer based cells. They are

Table 3.1 Timeline of historical development of solar technology

Sr. No.	Year	State-of-the-art development
1	1839	Edmond Becquerel, a French scientist revealed the process of photovoltaics with the help of electrolysis
2	1873	Willoughby Smith identified the property of photoconductivity in the semimetal selenium
3	1876	William G. Adams and Richard Evans demonstrated the ability of solid material to alter sunlight into electrical energy in the absence of moving heating system. They discovered it by exposing selenium solar cells into sunlight
4	1883	Charles Fritts developed the first kind of solar cells using selenium wafers
5	1887	Heinrich Hertz proved that ultraviolet radiation could cause electric sparkle between different metal electrodes at small voltage
6	1904	Photosensitivity in copper and cuprous oxide amalgamation—Wilhelm Hallwachs
7	1905	Photoelectric effect reported by Albert Einstein
8	1914	Barrier layer identified in the photovoltaic systems
9	1916	Robert Millikan—process of photoelectric effect
10	1918	Jan Czochralski developed method for producing single-crystal silicon
11	1932	The PV effect was found in cadmium sulfide (CdS) by Audobert and Stora
12	1953	The efficiency of materials with diverse band gaps corresponding to the solar band was demonstrated using theoretical calculations
13	1954	First solar cell competent of transferring sufficient solar energy into power with the efficiency of 4% was constructed at Bell Telephone Laboratories in America by Daryl Chapin, Calvin Fuller, and Gerald Pearson
14	1955	Commercial authorization for silicon PV systems
15	1957	PV cells with 8% efficiency fabricated by Hoffman Electronics
16	1958	np silicon PV for space cells developed by T. Mandelkorn at U.S. Signal Corps Laboratories PV cells with 9% efficiency fabricated by Hoffman Electronics The Vanguard I space satellite utilized a minute array of less than 1 W to power its radios Photovoltaics powered systems were used in Explorer III, Sputnik-3, and Vanguard II
17	1959	PV cells with 10% efficiency by Hoffman Electronics, reduction in series resistance by using grid contact was not achieved Satellite Explorer VI and Explorer VII were instigated with the photovoltaic collection of 9600 cells with the dimension of 1 × 2 cm
18	1960	PV cells with 14% efficiency fabricated by Hoffman Electronics
19	1962	The first telecommunications satellite (Telstar) of Bell Telephone Laboratories was launched with its initial power of 14 W
20	1963	Realistic silicon PV modules were developed Japan installed world's largest arrangement of PV module (242-W) on a lighthouse
21	1964	Satellite (first corona spacecraft) powered with 470-W PV array launched by NASA
22	1966	The first orbiting astronomical observatory was started by NASA with 1 kW PV array

(continued)

Table 3.1 (continued)

Sr. No.	Year	State-of-the-art development
23	1972	PV system fabricated with cadmium sulfide (CdS) was used in Nigeria's educational television
24	1976	83 PV power systems installed by Lewis Research Center, NASA Amorphous silicon PVC was fabricated for the first time by David Carlson and Christopher Wronski
25	1980	Thin film solar cell with the efficiency of more than 10% was developed with CuS_2/CdS at the University of Delaware
26	1981	The first solar-powered aircraft with 16,000 solar cells on its wings with 3000 W potential fabricated by Paul
27	1983	The production of PV technology exceeded 21.3 MW; sales more than $250 million
28	1985	The University of South Wales—more than 20% efficiency for silicon solar cells under 1-sun stipulations
29	1986	The first thin-coated power module (G-4000) commercialized by ARCO Solar
30	1988	Glass pane enclosed with a huge range of Al/Cu strips, less than a micron patented by Dr. Alvin Marks for Lepcon and Lumeloid (solar power technology) Lumeloid used low-priced films made of plastic. Conductive polymer was used to cover the plastic
31	1992	University of South Florida manufactured thin film PVC made of cadmium telluride with 15.9% efficiency
32	1993	The first grid-supported PV system was installed by Pacific Gas and Electric in Kerman, California having the power capacity 500-kW
33	1994	The National Renewable Energy Lab. developed solar cells of InGaP and GaAs with 30% conversion
34	1996	The ICARE, a solar-powered airplane with 3000 high efficient solar cells flew over Germany
35	1998	Flexible solar cells invented by Subhendu Guha for conversion of sunlight into electricity
36	1999	The conversion efficiency, as high as 32.3% was attained by compressing three layers of PV into a single solar cell—Spectrolab, Inc. in association with National Renewable Energy Laboratory
37	2001	• Helios, a solar-powered non-rocket driven aircraft developed by NASA • TerraSun LLC built up a technique of utilizing holographic pictures to focus sunlight against solar cell. Fresnel lenses or mirrors were used to concentrate sunlight
38	2002	A 100% degradation of ethylenediaminetetraacetic acid was achieved by solar irradiation using ferrioxalate process (Emilio et al. 2002)
39	2004	A sensor was developed using solar energy driven spectroscopic imager to investigate flexible X-ray solar radiance (Boerner et al. 2004)
40	2006	Chemical reactor powered by solar energy was designed for industrial manufacture of pure lime (Meier et al. 2006)
41	2011	Hybrid system embodying solar thermal technology and fossil fuel energy fabricated to increase the concentration of solar energy and sequester carbon dioxide (Ordorica-Garcia et al. 2011)
42	2013	The surface of silicon chip used in solar cell was patterned for low reflectivity using thermal nanoimprint lithographic technology (Landis et al. 2013)

(continued)

Table 3.1 (continued)

Sr. No.	Year	State-of-the-art development
43	2017	Colloidal quantum dots showed to be an excellent absorber of sunlight due to its high optoelectronic properties in solar cell technology (Zhao and Rosei 2017)

Source: Modified U.S. Department of Energy (EERE)

prepared from crystalline silicon unlike the second generation cells that are prepared from amorphous silicon, copper indium gallium selenide solar (CIGS) cell and CdTe cells. Second and third generation solar cells are based on thin film technologies. Third generation solar cells are also referred to as emerging photovoltaics (Bagher et al. 2015). Silicon (Si) is a widely accepted semiconductor used in PVC fabrication in crystalline, polycrystalline, or amorphous state. The other semiconductors including gallium arsenide (GaAs), indium gallium phosphide (GaInP), Cu(InGa)Se_2, cadmium telluride (CdTe) have also been used in PVC. Table 3.2 summarizes the materials used in the fabrication of photovoltaic cells. The efficiency of photovoltaic cells varies from material to material. For instance, crystalline silicon has undergone much modification to achieve maximum efficiency of around 16% covering international market of 86%. Similarly advanced materials and techniques such as dye sensitizers, ultra-thin wafers, and anti-reflection coatings have also been tested. The ultra-thin wafers have shown an efficiency of around 20%, which is very promising in this domain.

3.2.3 Mechanisms

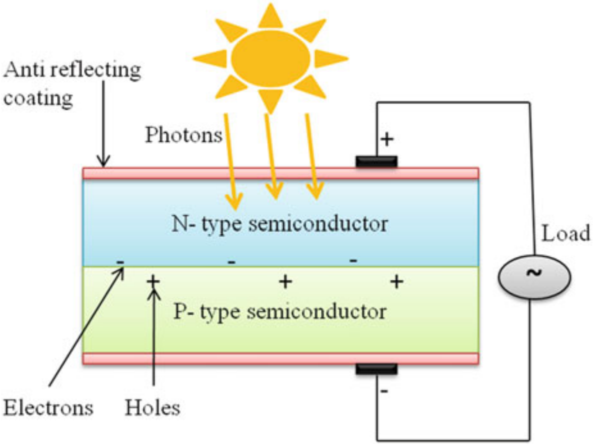
Solar cells and photodiode are essentially photovoltaic semiconductor appliances. Hence, the working of solar cell is nearly similar to that of photodiode with minute differences. Photodiode works in a narrow range unlike solar cells that work on wide spectral range. The metric for photodiode and solar cells is signal-to-noise ratio (quantum efficiency) and power output per incident sun energy (power conversion efficiency), respectively.

The photovoltaic effect is the physical source for the transformation of electromagnetic energy into electrical energy. It produces non-compensated space electric charge due to absorption of radiation by the given substance or medium. An electromotor energy which is equal to the potential difference of the two terminals of the discharged cell is developed as a result of the presence of this non-compensated charge. When the terminals of the cells are closed with the help of an external circuit, the conductor is packed with DC current, the intensity of which depends on the amount of the exterior resistance. Therefore, to produce electric current in cell, it is important to bring modification in the structure by separating the negative and positive charge carriers flowing in the conduction band. This division takes place due to the diffusion of carriers linking the regions of various carrier

Table 3.2 Common materials used in the fabrication of photovoltaic systems

Material used for solar cells	Descriptions		References
Silicon crystalline technology • Occupies 86% of PV market • Very stable • Module efficiencies 10–16%	Silicon (monocrystalline PV cells) • Fabricated from single cylindrical crystal of Si using saw cut method • 15–20% operating efficiency • Space-efficient panels • Longest operational life • Panels are most expensive		
	Silicon (multi-crystalline PV cells) • Fabricated from ingot of melted and recrystallized silicon • Cell efficiency ~13 to 16% • Occupies 90% of crystalline Si market • Simpler and economical • Low space-efficiency		
Thin film technology • Consists of silicon layers about 10µm thick, compared with 200–300 µm layers for crystalline silicon cells • Low cost substrate and fabrication process • Production modules operate at about 9%	Amorphous silicon PV cells • Most advanced thin film technology • Operating efficiency ~6 to 8% • Occupies about 13% of PV market • High end manufacturing technologies		Lee et al. (2015)
	Polycrystalline cells • Non-silicon based technology	Copper indium diselenide • 1 eV bandgap • High absorption coefficient—10^5 cm^{-1} • High efficiency—18% and >11% laboratory and module efficiency, respectively • Manufacturing process—Immature • Slow vacuum process	
		Cadmium telluride (CdTe) • Direct bandgap of 1.4 eV • High absorption coefficient • 20% and 6–9% laboratory and module efficiency, respectively	Green (2001)
Emerging technologies	Dye sensitizers • Cell efficiency ~7%		
	Ultra-thin wafer solar cells • Thickness ~45µm • Cell efficiency as high as 20.3%		
	Anti-reflection coating • Low cost deposition technique • Metal organic titanium or tantalum mixed with suitable organic additives used		

Fig. 3.1 Mechanism of photovoltaic cell



concentrations, with respect to electrochemical potential ascent and convection of charge in the cell's inner electric field. Silicon (crystalline semiconductor) is a best example of such a medium with a p-n junction. The arrangement of a region with the p-type conductivity occurs with the help of doping with atoms of acceptor elements from group III of the periodic table. The n-type conductivity can be formed by doping with atoms of donor elements from the group V elements of the periodic table. If such structure (crystalline silicon) once exposed to the solar radiation with quantum energy exceeds its energy gap ($E_g = 1.12$ eV), then electric charge and electron/hole pairs are created as a result of the light absorption. The pictorial representation of the photovoltaic effect depicted in Fig. 3.1 clearly illustrates how the electrons and holes are created when light energy falls on semiconductor materials.

The charge pairs are separated due to the effect of the electric field in the junction. The excess electrons are formed as a consequence on the n-side, while on the p-side, there is an excess of holes that result in the development of electric voltage (Boer 1992). In any solar cell based on crystalline silicon (P-type), the potential distribution, band configuration, and concentration of the electric field within the space charge of the p-n junction depend on the intensity of the donor and acceptor impurities.

3.3 Biological Photovoltaic System: An Introduction

Green vegetation and cyanobacteria through the natural process utilize water and convert it into electrons and protons. During the process, oxygen is expelled as waste product and the electrons and protons are used to fix carbon into complex carbon units which form the source for biomass production (Blankenship 2010). The major steps in the process of photosynthesis embody light capture, charge separation process, oxidation of water, and finally fuel production (Cogdell et al. 2010). In antenna complex, the light harvesting molecules (chlorophyll/carotenes) transfer the photonic energy through the reaction center, where the process of charge separation occurs. Once the charges are separated, the positive charges are drawn on for the oxidation of water molecules and the electrons are transferred to photosystem I through cytochrome b_6f and electron carriers (mobile). The fundamental reactions for oxidation of water and production of fuel are given in Eqs. (3.1) and (3.2).

$$2H_2O \overset{hv}{\rightarrow} O_2 + 4H^+ + 4e^- \tag{3.1}$$

$$CO_2 + 4H^+ + 4e^- \overset{hv}{\rightarrow} (H_2CO) + H_2O \tag{3.2}$$

The first half reaction (3.1) depicts water splitting, while the other half reaction (3.2) shows reduction of carbon dioxide to yield carbon based fuel. The process of photosynthesis is determined by the sum total insolation captured per unit time. There are two photosystems to perform above mentioned two reactions and for this, two photons are utilized per electrons, i.e. the reaction is driven relative to the amount of photons taken in Purchase and De Groot (2015).

Considering the total quantum of solar radiation hitting the earth surface, the photosynthesis efficiency for biomass production is less than 1% or a theoretical maximum of 4.5% (Barber 2009). Therefore, if biomass is used as feed for energy conversion, its efficiency would be invariably less compared to conventional photovoltaic devices (Tschörtner et al. 2019). The quantum/photon efficiency, however, for water splitting process occurring in the enzyme complex is much higher, which is 55% or theoretical maximum of 70% at a wavelength of 680 nm in the electromagnetic spectrum (Barber and Tran 2013). Therefore, an effective way to incorporate photosynthesis apparatus in any technology for improved energy production is to directly combine energy generation to the photosystem II (water splitting reaction).

3.4 Types and Configuration of Biophotovoltaic Systems

Several substantial configurations of biophotovoltaic cells have been designed to improve the energy conversion efficiency. In artificial photosynthesis, the steps such as incoming solar flux, electron/energy transmission, and catalysis of reaction occur separately in different time frames. Therefore, it is crucial to consider that the components operate in proximity with the theoretical limits of photonic energy to fuel conversion. The prominent types are discussed below.

3.4.1 Cellular Biophotovoltaics

In this particular configuration, oxygenic photosynthetic microorganisms are solely utilized for the photolysis of water and transport of electrons to the electrode for current generation. The microbes in cellular biophotovoltaics are also capable of producing electric energy in dark through respiratory break down of stored carbon, collected in the presence of sunlight. Therefore these types of BPVs are able to produce electric energy diurnally (Bombelli et al. 2011). Generally in cellular BPV, cyanobacteria are preferred over green algae because of the fact that they are physiologically simpler compared to complex eukaryotic algae, as they are more tough relative to sub-cellular sections of photosynthesis complex and their minimal basal requirement of energy. All these qualities collectively make them highly efficient for energy conversion.

The cyanobacterial strains used in earlier studies included *Anabaena* spp., *Phormidium* spp. (filamentous) and *Synechocystis* spp., *Synechococcus* spp. (unicellular) as they exhibited light dependent electric current generation. The cyanobacterial strains are used as anolyte. Earlier artificial electron mediators (AEM) such as 2,6-dimethyl-1,4-benzoquinone and hydroxyl-1,4-naphthoquinone were used, which produced power density of 288 mW/m^2 at current density of 600 mA/m^2 (Yagishita et al. 1999). Although AEMs were able to increase current densities, it was later found that it affected the viability of the microbes over a period of time. Therefore, considering the sustainability aspect, BPV in the absence of AEM was studied using *Synechocystis* species grown on carbon electrode (Zou et al. 2009). Its conversion efficiency was recorded to be much less, around 0.05–0.3%, but later higher power density of around 6 mW/m^2 was achieved demonstrating positive response of light, which was also maintained for months (Samsonoff et al. 2014). It was later proved through several studies that the efficiency in the form of power density depended on intensity and wavelength of light, temperature, species type, and anodic material. Several algal species have also demonstrated positive response in the absence of artificial mediators in BPV. A highest power density (100 mW/m^2) was achieved in microfluidized BPV in the absence of light (Bombelli et al. 2015).

3.4.2 Biological Hybrid Solar Cells

These types of solar cells utilize combination of both organic and inorganic material. The organic fraction includes photosystem I, which is a protein complex (photoactive) found in thylakoid membrane. Photosystem I was used to imitate the process of photosynthesis and to improve the efficiency of the solar cells (Ciesielski et al. 2010). Photosystem I is injected and harvested for several days in the inorganic portion of the solar cell. After several days of gathering photosystem I in gold layer, it becomes visible as thin greenish layer, which increases the energy conversion efficiency of the PV cell. Photosystem I was developed from spinach by extracting thylakoid membrane from it and subjecting it into further purification for the isolation of photosystem I from it. The different sheets of photosystem I harvest the sunlight (photonic energy) and convert it into chemical energy, which in turn creates a DC current flowing through the cell.

In one of the biohybrid photovoltaic systems, a biophotovoltaic cell with two terminals was constructed and the solution containing protein complex and charge transfer mediators was injected between (both terminals) working electrode and the counter electrode. However this system failed to give promising result because of disorientation of the protein complex arising from lack of control in protein attachment (Yaghoubi et al. 2015). To control attachment of protein, ZnO nanowires functionalized electrode was used for immobilization of the reaction center onto it, where cytochrome C was bound to the electrode surface (Yaghoubi et al. 2017). It showed high stable binding without denaturing protein. The use of nanoparticles in biophotovoltaic systems offer enhanced photon absorption due to larger surface area and also facilitates selective redox reactions. Selective electron pathways and increased bulk mobility of electrons can also be achieved in biohybrid photovoltaic systems.

3.5 Biophotovoltaic System Versus Photovoltaic System

The principle reason to shift from conventional photovoltaic cells to biophotovoltaic cells or solar cells is sustainable nature of BPV cells. Apart from being sustainable, there are various brilliant characteristics of BPV that differ from conventional PV cells, which will be illustrated in detail in this section.

The process of triggering electronic states in the respective photovoltaic system, both PVS and BPV requires photonic energy; however, there are a number of features that differentiate them. PV systems require no input of organic substance, while organic compound plays a significant role in BPV integrated MFCs. Unlike photovoltaic systems, the photoreceptive centers are collected and preserved by the oxygenic photosynthetic organisms which are able to reproduce, self-repair, and stock up energy for energy generation in absence of sunlight. The water which happens to be the source of electron in BPV restricts its far reaching exploitation unlike conventional PVS especially in those regions which suffer water crisis. PV technology is confined to specific solid state, whereas BPV can be operated with broad range of biological species and substrates. The major limiting factor of PVS is its inability to function in the absence of light. This shortcoming is successfully addressed in case of BPV devices. The biophotovoltaics based power plants installed offshore comprises algae community which produces excess electrons. These excessive electrons are stored in the cells and are utilized in the absence of light (Driver and Bombelli 2011). Thus BPV can also serve the purpose of capacitors.

3.6 Biological Photovoltaic Based Electrochemical System

Over the last 4–5 years, the research on bioelectrochemical systems has witnessed new heights owing to its ability to produce electric current/hydrogen while removing organic matter present in the substrate with the help of bioanode, i.e. via exoelectrogenic bacteria attached to the electrode (McCormick et al. 2015). The prominent bioelectrochemical systems (BES) utilize organic matter as fuel supply, but there are several BES which are fuelled by the light source and are termed as biophotovoltaic (BPV) systems. In the absence of organic substrate, algae and cyanobacteria (oxygenic photosynthetic life forms) present in BPV will harvest light energy (*hν*) and convert it into electric energy or fuel (Fig. 3.2).

BPV utilizes various cellular and sub-cellular photosynthetic complex including thylakoid membrane, reaction centers (photosystem I and PS II) and algae or cyanobacteria. For instance, the photosynthetic machinery in *Rhodobacter sphaeroides* consists of two antenna complexes (light harvesting), a reaction center, cytochrome bc$_1$ complex, and an adenosine triphosphate (ATP) synthase. The reaction center in *Rhodobacter sphaeroides* consists of three subunits of transmembrane protein that facilitate scaffolding of photosynthetic pigments and the cofactors, where the main reactions of energy conversion occur. The cofactors comprise primary donor unit which is bacteriochlorophyll dimer, two monomer units of bacteriochlorophyll A and B, two units of bacteriopheophytins A and B, two units of quinines A and B, one non-heme iron molecule, and one carotenoid. The pathway of primary electron transfer advances from bacteriochlorophyll A to quinone A and quinone B. The electron transfer pathway proceeds upon the absorption of photonic energy. This photosynthetic machinery is integrated in electrochemical devices for the purpose of charge separation and energy harvesting (Yaghoubi et al. 2015). In the reaction center, the primary charge separation process gives an internal quantum yield of around ~100% (Tan 2018). But when it is integrated with photovoltaic system, the internal quantum yield is relatively less giving low photonic electric current upon exposure to solar energy. Reduction in the photocurrent can be attributed to the course of charge recombination taking place at the surface of the

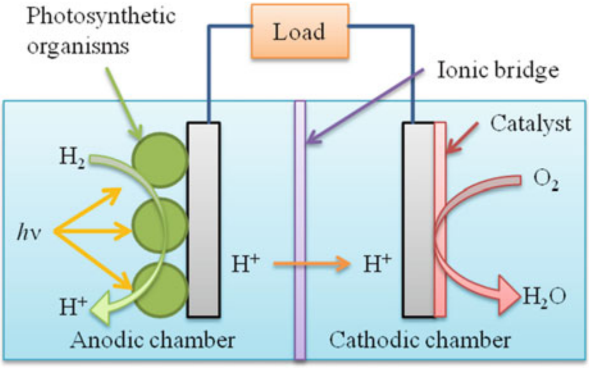


Fig. 3.2 Working mechanism of biophotovoltaic electrochemical system

electrode (Yaghoubi et al. 2017). The recombination of charge occurring at the surface of the metal electrodes leads to loss of electrons. This challenge was mainly encountered in case of metal electrode. Semiconductor electrodes have shown better result in charge transfer with proteins because of valance and conduction bands (Kamran et al. 2014). The energy band structure found in the semiconductors helps in selective transfer of charges using protein complex in biophotovoltaic systems.

The *Geobacter sulfurreducens* strains, also termed as electrogens because of their intrinsic property to conduct electric current are known to catalyze redox reaction in common BES. *Geobacter sulfurreducens* possess type IV pili (conductive) for electron transfer to extracellular insoluble electron acceptor and produce electric power. The power density of around 3.9 W/m^2 has been reported using pure culture of same strain which is highest of all tested microbes (Yi et al. 2009). The biofilm consisting of *Geobacter* spp. when grown on solid electron acceptor develops an extracellular matrix which is electrically conductive in nature and comprises exopolysaccharides, pili, and exocytochromes (Leang et al. 2013). The pili complex is important for electron transport within the biofilm to establish direct contact with the solid surface of electron acceptor. The cytochrome C of type IV pili (PilA) includes an aromatic amino acid moieties that help in transfer of electron through delocalized orbitals of electrons for production of current (Malvankar et al. 2014).

As per standard theory, the first step towards the release of electrons is oxidation of water molecule triggered by the absorption of light energy which occurs in photosystem II (oxygenic photosynthetic) reaction center. Thereafter, the flow of electron occurs from photosystem II to the external circuit depending upon the type of biological components involved.

3.7 Application of Biophotovoltaic Cell

The ability of photosynthetic bacteria and algae to capture light energy and produce direct current is utilized for various purposes. The process of photosynthesis in BPV, driven by microalgae and cyanobacteria utilizes two photons per single electron (feed). The electrons were obtained from water splitting which is then chemically converted to hydrogen. When carbon dioxide (CO_2) is utilized, the end product is carbon based fuel. Apart from the application of BPV cells for light capturing and charge separation process, the focus is also stressed much on the developing artificial photosynthesis for the production of different types of fuel to ensure its extensive and prompt availability.

Microalgae–microbial fuel cell (MFC), a kind of biophotovoltaic BES utilizes microalgae as a catalyst in cathode of MFC and produces bioelectricity with the help of exoelectrogenic microbes in dark reaction. Along with bioelectricity, microalgae–microbial fuel cell produces useful chemicals, oxygen, and biomass. The microalgae in MFC are principally used to produce oxygen and diffuse it into the cathodic aqueous media for the production of electric energy. Microalgae in MFC replace aeration process required for the reduction of protons. It will therefore bring down

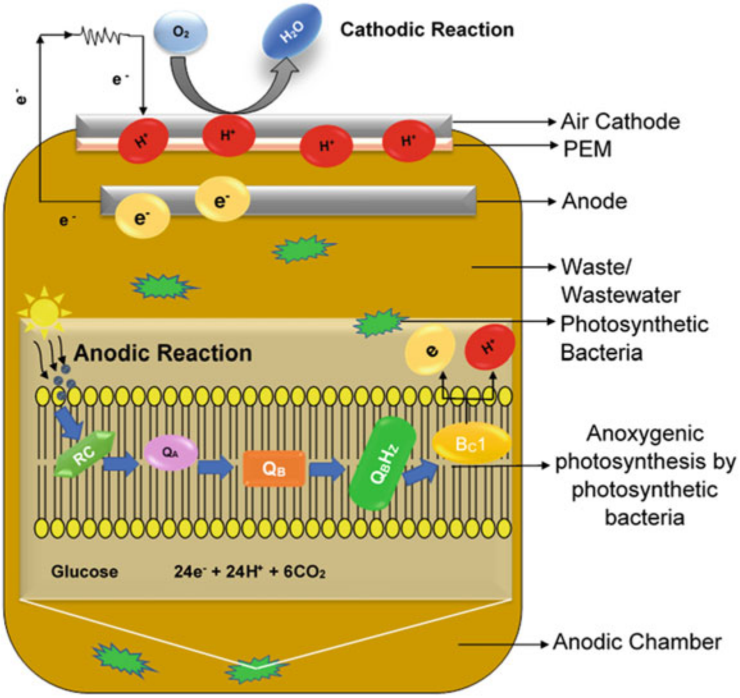


Fig. 3.3 Schematic of anoxygenic photosynthesis within microbial fuel cell

the cost of aeration and electric current production (Lobato et al. 2013). Photosynthetic purple bacteria in MFC produced H_2 gas from the organic matter and power density of around $120 mW/m^2$ through the process of anoxygenic photosynthesis (Chandra et al. 2012). A complete reaction of anoxygenic photosynthesis in an MFC reactor is depicted in Fig. 3.3. The bacterial chlorophyll a (Bchl a) found in anoxygenic photosynthetic organisms traps sunlight as source of energy and utilizes organic waste/CO_2 as an electron source from atmosphere or wastewater/substrate. The efficiency of Bchl a to transfer energy is nearly 95–99%. The conversion of solar energy in light harvesting system occurs through quinone pool and cytochrome (cyt) bc1 complex. Moreover, anoxygenic photosynthesis have also shown potential in treating acid rich wastewater while producing bioelectricity (Chandra et al. 2017).

It was demonstrated that algae grown in clusters over 60 m in oceanic stretch could produce more energy output and provide huge energy storage than graphite and silicon based standard commercial electrodes (Kovalenko et al. 2011). The nutrients required for the algal growth can be reused in sustainable fashion; therefore, it can easily convert atmospheric carbon dioxide into carbon based fuels and other alkanes while MFC can consistently operate in an independent fashion (Lee et al. 2015). The huge amount of CO_2 released during the production of bioethanol wine preparation was captured using microalgae and transferred from anode to cathode to generate electric current (Powell et al. 2011). The microalgal biomass also works as energy storage system which can be digested in MFC for current generation. Algae not suitable for biofuel production can be chosen for the development of BPVs by growing it in large stretch of land or sea. They can even work as solar collector which can help in refueling automobiles in the absence of sunlight.

One of the biggest roles played by the photosynthetic electrochemical system from an environmental perspective is the removal of atmospheric CO_2 (Rosenbaum et al. 2010). Hydrogen production is another popular feature of these kinds of reactors. However, molecular hydrogen may cause an inhibitory effect in the photosynthetic electrocatalytic process. Therefore, it is crucial to rapidly convert molecular hydrogen gas into electrical energy for maintaining mild hydrogen partial pressure. It can be achieved by incorporating photosynthetic H_2 production along with rapid oxidation of H_2 molecules with the help of electrocatalytic photoconversion, where hydrogen/ protons serve as a mediator of electrons between the anode and microbial metabolism (Rosenbaum et al. 2010).

3.8 Implication

The primary challenge for achieving measured conversion of photonic energy into chemical products remains the fabrication of ideal materials within the physical framework relevant to internal potential of 2.9 V. While converting electric charge into hydrogen and then back to electric energy, only 1.23 V of energy is saved and recovered in the fuel. It is therefore important to make use of remaining 1.7 V for effective energy conversion (Purchase and De Groot 2015). It can be done by devising methods to avoid catalytic species kinetic stabilization and recombination of formed species. Further, better oxygen evolution creating sufficient heat is also essential. The absorption of photons can be enhanced by preventing absorption of the same energy by both the photosystems so that there is no competition for photons. It can be done by incorporating two different absorbers, one for infra-red region and the other for visible spectra. The current generation in microbe assisted solar electrodes is dependent on two types of microbial community, viz. cyanobacteria for current flow in anode and oxygen reducer microbes with significantly high potential for current flow in the cathode. It is important to drive biocatalytic reduction of oxygen at a high potential to produce cathodic electric current. Another limiting factor is to improve the capacity of BPV based BES to function as a sustainable body. It can be achieved by refining the aspect of photon absorption, improving charge transfer between biomolecules without the loss of energy and incorporating suitable electrode materials and redox mediators.

3.9 Conclusions

Biophotovoltaic systems are promising devices, which enables production of numerous bioenergy source and byproduct such as biohydrogen, biomethane, algal biofuels, biodiesel, and biomass. Integration of photosynthetic machinery with the bioelectric systems has opened wide avenue for number of possibilities such as wastewater treatment, electricity generation, and useful chemical production (methanol and formic acid). Numerous studies are done in this field to explore wide range of substrate and electrode materials. To make the process economically sound, extensive research has to be done on end products and protein complex. The commercialization of biophotovoltaic devices/solar cells is speeding up because of its bright features and maximum output. Also the feedstock required in its operation is either algae or cyanobacteria, which is abundant to grow when food security is considered. The best part is these systems are self-sustainable and self-renewable. Apart from being adapted to grow in saline water and freshwaters, some strains can also grow in wastewaters. Therefore, it can also be operated for the treatment of wastewater.

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Chapter 4
Electro-Fermentation Technology: Emerging Platform

Maninder Singh, Gargi Joshi, and Mohammad Asif Ali

Abstract The technique of electro-fermentation (EF), with industries utilizing electrochemistry as a tool, has paved a new direction in the field of biotechnology. The use of electrochemical cells can easily influence the microbial metabolism and in turn the electrocatalytic activities during fermentation. EF approach is beneficial in optimizing existing fermentation technologies as it significantly helps in enhancing carbon efficiency, microbial growth, and ease of product recovery. Also, it can be efficient in controlling fermentation-based complex microbial actions and thus provides an advantage over residue utilization in agri-foods, which gives added benefit towards biobased materials. In this chapter, we have described the emerging EF technology and its immense potential in the commercial world.

Keywords Electro-fermentation · Electrodes · Electricity · Microbial fuel cells

4.1 Introduction

Fermentation has a long history in food production, which played a fundamental role in transforming food and production over the centuries to improve the preservation time and textural properties (Raveendran et al. 2018). Lately, fermentation technology has been the key platform to obtain various products such as fermented foods like yogurt, soy sauce, amino acids, bread; beverages such as beer, perfumes, solvents (acetone, butanol, etc.), enzymes, (Lee et al. 2018; Xiang et al. 2019) pickles, and biopolymers (Babu et al. 2013). On the other hand, industrial-scale fermentation, due to several constraints, has limited commercial viability. For

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instance, upstream processes require purified substrates with the help of both initial and fermenting conditions, which can cost up to 50% of the total cost (including downstream operations). Another primary concern in conventional fermentation is limited product selectivity from a given substrate due to the redox imbalance in the metabolic pathways. Altering redox balance requires different criteria or strategies like gas sparging (air, N_2, O_2, and H_2), pH control, and addition of co-substrates (Williams-Rhaesa et al. 2018). Nitrogen helps anaerobic fermentation by controlling the accumulation of volatile metabolites (Sterling et al. 2001), whereas oxygen or pure air helps as an electron acceptor to balance the redox equivalents of microbial metabolism in the culture media (Kang et al. 2013) (Tamagnini et al. 2002). After the addition of electrons donors, di-hydrogen to increase the reduction of metabolism (Tamagnini et al. 2002). For the mentioned constraints, some alternatives to overcome such kinds of shortcomings. Thus, electrostimulation of microbial metabolism presents as one of the other options. The use of electrodes to get products with high purity is necessary for Fermentation environments (Moscoviz et al. 2018). This approach is known as the Electro-fermentation (EF) given by Rabaey and Rozendal (2010), Rabaey and Ragauskas (2014) and which was revised recently Schievano et al. (2016).

EF can help in stabilizing and optimizing the fermentation processes by controlling the redox and pH imbalances and stimulate carbon chain break down. It also improves the yield of the biomass extracted from the microbes and the required products by the use of selective membranes. Moreover, it assists in driving the fermentation process unidirectionally towards a single specific product (Moscoviz et al. 2016). A schematic illustration demonstrating the EF process is shown in Fig. 4.1.

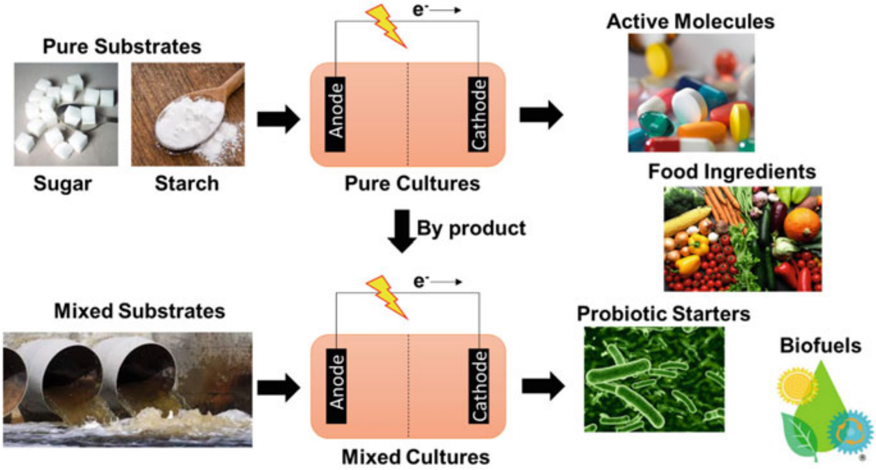

Fig. 4.1 Schematic illustration showing the electro-fermentation process

4.2 Principles of Electro-Fermentation

In the electro-fermentation (EF) technique of energy-rich materials such as carbohydrate or alcohol is carried out using electrodes where electrodes act as an electron source or sink.

When the working electrode (WE) acts as a cathode, the process is known as cathodic electro-fermentation (CEF) and the final product is reduced more than the initial material. Whereas, when the WE act as an anode, the fermentation is known as anodic electro-fermentation (AEF) and the final product is more oxidized than the initial material. Thus, the electron provided triggers the fermentation process in unbalanced conditions and also maintains the fermentation reaction conditions. Small current densities can affect the extracellular and intracellular oxidation-reduction potential (ORP) and thus an overall biological regulation. Electro-fermentation efficiency is represented mathematically as,

$$\eta_{\mathrm{EF}} = \frac{Q_{\mathrm{e}^-}}{Q_{\mathrm{product}}} \tag{4.1}$$

where η_{EF} is the EF efficiency, $Q_{\mathrm{e}}{}^{-}$ is the charge transferred through the electric current, and the Q_{product} is the total charge of the product. $Q_{\mathrm{e}}{}^{-}$ is the integral of the electric current (I) over the total time of EF and can be easily calculated using chronoamperometry. It is defined as,

$$Q_{\mathrm{e}^-} = \int I dt \tag{4.2}$$

For Q_{product} calculations, moles of electrons available per mole of the product (N_{product}) and the number of moles of product (n_{product}) are as follows:

$$N\left(\mathrm{C}_w\mathrm{N}_x\mathrm{O}_y\mathrm{H}_z\right) = 4w - 3x - 2y + z \tag{4.3}$$

$$Q_{\mathrm{product}} = n_{\mathrm{product}} \times N_{\mathrm{product}} \times F \tag{4.4}$$

where F is the Faraday constant (96,485 C/mol e^-). The η_{EF} describes the electricity consumption or generation over the production of the required molecule during the EF process. If the value is greater than 1, then AEF is near to the electricity production or CEF is near to the electrosynthesis. Whereas if the value is between 0 and 1, electrons recovered will be more in the product than provided as in AEF or consumed as in (CEF). Thus, η_{EF} can help in assessing the overall performance of the EF process.

4.3 Separation Technologies Using Electro-Fermentation

In the bio-processes, the production and the recovery of the product are interrelated to each other; for example, in the case of organic waste requires conversion of a very complex substrate. Similarly. in every ideal production strategy, a single substrate is transformed into a single product using a defined pathway (Sadh et al. 2018). In a perfect recovery, depending on some physicochemical properties of the targeted product, good separation can be achieved.

In fermentation technology, volatile fatty acids and alcohols can be easily differentiated based on their hydrophobicity and volatility. Thus, they are prime candidates as target products for physicochemical separations but pose problems in the production and titer. It can be costly, as low-titer production needs excessive financial funding and operational costs can become very high, with respect to energy and the required chemicals. Even the cost of extraction becomes higher, in order to get a high concentration of targeted products without impurity due to trace organic compounds having similar physicochemical properties. In the case of succinic acid, the recovery and purification can cost up to 60% of the total cost (Handojo et al. 2019).

Volatile fatty acids have charged anions and short to mid-chain volatile components, for example, butyric, acetic, lactic, and caproic acids can quickly move across an ion exchange membrane, like in one of the cases, it was an anion exchange membrane (AEM) (Wang et al. 2018). This approach was used for the microbial electrosynthesis to elongate C6-VFA to C8 (Varcoe et al. 2014). AEMs can be used for a variety of charged species like succinic acid as a chemical precursor. Product inhibition can be prevented under this circumstance, as the electrochemical extraction can be done directly from the fermentation broth (Andersen et al. 2015). Generally, the efficiency of the extraction process is directly proportional to the concentration of the obtained product.

One of the key challenges in the electrochemical extraction is that the product concentration must be sufficiently high to continue the removal with ease, however should be satisfactorily below the inhibition thresholds. Concentration inhibition for fatty acids in an anaerobic sludge has an inhibition threshold of around 15 g/L for short-chain volatile fatty acids and longer chain volatile fatty acids are more generally toxic (Yuan et al. 2019). With such high toxic values, extraction for mid-chain volatile fatty acids is problematic for practical bioproduction and thus requires a balance between production and recovery of these fatty acids (Reyhanitash et al. 2017, 2016; Ma et al. 2015; Outram and Zhang 2018).

4.4 Electro-Fermentation Based on Microbes

The EF technique can readily bag electrons for microbial fermentation using specialized electrodes. After oxidation of organic and inorganic acids, the electrons are already present in the waste matter through self-sustaining anodifilic microbes, and so the cost is not much (Kumar et al. 2018). Because of the redox reactions occurring, chemical energy can be brought back as the electrical power or utilized through other by-products (anode and cathode chambers can be separated using specific membranes) (Naqvi et al. 2018; Butti et al. 2019).

For all EF operations, the familiar principle is as followed. In the anode chamber, proton and electrons are generated through electrochemically active bacteria utilizing organic matter. And, via proton exchange membrane, protons are transferred to the cathode chamber where reduction occurs. In anode chamber, electrons are trapped by the anode and are transferred to the cathode compartment using an electrical circuit. In the cathode chamber, both protons and electrons are reduced by the oxygen molecule, acting as a terminal electron acceptor. Based on the number of compartments and operation types, EF can be well-thought-out into single-chambered, double-chambered (Fig. 4.2) tubular, stacked, baffled, up-flow system (Awate et al. 2017; Ishizaki et al. 2019; Rabaey et al. 2005; Liu et al. 2005). By doing other modifications, EFs can be more well-thought-out into the microbial desalination system (MDS), microbial electrolysis cell (MEC), and bioelectrochemical treatment system (BES) (Al-Mamun et al. 2018; Jain and He 2018; Kadier et al. 2016, 2017; Mohan and Chandrasekhar 2011; Kim 2010; Chandrasekhar and Ahn 2017; Animesh et al. 2016). Chandrasekar et al. studied the feasibility in the enhancement of the power generation using MFCs along with the acid elutriation fermentation and found that these are the promising processes for the simultaneous production of bioelectricity and waste treatment using piggery waste (Mohan and Chandrasekhar 2011; Chandrasekhar and Ahn 2017).

Specialized microbes are explored based on their electrochemical activities and divided into to, electrigen, exoelectrogen, and anode-respiring bacteria. It asserts that the microbes are capable of transferring electrons outside the cell membrane.

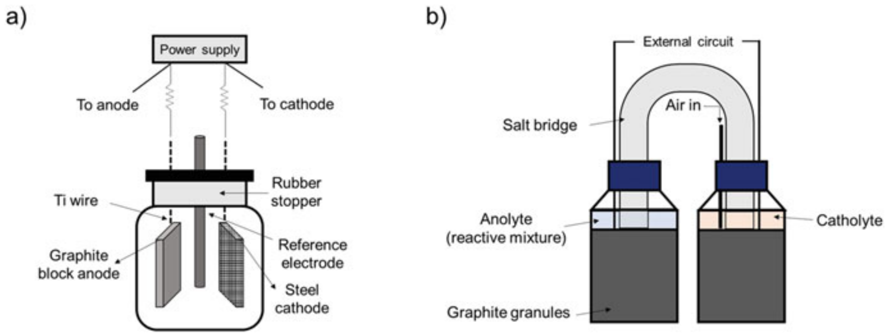

Fig. 4.2 Microbial fuel cells (MFCs). (**a**) Single chamber and (**b**) double chamber

Microbes classified as the exoelectrogen type are capable of producing electricity at the anode through electron generation. Here, it is apparently due to their ability to reduce metal. Endoelectrogen, in contrast, consumes electric current at cathode through oxidation of metals**.** They preferentially use either the membrane proteins/ structures like pili, filaments, cytochromes or otherwise secrete mediators; for example, *Geobacter sulfurreducens* takes advantage of its pili structures like conductive wires for inter-cellular transmission of electrons.

4.5 Potential Role of Electro-Fermentation

In the future, fermentation operations at the industrial scale do need to use the waste material as a potential substrate to obtain high energy products. It will also help to establish a significant platform for biomass utilization. The massive amount of residual solid biomass and wastewater, food waste remain in agro-food chains. In the biomass industry (sugar platforms), enzymes convert the biomass into five and six-carbon sugars.

Further, the remaining intermediate feedstock waste is processed using catalytic/ chemical/enzymatic/microbial/ operations to increase chemical and fuel production. Mostly, these processes demand high purity of the substrates, and thus, EF plays a vital role in this context. At the industrial scale, large amounts of organic waste is generated, which needs to be purified to maintain the sustainability of the entire chain.

Electrical current and potential can affect the metabolic activity of the organisms. Another vital point to look out for, is to solve the problem of fewer fermenters available, including electrodes for efficient EF. It is also essential to take note that for long term opertaions the use of surface-based technology when brought in close contact with complex culture broths, is more feasible. Additionally, a more cost-effective technology has to be devised to gain maximum efficiency out of EF. Recent advancements in the EF technology involving generation of electricity using EF from various wastes such as organic seems like an exciting avenue for future investigation. Such developments in the microbial synthesis, have opened the doors to produce useful chemicals from pure materials as well as bio wastes. To implement it successfully, a few issues regarding different range of pH, potential, and high resistance are necessary to be studied in more detail.

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Chapter 5
Electrochemical Losses and Its Role in Power Generation of Microbial Fuel Cells

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Abstract Microbial fuel cells (MFC) is an emergent source of renewable energy technology, where the microbes are incorporated in an electrochemical system with organic loads, for controlled production of electricity from wastewater due to the catalytic action of anode respiring microbes under the anaerobic condition. Considering the redox potential of electrochemical reactions, MFC with oxygen as terminal electron acceptor is capable to produce the potential of 1.2 V theoretically However, in real practice, the obtained voltage from MFC is too low s(500–650 mV) due to various electrochemical losses encountered in the MFC, which further affects the power density. The state of the art review focused in this chapter is on the electrochemical losses related hurdles and its role in power generation in the MFCs, which is limiting this technology to be adopted widely and considerably efficient. Though the power production from MFC is being impelled by various governing aspects such as the selection of microbial strains, substrate conditions, electrodes materials, and operating conditions, researchers have attempted various studies to overcome the electrochemical losses thus making MFCs ideal for real-time applications.

Initially, core fundamentals of electrochemistry associated with MFCs are discussed along with the concepts of electrochemical losses and its various possible forms, which edges the performance of MFCs with reduced output in much elaborate manner. Secondly, the concepts of electrochemical overpotential, power generation, different techniques for estimating the electrochemical losses along with the possible affecting factors, and strategies to reduce it are presented. The current perspectives and outlook of research studies focused on to overcome the challenges with the electrochemical losses are also presented.

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5.1 Introduction

The availability of a finite amount of fossil fuels is fast depleting and has become an unreliable and unsustainable source of energy. Research toward alternative, renewable, and sustainable sources of energy is carried out nowadays. One such technology is the Bio-electrochemical systems (BES), which uses bacteria for electricity generation using wastewater as a substrate. Among the Bio-electrochemical systems (BES), Microbial fuel cells (MFCs) have been regularly seen as one of the confident elective wellsprings of energy.

MFCs are bioreactors that transform the stored chemical form of energy from wastewater into electrical energy through electrochemical reactions carried out by microbes as biocatalysts (Jadhav et al. 2019). The fascinating property of MFC is that it can generate electricity and treat wastewater simultaneously (Zhao et al. 2009a; Bhunia and Dutta 2018).

A classic MFC comprises a positive electrode (anode) and a negative electrode (cathode), which are inserted in an aqueous solution in different compartments separated by placing the ion exchange membrane. Anodophilic bacteria, which have accumulated over the anodic surface forming a biofilm, oxidize the organic content of the feed/substrate, producing free electrons and protons. The electrons move toward the cathode through an external load, the protons are transfered via the ion exchange membrane and gets reduced in the cathodic compartment, by reducing the oxygen into water and current (Scott and Yu 2016). There is a group of microorganism that has the ability to transfer electrons extracellularly called the exoelectrogens or anode respiring bacteria or anodophiles.

When these exoelectrogens come in contact with the anode surface, extracellular electrons move through a couple of potential components such as mediated or indirect transfer of an electron, direct transfer of electrons, and transfer of electrons through microbial nanowires or pili (Harnisch and Freguia 2012).

A lot of approaches have been made to increase the rate of growth of the microbes and to decrease the start-up time of the MFCs, which in turn is believed to enhance the functioning of the reactor as a whole. Performance enhancement also includes the choice of the consortia, electrode positioning, and variations in the external load. Another way to amplify the power output is to change the external resistance, although this method has not been well exploited (Mathuriya et al. 2018). Hence the external load should be accordingly adjusted to acquire a maximum power density (Woodward et al. 2010).

The power generation of MFCs could be governed by numerous factors, namely reaction kinetics, operating temperature, internal resistance, the flow rate of fuel, and load. Electrochemical analysis is required to know the efficiency of the electrode and electrolyte used in a MFC construction. Many techniques are available to check the various electrochemical parameters in the working of the MFC, as mentioned in the following sections. Various electrochemical analytical techniques are used namely, Polarization curve, Cyclic Voltammetry (CV), Chronoamperometry (CA), Chronopotentiometry (CP), Current Interruption(CI), and Electrochemical Impedance Spectroscopy (EIS).

Internal resistance in MFC is possibly caused by various aspects like electrode resistance, resistance offered by the electrolyte, mass transfer resistance, charge transfer resistance, and Ohmic loss. A better perspective of the functioning of MFC is required to get rid of all the bottlenecks in MFC performance. In MFCs, bio-catalysis proceeds through microbial metabolic activity. The external resistance generally affects the bacterial population on the surface of the anode which brings about the changes in the metabolism. Recent researchers have found that the greater the external resistance the greater will be the biomass growth but lower will be the current generation.

A persistent problem encountered while evaluating the functioning of the MFCs through the polarization curve is power overshoot. This phenomenon occurs at higher current densities at the power density curve with the voltage and current descending rapidly resulting in low power than previously determined with the lower current densities (Ieropoulos et al. 2010). The overshoot of power could be overcome by permitting adequate time for the microbial biofilm to fine-tune to a variation in resistance via a single constant value of resistance for every cycle. MFC with matured biofilm may also show overshoot and this may be because the inoculum that was used might be with low conductive capacity (Winfield et al. 2011).

A range of struggles have been taken to advance the voltage of MFCs. A series connection with the MFC reactors can yield a good amount of voltage as done in the case of batteries. However, steady power cannot be obtained because of the voltage reversal. These limitations restrict the practical power applications using MFCs. But by using capacitors voltage output can be increased. Multiple capacitors are charged by MFCs in series and then discharged to achieve two goals: increasing MFC voltages and avoiding voltage reversal. By doing so there can be negligible power losses which in turn results in high energy production at higher potentials (Logan 2008).

Another approach may be the use of nanomaterials to reduce the detrimental effects of high internal resistances, voltage reversal, overpotential loss, activation loss, etc., and at the same time to increase the power generation which could probably make the Microbial Fuel Cell technology emerge into a vast source of electricity and wastewater treatment in the coming future.

5.2 Electrochemistry Associated with MFC

MFC system is one of the collaborative bridges between electrochemistry and microbiology sciences. It provides a flexible platform for both oxidation and redox reactions contributing to organic matter removal, pollutant degradation, and energy recovery. Advances in understanding the microorganisms and electron transfer phenomenon have increased the efficiency of the reactions.

In MFCs, the ion exchange membrane separates both the anode and cathode. The fuel is the solution comprising the microorganisms (Jadhav et al. 2017). Electrons transfer to the anode through the membrane or electron mediators, or by pili or nanowires synthesized by bacterial communities, or unknown (Logan and Regan 2006).

$$\text{Anode}: C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^- \ \left(E^0 = 0.3\ \text{V}\right)$$

$$\text{Cathode}: 6O_2 + 24H^+ + 24e^- \rightarrow 12H_2O \ \left(E^0 = 0.8\ \text{V}\right)$$

$$C_6H_{12}O_6 + 6O_2 \rightarrow 6CO_2 + 6H_2O + \text{Electric Energy}\ (E_{\text{cell}} = 1.1\ \text{V})$$

Electricity can be generated from the substrate in MFC when the reactions are thermodynamically favorable with negative Gibbs free energy. The difference in electric potential is due to different solutions and the flow of electron gives extra current and thus electrical power. Theoretically, MFC is capable to produce the maximum voltage of 1.1 V with an oxygen molecule as a cathodic terminal electrons acceptor. Due to these shortfalls, the actual potential output (V) is continually less than the theoretical potential (V) due to the various kinds of voltage losses contributed during electrochemical redox reactions.

$$V = E^0 - IR_{\text{ext}} - \eta a - \eta c$$

where, ηa and ηc are anodic and cathodic overpotential loss, R_{ext} external resistance, E^0 theoretical redox potential.

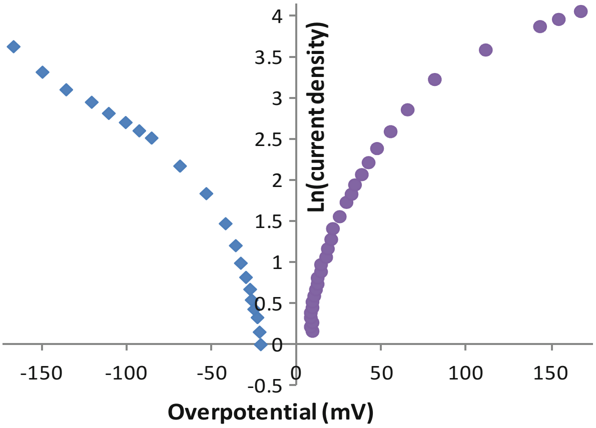
The flow of electrons in MFC allows us to drive the electrochemical reactions and leads to wastewater treatment as well as energy recovery. The electron transfer also regulates the metabolic activities of the microbial population in an anodic chamber and hence affects the performance of MFC.

5.2.1 Electrochemical Kinetic Model Equations

5.2.1.1 Butler–Volmer and Tafel Equations

The overpotential due to the charge transfer phenomenon can be regulated by the kinetics processes and the rate of heterogeneous electron transfer mechanism can be explained by Butler–Volmer equation. The condition where the reactant species remains profuse and the current is less means the concentration overpotential and Ohmic losses will be neglected (Jadhav et al. 2020b).

Fig. 5.1 Schematic diagram of Tafel plot



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

Here, I is current,

A, the projected surface area of the electrode
I_o, the exchange current density
a denotes charge transfer hurdle
n is the number of electrons that take part in the electrode reactions, and
Z_{act} is the charge transfer overpotential

Usually, the reactions over the electrode may follow more than a single simple step, and always there will be overpotential related to all the steps. The I_o is a key factor for the degree of electrooxidation or reduction of the chemical species at the region of the electrode under equilibrium condition; a high I_o denotes a faster reaction rate, while a lower I_o denotes a slow reaction rate.

The Butler–Volmer equation could be further shortened into the modified equation given below at the prevailing condition with the high overpotential region at '418/n' mV, where 'n' is the number of exchanged electrons which yield into the Tafel equation. The current density (I) and Tafel slope (b) are the most significant experimental parameters generally used to investigate the electrode reaction mechanism. The plot for overpotential versus log I_o is renowned as Tafel plot in Fig. 5.1

$$\forall_{\text{act}} = b\log_{10}(\frac{i}{i_o})$$

The values of I_o and b can be attained from the Tafel plots by extrapolating the linear region curve by assuming Z_{act} as zero (Liu et al. 2007; Zhao et al. 2009b).

There are various approaches that have been recommended with the goal of increasing the exchange current density (I_o) by decreasing the charge transfer overpotential. Some of them are (1) enhanced catalysts or biocatalysts, (2) the modification of the electrode to facilitate the direct electron transfer, (3) selection of electrode materials with high surface area, (4) optimum operational conditions to raise the rates of chemical reactions, (5) utilization of genetically engineered and effective exoelectrogenic microbial species after adaptation to enhance the electrode performance (Zhao et al. 2009b).

5.3 Electrochemical Losses

5.3.1 Transition/Temperature Losses

The performance of the MFCs can be reduced by temperature due to its influence on oxygen reaction degrees catalyzed by Pt over the cathode, microbial kinetics, and the mass transfer degree of protons across the solution.

Generally, the investigations on MFCs will be performed at higher temperatures of about 30–37 °C (Liu et al. 2005). If the MFCs reactors are used for treating the wastewater at the lower temperature, the operating cost will be reduced drastically. So, the information on the performance of the MFCs related to the temperature as a key function is insufficient.

The performance of anaerobic digestion process in the conventional anaerobic reactor is badly affected at the temperature below 30 °C. The Bruce effect of temperature study was done on the performance of the MFCs, surprisingly it was noted that there was only a minor drop in the power density by 9% while reducing the temperature from 32 to 20 °C (Liu et al. 2005).

The strategy of sampled-time digital control was employed on the MFC stacks operating on continuous mode with similar electrical and hydraulic connections, which got the benefit of intermittent operation mode with power-saving and it can also be used with the hybrid-series stack MFCs to avoid the voltage reversal issues. This controller got the scope of avoiding the perturbations and disturbances due to temperature effect, electrical loads, and concentration of substrate (Boghani et al. 2017).

5.3.2 Activation Losses

The charges that are released during the oxidation of fuel, as well as the reduction on the anode, require to overcome an energy barrier to initiate the transport. The activation overpotential is essential for the transmission of electrons released to the anode from the substrate through microbial shuttling phenomenon at the bacterial surface, which can either serve as a mediator for the transportation of electron to the anodic surface from the anolyte solution or as an electron acceptor in the cathodic surface. This activation overpotential can be delivered through the energy barrier.

At low current densities, activation losses always result in low polarization. However, at intermediate and high current densities, activation loss is dominated by the concentration losses and Ohmic losses. Though the activation losses can arise on the cathode and anode as well, the overpotential at the cathode is comparatively higher. Still, by increasing the exchanged current densities, it can be reduced. The below mentioned Tafel equation could be used for calculating activation overpotential.

$$\Delta V_{\text{act}} = A \times \log \left(i/i_0\right)$$

where, the activation overpotential (ΔV_{act}), the Tafel slope (A), the current density (I), and the current exchange density (i_0).

To achieve low activation loss, the following steps might be adopted: (1) increasing surface area and roughness of the anode; (2) by employing suitable mediators, which can pass through the cell membrane thus reducing intracellular activation losses or by using the microbes with conducting pili; (3) rising the operational temperature; and (4) enriching the biofilm over the electrodes (Logan et al. 2006a).

5.3.3 *Ohmic Losses*

Ohmic losses (also known as Ohmic polarization) refer to the resistance offered during the flow of charges across the (1) cation ion exchange membrane and electrolytes; and (2) electrodes and interconnections. The Ohm's law used to calculate the Ohmic losses is as follows:

$$\Delta V = I\, R_{\text{int}}$$

where current (I) and the total internal resistance (R_{int}). This phenomenon could be diminished through the usage of membrane with low resistivity or high conductivity (such as a 3D architecture graphite felt electrode which is reported to have comparatively low contact resistance than ionic resistance). Reducing the space between the electrodes can also be employed and if possible, a solution with a conductivity of maximum tolerable by the bacteria may also be adopted (Logan et al. 2006b).

5.3.4 *Bacterial Metabolic Losses*

For the process of metabolism, the transport of bacterial electron from the substrate which is at a lower potential to the electron acceptors namely oxygen or nitrate at a greater potential through the electron transport mechanism occurs. The anode in the MFC act as the acceptor of electrons, and its capability governs the energy gain intended for the microbes through its metabolic process.

The greater the dissimilarity in the redox potentials of the anolytic solution and the anodic half-cell potential, the possibility in metabolic energy gain will be higher for the microbes; unfortunately, the extremely achievable voltage from MFC will be lower. Consequently, the anode half-cell potential has to be retained lower for attaining a maximum voltage. The transport of electrons will be stopped if the anodic potential happens to be excessively low and a massive loss of electrons occurs during the high energy product generation. Several elements that can influence the microbial losses are (i) inoculum type, (2) microbe's composition, (3) microbe–anode interaction, (4) rate of substrate degradation by the microbial populations, and (5) number of active-substrate degrading microbes. The effect of a lower anodic potential and its probable impacts on the steadiness of power production require more attention for the future research (Logan and Regan 2006).

In MFC with electrochemically improved mixed bacterial strains, the various electron transfer interaction phenomenon and redox species could tip to the multifaceted electrochemical behavior. Further, studies indicate the capability of the microbial diversity on the anodic biofilm to acclimatize their metabolism techniques and the methods for the transfer of electrons due to the alterations in the smeared anodic potential for gaining efficiently the available microbiological energy (Schröder 2007).

The commonly used microorganisms in the MFCs studies are *Shewanella* sp., *Proteobactoria* sp., and *Pseudomonas* sp. In MFCs, for wastewater treatment, mixed cultures of microbes are preferably used (Sharma and Kundu 2010).

Substrate concentration has a substantial effect on the electrochemical functioning of MFC. At higher concentrations of substrate/feed, the substrate inhibition was found to reduce the electrochemical performance of the MFCs. To analyze the substrate inhibition effect due to substrate concentration, three bio-kinetic models such as Haldane, Tessier, and Aiba were preferred to fit the obtained experimental data ($R^2 = 0.98$–0.99).

While the performance of the MFC was highly reproducible, the phylogenetic studies of the exoelectrogenic biofilm associated with the anode had shown the population of microbes temporarily varied and sustained with higher biodiversity for an extended period. These outcomes suggested that the exoelectrogenic microbes in the MFC are both self-sustaining and optimizing. Due to that, they are capable to advance and continue with functional steadiness irrespective of instabilities in the carbon source and the consistent launch of competitive microbes (Ishii et al. 2012).

5.3.5 Concentration/Mass Transfer Losses

Concentration polarization or concentration loss arises while the mass transport rate of a chemical species from or to the electrodes hampers the generation of current. The reason for concentration losses may be due to the imbalance in the mass transfer of the feed/substrate and the product from the anode. Also, the overall generated current from the system may end up with the improved anodic potential and decreased cathodic potential or even vice versa. Typically it occurs at higher current density because of restricted mass transfer of chemical species which diffuse toward the surface of the electrode. The anode may experience concentration losses, either through the shortage of discharged oxidizing species from the surface of the anode or a meager source of reduced species over the electrode.

Consequently, the increase in the anodic surface area due to the ratio among the oxidized and reduced species may result in an increased anodic potential. But, on the cathodic side, a fall in cathodic potential may occur. Further, the buildup of cations and the oxidized products in the biofilm, can vary the redox condition and the microbial metabolic activity. The interruption in the transport of cations could develop higher pH gradients further among the electrodes which may end up with a substantial decrease in the output power. The most prominent contributing elements toward the concentration losses such as operational parameters and anodic designs can be characterized through the maximum polarization techniques (Logan et al. 2006a).

5.3.6 Voltage Reversal During Stacked Arrangement of MFCs

Focusing on the scaling-up process, the electrical energy generated by the MFCs may not be substantially improved through connecting all the reactors in series or parallel, which end up with the reversal of voltage and may result in reduced stack voltages (Jadhav et al. 2020a). For optimum and real-time applications multiple numbers of MFCs are essential, the MFCs stacks may produce worthwhile output energy but regrettably result in new challenges named voltage reversal. To reach the optimum level of performance, a huge number of research works has been attempted to know and address this challenge.

Through extensive research, among the three structural materials, Poly C was better in terms of suppleness to cell-reversal and power output. The PEM that utmost resulted with high energy output ranges to be generated for a single MFC was, in fact, that type brought about the utmost cell-reversal occurrences in stack MFCs (Ieropoulos et al. 2010).

The innovative idea of this work deals with making compartmental arrangements with the numerous units of MFCs instead of making single reactor of large volume. Hence, reduction in size of individual MFC unit resulted in high efficiency in harvesting the power with minimum power losses phenomenon. Through the series/parallel electrical arrangements of multiple MFC units can ensure the possibility to power the electrical and electronic gadgets for real-life applications (Winfield et al. 2011).

It is evident that the selection of miniature and multiple numbers of MFC reactors is an efficacious strategy for scaling-up. Through this attempt, the robustness and efficiency of this technology had been established by functioning concurrently 112 reactors with highly remarkable power generation for an extended period of time. The power output of the stack was decreased by electrical connections but enhanced by connecting the cells in parallel. In fact, the smart strategy of combining the cells in parallel and series is essential for real-time applications (Mateo et al. 2018).

As an attempt to intensify the whole stack voltage and current, six individual MFCs were coupled in serial or parallel connections. It ended with an open-circuit voltage of 670 mV and 4160 mV for parallel and serial connections, respectively. Nevertheless, the serially connected MFCs stack generated the power at a high voltage of 2.279 V (0.049 A), while the parallel MFCs stack provided an improved current of 0.269 A at 0.354 V (Aelterman et al. 2006).

An investigation was carried and reported on the reasons for reversal of charge and the influence of extended reversal on power production aspects by means of a single air-cathode MFC stack extensively. The reason behind the reversal of voltage was due to starvation of fuel and followed with the bacterial activity losses. The reversal of voltage mainly influenced the anode bacterial communities over the MFCs through a relative reduction in the performance of MFCs ensuing starvation cycle (Cheng et al. 2006).

A piece of better and detailed knowledge on the long-term influences related to the reversal of voltage on the bacterial biofilm for power production by MFCs stacks is very essential to enhance the generation of huge voltage in the MFC stack systems efficiently.

5.4 Polarization Curves and Overpotential for Power Generation

A perfect polarization curve of a power generating device like MFC consists of three major distinctive regions situated at various ranges of current either well varying, segregated, and substantially overlapped. The overpotential of MFC is the summation of non-linear overpotential of the anode and cathode and also the Ohmic linear internal overpotential of the MFCs (Figs. 5.2 and 5.3). Through examining the probable variations of both the cathode and anode at variable currents, the restrictive factor for performance can be obtained (Zhao et al. 2009b). The various overpotentials is shown in the Fig. 5.2.

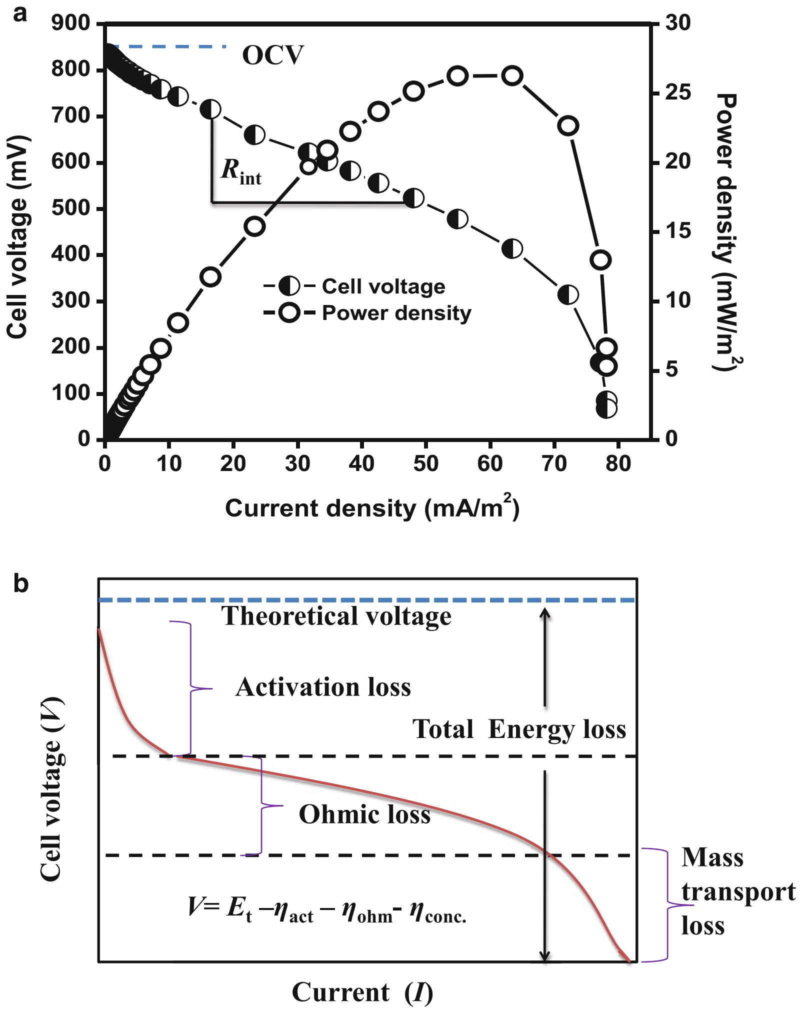


Fig. 5.2 (**a**) The ideal current–voltage polarization curve of MFC, and (**b**) the polarization curves representing various voltage losses

5.4.1 Charge Transfer Overpotentials

The region of charge transfer overpotential ($Z_{act,a}$ + $Z_{act,c}$) is positioned at lower current and arises after the irreversibility (slowness) of the reactions which take place over the surface of the electrode. This phenomenon relies on the electrode material's nature, activities of reactants, electrocatalysts, electrolyte with the electrons mediator, spectator species, biofilm, electrodes with microstructures, biocatalysts, and their metabolic breakdowns along with the operating condition like temperature (Zhao et al. 2009b).

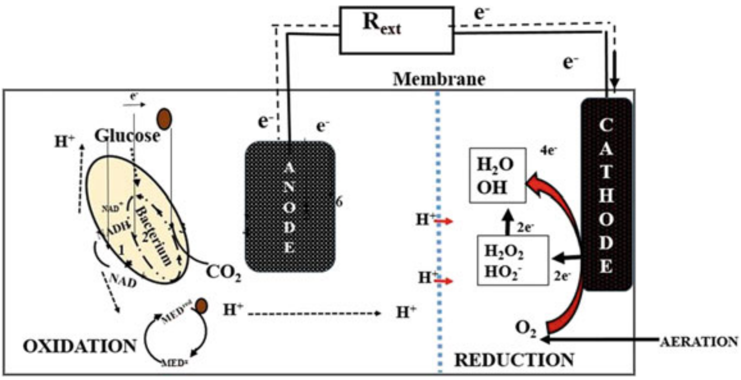


Fig. 5.3 Schematic diagram of overpotential losses in MFC during the transfer of electrons (1) Activation losses, (2) Ohmic overpotential, (3) Concentration overpotential, (4) Membrane resistance losses, (5) Electron acceptor-reduction losses, (6) Losses at cathode

5.4.2 *Ohmic Overpotential*

Ohmic overpotential usually evident themselves near the intermediate current region at the polarization curvature. These losses are triggered by the electrolyte's ionic resistances due to lower concentration of ions, biofilm, ion exchange membrane, interconnects, electrode's internal resistances, current collectors, the electronic gadgets used for measuring the performance and also the design of MFCs reactors (Zhao et al. 2009b).

5.4.3 *Mass Transport Overpotential*

Mass transport overpotential is the consequence of the alterations in the concentration of reactants or products near the interface among the surface of the electrode and bulk of the electrolyte. This phenomenon is predominant with greater current densities, in case of lack in the supply of reactants to the reaction zones of the electrode at the degree mandatory to endure the current production.

The occurrence of high concentrations in product species may end up with the decline of the reactant's transport during the process. Mass transfer related overpotential can be also declined on the basis of the geometry of MFCs, electrode's structure with biofilm, electrolyte, metabolites, and its products (Zhao et al. 2009b).

5.5 Techniques Used for Estimating the Electrochemical Losses

5.5.1 Electrochemical–Voltammetric Techniques

Voltammetry is an imperative technique used to examine the electrochemical reactions in MFCs. As a promising technique, there are several forms of voltammetry which include Linear sweep voltammetry (LSV), square wave voltammetry (SWV), different pulse voltammetry (DPV), and cyclic voltammetry (CV). Technically, the series of voltages or voltage may be applied to the electrodes and their responding current will be measured (Scott and Yu 2016).

5.5.1.1 Linear Sweep Voltage (LSV)

LSV encompasses the ramping or scanning of the working electrode's potential linearly at the time rates between 1.0–1000 mV/s. Any species like electrode's surface or solution which can be set to reduce or oxidize through the scanning potential range resulted into the waveform with a peak current (i_p) in the forward scan. This technique can be used to find the electrochemical reaction's kinetics on the basis of temperature dependency, the reaction rate constant, and electrocatalytic improvement kinetics.

Through LSV technique, three well-defined regions are witnessed namely (1) kinetically regulated charge transfer region with the current density values not influenced by the rate of mass transfer and also with independent rotation rate; (2) sloppy-mixed kinetic value and diffusion regulated region along with the current is partly regulated by the mass transport and electron transfer kinetics; and (3) mass transfer/diffusion regulated region. It is also known as restrictive current region which increases linearly (Narayanamoorthy et al. 2012).

This method is preferably employed with the slower sweep rate of less than 1 mV/s for delivering polarization data for the reactions at the pseudo-steady condition. Due to these reasons, this potentiodynamic technique is less used than the cyclic voltammetry (CV) technique.

5.5.1.2 Cyclic Voltammetry

Cyclic voltammetry (CV) is the universally used technique to examine the reduction and oxidation reactions in the MFCs. In this method, the direction of the potentials will be swiped among fixed two points and the resulting current will be documented (Table 5.1). The possibility of affecting the sensitivity of cyclic voltammetry outputs by the capacitive currents due to these normal and DPV techniques got evolved to decrease the issues related to capacitive current (Narayanamoorthy et al. 2012; Katz and MacVittie 2013; Vielstich 2014).

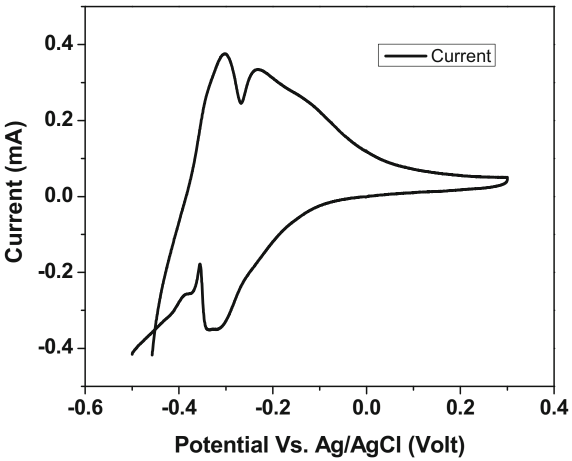
Table 5.1 Comparison of existing techniques for electrochemical analysis

S. No	Techniques	Advantages	Limitations
1	Polarization curve	Provides details related to losses in performances	The mechanism of underlying is challenge to analyze the various contributors for voltage drop overlapping and its estimation (Kashyap et al. 2014)
2	Current interruption	It can be used to find the internal resistances, simple to identify the magnetic and galvanic coupling effects which lead to errors during measurement (Kashyap et al. 2014)	The total Ohmic loss near the electrodes can be found, but unable to distinguish the mass transport, transfer of charge, and other types of Ohmic losses (Kashyap et al. 2014)
3	Cyclic voltammetry	Used extensively for calculating the electrochemical redox reactions	Capacitance and background charging currents are the major challenges and peak Faradaic current against the charging current declines with an increase in voltages (Kashyap et al. 2014)
4	Square wave voltammetry	It can distinguish against the charging current and eliminate a large capacitive background current than the rapid scanning CV (Scott and Yu 2016)	It can detect range as lower as 108 mol/dm^3 (Zhao et al. 2009b)
5	Different pulse voltammetry	This technique may overcome the major limitation in both CV and LSV techniques due to significant background intensities from non-Faradaic and capacitive currents (Zhao et al. 2009b)	It creates practical problems related to the stability of the materials and cell operation at any larger scale (Scott and Yu 2016)
6	EIS	Distinguihes the various contributors like mass transport, charge transfer, and electrolytic resistance which leads to internal resistances (Kashyap et al. 2014)	Suitable for steady or quasi-steady state MFCs and not suitable for the simple fitting models (Kashyap et al. 2014)

For CV studies over the MFCs could be extensively employed to examine: (a) the phenomenon of electron transfer among the biofilm and electrode, (b) the redox potentials among the biological and chemical species, and (c) electrocatalyst's performance (Zhao et al. 2009b).

For CV analysis over the electrodes microbial biofilm of the MFCs, it is necessary to have a triple-electrodes arrangement which includes reference electrode (RE), working electrode (WE), and counter electrode (CE). A linearly reliant potential in relation to RE can be imposed over the working electrode (WE). A CV scan may begin at the initial potential (E_1), then continues to a final potential (E_2) and once again it will reach the initial potentials. The scan rate (v) is the variation of potentials with time as an element through the gradients curves shown in Fig. 5.4.

Fig. 5.4 Schematic of cyclic voltammetry



Initially, there will not be any current flow except the capacitive background current while we increase the voltages from E_1 to E_2. The current starts to rise up to extreme value from a definite potential due to the oxidation process over the surface of the electrode and finally falls down. For an anodic oxidation process, i_{pA} is the peak current during maximum current and hence the anode peak potential (E_{pA}) is the peak potential where the maximum current ensues with the respective potential.

Later reaching E_2 along with the reduced potential return to E_1, the corresponding reduction reactions may lead to progress. Finally, the cathodic peak current (i_{pC}) and the related peak cathode potential (E_{pC}) could be found from the graph (Harnisch and Freguia 2012).

5.5.1.3 Square Wave Voltammetry (SWV)

The technique of Square wave voltammetry (SWV) is being used to understand the mechanistic and the kinetic enquiries. This technique is adopted from the principle of well-known Oster young square wave. By means of the waveform, the response of current is tested to the excited potential, during every forward pulse and reverse pulse. By using this method, three conceivable current potential plots could be made, which are (1) forward current against potential, (2) reverse current against potential, and (3) difference current against potential, which are usually employed in the majority of the analytical purposes.

The subsequent voltammetry graphs produce peaks for every electrically active species that are existing and also relatively proportionate to the concentration of the available species in the bulk of the solution. The major advantage of this technique is its ability in distinguishing the charging current and also can eradicate a large capacitive background current than the rapid scanning CV (Scott and Yu 2016).

The SWV technique can detect a range as low as 108 mol/dm^3 when employed as an electrochemical analytical method to find the characteristics and intensities of electrochemically active metabolites even at trace levels (Zhao et al. 2009b).

5.5.1.4 Different Pulse Voltammetry (DPV)

This is a modified and advanced voltammetry technique with a developed sensitivity than the CV and LSV techniques. Most remarkably, these techniques can be used to overcome the major limitation in both CV and LSV techniques due to significant background intensities from non-Faradaic and capacitive currents (Zhao et al. 2009b).

Improved refinement of Faradaic currents which are the transfer of electrons to and from electrodes could be achieved by means of DPV. Further, the possible perturbation comprising smaller pulses will be overlaid like a staircase waveform. DPV analysis can also be used for enhanced selectivity in perceiving diverse redox processes than the LSV and CV techniques (Zhao et al. 2009b).

The CV technique is engaged to evaluate the electrochemical catalytic nature of different electrode materials at various phases of microbial growth and fermentation with polyaniline modified platinum anode dipped in a stirred anaerobic culture of *Escherichia coli K* strain. Under the fermentative situations, the electrode had shown high electrochemical catalytic activity, virtually with constant potential in the range of 100–600 mV. Unfortunately, due to the development of a PtO layer at the Pt surface the activity was reduced with the resultant positive potentials. The DPV method exhibit practical issues related to the material's stability and the operation of the MFCs at several larger volumes (Scott and Yu 2016).

5.5.2 *Electrochemical Impedance Spectroscopy (EIS)*

EIS is an influential, non-noisy, and semi-quantitative strategy to analyze the proper functioning of bio-electrochemical frameworks like MFC. Certainly, by using meaningful equivalent circuits the quantitative understanding of the impedance information can be acquired. Determination of power using this systems offer higher internal resistance. The influence of various resistances to the general internal resistance of the system can be determined by using EIS. EIS has been utilized exhaustively in the majority of the MFC reactors which helps in novel designs of MFCs in achieving higher power generating capacity (Sekar and Ramasamy 2013).

EIS exploits the enormous range of time scales over which various procedures happen in the MFC being considered. It is a tool for inspecting substance and physical procedures in arrangements, at solid-liquid and at solid-solid interfaces, as it permits separation of the adaptable voltage loss marvels. Intensive data on the internal Ohmic losses, charge, and mass exchange impedances can be obtained from the EIS spectra (Zhao et al. 2009b).

5.5.2.1 Impedance Based on Electrochemical Parameters

The role of impedance is high particularly in a profoundly heterogeneous framework like MFCs. The electrochemical interface in MFC is incredibly multifaceted where the microorganisms colonize over the anode, creating the electrochemical response to be convoluted. EIS is an efficient strategy to inspect the elements of the bound or portable charges in the mass or interfacial area of the framework. The fundamentals and essential hypothesis of impedance depend on the electrochemical parameters which are discussed in the following sections.

Electrolyte Resistance

Electrolyte resistance in an electrochemical cell alludes to the resistance created by the electrolytic solution. The electrolyte resistance relies upon the particle type, temperature, ionic fixation, and the anticipated surface area over where the current gets conveyed. Then again, the suspended microorganisms accessible in the anolyte can likewise prompt the electrolyte resistance. However, this can be illuminated by associating two resistors in parallel associations at the electrolytic level (Laurent et al. 2001; Sekar and Ramasamy 2013).

Double Layer Capacitance

The electrical double layer is also referred to as charge separation which may occur at any interface of the polarized systems like the electrode's interface, electrolytic solution, and proton exchange membranes, etc., similar to the capacitor present in the electrical circuits. Similarly, the division of charges additionally occurs over the film of microbes for keeping up the proton motive force process for the metabolism. The articulation for the double layer capacitance had been exhibited by Helmholtz's, Stern, Guoy–Chapman, Grahame models, and so on (Marke and Tribollet 2008; Sekar and Ramasamy 2013).

Polarization Resistance

The electrode is viewed as polarized at the condition where the applied electrode's potential is unique from the equilibrium capability of the electrochemical responses at the highly polarized electrode which may prompt oxidation/reduction of the species close to the anode surface. If there should arise an occurrence of two basic actively managed responses, the cell's potential is identified with current and it very well may be effectively determined by utilizing the easy Butler–Volmer connection. (Sekar and Ramasamy 2013).

Charge Transfer Resistance

The exchange of electrons from the ions accessible in the electrolytic solution for the solid metal electrode is referred to as charge transfer response; it depends on the concentration, chemical reactions, temperature, and the reactants potential. The charge transfer resistance of electrochemical systems can be computed by using the Butler–Volmer equation at the condition of complete stirring in the systems which favor the charge transfer kinetics (Sekar and Ramasamy 2013).

Warburg Resistance

In an electrochemical system, the role of diffusion is crucial because of the mass transfer from the electrolytic solution (bulk) through the microbial biofilm. During the electrochemical processes the provided substrates should pass through the anodic biofilm, undergo oxidation, and the generated products must be diffused back to the bulk part of the electrolytic solution (Sekar and Ramasamy 2013).

At the low AC frequency, the diffusion is significant, while at high AC frequency, the impedance because of crossover/diffusion can be neglected, in the meantime the reactants could not have an adequate time frame to go from bulk to the outside of the electrochemical responses. Subsequently, the estimation of the impedance determined by diffusion requires the amendment of a diffusion element in the equivalent circuit model. The component which represent the semi-infinite linear diffusion is referred to Warburg impedance (Marke and Tribollet 2008; Sekar and Ramasamy 2013; Michalska et al. 2017).

5.5.2.2 Various Plotting Techniques

Nyquist Plot

Nyquist plot and Bode plot are utilized as plotting techniques for calculating the prevailing impedances in an electrochemical system. In the case of Nyquist plots, the X-axis is the actual area for impedance, Y-axis is the imaginary area of the impedances, and finally all the points in Nyquist plot relate to the impedance at a single frequency (Sekar and Ramasamy 2013). The major constraint of Nyquist plots is that they cannot display the denoted frequency by individual data points, which are the portrayal of the impedance vector in the complex planes at a particular recurrence (frequency) (Zhao et al. 2009b).

Bode Plot

One of the drawbacks of the above referred Nyquist plot is that the recurring frequency cannot be known from the graph. By utilizing the Bode plot the above said constraint can be evaded, where the impedance can be plotted alongside the logarithmic recurring frequency on the X-axis against the absolute values of both the impedance and the Y-axis phase angles (Sekar and Ramasamy 2013).

Through the Bode plots, frequency data are represented as a plot with the magnitude and the impedance vectors with the phase angle against frequency (Zhao et al. 2009b).

5.5.2.3 Equivalent Circuits for Impedance

The optimal power outcome from the MFCs is mainly restricted by the developed high internal resistance within the electrochemical systems. To analyze the various contributing components for the internal resistance, a suitable method like EIS could be utilized. The EIS of reactors can be examined through the equivalent circuits comprising the components like resistors, inductor, capacitor, Warburg constant, and constant phase element (CPE), etc. (Sekar and Ramasamy 2013).

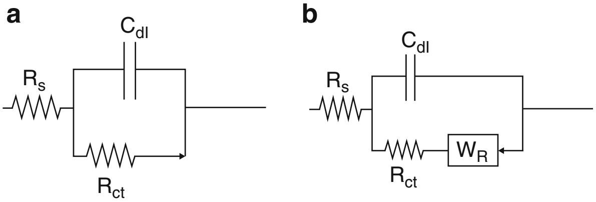
Randles Cell Circuit

A simplified cell model which is being used commonly is the Randles circuit model and it is the basic model to start the other unknown complex models. The basic Randles model comprises a double layer capacitance, solution or Ohmic resistance, and a polarization transfer resistance (Sekar and Ramasamy 2013).

The equivalent circuit for an electrode inserted in the electrolytic solution will be the double layer capacitance (C_{dl}) that is parallel to the charge transfer resistance (R_{CT}) and then, these parallel combinations will be connected in series for solution resistances (R_{Ω}) (Fig. 5.5a).

The resulting Nyquist plot may consist of a semicircular arc that intersects at the real axis (Z′) of the plot at two places. The intercept nearby the origin provides the solution resistance (R_{Ω}) and the farthest intercept away from origin provides the entire resistance ($R_{\Omega} + R_{\mathrm{CT}}$) of MFCs (Sekar and Ramasamy 2013).

Fig. 5.5 Schematic diagram of (**a**) Randles cell circuit; (**b**) mixed control circuit



Mixed Control Circuit

The equivalent circuit for an electrode inserted in the electrolytic solution and assumption of diffusion of reactants through the biofilm will be the double layer capacitances (C_{dl}). It is parallel to the charge transfer resistance (R_{CT}) along with Warburg impedance (W_R). These parallel combinations will be arranged in series in case of Ohmic or solution resistance (R_{Ω}) (Sekar and Ramasamy 2013) as shown in the Fig. 5.5b. Likewise the insertion of Warburg component to this basic Randles circuit model may end up like a semicircle with an extended arm.

5.5.3 Chronoamperometry

The chronoamperometric technique is convenient to calculate the diffusion coefficients in order to investigate kinetics mechanisms. Through CA all this information can be obtained precisely than the resultant CV technique. The working electrode's potential will get stepped and at the electrode region, the current generated through the Faradaic process can be monitored as an element of time. The relation of peak oxidation current against the peak reduction current can yield the inadequate information on the distinctiveness of the electrolyzed species. Nevertheless, as like other pulsed systems, CA produces excessive charging currents, which decline with time exponentially to the resistance–capacitance circuit.

CA can be employed often to produce factual electrochemical polarization information at a steady state by altering the applied potentials and recording the resultant current once the steady state is achieved. This phenomenon is overwhelmingly crucial for reactors with moderate reaction times, on account of MFC reactors (Scott and Yu 2016).

The Faradaic current, which is due to electron transfer mechanism decreases as clarified through Cottrell condition.

Through an extensive study, MFC reactor with *Geobacter sulfurreducens* inoculum, electron donor, and with a graphitic anode yielded an oxidizing electrode potential at 0.2 V against the silver electrode. The cells attached to the electrodes generated current for weeks even when the substrate was substituted by an anaerobic buffer deficient in nutrients. This is the first study on the microbial electricity production solely by cells attached to an electrode (Bond and Lovely 2003).

During a study, activated carbon cloth (ACC) and carbon fiber veil anodes were compared to understand the oxidation currents for sulfide created at anode potential of +0.2 V against silver electrode. Finally, the ACC anode with high porosity gave significantly enhanced performances than other electrodes (Zhao et al. 2009a, b).

The other dynamic techniques like chronopotentiometry and Chronocoulometry are similar to Chronoamperometry; however, can yield excessive details about the electrochemical systems (Cheng et al. 2006; Scott and Yu 2016).

5.5.4 Chronopotentiometry

Chronopotentiometry (CP) technique is used to investigate the developing potential with time as an element at an electrode operative with a persistent current which is just reverse to Chronoamperometry. Simple and effective research was done by using this edifying technique on air-cathode carbon cloth electrodes coated with layers of polytetrafluoroethylene (PTFE). A constant current was applied over the layered carbon cloth electrodes to measure the electrode potentials. Subsequently resulted potentials were then plotted to get the electrode performance as an element of current density. Coulombic efficiency of about 42% was obtained when employed with a four-layer-PTFE coated electrode (Cheng et al. 2006; Zhao et al. 2009b), which is deemed to be a reasonably good performance.

5.5.5 Current Interruption

Current Interruption (CI) methods can be applied significantly for determining the Ohmic resistances of MFCs. The main principle of CI method is to detect the resulting voltage transients by interrupting the current flow.

By this method, the Ohmic overpotential will be parted from other overpotential losses since the previous is a close instant process, while the resting time for other types of voltage losses occurrence is pointedly longer. The major drawback of this technique is that very less period (<10 ms) measurement of the perturbations in the MFCs is essential for the precise and definite outcomes. Also if the data is not collected rapidly, then there is possiblity of overestimation of the voltage change (Zhao et al. 2009b).

The MFC must be worked in a relentless state conditions where there ought not to be concentration loss. So to apply the method, the electrical circuits must be opened, delivering zero flow of current. This is sought after by an increasingly slow ascent of the potential which may in the end achieve the Open-circuit voltage (OCV). Ohmic losses are proportional to current, because of this if there is interference of current. Ohmic loss disppear instantaneously.

This yields the steep rise in the electrode potential (E_R) which is proportionate to resistance (R_Ω) and the current (I) which was generated before the current interruption. The electrode overpotential which occurred through current generation can be witnessed by the slow increment of the additional potential (EA) as it advances the OCV. This yields the precarious ascent in the electrode potential (ER) which is corresponding to obstruction (R_Ω) and the current (I) which was created before the current interference. The electrode overpotentials during current production is indicated by the slow increment in the potential as it progresses the OCV (Logan 2008).

5.5.6 Rotating Disk Electrodes (RDE) and Rotating Ring Disk Electrode (RRDE)

RDE and RRDE are the Hydrodynamic voltammogram techniques used to perform the electrochemical experiments which are vital to examine the accurate kinetics parameter of electron transfer and of electrochemical reaction processes with enzyme electrodes. The advancements in the voltammetry techniques were attained by Levich et al. with these novel Rotating Disk Electrodes (RDE) and Rotating Ring Disk Electrode (RRDE) for electrochemical studies.

These techniques are being applied to evaluate the electrocatalyst or tailored electrode's performance and also for calculating the number of electrons that have participated in Oxidation-Reduction Reaction (ORR). The preferred cathode process is the reduction of oxygen molecules to water, unfortunately partial ($2e^-$) ORR may remain over carbon-based electrode and finally result with the production of highly reactive species of hydrogen peroxide (H_2O_2), which might disturb microorganism's metabolism. RRDE setup include a RDE with an added ring-type electrode balanced at an independently regulated potential to find the products including peroxide species of the electrochemical responses taking place at the Centre of electrode's disk (Zhao et al. 2009b).

5.5.6.1 Rotating Disk Electrodes (RDE)

The Rotating Disk Electrode (RDE) contains a planar plate made of the electrode material which might be spinning at a steady speed around a focal axis vertical to the plate (disk). At the hour of examination the pivot of the plate may draw the liquid up and throw it out over its surface radially. Therefore, laminar stream at a RDE delivers constant flows of material from the bulk solution to the outside of the electrode. The bulk solution at a distance from the electrode remain mixed due to rotation whereas the fraction of the solution near to surface of the RDE rotate along with the electrode (Scott and Yu 2016).

A study was reported with the electrochemistry details of a single microbe which was electrochemically not active but conductive electronically over the surface of the electrode. The oxidation of hydrogen has been analyzed by using electrode tips comprising of the well-defined size of Pt nanoparticles (Chen and Kucernak 2004; Zhao et al. 2009b).

5.5.6.2 Rotating Ring Disk Electrode (RRDE)

RRDE is comparative like the rotating disk electrode, yet it will have a second electrode which is a concentric ring electrode situated outside the plate and used to locate the "seemingly perpetual" species delivered over the plate. The ring is by and large electrically protected away from the plate so that the possibilities can be controlled freely. The RRDE is utilized to sense and discover the improvement of any intermediates, which are electrochemically dynamic themselves over the span of electrochemical reaction (Albery et al. 1969).

The collection efficiency and transit time are two major parameters which characterize the geometry of RRDE. From the magnitude of ring and current generated at the disk electrode, the details of the collection efficiency of the electrode reactions may be obtained (Albery et al. 1969).

This technique may not be always used to enquire the electrochemical behavior of biofilm over electrode surface only due to the fragile nature of biofilm and destined to be harmed under the state of high revolution speed (Chen and Kucernak 2004; Zhao et al. 2009b).

5.5.7 Assorted Techniques

There is a wide scope of a few other electro analytical procedures which could be conceivably of help in the investigation of MFCs electrochemical properties. The understanding of the fundamental processes happening in the Square wave voltammetry (SWV), which has detection range as lesser as 10^{-8} can be determined by the nature and concentration of the available electroactive metabolites even at trace levels (Scott and Yu 2016).

AC voltammetry is similarly another method, which is accepting immense interest in the field of electrochemical examination and may eventually get wide uses in MFCs (Zhao et al. 2009b; Scott and Yu 2016). Some of the most crucial and highly potential assorted techniques are mentioned in the following sections.

5.5.7.1 Electro Analytical Technique

Electrochemistry gets combined with other analytical techniques like fluorescent in situ hybridization, microarrays, and microscopy analysis which can deliver noteworthy insights regarding the behavior of DNA molecules, electroactive microorganisms, and other microbes species which are existing in the MFC system being explored (Zhao et al. 2009b; Scott and Yu 2016).

5.5.7.2 Electrochemical-Quartz crystal Microbalance Technique (EQCM)

This is a non-destructive online monitoring technique which can be used to find the most essential details of the biofilm development over the provided surface of the electrode, and also be used to get the very precise information on the biofilm–electrode interfaces (Scott and Yu 2016).

5.5.7.3 Electrochemical-Spectroscopy Techniques

Electrochemical-Spectroscopy Techniques facilitate an in vivo understanding of the structure of the molecule, electrons transfer phenomenon, and the details of interactions between the microbes and electrodes. The direct surface current improved the interface among a gold electrode and *Geobacter sulfurreducens* bacteria which was studied through the infrared absorption spectroscopy and subtractive interfacial Fourier transform infrared spectroscopy. Through this analysis, the IR spectral bands reveal the information about the presence of an increased amount of proteins at the interface with time to the ascent in the current. It consequently showed that the extracellular cytochromes were liable for the exchange of electrons to the electrode (Zhao et al. 2009b).

5.5.7.4 Microelectrode Technique

Microelectrode technique is one of the most potential method that is capable of eliminating the influences of Ohmic resistance and mass transport restrictions phenomenon during an electrochemical analysis. This technique can be generally well suited for the in-situ assessments of the accessibility of the conductive pili (nanowires) which favors the direct electrons transfer in MFCs (Zhao et al. 2009b; Scott and Yu 2016).

The oxygen reduction reaction (ORR) at the area of the anode in a MFC is distinguished to be an explanation behind a restricted increment in the pH. For all intents and purposes, it is trying to find the definite separation between the estimation point and the cathodic electrode. A study was detailed by utilizing location sensor appended with a miniaturized scale pH electrode for deciding the separation during the pH circulation estimations. It was additionally expressed that, without the anolyte/substrate, the pH expanded to a degree that falls in the scope of 0 to 0.8 mm from the cathode material and arrived at a pH of 7.2, while with the anolyte/substrate, the pH unexpectedly improved inside the scope of 0 to 1.4 mm and accomplished 9.3 (Motoyama et al. 2016).

5.5.7.5 Denaturing Gradient Gel Electrophoresis (DGGE) and Polymerase Chain Reactor (PCR)

DGGE is one of the electrophoretic system which has become a standard procedure in the territory of environmental microbiology for portraying the microbial populace arrangements and its biology. In MFCs the development of microbial networks over a period has been seen by utilizing DGGE alongside the polymerase chain reaction (PCR) for intensifying the 16S rRNA fragments of microbes (Zhao et al. 2009b).

Several studies had reported that *Geobacter* and *Shewanella* species had been confirmed to be an electrically active microorganism through direct transfer of electron. Most interestingly, the examination of the bacterial communities present in the anodic biofilm showed a variety of microbes which are maximum in numbers than the iron reducing bacteria. Further, it was shown that, there are possibilities of existence of many other unique microorganisms to be augmented in the reactor. The forms of microbial communities which are found in MFC reactors depend intensely on the available feed and other ambient parameters and no other distinctive consortia of microbes has been identified from the clones archives till now (Logan and Regan 2006; Clauwaert et al. 2008; Zhao et al. 2009b).

5.5.7.6 Confocal Laser Scanning and Atomic Force Microscopy

Confocal Laser Scanning Microscopy (CLSM) and Atomic Force Microscopy (AFM) are the high-resolution and most dominant sorts of microscopy. In fact these techniques are not coming under the category of direct electrochemical analytical techniques but they are adding merits because they are highly capable of providing the complementary information on electrochemical nature of electrode materials.

It is evident that through the CLSM and AFM techniques, the images of the conducting substrates and pili of the microbes can be seen in MFC research works, specially, the pilus or pili of *Geobacter* species were recognized (Reguera et al. 2006). Due to these methods, the occurrence of nanowires or pili ensures the chances of longer-range direct electron transfer phenomenon from the microbes to the anodic region.

A combination of both AFM and electrochemical analytical techniques has been established in the recent days to know the role of conductive channels for ions available in the ion exchange membrane (Zhao et al. 2009b).

EIS and CLSM has led in the remarkable understandings of the process of biofilm development of both mixed and co-culture through various staining techniques (Stöckl et al. 2016).

5.5.7.7 Twin: Working Electrode System (Twin-WE)

The twin-working electrode system helps in understanding the mediator-dependent transfer of electrons from microbes or creating the redox slopes which occur due to biofilm development (Hassan et al. 2017).

This technique is used for detecting and estimating the different quantities of electron arbiters present in a small scale microbial systems.

This methodology encouraged the creation of relentless state current sign which encourages the more point by point data on the mid-potentials and concentrations of two model chemical substance like hexacyanoferrate (HCF) and riboflavin (RF) inside the slender layer system. This Twin-WE strategy was used for sensing the soluble mediators removed by live *Pseudomonas aeruginosa* strain (Hassan et al. 2017).

5.6 Factors Influencing on Electrochemical Losses

There are various elements affecting the ideal functioning of MFC such as selection of substrate, a better understanding of biofilm formation with the exoelectrogenic bacterial communities, reactors design which ensures less internal resistance, and the operational condition parameters.

5.6.1 Based on Operational Conditions

The optimal performance of MFC is reliant mainly on the operational conditions like temperature, pH of the anolyte and catholyte, electrode's potential effects, and the connected external loads to the system.

5.6.1.1 Temperature Effects

In an investigation on effect of temperature, it was revealed that biofilm was not formed at lower temperatures like 15 °C. Eventhough it is demostrated that the favorable condition for biofilm is 35 °C it is reported that it is also formed at 22 °C. It was further revealed that temperatures above 50 °C lead to irreversible biofilm deactivation (Patil et al. 2010; Sun et al. 2014).

An investigation revealed that there was a rise in the current density with increase in temperature and also there was diminishing of the lag phase with the temperature's increments. Further, temperatures from 0–10 °C came about with reversible inactivation of microbial biofilm of almost 90%; however, temperatures above 50 °C brought about irreversible inactivation phenomenon (Sun et al. 2014).

5.6.1.2 pH Effects

The influence of pH on the anodic chamber has been studied by using as microelectrode measurement system for biofilm development by changing the pH of the anolyte and various microbial communities namely *S. oneidensis* and *G. sulfurreducens*. It was reported that, pH in the bottommost of the microbial biofilm reduced against the development time because of escalation of proton movement at the biofilm and the impedance values at the anode with open-circuit voltage exposed that the two microbial communities desired a pH of 7 ± 1 (Babauta et al. 2011).

MFCs operated in a neutral pH environment resulted in an power overshoot phenomenon once the anode had been subjected to acidic pH of 3 for around 24 h. The variations in the conductivity of the catholyte and the pH had also affected the power output but there was no overshoot as in anodic phenomena (Winfield et al. 2011).

It was reported with a power density of 5000 mW/m^2 and by maintaining at pH of 1 in the cathodic chamber was 2.5-fold higher power output achieved with the same electrodes in an air-cathode MFC at pH 7.5. This work further illustrated the advantages of maintaining catholyte chamber with lower pH where the hindrance by mass transfer can be counterbalanced (Erable et al. 2009).

The influence of the anode's pH in the primary sludge aimed at the power generation was confirmed and further said that, the prolonged current generation with the higher power density was noticed by the supplement of buffer solution to the primary sludge (Vologni et al. 2013).

5.6.1.3 Anode Potential Effects

The anodic half-cell potential effects in MFC reactors can be studied by using a potentiostat to understand the electrochemical abilities of the anodic biofilm with the bacteria reducing the ions. It was reported that the higher current with quick start-up time may ensure the optimum anodic potentials. Further, a review stated that 14 studies have exposed an enhanced performance of 71% against the anode potential, while 14% with reduced performance. The performance was unrelated to the genera of microbes and the electrode materials (Wagner et al. 2010).

The electron liberating capability of bacteria can be influenced by the anodic potential, because the extracellular electrons transferal rate is regulated through the variance in the potentials among the final electrons transporter and the anode irrespective of the mechanisms. The applied potential over the anode material may favor the development of microbial communities that may result in higher current output. It was further reported that the usage of poised potentials in the course of the initial start-up phase facilitated to improve the functioning of the reactor by enhanced outputs of power and efficiency of COD removal (Srikanth et al. 2010).

The influence of the anodic half-cell potential over the power shoot phenomenon was investigated and reported that reduced external resistance resulted with decreased power and current. It is also stated that a deficiency in electron transfer mechanisms may favor the power overshoot issues and can be prevented by an anodic potential start-up above −0.03 V (Zhu et al. 2013; Sun et al. 2014).

5.6.1.4 External Loads

To emphasize the crucial role of the external loads, a study was conducted and reported that lowered external resistance had ended up with the steady rise of both the kinetic capabilities of the microbes and the incessant current production. The current generation can be increased with the increase in loading rates, the low external resistance is preferable which might be equal to its internal resistance of the cell (Aelterman et al. 2008).

A highly projectable work was reported regarding the necessity of connecting to a fixed resistance for a long time to ensure the highly stable current production by microbes in MFCs. Further, even moderately slower linear sweep voltammetry scan rates with the prolonged time among switching the circuit load during the fed-batch of operation could yield imprecise polarization and power density curve of the MFC reactors (Watson and Logan 2011).

The most promising study on the influence of the various external loads over the anodic microbial communities was revealed by employing Denaturing gradient gel electrophoresis (DGGE) and Polymerase chain reaction (PCR) amplification techniques. By these techniques the 16S rRNA gene fragments were amplified and exposed the availability of distinct variances in the anode electrogenic bacterial communities, mainly due to the higher current densities, in other words, the low external loads. The yield of biomass in the cell was lower than other anaerobic treatment processes, when it is operated at low external loads. The external load directly influences the biomass yields, COD elimination efficacy, and the production of current (Katuri et al. 2011).

5.7 Strategies to Reduce Electrochemical Losses

5.7.1 Selection of Electrode Materials and Assembly

The stable functioning of the MFC reactors rely on the selection of electrodes materials and assembly due to the fact that the anode materials favor the bacterial growth for the enhanced transfer of electrons with major requirements like high conductance, bio-compatibility, chemically non-reactive, and less costlier. On the other hand, the cathodic electrode materials are also similar to the anodic materials, but it should possess higher overpotential losses because of prevailing lower redox reactions while using the dissolved oxygen as an electron acceptor. The most conventional method to decrease the overpotential losses is to paint the carbon electrode surface with metallic electrocatalysts like Pt (Liu et al. 2005).

An extensive research work on the carbon electrodes modification for enhancing the MFC performance had reported that coating 4(N, N-dimethylamino) benzene-diazonium-tetrafluoroborate over the carbon cloth will lead to enhanced adhesion of the bacterial communities on the electrode’s surface.

The importance of gas diffusion layers over the cathode material and its influence on the bacterial adhesion on the anodic material was experimented and reported that the MFCs with multiple diffusion-layer coatings over the cathode had resulted with the richness of exoelectrogenic bacteria on anodes due to less oxygen crossover in the anode chamber (Butler and Nerenberg 2010).

An noticable work have been carried out on connecting multiple membrane electrodes assemblies of MFCs in series connection. This study reported that while connecting several membrane electrodes in the arrangement of microbial fuel cells (mMEA-MFCs) with the external load, even though shown the voltage losses but all the individually functioned reactors resulted with an improved output voltages (Kim et al. 2013).

5.7.2 Selection of Ion Exchange Membranes

The ion exchange membrane (IEM) is capable of influencing the performance of MFC to a greater level due to its direct relation with the development of internal resistance which leads to poor power production. The commercially available IEM named Nafion® (DuPont, Wilmington, Delaware) is the utmost commonly used membrane which favors the high selective protons permeability for better performance. Despite several attempts by the researchers in developing a low cost and durable membranes to replace this very expensive Nafion, but it is still in the market with outstanding performance (De Juan and Nixon 2013; Gunaseelan Kuppurangam et al. 2018).

A highly projectable work was done to know the behavior of the protons-specific membranes like Nafion through a batch mode study and reported that the cation species other than the protons likewise got moved through the Nafion membranes which came about with the charge balance among the anodic and cathodic chambers because of the higher concentrations of NH_4^+, Na^+, Ca^{2+}, K^+, and Mg^{2+} than the protons concentrations in the catholyte and anolyte of MFCs. Because of this key reason, Nafion and other various PEMs employed in the MFC reactors are not certain to be protons-specific but essentially be cations-specific membrane (Oh and Logan 2006).

The optimal performance through the power output can be achieved by keeping up the proper proportion of PEM’s surface area to the working volume of the reactor so as to diminish the inner resistance of the reactors with moderately bigger size (Oh et al. 2004).

5.7.3 Reactor’s Design

The MFC reactors can be fabricated with several materials into various designs as per the requirements like the H-Type, tubular, stacks, solid phase, sediment, and cube-shaped reactors. The major constituents of the reactors are the anode, cathode, IEM, and the main construction frame materials (Mohan and Chandrasekhar 2011; Gunaseelan Kuppurangam et al. 2018).

Though the internal resistance is believed to be a key factor in evaluating the MFCs performance, the configuration of the MFC reactors will be based on the idea of attaining low internal resistance which correspondingly results with a higher power density (Rabaey et al. 2005).

The most commonly used MFC reactors are H-Type and Cube-Type, due to the higher internal resistance in the H-Type because of the higher electrode distances from membranes, the cube–type reactors with less electrode-membrane distance are being extensively used to examine the new substrates, biocatalysts, and electrode material's compatibilities (Oh and Logan 2006; Zhang et al. 2011; Sun et al. 2014; Ren et al. 2011).

Alternatively, in recent days the membrane-less air-cathode MFC is extensively being used due to its less internal resistances and higher power density even with the relatively higher level of oxygen transmission near the anodic compartment. Further, the usage of membrane-less cells ends up with the reduction of the membrane costs and maintenance of the membrane, which ensures the MFCs for the commercialization and practical applications (Sun et al. 2014).

5.7.4 Substrate and Its Concentration

Indeed, even with the difficulties and key factors, the choice of substrate assumes the significant job in the production of power from MFCs. The substrates or fuel can comprise of easy to complex blends of organic materials which supports the development of the microbial populace.

The substrates like acetate, sucrose, and glucose come under the category of simple mixture substrate which can be suggested for instantaneous results and the complex mixture substrates are recommended for the diverse microbial communities. The most commonly used and promising source of substrate for better growth and high electricity production is the brewery wastewater due to the abundance of organic growth-promoting matters with less inhibitory constituents (Feng et al. 2008). Complex substrates to be specific like synthetic wastewaters, sewerage wastewater, landfills leachate, and textile wastewaters can likewise be utilized for the power production (Das and Mangwani 2010; Pant et al. 2010; Deval et al. 2017).

Recently, it was reported in a review that, the concentration gradients transport phenomenon should take place at the same rate that the microbes generate or consume the currents in Microbial Electrochemical Technologies (METs) and there should be essential knowledge regarding the need for matching the transport gradients rate for all the species that ought to be considered (Torres 2016).

5.7.5 Selection of Biocatalysts and Its Enrichments

Usually, microbes facilitate the degradation (oxidation) of the feed/substrate, transfer of electrons to the anodes by electron shuttling or electron transfer mechanism, and this phenomenon is widely accepted as bioelectrocatalysis. The optimal functioning of the MFCs mostly depend on the chosen electrode's projected surface area and the associated microbial biofilm which favors the direct electron transfer mechanism.

There are some specific bacterial species which possess the capability to release the free electrons from their outer cell wall or extracellularly which are named exoelectrogens. The exoelectrogenic bacteria have huge potential to decompose the provided substrate in the form of wastewater and transfer the released electrons directly or indirectly toward the anode better than any other bacterial species.

Our knowledge about the bio-electrochemically dynamic exoelectrogens is still in its beginning level. Understanding about the mechanism of the electron transfers by the bacterial communities to the electrodes came to light by the research reported on the metal ions-reducing bacteria namely *Geobacter* and *Shewanella* species (Bond and Lovely 2003; Gil et al. 2003).

To ensure the proper functioning of the microbial fuel cells (MFC) with microbial enrichment techniques namely in situ immobilized anode with agarose gel (Luo et al. 2016), microbes immobilized in alginate along with activated carbon mixture (GAC) (Mesran et al. 2014), latex coating (Wagner et al. 2012), and bacteria/multi-walled carbon nanotube (MWCNT) hybrid biofilm were employed (Zhang et al. 2017).

By using the microbes enrichment techniques like immobilization on an anode favors the direct transfer of electrons among the exoelectrogens and the anode with decreased start-up time which leads to better performance of MFC (Wagner et al. 2012).

5.8 Strategies on Trend and the Outlook

It is evident that MFCs can generate electricity as clean energy practices through the bio-electrochemical conversion process from the provided organic loads in wastewater. There are several aspects which clearly show the necessity for further substantial developmental research to understand the bio-electrochemical energy conversion processes and also considering the potential of this technology to replace the currently existing non-renewable energy generation processes which lead to huge pollution.

5.8.1 Maximum Power Point Approach

Earlier, the energy generation from the MFCs was performed by using the external loads or the charge pumps for current boosting, but ended up with the dissipation of energy or received flow of electrons passively without any control. An edifying new methodology and system can harvest the energy from the MFCs actively at any given operating conditions without the resistors or charge pumps, exclusively at the highest peak power to exploit more power generation (Pant et al. 2010).

The highly projectable progress has been achieved in the approach of maximum power point tracking (MPPT) harvesting framework from the refinement in the gradient method model or perturbation and observation which was employed to track and optimizing the external loads or resistance (Pant et al. 2010).

Most fascinatingly, a recirculating-flow MFC was controlled by the maximum power point circuit (MPPC) at its peak power point condition, where the charge pump cannot be used due to its current constraint. Within an assessment period of 18 h test, the energy acquired from the MPPC was 76-folds greater than the charge pump. Further, both conditions lead to similar efficiency in organics removal, but 21 times higher Coulombic efficiency was attained with MPPC than the charge pump (Pant et al. 2010).

A simple and effective work was done and reported with using MPPT as a control against the MFC with fixed resistance for treating the swine wastewater with respect to long-term performance and start-up time. This study reported with 40% improved Coulombic efficiency, improved exoelectrogenic microbial activity, minimal energy losses in anodic and cathodic reactions, and restricted activity of methanogens in the anodic chamber. There was no negative influence on the production of power and organic loads removal by using this MPPT control system (Molognoni et al. 2014).

The use of MPPT and the reactors assembly strategy may lead to increased stack voltages and also dodge the voltage reversal along with the application of a control mechanism which favors the peak power withdrawal from the MFCs. Further, this strategy can be transferable among the different designs of MFCs with the diverse variations in substrate conditions (Boghani et al. 2014).

5.8.2 Controller Based Energy Harvesting/Booster Circuits

It is a known fact that the electricity generated from the MFCs will be very less, and therefore any kind of electronic devices cannot be operated directly. Due to this, a power management system (PMS) is very much essential for energy accumulation generated from the MFC initially and used to drive the devices without any fluctuation in the output. In a recent endeavor, a study was done with the transformer-based PMS with two super-capacitors, which is capable of functioning under a very less voltage than any other existing MFC–PMS reactors. It is reported that an IEEE 802.15.4 wireless sensor framework was successfully driven with this PMS connected with a continuous-mode MFC even with the low input of 0.18 V (Yang et al. 2012).

A study was done with a synchronous boost converter energy harvester with MFC by using a P-channel metal oxide semiconductor field effect transistor (MOSFET). This harvester setup resulted in high converter efficiency of 73%, from 43.8% to 75.9% and also prohibited the reverse current flows (Pant et al. 2010).

In another investigation, MFC stack with the voltage lesser than 0.3 V was supported up into 2.5 V by utilizing a PMS, which was used to turn on light with different LEDs. One of the significant finding from this novel investigation is that a deficient power supply to the PMS in arrangement from the MFC could prompt voltage inversion of PMS capacitor in series electrical arrangement (Kim et al. 2019).

An exceptionally adaptable work was accounted for by utilizing an ultra-low-power energy harvester (UPEM) for single MFC at low cost. UPEM is used to charge a supercapacitor of 3.3 V with 2.09 mW contribution from the MFC to power a real-time framework containing temperature, CO_2 sensor, moistness, iBeacon™ transmission, and long-extend remote information transmission more than 5 km at interim shorter than 3 h, utilizing a solitary MFC with a most extreme intensity of 0.52 mW (Yamashita et al. 2019).

5.8.3 Time Control Strategy

Due to the modifications and advancements, the Parsimonious control was implemented with the MFC arrays to minimize the costs. The controller parameterization was employed with various linearized models against the MFCs with the dynamic operating conditions. This work had demonstrated the potential usage of simple digital potentiometer approaches to control the MFCs with relatively less expensive microcontrollers which may lead to the effective utilization of MFCs in a huge scale arrays (Boghani et al. 2016).

The most remarkable similar kind of study was done on the usage of sampled-time digital control strategy over the MFC stack with an improvement in intermittent operation with resultant power-saving facility which can ensure the avoidance of voltage reversals. An independent controller was coupled with one MFC reactor and the stacks were coupled in the series arrangement for making a hybrid-series connection. The voltage of the stack was directed with resulting total series stack arrangement voltages of 1.26 V from the single MFC reactor's voltages of 0.32, 0.32, 0.32, and 0.3 V, respectively (Boghani et al. 2017).

5.9 Summary

MFCs are one of the most encouraging manageable green practice to meet the developing future demands for the treatment of wastewater and sustaining nature by power generation from the organic contaminations. The enhancements in the MFCs performance with the aspects of power intensity were stale for a very long while until the implementation of significant advancements in the electrodes materials modification through the nanotechnology, reactors design, proper knowledge on the exoelectrogens and operational parameters optimization. This chapter has offered an overview from the fundamentals of the electrochemical losses which reduce the performances of the MFCs to the trending current strategies to guarantee the optimal performance of MFCs, which includes the application of nanotechnology for modification of anodes to enhance the growth of the microbial biofilm with the extended surface area and high electron transfer mechanism due to less resistance nanomaterials like the nano-rods which mimics the role of bacterial pili. Meanwhile, the nano-electrocatalysts with the high oxidation-reduction reaction (ORR) values can be used for the cathode modifications for maximum reduction which leads to high performance.

Obviously, more understanding is needed on the operating parameters such as pH, temperature effect, biofilm formation, and substrate/nutrient concentration which ensure the extraction of maximum energy from the provided organic loads in the MFCs. Even after all the above-discussed challenges in operating the MFCs stack with voltage reversal which hinders it from the real-time applications, the promising energy harvesting control strategies like maximum power point approach, controller based energy harvesting/booster circuits, and time control strategy may ensure a new prospects in energy harvesting and steady supply which might make the MFCs technology viable for the commercialization and real-time applications with less carbon footprints.

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Chapter 6
Electricity-Driven Microbial Factory for Value-Added Resources Recovery from Waste Streams

Mohamed Mahmoud

Abstract Over the past few decades, there is a paradigm shift towards recovering value-added products from contaminated water bodies due to the high cost and energy consumption associated with their treatment and disposal. Microbial electrochemical technologies, including electrofermentation (EF), represent a promising option for the production of a wide range of useful products from waste streams. EF technology holds a great promise to improve the output of traditional fermentation by controlling the microbial metabolism through regulating the intracellular and extracellular redox balance, leading to produce chemicals of interest with improved selectivity, specificity, and product recovery. This chapter provides a state-of-the-art analysis for the recent research advancement and technology development. This chapter also discusses the possible microbial community interactions and how it might affect the overall efficiency of EF systems. An overview is given on the integration possibilities of EF with the existing wastewater treatment process that most likely will lead to successful utilization of waste streams and biomass treatment towards developing value-added biorefinery for sustainable circular economy.

Keywords Electrofermentation · Electrochemically-active bacteria · Microbial electrochemical technology; · Microbial competition · Value-added products

6.1 Background

The global energy demand is currently about 13,864 million tons of oil equivalent (Mtoe) annually with over 85% of this demand is being provided from fossil fuels combustion (BP 2019), leading to ~35 gigatons of carbon dioxide (CO_2) emission released into the atmosphere (Dowell et al. 2017; IPCC 2014). Replacing the current means for energy production with more sustainable, carbon-neutral energy sources

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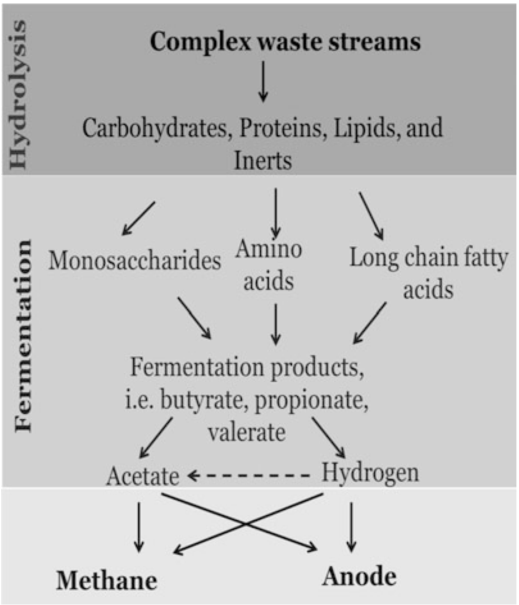

Fig. 6.1 Anaerobic food web, which involves several groups of microorganisms to mediate the biotransformation of complex waste streams into value-added products (Adopted from Mahmoud 2016)

remains a challenge that is facing our society (Brockway et al. 2019; Liu and Rajagopal 2019). Fortunately, this demand can be met from the bioconversion of waste streams to value-added products, such as biochemicals and biofuels. For example, approximately 2.2 million m^3 of wastewater and 2 billion tons of municipal solid waste (MSW) are globally released to the environment every year (Kaza et al. 2018; WWAP (United Nations World Water Assessment Programme) 2017). The release of these potentially harmful waste streams into environment without proper treatment has been a serious cause for concern over the past few decades. Therefore, converting waste streams into various forms of renewable energy creates a "win-win" scenario that solves a wicked waste-management challenge, reduces the cost associated with conventional waste streams treatment, produces renewable energy, and recovers value-added products (Rittmann 2008). Theoretically, energy produced from organic-rich waste streams is approximately a few order of magnitude higher than energy required for wastewater treatment processes (Dubrawski et al. 2019; Logan and Rabaey 2012; Heidrich et al. 2011).

One option for bioenergy production from waste streams is the "anaerobic digestion (AD)," in which different microbial groups convert complex organic compounds under strict anaerobic condition to organic acids and hydrogen gas (H_2), which are subsequently consumed by methanogens to generate methane gas (CH_4) (Fig. 6.1) (Li et al. 2015; Metcalf and Eddy 2003; Rittmann and McCarty 2001). Despite the benefit of producing CH_4 from waste streams digestion, the low conversion yield of CH_4 to electricity (i.e., 30–40%) limits the application of AD to treat low- and medium-strength wastewater. In addition, AD technology is susceptible to process instability and low biogas production, mainly due to the low organic contents in donor substrates, organic acids accumulation, decrease in the reactor pH, and/or high-level of free ammonia (Mao et al. 2015; Rajagopal et al. 2013; Chen et al. 2008). Thus, AD has often been used to stabilize concentrated waste streams, such as waste activated sludge generated during aerobic domestic wastewater treatment and food wastes (Peccia and Westerhoff 2015; McCarty et al. 2011). Although the coupling of aerobic treatment of wastewater with anaerobic stabilization of waste activated sludge to generate biogas seems to be beneficial, this integration allows only a tiny fraction of this organic matter to be recovered, making the current wastewater treatment practices energy-negative processes (McCarty et al. 2011).

A more recent technology for waste valorization is the "microbial electrochemical technologies (METs)," which are unique platforms that utilize electrochemically-active bacteria (EAB) to catalyze bioelectrochemical reactions. EAB have the capability to exchange electrons beyond their outermost membranes with an electron acceptor (i.e., anode) or an electron donor (i.e., cathode), leading to convert organic compounds into electricity, methane, hydrogen, hydrogen peroxide, or other value-added products (Zou and He 2018; Malvankar and Lovley 2014; Rittmann 2008; Lovley 2008). Despite the growing interest in METs research, only a few studies have addressed the scaling-up of METs with the majority of published research were performed using laboratory-scale MET reactors (i.e., $<<1$ liter) (Heidrich et al. 2013; Cusick et al. 2011; Logan 2010). Therefore, the main challenge to commercialize the METs is to improve the electron recovery and the productivity of value-added products, while reducing its high capital, and operation and maintenance (O&M) cost, especially when complex waste streams used as donor substrates (Logan 2010; Rittmann 2008).

Similar to the biodegradation of complex organic matter in AD (Fig. 6.1), the biodegradation of organic matter in METs must be occurred through a cascade of anaerobic reactions, including fermentation and anode respiration. Given that EAB have limited ecological capability to consume a limited number of donor substrates (such as acetate), fermentation represents a crucial step to generate simple products that EAB can efficiently consume (Pant et al. 2010). For example, Ge et al. (2014) showed that the energy recovery of MET systems was inversely proportional to the degree of substrate complexity (expressed as chemical oxygen demand (COD)). They observed that the highest energy recovery was achieved when acetate used as the main sole donor substrate (i.e., 0.40 $\text{kWh/kg}_{\text{COD}}$ compared to only 0.17 $\text{kWh/kg}_{\text{COD}}$ for domestic wastewater and 0.04 $\text{kWh/kg}_{\text{COD}}$ for industrial wastewater). The main cause for this low energy recovery, especially for substrate of low solid contents (e.g., landfill leachate), is the inhibition of fermentation not anode respiration (Mahmoud et al. 2016). Despite the fact that fermentation and anode respiration can be occurred in the same reactor (Mahmoud et al. 2014), the integration of METs with anaerobic digestion to perform some or the majority of fermentation in a separate reactor seems to be beneficial for METs (Katuri et al. 2019; Escapa et al. 2016; Mahmoud et al. 2014).

6.2 Fermentation as an Essential Step in Wastewater Biodegradation

Fermentation represents a crucial step in anaerobic food web, in which soluble organic matter (i.e., the products of particulate organic matter hydrolysis) is converted to organic acids, alcohols, and H_2 (Temudo et al. 2007; Bolzonella et al. 2005). It is considered a central step whether the final product is CH_4 in AD, electric current or H_2 in METs (Rittmann 2008). The hallmark of the fermentation process is that fermenting bacteria extract energy from biodegradable donor substrates without the need of external electron acceptors (e.g., nitrate and oxygen), where the electron acceptors are originated from the initial donor substrates. Fermentation often relies on substrate-level phosphorylation to drive adenosine triphosphate (ATP) generation (Rodríguez et al. 2006; Metcalf and Eddy 2003; Rittmann and McCarty 2001). Thus, fermentation involves a rearrangement of donor substrate molecules into simpler products (i.e., organic acids, alcohols, and H_2) (Fig. 6.2).

Considering the mixed-culture fermentation of glucose, one mole of glucose could theoretically produce 2 moles of acetate, and 4 moles of H_2 (Eq. (6.1)) that its partial pressure should be maintained at a very low level in order to make fermentation thermodynamically favorable (Mahmoud et al. 2017; Angelidaki et al. 2011; Rodríguez et al. 2006; Thauer et al. 1977).

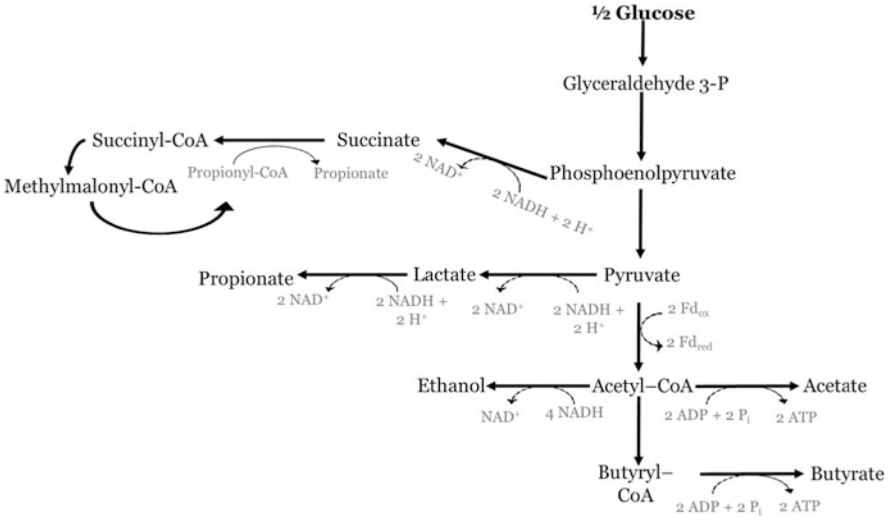

Fig. 6.2 Selected fermentation pathways involved in the mixed-culture glucose fermentation

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2\,CH_3COO^- + 4H_2 + 2CO_2 + 2H^+ \qquad \Delta G^{\circ}_{rxn} = -8.59\ \mathrm{kJ/e^-} \tag{6.1}$$

In order to make fermentation thermodynamically feasible, hydrogen partial pressure must be maintained at a low level (Hallenbeck 2009; Stams and Plugge 2009; McInerney et al. 2008). However, increasing the hydrogen partial pressure would induce a metabolic shift in the fermentation pathways and stoichiometry towards producing more reduced organic acids (e.g., butyrate, lactate, and propionate) instead of producing acetate and H_2. The main reason for this detouring is that at high hydrogen partial pressure microbes tend to replenish the NAD^+ and oxidized ferredoxin pools to continue fermentation, resulting in production of more reduced organic acids (Angenent et al. 2004).

Another major challenge for fermentations is that the product spectrum as well as microbial population structure and diversity can be significantly altered by changing the operating conditions, including pH (Lu et al. 2011; Metcalf and Eddy 2003), organic matter loading (Temudo et al. 2008), the degree of substrate complexity (Saint-amans et al. 2001; Himmi et al. 2000), the presence of inhibitory compounds (Mahmoud et al. 2017), and temperature (Batstone et al. 2002). For instance, Velasquez-Orta et al. (2011) showed that the microbial fuel cell (MFC) performance, in terms of COD removal and power density generation, was significantly affected by the degree of substrate complexity. The highest power density was reported when acetate used as the sole donor substrate (99 ± 2 $\mathrm{mW/m^2}$) compared to only 4 ± 2 $\mathrm{mW/m^2}$ for starch, mainly due to that different initial donor substrates have distinct degree of substrate degradation and fermentation pathways. In another study, Zhang et al. (2014) revealed that the microbial community structure of AD bioreactors significantly changes as a function of the influent donor substrate composition, probably due to the inability of many microbes to use certain substrates to grow, leading to a dramatic change in mixed-culture community structure towards species that have the ability to consume these substrates.

6.3 Overcoming the Fermentation Bottlenecks Through Electricity-Driven Fermentation

In an early review, Rabaey and Rozendal (2010) discussed the possibility to alter the fermentation pathways towards the production of targeted chemicals and bioproducts by inserting polarized electrodes in the bulk solution of AD reactors, which was later called “electrofermentation (EF)” (Rabaey and Rozendal 2010). The role of polarized electrodes is to provide an external source of either oxidizing or reducing power, leading to stimulate microbial metabolism in traditional fermentation bioreactor towards producing targeted chemicals and enhancing the microbial growth (Agler et al. 2011). In this platform, the supplied electric current allows the organic matter fermentation to proceed under imbalanced redox conditions by altering the extracellular and intracellular $NAD^+/NADH$ balance and oxidation-reduction potential (ORP) (Moscoviz et al. 2016).

In anodic EF, organic-rich substrates (e.g., carbohydrate and protein), which are the main source of electrons and energy, are fermented into more oxidized final products. In this case, the anode acts as the terminal electron acceptor. In contrast, the working electrode in cathodic EF (i.e., cathode) supplies electrons to the microbial cells, leading to convert the initial substrates into more reduced final products (Kracke and Krömer 2014).

EF systems have been commonly used to improve the production of a wide spectrum of value-added products from different waste streams (Table 6.1). Similar to AD, the complex organic matter in METs is biodegraded through a cascade of bioelectrochemical reactions under strict anaerobic condition. First, the particulate organic matter is hydrolyzed to soluble monomer, which is then converted into organic acids, alcohols, and H_2 by fermenting bacteria. Then, the majority of fermentation by-products are further converted into acetate and H_2. Finally, acetate and H_2 are consumed by either EAB (the desired pathway) or methanogens to generate CH_4 (the undesired pathway) (McCarty et al. 2011; Parameswaran et al. 2010; Thauer et al. 2008; Rittmann and McCarty 2001). Generally, there are two main H_2-consumers in the anode of MET systems rather than EAB: hydrogenotrophic methanogens and homoacetogens (Mahmoud et al. 2017). Hydrogenotrophic methanogens consume H_2 as the main donor substrate to produce CH_4 (Eq. (6.2)) (Stams and Plugge 2009; Thauer et al. 2008), while homoacetogens also consume H_2 to yield acetate (Eq. (6.3)) (Schuchmann and Müller 2014). Thus, it is a challenge to minimize the conversion of H_2 to CH_4, in the presence of hydrogenotrophic methanogens; however, there are several attempts to limit or inhibit the activity of methanogens, such as using chemical inhibitors (Zhu et al. 2015; Parameswaran et al. 2010), employing active harvesting of H_2 (Lu et al. 2016), altering operational conditions (Mahmoud et al. 2017), and genetically modifying EAB (Awate et al. 2017).

$$\mathrm{HCO_3}^- + 4\mathrm{H_2} + \mathrm{H}^+ \rightarrow \mathrm{CH_4} + 3\mathrm{H_2O} \qquad \Delta \mathrm{G}^{\circ}{}_{\mathrm{rxn}} = -16.38\ \mathrm{kJ/e^-} \tag{6.2}$$

$$2\mathrm{CO_2} + 4\mathrm{H_2} \rightarrow \mathrm{CH_3COO^-} + \mathrm{H}^+ + 2\mathrm{H_2O} \qquad \Delta G^{\circ}{}_{\mathrm{rxn}} = -11.88\ \mathrm{kJ/e^-} \tag{6.3}$$

Recently, Zhao et al. (2015) studied the role of polarized electrodes for enhancing CH_4 production in AD bioreactor fed with waste activated sludge. Despite the obvious increase in CH_4 production compared to control experiments (without polarized anodes), mass balance revealed that $>50\%$ of CH_4 production was originated from unknown pathway. Microbial community analysis as well as fluorescence in situ hybridization (FISH) revealed the dominance of *Methanosaeta* and *Geobacter* species in electric-anaerobic sludge digester bioreactors. Owing to the increase in biofilm conductivity, they concluded that polarized anode facilitated organic matter degradation and electron exchange between methanogens and

Table 6.1 Application of EF for improving the value-added products recovery from wastes streams

EF reactor architecture (reactor volume)	Mode of operation	Substrate	Donor substrate concentration	Inoculum	Electrode type	Applied potential	Organic matter removal	Desired final product	Reference
(1) Anodic EF									
Single-chamber reactor (1 L)	Batch	Composite food waste	10 g COD/L	Mixed-culture	Graphite	−0.6 V[a]	57%	Mixed organic acids	Sravan et al. (2018)
Single-chamber reactor (0.126 L)	Batch	Fermented food waste	N.A.[b]	Mixed-culture	Carbon fiber	+0.8 V[b]	70–87%	H_2 and CH_4	Jia et al. (2020)
Dual-chamber reactor (16 mL)	Continuous	Pre-treated switchgrass waste	0.026 g /L	Mixed-culture	Carbon felt	−0.005[a]	Up to 74.2%	H_2	Lewis and Borole (2016)
Dual-chamber reactor (0.32 L)	Continuous	Pre-treated landfill leachate	1.23 g COD/L	Mixed-culture	Graphite rods	−0.046[a]	28%	H_2	Mahmoud et al. (2016)
Dual-chamber reactor (90 mL)	Batch	Glycerol	Up to 100 g glycerol/L	*G. sulfurreducens* + *C. cellobioparum*	N.A.[b]	+0.445[a]	100%	Mixed product (i.e., acetate, lactate, ethanol, and H_2)	Speers et al. (2014)
Dual-chamber reactor (0.2 L)	Batch	A mixture of phenolic compounds	200−1200 mg/L	Mixed-culture	Carbon felt	+0.6[c]	49–61%	H_2	Zeng et al. (2015)
Dual-chamber reactor (25 mL)	Batch	Glycerol	1 mmol glycerol/L	Engineered *E. coli*	Graphite felt	+0.2[a]	80%	Ethanol and acetate	Sturm-Richter et al. (2015)
Dual-chamber reactor (0.31 L)	Batch	Glycerol	11.05 g glycerol/L	Recombinant *Klebsiella pneumoniae* L17	Carbon cloth	+0.705[a]	100%	3-hydroxypropionic acid	Kim et al. (2017)
Dual-chamber reactor (0.145 L)	Batch	Dye wastewater	Up to 300 mg dye/L	Mixed-culture	Graphite brush	+ 0.8 V[c]	Up to 96%	H_2	Hou et al. (2017)
Dual-chamber reactor (88 L)	Continuous	Domestic wastewater	~400 mg COD/L	Mixed-culture	Cardon felt	+1.1 V[c]	34%	H_2	Heidrich et al. (2013)

(continued)

Table 6.1 (continued)

EF reactor architecture (reactor volume)	Mode of operation	Substrate	Donor substrate concentration	Inoculum	Electrode type	Applied potential	Organic matter removal	Desired final product	Reference
(2) Cathodic EF									
Dual-chamber reactor (0.9 L)	Batch	Glycerol	17.5 g glycerol/L	Mixed-culture + *G. sulfurreducens*	Planar graphite	−0.656[a]	100%	1,3-propanediol	Moscoviz et al. (2018)
Dual-chamber reactor (0.86 L)	Continuous	H_2	N.A.[b]	Mixed-culture	Graphite granules	−0.850[a]	N.A.[b]	CH_4	Villano et al. (2011)
Dual-chamber reactor (0.45 L)	Batch	Glycerol	0.3 mmol/L	*Clostridium pasteurianum* DSM 525	Graphite felt	+0.045[a]	100%	Butanol and 1,3-propanediol	Choi et al. (2014)
Dual-chamber reactor (0.45 L)	Batch	Sucrose	5 g Sucrose /L	*Clostridium tyrobutyricum*	Graphite felt	−0.545[a]	100%	Butyrate	Choi et al. (2012)
Dual-chamber reactor (0.65 L)	Batch	Glucose	3 g glucose/L	Mixed-culture	Graphite plate	–	76.52%	Polyhydroxyalkanoates	Srikanth et al. (2012)
Dual-chamber reactor (0.25 L)	Batch	Glucose	3 g glucose/L	*Corynebacteriumglutamicum*	Graphite plate	−0.401[a]	100%	Lactate	Sasaki et al. (2014)
Dual-chamber reactor (0.87 L)	Batch	Acetate	50 mmol/L	Mixed-culture	Graphite felt	−0.433[a]	100%	Ethanol, butyrate, propionate, and methane	Steinbusch et al. (2010)
Dual-chamber reactor (0.28 L)	Batch	Corncob hydrolysate	15 g sugar/L	*A. Succinogenes* NJ113	Carbon felt	−0.8[a]	N.A.[b]	Succinate	Zhao et al. (2016)
Dual-chamber reactor (0.1 L)	Batch	Glycerol	20 g glycerol/L	*Klebsiella pneumoniae*	Graphite felt	−0.45[a]	~60%	Ethanol, succinate, lactate, acetate, and 1,3-propanediol	Harrington et al. (2015)

[a] Potential is reported versus standard hydrogen electrode (SHE)
[b] *N.A.* not available
[c] Potential applied between anode and cathode

Geobacter species. In more recent study, Luo et al. (2016) documented the positive role of polarized electrode for enhancing CH_4 production under ammonia stress.

Taken together, these available laboratory-scale studies demonstrate that EF platform opens up new opportunities to integrate METs with the existing AD technology in order to solve the problems associated with conventional AD technology, such as low product yield, slow hydrolysis/fermentation rate (Park et al. 2018), the requirement of long sludge and hydraulic retention times (Song et al. 2016), and the process instability at low temperature (Liu et al. 2016).

In cathodic EF, the electrode acts as the source of electrons to stimulate EF towards production of more reduced final products, including 1,3-propandiol (1,3-PDO), butanol, and polyhydroxyalkanoates (PHA), with high purity and rate (Xue et al. 2017; Moscoviz et al. 2016; Kracke and Krömer 2014). For instance, Choi et al. (2014) showed that a positive working potential (i.e., +0.045 V vs. standard hydrogen electrode (SHE)) triggered a metabolic shift in *Clostridium pasteurianum* towards production of NADH-consuming metabolite, such as 1,3-PDO from glycerol fermentation and butanol from glucose. More recently, there are efforts to use mixed-culture microbial community for glycerol electrofermentation to selectively produce 1,3-PDO (Roume et al. 2016; Xafenias et al. 2015; Zhou et al. 2013).

Although the use of electric current has been proved to be effective tool to drive the microbial, allowing the production of targeted chemicals of interest, it seems to be a challenge to build successful mixed-culture microbiomes that are resilient to improve the EF selectivity and specificity (Schievano et al. 2016). Dennis et al. (2013) revealed that change in microbial community structure was significantly associated with change in electrofermentation pathway of glycerol and product spectrum. Zhou et al. (2015) observed that the sharp decrease of 1,3-PDO production from glycerol over long time of operation (>150 days) was associated with loss of the dominant *Citrobacter* spp.

Polyhydroxyalkanoates (PHA) is another targeted chemical that can be produced during the cathodic EF of glucose. In a proof-of-concept study, Srikanth et al. (2012) observed high accumulation of PHA (19% of dry cell weight) with high hydroxybutyrate concentration(~89%) by providing a microaerophilic environment in the cathode of an MET with glucose as the sole carbon source.

6.4 Towards Building Successful Microbiome: Teamwork or Coexistence?

Despite the fact that the EET mechanism in METs has not yet been fully elucidated, there are 2 main mechanisms through which EAB can exchange electrons with electrodes: direct electron transfer and indirect (or mediated) electron transfer (Fig. 6.3) (Torres et al. 2010). Indirect electron transfer relies on redox active shuttles that transfer electrons between EAB and solid surfaces by altering their oxidation states. The extracellular shuttles can be either a secondary microbial metabolite (e.g.,

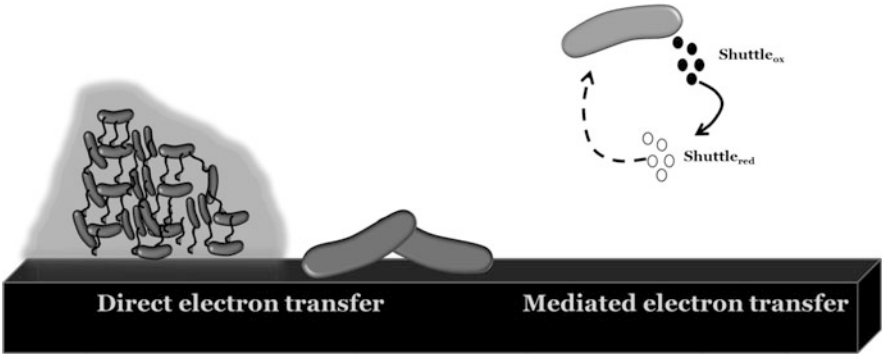

Fig. 6.3 Proposed electron transport mechanisms used by EAB (Adopted from Mahmoud 2016; Torres et al. 2010)

phenazine and flavins) or synthetic molecules (e.g., Anthraquinone-2,6-disulfonic acid, neutral red, and cobalt(III) sepulchrate) (Kracke et al. 2018; Torres et al. 2010; Marsili et al. 2008; Emde and Schink 1990). For direct electron transfer, EAB community has the ability to exchange electrons with solid surfaces via direct contact of redox proteins embedded within the EAB outer membrane (e.g., nanowires) (Malvankar and Lovley 2014; Lovley 2008). These mechanisms have been commonly postulated in anodic and cathodic EF systems (Moscoviz et al. 2016; Torres et al. 2010; Rabaey and Rozendal 2010). Although the study of EET in the anode of METs in early studies has focused only on two Gram-negative, mesophilic EAB: *Shewanella oneidensis* MR-1 and *Geobacter sulfurreducens*, there are so far over 100 isolated EAB that have the ability to transfer the electron to/from solid surfaces (Logan et al. 2019; Doyle and Marsili 2018).

EAB community performing indirect electron transfer (e.g., *S. oneidensis* MR-1) is often characterized with low current density generation (i.e., ≤ 1 A/m^2) mainly due to the slow diffusion of redox shuttles, although they are capable of using fermentable donor substrates, such as glucose, as the main source of energy and electrons. On the other hand, EAB community performing respiration via solid-conductive mechanism (e.g., *G. sulfurreducens*) are capable of producing much higher current density; however, their metabolic capability is limited to only consume simple substrates, including acetate and H_2 (Torres et al. 2010; Marsili et al. 2008). In order to overcome this limited metabolic capability, Speers et al. (2014) proposed a successful strategy to use a co-culture of an EAB (*G. sulfurreducens*) and a fermenting bacterium (*Clostridium cellobioparum*) to enhance glycerol fermentation into ethanol. Interestingly, Lusk et al. (2015) used a pure-culture thermophilic bacterium—*Thermoanaerobacter pseudethanolicus*—that has the ability to ferment complex donor substrates (e.g., xylose, glucose, and cellobiose) and perform anode respiration without the addition of redox mediators.

Despite the benefits of using pure cultures in EF systems for higher selectivity and specificity of fermentation reactions, mixed-culture EF systems may be advantageous to simplify the fermentation process. Owing to the high robustness and functional stability of mixed-culture microbial community compared to pure cultures, mixed-culture EF systems can handle a wide range of complex waste streams, such as real wastewater. It was previously demonstrated in different MET configurations that EAB rely on fermenting bacteria to provide their "fuel" by converting complex organic matter into simple donor substrates (Logan et al. 2019; Mahmoud et al. 2017; Parameswaran et al. 2010). These syntrophic interactions—either by mediated interspecies electron transfer (MIET) via the diffusion of electron carriers (i.e., H_2 and formate) (Parameswaran et al. 2010) or direct interspecies electron transfer (DIET) in presence of conductive materials (Lovley 2017)—are required to maintain the concentrations of fermentation by-products below a threshold limit to make the fermentation thermodynamically favorable (Kiely et al. 2011).

Although methanogens represent the main trophic guild in anaerobic digesters to produce $CH_4$from organic wastes, methanogens represent undesired competitors for EAB, since they can compete for space and food (Siegert et al. 2015). They can produce CH_4 by two pathways: (1) aceticlastic methanogenesis by oxidizing acetate (Eq. (6.4)) and (2) hydrogenotrophic methanogenesis by oxidizing H_2 (Eq. (6.2)). Owing to the thermodynamic and kinetic advantages of EAB over aceticlastic methanogens, EAB usually outcompete aceticlastic methanogens (Parameswaran et al. 2010; Esteve-Nunéz et al. 2005); hence, they are not a competitor for acetate-consuming EAB. However, hydrogenotrophic methanogens have metabolic advantage over EAB for H_2 consumption, thereby minimizing the possibility of H_2 harvesting or its conversion into another useful product, such as electric current (Mahmoud et al. 2017). Among several possibility to inhibit methanogens, chemical inhibitors (e.g., 2-bromoethanesulfonate) seem to be the most effective option for inhibiting methanogens, although using chemical inhibitors is not practically feasible for industrial applications of EF and other MET as well as they are toxic (Karthikeyan et al. 2017; Mahmoud et al. 2017; Lu et al. 2016; Zhu et al. 2015; Parameswaran et al. 2010; Chae et al. 2010; Freguia et al. 2008).

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3{}^- \qquad \Delta G^{\circ} = -3.88\ \mathrm{kJ/e^-} \tag{6.4}$$

Another potential competitor for EAB is sulfate-reducing bacteria (SRB). Sulfate reduction process in the anodic EF systems is likely to occur, particularly for sulfate-rich organic waste streams, such as food wastewater, petrochemical effluents, and pulp and paper wastewater (Hao et al. 2014). Although there is no comprehensive study showing the impact of sulfate on the performance of EF systems, a recent study revealed the applied current in a microbial electrolysis cell favored sulfate removal from sulfate-rich wastewater (Wang et al. 2017). Their results suggest that EAB can integrate with SRB to remove organic matter and sulfate, although they did not study the effect of sulfate on anode respiration. In another study, Lee et al. (2012) showed that increasing the sulfate concentration had a negative effect on the performance of MFCs as indicated from low power density generation and electron recovery.

Given that nitrate can be reduced in strict anaerobic conditions, it represents a real risk for EAB in the anode of MET reactors, including anodic EF systems. Nitrate reduction by nitrate-reducing bacteria (NRB) (or denitrifiers) is an undesired process, since it would limit the substrate availability for EAB (Sukkasem et al. 2008). For instance, Jin et al. (2019) showed that supplementing the anode of an MFC with nitrate (100 mg-N/L) decreased Coulombic efficiency (CE) by ~2.2-fold (from 63.9% to 29.4%). In another study, Kashima and Regan (2015) studied the impact of nitrate on the efficiency of pure-culture electrochemically-active bacterium (i.e., *Geobacter metallireducens*). The addition of nitrate (10 mM) resulted in a remarkable reduction of CE (from ~78% to ~4%). A likely reason for low CE and electron losses is the competition between EAB and NRB for substrate and space.

6.5 EAB–Electrode Interaction and EF Systems Architecture

So far, the majority of published research were performed using small-scale EF systems. Thus, the successful scaling-up of EF systems (and other MET as well) will greatly depend on the selection of biocompatible electrodes that favor the microbe-electrode interactions as well as the system design and architecture (Logan et al. 2006). The ideal electrodes for EF systems should have: (1) a relatively high electrical conductivity; (2) high chemical stability; (3) low cost; (4) large accessible specific surface area; and (5) high mechanical strength (Hindatu et al. 2017; Xie et al. 2015).

Owing to their biocompatibility, low cost, and high electrical conductivity, carbon-based electrodes, including graphite brush, carbon cloth, graphite felt, carbon brush, granular activated carbon, and carbon fibers, have been widely used for METs research (ElMekawy et al. 2017; Xie et al. 2015). In addition, altering the surface chemistry of electrodes by either adding conductive catalysts (e.g., carbon nanotube, graphene, and iron oxide) or conducting polymers (e.g., polyaniline and hydrogels) has resulted in enhancing the bacterial colonization and microbe-electrode interactions, thereby reducing the surface electron transfer resistance and improving the extracellular electron (EET) rate (Hindatu et al. 2017). Other surface treatment approaches (e.g., acid treatment (Feng et al. 2010), ammonia treatment (Call et al. 2009), surfactant treatment (Guo et al. 2014a), heat treatment (Wang et al. 2009), and flame oxidation (Guo et al. 2014b) have been applied to alter the surface chemistry of electrodes, facilitating the microbe-electrode interaction and enhancing biocompatibility properties of electrodes. In addition, other non-carbon electrodes (e.g., stainless steel, gold, and titanium) have also been used for METs research; however, their small accessible specific surface area and relatively high cost would limit their application for large-scale MET reactors (Fan and Liu 2014; Richter et al. 2008; Dumas et al. 2008).

Most of recent EF studies have paid more attention to understand the fundamental aspects of EF systems rather than the reactor design and architecture. Single-chamber EF system (i.e., without using ion-exchange membranes) seems to be ideal to upgrade the existing AD technologies for wastewater treatment, such as municipal sludge and food-processing wastewater. This approach would enhance the efficiency of AD, while keeping its O&M relatively low. However, if the purpose of EF system is to recover high-purity chemicals, EF should occur in multi-chambered EF systems equipped with of ion-exchange membranes (bipolar membrane, anion-exchange membrane, and cation-exchange membrane). For example, Roume et al. (2016) used a 3-chambered cathodic EF system to enhance the bioelectrochemical reduction of glycerol to 1,3-PDO. Using arrays of selective membranes, they reported a high production yield of 1,3-PDO (i.e., 0.72 mole of 1,3-PDO per 1 mole of glycerol). Similarly, Andersen et al. (2014) revealed that using an anion-exchange membrane remarkably enhanced the extraction and upgrading of short-chain carboxylates into esters during bioelectrochemical fermentation.

6.6 Conclusion and Future Perspectives

EF is an emerging platform that integrates electrochemistry with traditional fermentation. In EF systems, the polarized electrodes can act as either electron acceptor (i.e., anodic EF) or electron donor (i.e., cathodic EF), leading to stimulate microbial metabolism in traditional fermentation bioreactor to produce targeted chemicals with high purity, to enhance microbial growth, to improve the microbial interspecies interactions, and/or to achieve carbon breakdown or chain elongation. Despite the great promise of this hybrid technology, it is still in its infancy. Multidisciplinary studies are required:

1. to understand the microbial community interaction and how it affects the microbial metabolism as well as cultivating new microbial isolates that are capable of improving the selectivity and specificity of EF. Owing to the recent advances in molecular biology and culture-independent tools and techniques, both options seem to be easily achievable,
2. to improve the EF system architecture and design, including the electrode materials and shapes. This remarkably improvement of EF efficiency has to be accompanied with novel reactors design that should have a relatively low cost and high availability,
3. to investigate the possibility of integration with other existing wastewater treatment processes, such as anaerobic digestion, and
4. to find a practical way to improve the extraction of the produced chemicals.

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Chapter 7

Essential Factors for Performance Improvement and the Implementation of Microbial Electrolysis Cells (MECs)

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Abstract The microbial electrolysis cell (MEC) is a novel biotechnological process that can translate the chemical energy (organics/nutrients) present in wastewater or biomass into hydrogen gas and other valuable products. For the realization of real-world MEC application, achieving optimal hydrogen production is necessary. To obtain this optimum hydrogen production, significant factors about the MECs must be realized. Therefore, in this chapter, key factors affecting H_2 production and yield in MECs are explored and the important factors are fully discussed. The performance of an MEC depends on many factors and operating parameters: the types of microorganisms and their metabolic behavior, anodic e^- (electron) transport and its mechanism, energy sources (substrate, fuel, nutrients), physiochemical properties of electrode and membrane materials, various separators, operating parameters such as pH, temperature (T), E_{ap} (applied voltage), or external power source, organic loading rate, conductivity, and the salinity or ionic strength of electrolytes and solutions are considered to be the critical parameters. The geometric design of MECs reactors is also considered to include influencing parameters. Overall, advances in MEC technology in terms of operating costs, substrates, and use of low-cost electrode materials are required to make MEC systems sustainable, economical, and a commercially viable technology.

Keywords Biohydrogen gas production · Exoelectrogens · Anodic electron transport · Applied voltage in MEC · Operating conditions · MEC reactor geometry · Anode and cathode materials

7.1 Introduction

In 2005, the first laboratory-scale electrolysis-type process based on microbial fuel cells (MFC) to produce H_2 from organic matter was developed independently by two research groups: one at Penn State University, USA (Liu et al. 2005a), and the second at Wageningen University, Netherlands (Rozendal et al. 2006a). This technology, initially named the bioelectrochemically assisted microbial reactor (BEAMR), was reported by Liu et al. (2005a) and Ditzig et al. (2007), then reported as the biocatalyzed electrolysis cell (BEC) by Rozendal et al. (2006b), and further described as electrohydrogenesis by Logan et al. (2008), Lalaurette et al. (2009), and Lu et al. (2010).

However, the process is presently recognized as microbial electrolysis, and where these process are carried out are named microbial electrolysis cells or MEC reactors (Call and Logan 2008; Sim et al. 2018). MECs are bioelectrochemical reactors that are extended versions of microbial fuel cells (MFCs), which have a configuration

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exactly analogous to a battery or fuel cell. MEC systems are constructed of an anode electrode with a cathode electrode, which are connected together by a wire to complete an electric circuit (Logan et al. 2006). In principle, the MEC process is analogous to that of the MFCs because the arrangement of the electrodes and the electrogenic reactions are similar, but a key difference between the MEC and MFC processes is in the end products as a result of the biologically catalyzed reaction (Varanasi et al. 2019; Kuppam et al. 2017). In MFCs, the anodic chamber is anaerobic and bioelectricity is directly harvested, whereas in MECs, the entire reactor system including the anode and cathode is in an anaerobic environment and biohydrogen is formed at the surface of the cathode (Chookaew et al. 2014; Zhou et al. 2013). Notably, the research on MEC technology is greatly multidisciplinary in nature (Kumar et al. 2017a, b), including bioelectrochemistry, microbiology, and molecular biology, physiochemical properties of materials, chemical processes, and environmental engineering.

7.2 Main Components and Constituents of MECs

7.2.1 Anode and Anode Materials

Anode materials are a key constituent of MECs, because the microorganism (electrochemically active bacteria, EAB) sticks to the anode surfaces, known as anode acclimation, to form the anode biofilm that is primarily accountable for the transfer of electrons from the electrode (electron generation). Thus, MEC development requires the selection of high catalytic anode materials because the catalytic reactions on the anode of MECs are basically the same as those on the MFC anodes. Thus, the anode or anode electrode materials of MFCs can also be used as anodes in MECs. The literature contains reports of various materials used as anode materials in MECs; their hydrogen production rate (HPR) is compared and tabulated in Table 7.1. As Table 7.1 clearly indicates, the most common anode materials used in laboratory-scale MECs or MFCs are carbon-based electrodes such as graphite or carbon cloth. These carbon-based materials are used so far as anodes because of possessing higher electrical conductivity, flexibility in shape and size, biological compatibility, a large surface area for reactions, and economic and chemical stability with low overpotential (Logan et al. 2008; Rozendal et al. 2008a; Kadier et al. 2016a; Escapa et al. 2016).

Some reports on MECs have described heat-treated carbon- or graphite-based electrode (anode and cathode) materials; for instance, carbon or graphite anode materials can enhance anode electrode performance in MECs, probably because of the cleaning effect at the anode surface. Wang et al. (2009) successfully demonstrated that inexpensive heat-treated electrode materials, such as carbon mesh-type anodes, can enhance the yield and overall performance of MFCs and MECs. Some studies reported that the NH_3 or N_2 gas treatment method can also enhance MEC or MFC performance, but heat treatment (450 °C, 30 min) offers relatively better

Table 7.1 Performance of MECs with different anodes, cathodes, feedstocks, applied voltages, vector configurations, and operation modes

Anode	Cathode	Feedstock type/ concentration (g/l)	E_{ap} (V)	Operation mode	Employed volume (ml)	HPR (H_2 m^3/ m^3 day)	Reference
CC	Pt/CC	Acetate	0.25–0.85	Batch[a]	400	0.37	Liu et al. (2005)
GF	Ti/Pt	CH_3COO^-/10 Mm	0.5	Fed-batch[a]	6 l	0.02	Rozendal et al. (2006a)
NH_3 pre-treated graphite granule	Pt/CC	CH_3COOH/1	0.6	Fed-batch[a]	14	1.1	Cheng and Logan (2007)
		C6$H_{12}O_6$/1				1.23	
		Lactic acid/1				1.04	
		Propionic acid/1				0.72	
		Butyric acid/1				0.45	
		Valeric acid/1				0.14	
		Cellulose/1				0.11	
Carbon paper	Pt/CP	DWW/1	0.5	Continuous-flow[a] (0.368 g/l)	292	0.154 H_2 g COD^{-1}	Ditzig et al. (2007)
Graphite brush	Pt/CC	CH_3COO^-/l	0.8	Fed batch[b]	28	3.12	Call and Logan (2008)
		CH_3COO^-/1	0.6			1.99	
GF	Ti plate/Pt	CH_3COO^-/2 mM	0.8	Fed-batch[a]	28	0.052	Chae et al. (2008)
Carbon felt	Pt/CP	Sodium acetate/90.7	1	Continuously fed[a]	50	6.3	Tartakovsky et al. (2009)
CC	Pt/CC	Acetate/5	0.6	Single-chamber	28	2.3	Hu et al. (2009)
	NiMo/CC	Acetate/5				2.0	Hu et al. (2009)
	NiW/CC		0.6			1.5	

GFB	SS brush	Acetate/1	0.5	Single chamber	28	1.7	Call et al. (2009)
GB	CC	Fermentation effluent/6.5	0.6	Fed-batch[b]	26	2.11	Lu et al. (2009)
		Buffered effluent/6.5				1.41	
GFB	SS A286	Acetate/1	0.9	Fed-batch[b]	28	1.5	Selembo et al. (2009a)
	SS 304	$CH_3COO^-/1$	0.9			0.59	
	SS420	$CH_3COO^-/1$	0.9			0.58	
	SS316	$CH_3COO^-/1$	0.9			0.35	
NH_3-treated GB	Pt/CC	P-Glycerol/1	0.5	Fed-batch[b]	28	0.80	Selembo et al. (2009b)
		P-Glycerol/1	0.9			2.01	
		Glucose/1	0.5			0.83	
		Glucose/1	0.9			1.87	
		B-Glycerol/1	0.5			0.14	
		B-Glycerol/1	0.9			0.41	
NH_3-treated GB	Ni powder Ni 210	Acetate/1	0.6	Fed-batch[b]	28	1.3	Selembo et al. (2010)
GFB	Pt	Swine WW/2	0.55	Fed-batch[b]	28	1	
GFB	Pt/CC	Synthetic effluent/5	0.5	Fed-batch[b]	28	1.11 ± 0.13	Lalaurette et al. (2009)
		Cellobiose/5	0.5			0.96	
		Lignocellulose/5	0.5			0.11	
Graphite granules	Carbon felt	$CH_3COO^-/10$ mM	1.06	Single-chamber continuous flow[b] (0.88 ml/min)	140	0.57	Lee et al. (2009)

(continued)

Table 7.1 (continued)

Anode	Cathode	Feedstock type/ concentration (g/l)	E_{ap} (V)	Operation mode	Employed volume (ml)	HPR (H_2 m^3/ m^3 day)	Reference
Carbon brush	CNT	CH_3COO^-/30 mM	1.06	Fed-batch[a]	120	NA	
Carbon brush	CNT	CH_3COO^-/80 mM	1.06	Fed-batch[a]	120	NA	
Graphite granules	Ti tube/Pt	CH_3COO^-/0.5	1.0	Batch[b]	400	1.58	Guo et al. (2010)
GB	Pt/CC	Residue of an ethanol–H_2– fermentation /50:50, v:v	0.5–0.8	Fed-batch[b]	26	2.11	Lu et al. (2009)
GB	Pt/CC	Proteins-bovine serum albumin/0.7	0.6	Fed-batch[b]	26	0.42	Lu et al. (2010)
GF	Co-Mo alloy	Acetate/2.72	1.0	Continuous flow[a] (2.6 ml min^{-1})	200	50	Jeremiasse et al. (2010a)
HTGB	Pt/CC	Acetate/1.5	0.6	Fed-batch[b]	28	3.6	Nam et al. (2011)
NH_3-treated GB	SS	Acetate/1	0.9	Fed-batch[b]	28	1.4	Ambler and Logan (2011)
NH_3-treated GB	Pt/CC	Potato WW/1.9–2.5	0.9	Fed-batch[b]	28	0.74	
GB	Pt/CC	Sodium acetate/1	1	Batch[b] cylinder	28	17.8	Cheng and Logan (2011)
HTGB	SS/Pt	CH_3COO^-/1.5 g/l	0.9	Fed-batch[a]	28	1.6 ± 0.2	Nam and Logan (2011)
GF	Pt/CC	Acetate/1	0.8	Fed-batch[b]	45	5.56	Liang et al. (2011)
GB	Pt/CC	Alkaline WAS/2.4	0.6	Fed-batch[a]	26	0.91	Lu et al. (2012)
GB	Pt/CC	Glucose/2	0.8	Fed-batch[b]	26	0.37	
Carbon brush	Pt/C Fe/Fe_3C@C	Acetate/1 Acetate/1	0.8 0.8	Fed-batch[a] Fed-batch[a]	120 120	0.0231 0.0182	Xiao et al. (2012)

	CNT		0.8			0.0076	
HTGB	Pt/CC	Food processing WW/8.1 kg/m^3	0.9	Fed-batch[b]	28	1.8	Tenca et al. (2013)
HTGB	MoS_2	Industrial WW/4.1 kg/m^3	0.7	Fed-batch[b]	28	0.17	
	SS304 sheet	Industrial WW/4.1 kg/m^3	0.7	Fed-batch[b]	28	0.12	
CF	SS	Urban WW	1.1	Continuous flow[b]	120 l	0.015	Heidrich et al. (2013)
GB	Pt/CC	Trehalose (50 mM)	0.8	Fed-batch[b]	38	0.25	Xu et al. (2014)
Carbon brush	CC/MoS_2/CNT-90	Acetate/1	0.8	Fed-batch[a]	130	0.01	Yuan et al. (2014)
GFB	Pt/CC	Acetate	0.8	Fed-batch[b]	130	3.7M H_2/M acetate	Hou et al. (2014)
GFB	SS/Pt	Cellulosic fermentation WW	0.9	Continuous flow[a] (2.6 ml/min)	302	0.49	Nam et al. (2014)
Carbon felt	Ti/Pt	Acetate/1.5	–	Fed-batch[a]	200	0.013	Lee et al. (2015)
CC	Polyaniline/multiwalled CNT biocathode	CH_3COO^-/1.28	0.9	Batch[b]		0.67	Chen et al. (2015)
GF	Biocathode	Acetate/1	0.72	Fed-batch[a]	40	0.301 ± 0.005 (CH_3COO^-) 0.127 ± 0.024 (sodium bicarbonate)	Chen et al. (2016a)
Carbon in brush shape	3D-Microporous (SSFF)	Sodium acetate/1	0.9	Batch[b]	28	3.66 ± 0.43	Su et al. (2016)
Carbon felt	Nano Mg $(OH)_2$/Gr-CP	NaAc $3H_2O$/2000	0.7	Batch[b]	80	0.63 ± 0.11	

(continued)

Table 7.1 (continued)

Anode	Cathode	Feedstock type/ concentration (g/l)	E_{ap} (V)	Operation mode	Employed volume (ml)	HPR (H_2 m^3/ m^3 day)	Reference
GF	SS	Urban WW	0.9	Continuous flow[a]	175 l	0.005	Cotterill et al. (2017)
CC	Pt/CC	Rice straw	0.8	Fed-batch[a]	358	2.46 mmol/l day	Wang et al. (2017b)
CF	SS	Urban WW	1.5	Continuous flow[a]	130 l	0.031	Baeza et al. (2017)
SS felt tube	Ti mesh tube/Pt	Sodium acetate/4	1	Fed-batch[a] Tubular	1000	7.10	Guo et al. (2017)
Carbon felt	SS plate	Simulated landfill Leachate	1.0	Batch and continuous[a]	1000	0.04 ± 0.06	
Carbon felt	Pt/CC	Catalytic pyrolysis aqueous phase (CPAP) generated from pine sawdust	−0.2 V Vs. Ag/AgCl	Batch and continuous[a]	32	5.8 ± 0.18	

CC carbon cloth, *CNT* carbon nanotubes, *GFB* graphite fiber brush, *Pt/CC* carbon cloth with 0.5 mg Pt/cm^2 cathode, *SS* stainless steel, *WW* wastewater, *CF* carbon fibers, *HTGB* heat-treated graphite brush, *GF* graphite felt, *GB* graphite brush

[a] Two chamber

[b] Single chamber

performance for larger-scale MEC or MFC operations (Escapa et al. 2016). Some recent reports (Fan et al. 2011) claimed that carbon nanotubes (CNTs) can enhance the e^- transport rate on the surface of anode electrodes as well as offer larger anode surface area, and thus this is a good alternative for anode materials in MECs. Carbon-based electrode materials are produced worldwide: major suppliers are Graphite Electrode Sales and E-TEK from USA, FMI Composites Ltd. from UK, Alfa Aesar from Germany, G.G. Products Company Limited from China, and some Netherlands-based companies.

7.2.2 Cathodes and Catalysts

The overall performance of MFCs and MECs is largely dependent on the cathode materials, but in the MECs, the performance of the cathode is more critical because hydrogen is produced at the cathode surface. Cathode materials in MECs are reported to account for 47% or more of the capital costs of the systems. So, the cathode has a significant role in the economic viability of real-world MEC operations (Rozendal et al. 2008a). Various reports state that the hydrogen evolution reaction (HER) on graphite- or carbon-based cathode materials is not feasible because of the low rate of activity; thus, it is essential to dope, coat, or polish these materials with a layer of catalyst by electroplating or electrodeposition. A high electrocatalytic catalyst must be employed over the cathode to accelerate the HER rate and to decrease the overpotential of electrodes or overcome the thermodynamic barrier during the reduction of protons to H_2 gas (Chen et al. 2015; Kadier et al. 2016a). Some reported cathode catalysts and the coatings on different cathode surfaces that increase the HER with HPR are shown in Table 7.1. Liu et al. (2014) described the effect of Pt catalyst filling rate at the cathode surface over the power output of MECs as 0.2 g/cm^2 of Pt catalyst on the cathode surface, which can be sufficient for maximizing the power generation in MECs. Reduction in the catalyst layering (coating) on the cathode surface will reduce the energy efficiency, energy recovery, and power density of the MEC system. So, the optimum and uniform catalyst coating is necessary for desirable MEC performance.

In most reports, the expensive metal catalyst Pt is used as the cathode catalyst for HER in MECs, mainly because of its outstanding electrochemical catalytic characteristics and low overpotential coupled with its inert nature (Chen et al. 2015; Kadier et al. 2017b). Despite all these advantages of Pt, such as exceptional catalytic activity as the cathode in HER for MEC operations, Pt cannot be utilized as the cathode catalyst because of its higher cost, and destruction by chemicals such as sulfides and phosphate anions, causing unsuitability for sustainable development (Kadier et al. 2017b; Munoz et al. 2010). Further, pH sensitivity hinders the process for using Pt for wider or practical MEC applications in real wastewater conditions, which has encouraged researchers to search for alternative cathode catalysts. Some recent reports for alternatives to Pt as the cathode catalyst have included metal nanoparticle-loaded cathode materials, electrodeposits, electroplated materials, and combinations of two to three metal alloys (stainless steel, nickel, MoS_2), which have been developed and tested for large-scale MEC operations (Kundu et al. 2013; Escapa et al. 2016; Yuan and He 2017; Zhen et al. 2017; Wang et al. 2017a; Kadier et al. 2017b; Choi et al. 2019); these and some others are presented in Table 7.1. One good attempt by Selembo et al. (2009a) utilized nickel oxide (NiO_X) catalyst as the cathode catalyst in MECs to increase HER, HPR, and energy recovery, but reduction in catalytic activity was seen with retention time. An inexpensive NiMo alloy as cathode catalyst shows higher HPR with retaining catalytic activity, although it is toxic and has adverse environmental impacts (Hu et al. 2009). Chaurasia et al. (2019) showed that Ni-Co-P on SS316 is excellent cathode catalyst in MECs. All these ideas have drawn research attention toward the development of suitable H_2-producing biocathodes.

In comparisons of Pt-based chemical cathode catalysts, a biocathode or biocathode catalyst has advantages of low cost and self-generation without causing any environmental impact or secondary pollution (Jafary et al. 2015; Jafary et al. 2017). Rozendal et al. (2008c) reported the first development and use of a biocathode or biological cathode for H_2 generation from an in vivo isolated mixed-culture microorganism (EAB) and also explored the triple-stage start-up processes to enrich H_2-producing biocathodes. An MEC containing graphite felt electrodes has been constructed with a biological anode (anode biofilm) and acetate as substrate, first operated in a batch process and after that converted into continuous process mode. Jeremiasse et al. (2010b) demonstrated a fully biological MEC wherein both electrode (anode and cathode) reactions in the MEC were catalyzed by EAB, to differentiate and realize the performance differences from the half-cell electrochemical cell in MEC reported by Rozendal et al. (2008c) with a similar experimentation procedure. Huang et al. (2014) explored the biocathode in MECs for the energy recovery with some metals and found that cobalt (Co) was efficiently separated and obtained acetate and CH_4 yield at the E_{ap} of 0.2 V. In this MEC system, approximately 88.10% of Co(II) was recovered by concurrently obtaining the yields of 0.113 ± 0.000 mol CH_4/mol COD, 0.103 ± 0.003 mol acetate/mol COD, and 0.266 ± 0.001 mol Co/mol COD (Huang et al. 2014).

7.2.3 Membranes or Separators

The membrane or separator is another key component for desirable MEC operation. Cathode and anode chambers generally are physically separated by membranes or separators in MECs. A membrane selectively allows the ions or particles to pass as per their specified pore size. Membranes are also used to prevent mass, hydrogen, CH_4, transport of substrate, and microorganisms moving among anode or cathode chambers, except for H^+.

Membranes also act as a salt bridge or as a partition to prevent any short-circuit or unwanted mixing in MEC reactors (Escapa et al. 2016; Kadier et al. 2016a; Park et al. 2017). Various membranes or membrane materials have been explored in

Table 7.2 Performance of MECs with various kinds of membranes or separator materials

Types of membrane	HPR (H_2 m^3/m^3 day)	Current density (A/m^3)	Reference
CEM[a] (256 cm^2)	0.33	2.25	Rozendal et al. (2007)
Nafion 115 membrane[b]	5.8 ± 0.18	6.8 ± 0.1 A/m^2	
Sulfonated poly(arylene ether sulfone) (SPAES)/ polyimide nanofiber (PIN)	Efficiency of MEC: 90.3% for SPAES/ PIN	–	Park et al. (2017)
AEM[b]	7.10 ± 0.01	654 ± 22	Guo et al. (2017)
CEM[b] (Nafion)	0.5 ml/h	–	Cheng and Logan (2007)
AEM[b]	2.0 ml/h	–	
CEM[a]	1.22	1.8	Tartakovsky et al. (2009)
CEM[b]	0.36	92	Lu et al. (2010)
CEM[b]	0.12	2.8 A/m^2	Jia et al. (2012)
AEM[b]	0.43	109	Lu et al. (2010)
CEM[b]	12.9 ml	–	Chae et al. (2014)
CEM[b] (25 cm^2)	14.4 ml	–	Chae et al. (2014)
AEM[b]	3.6 mol H_2/mol Acetate	40.1	Yasri and Nakhla (2017)
Anion-exchange membrane[a] (256 cm^2)	0.31	2.37	Rozendal et al. (2007)
Composite proton-exchange membrane[c]	vs. 61.8% for Nafion-211		Park et al. (2017)
Bipolar membrane (8 cm^2)	0.018		Wang et al. (2013)
AEM[b] (30 mm)	1.10		Logan (2008)

CEM cation-exchange membrane, *AEM* anion-exchange membrane
[a] Single chamber
[b] Two chamber
[c] Three chamber

practical operation of MECs so far: anion-exchange membranes (AEM) and proton-exchange membranes (PEM) are widely used examples of common membranes in MECs and MFCs. Some researchers reported utilization of other membranes in MECs, including bipolar membranes, nanofiber-reinforced composite proton-exchange membrane (NFR-PEM), anion-exchange membrane (AEM), forward osmosis membranes, and charge-mosaic membranes (Table 7.2).

Nonetheless, the major weakness of the membranes is that a pH gradient is created through the membrane, which affects overall MEC performances by lowering the pH inside the anode chamber with a higher pH inside the cathode chamber or at the cathode as well as a reduction in energy recovery. Consequently, the pH gradient caused by the membrane increases losses and reduces the energy recovery of the MECs, which reduces overall MEC performance. These parameters are well demonstrated in MECs by Rozendal et al. (2008b) by utilizing a Nafion membrane, finding that a 0.38 V loss at the E_{ap} of 1.0 V is caused by a pH increase by 6.4 units. Also, several researchers reported that, from an economic perspective, membranes are expensive, and contribute to extensive cost in practical or large-scale MEC operations (Hu et al. 2008; Arends et al. 2014; Catal et al. 2015).

7.2.4 Power Supply (PS) or Driving Force of MEC

External power sources as applied voltage at cathodes in the MEC system trigger hydrogen production in MECs, which is the major driving force of MECs for various uses, and this differentiates the MECs among other bioelectrochemical systems (BES) or fuel cell systems. A conventional DC power supply source supplies constant DC power as applied voltage at cathodes to the MEC system. A potentiostat can be used as an external power source as E_{ap} as a substitute to the DC power supply in the MEC system (Nam et al. 2011). The potentiostat is a device that regulates voltage output to an operational and a reference electrode for conducting electro-catalyzed reaction measurements. Although the E_{ap} needed for MECs is very much less in comparison to water electrolysis, at 1.7–2.0 V (Zhang and Angelidaki 2014), but the overall external power utilization is still high, especially for those remote regions where electricity delivery to the extent needed for long-term MEC operation is challenging. Thus, numerous attempts have explored an appropriate substitute for the conventional DC energy sources or power supply to decrease the energy requirement of MEC reactions (Zhang and Angelidaki 2014; Varanasi et al. 2019).

Based on the existing literature, MFCs are the conventional BES system capable of generating bioelectricity from waste and wastewater; thus, MFCs were hypothesized as a potential renewable PS for MECs. Such a system was first reported by Sun et al. (2008): use of a single-chamber MFC directly feeds the power in the MEC and obtains a maximum HPR of 0.0149 m^3 H_2/m^3 day. In similar reports, to enhance voltage output, three MFCs of 350 ml each were incorporated in a series circuit pattern of a double-chamber MEC; the HPR was not enhanced (0.0145 m^3 H_2/m^3 day), even though E_{ap} increased to 0.807 V (Sun et al. 2009). Moreover, it was found that E_{ap} of the MEC system could be reduced by external resistance (R_{ex}) when MFCs were employed in a series circuit pattern, as described by Sun et al. (2010). Zhao et al. (2012) successfully showed that the MECs can also be utilized to feed the power by CO_2 reduction at the cathode; MFCs can be utilized to feed the external power requirement of MECs. Further, many important challenges need to be explored before the large-scale or practical applications. For instance, the external power sources or applied voltage required for the MECs system are less with the maximum detected voltage requirement in a usual MEC system, approximately 0.8 V (Cheng and Logan 2007). External power sources as applied voltage requirement of MECs may be adjusted by using multiple MFCs in a series circuit pattern; moreover, this theory and arrangement could not work properly for practical and long-term use of a MEC because of the voltage reversal phenomenon of MFCs (Oh and Logan 2007; Zhang and Angelidaki 2014). An additional constraint in such an arrangement is that the interactions of MFCs and MECs can make the system unstable with reduction in overall system performance. These challenges can be addressed by employing capacitors in the circuit to boost the voltage output and electrical energy transport efficiency of the MFC system, as clearly demonstrated by Hatzell et al. (2013). In this approach, MFCs are used in a parallel circuit pattern for the charging of the capacitors and then capacitors were designated to discharge in a series circuit to increase the voltage output applied to MECs. Such a circuit arrangement can increase overall system energy recovery by 9% to 13% and HPR to 0.72 m^3 from 0.31 m^3 H_2/m^3 day.

Among other renewable energy sources, Ajayi et al. (2009) showed the use of a dye-sensitized solar cell (DSSC) to feed the applied voltage of an MEC system. In this study, an open circuit voltage of 0.6 V was generated by this system, then provided to the MEC. This arrangement yielded 0.4 mol H_2 in the 5 h retention time and achieved 78% of cathode energy efficiency. An effort was made to decrease the cost of such an MEC system by utilizing non-Pt cathode materials, demonstrated by Chae et al. (2009). Chae et al. (2009) also showed a considerable increase in H_2 production by utilizing a carbon nano-powder-coated electrode as an alternative to the Pt cathode.

Nevertheless, extensive research is required to explore the scale-up possibility of a DSSC-MEC system for large-scale or industrial-scale H_2 production. More recently, Chen et al. (2016b) used the electricity harvested by the thermoelectric microconverter (TEMC) as a potential power source for MECs. The TEMC is an effective device to convert waste heat energy to electric power. In addition to the external power sources already stated, other potential renewable electricity or energy sources, including wind, hydropower (HP), and geothermal resources, could be an alternative PS for MECs. The electricity from those energy sources can be utilized completely in case of higher load periods whereas it is not utilized during lower loads. The additional electricity can be utilized by MECs for H_2, biochemical, and value-added product production. In such cases, the additional electricity or electrical energy is stored and kept in liquid and gas fuels as transportable fuels such as hydrogen gas or ethanol. However, many critical challenges must be addressed before operating such integrated systems.

7.3 Electrochemically Active Bacteria (EAB) and Extracellular Electron Transfer (EET) Mechanisms in MEC

The overall energy recovery of the MEC system is considerably affected by the activity of electrochemically active bacteria (EAB) by facilitating the transport of electron or electron generation from the substrate to the anode surface. EAB are the

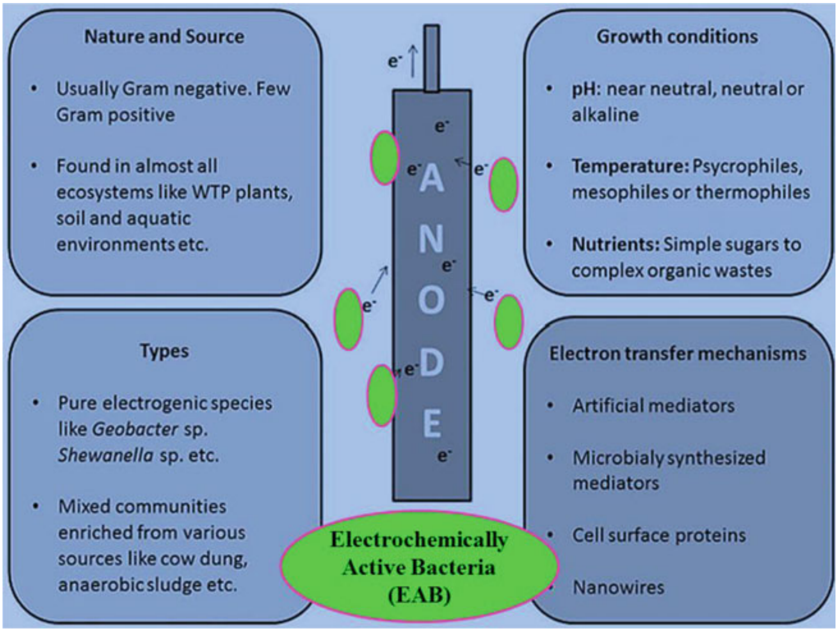

Fig. 7.1 Characteristics of EAB in MECs or MFCs

group of microorganisms called exoelectrogens employed at anode or anode chambers of MFCs or MECs that can transport the electrons from feedstocks to extracellular electron acceptors (EEA) (Kadier et al. 2016a, 2019; Saratale et al. 2017).

Lovley (2006) reported that EAB could be electricigens, exoelectrogens, and anode-respiring bacteria (Chen et al. 2016a). Interestingly, EAB have been found in mostly in vivo conditions (all natural environments) and are well described (Chabert et al. 2015; Varanasi et al. 2019), and in soil and domestic wastewater (DWW) (Heidrich et al. 2013; Escapa et al. 2014; Heidrich et al. 2014), and also in industrial wastewater and anaerobic sewage sludge (Guo et al. 2013). Tender et al. and Reimers et al. described and reported EAB in ocean waters, marine sediments, and municipal sewage/treated wastewater.

Various characteristics and physiological properties of EAB, such as morphology, genotype, nature and source, type, various growth environments, nutrient medium, and extracellular electron transfer (EET) or transport mechanisms are displayed in Fig. 7.1.

However, very few species and cell lines of EAB have been isolated or well characterized by detailed study, and thus further exploration is needed. Detailed reviews of EAB were done by Semenec and Franks (2014), Chabert et al. (2015), and Saratale et al. (2017), but most reported EAB have been found, for example, as gram-negative and anaerobic by utilizing Fe(III) or acetate as electron donors. Furthermore, substrate source and inoculum source are the crucial biological parameters that control the overall performance of MFC and MEC systems, as justified by many studies (Saratale et al. 2017).

Further, the effects of different anode biofilms or acclimation of anode procedures for improving energy recovery or H_2 yield in MECs have been well described by Ullery and Logan (2015), Doyle and Marsili (2015), and Li et al. (2017). Various inoculation and enrichment microorganism techniques are often utilized in the MECs (Chabert et al. 2015; Kadier et al. 2016a). (1) Initially, reactor systems were operated in MFC mode until reaching repeated stable current density or power generation, and then converted in MECs by transferring the anode biofilm to anode chambers of MECs (Liu et al. 2005b; Hu et al. 2008; Call and Logan 2008). (2) Utilizing discarded running MFCs/MECs or effluent from similar system or scraping the microorganism from bioanodes was reported by Cheng and Logan (2007) and Rozendal et al. (2008a). (3) Activated sludge of municipal sewage treatment plants, sewage wastewater, and anaerobic sludge are used as seed (inoculum) (Sun et al. 2014; Sosa-Hernández et al. 2016; Heidrich et al. 2014). The major drawback of using wastewater as inocula is the possible competition between the various microbial communities (Kumar et al. 2017b). (4) Use of pure culture or pre-cultured pure bacterial strains, cell lines, and species is also reported (Hu et al. 2008; Kadier et al. 2015, 2017a).

Moreover, EAB or other microorganisms act as biocatalysts at the anode or in anode chambers of MFCs/MECs systems. Good understanding of the mechanism of microbial EET is essential to increase electron transfer at anode or electrode. However, the actions and mechanism of EET are not well described in the literature. Three well-described EET mechanisms have been reported (Semenec and Franks 2014; Kadier et al. 2016a) (Fig. 7.2). (1) Direct electron transfer (DET): bacteria or microorganisms use their outer membrane for electron transfer (cytochrome *c*) and adhere on the anode surface or are physically associated to the anode surface (e.g., *Geobacter sulfurreducens*, *G. metallireducens*, *Clostridium* spp.). (2) Electron transfer or transport of electrons occurs by biogenic soluble mediators (electron transport by cells at anode surface) present in biological cell fluids and also produced by microorganisms (*Pseudomonas* spp.). (3) Transfer or transport of electrons occurs by bacterial appendages or pili as electrically conductive nanowires.

7.4 Main Operational Factors Affecting MEC Performance

Bio-H_2 production in the MECs is a multifaceted process that is greatly affected by the major operating parameters including applied voltage, pH, feedstocks, conductivity of nutrient medium, operating temperature, and electrode materials that influence Y_{H_2} and energy recovery (Parkhey and Gupta 2017; Kadier et al. 2016a, 2019) (Fig. 7.3).

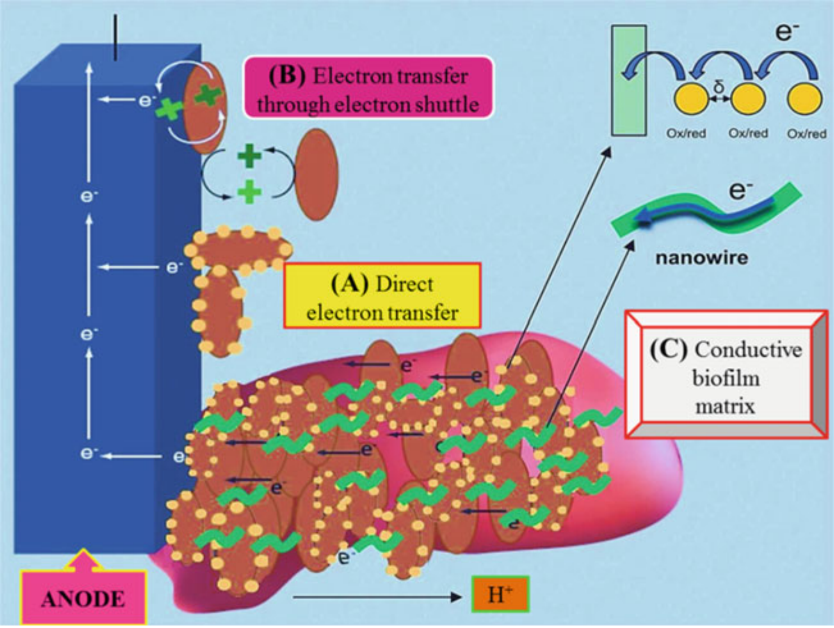

Fig. 7.2 Electron transport or electron transfer mechanisms of anode surface in an MEC. (**a**) Direct electron transport with aid of surface *c*-type cytochromes as microbial cells with direct contact with electron acceptor. (**b**) Electron shuttle or e$^-$ transport through e$^-$ shuttle. (**c**) Electron transport or transfer by conductive pili as a conductive biofilm

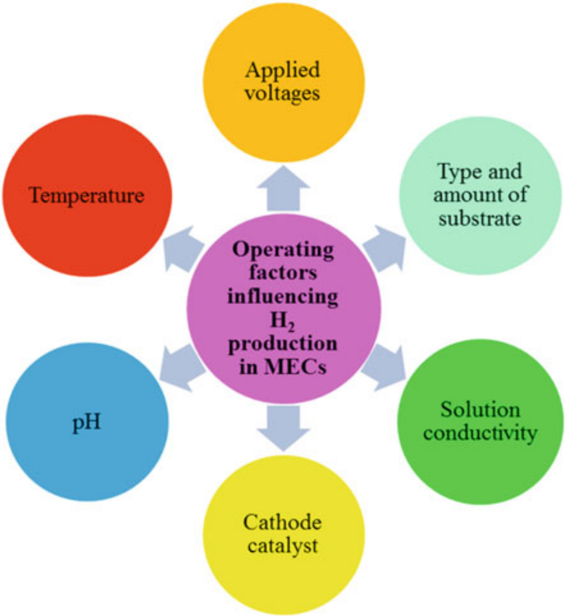

Fig. 7.3 Schematic representation of important operational parameters influencing the performance of MECs and energy recovery

7.4.1 pH

EAB are pH sensitive, and thus operating MECs at a constant pH or maintaining constant pH inside an MEC system is difficult. So, controlling pH for the desirable or optimal hydrogen production in MECs is a crucial factor. A neutral or high pH is always favorable for bacteria/exoelectrogens, whereas in fungi it is conflicting and an acidic or low pH range is required for optimum microorganism growth and activity (Logan 2008; Varanasi et al. 2019). Khan et al. (2012) reported that EAB growth and activity required a higher pH or basic range for electron transport to the anode electrode. Additionally, Nimje et al. (2011) studied the cyclic voltammetry for evaluation of pH effects on the energy recovery of the MECs. The observed experimental research reported an upsurge in microbial catalytic activity as a rise in pH rate of the nutrient medium or anolyte from 7 to 9. A similar experimental result was indicated by Rozendal et al. (2008c), that is, the increase of pH by approximately 6 units results in a reduction in the output of the cell potential of 0.38 V.

Moreover, the pH of the electrolyte solutions or nutrient medium is an important parameter that primarily affects the performance of MECs, as it controls the combined thermodynamics and kinetics of the process as well as the anode and cathode reactions. Liu et al. (2014) reported that the E_{cell} is inversely proportional to the pH, and at any time per unit increase in pH led to a decrease in anodic potential by 0.059 V unit. The hydrolysis of acetate (CH_3COO^-) in the MECs system for producing H^+, HCO_3^-, and electrons has the associated potential of approximately −0.28 V at pH 7 (Logan et al. 2006). Difference in pH as a general phenomenon was observed in the case of ion-exchange membranes (IEM) utilizing MECs, and it results in MEC below performance and reduction in the yield of hydrogen. Liu et al. (2014) described that pH 9 is the optimal pH in MECs for chemical oxygen demand (COD) degradation and increases in hydrogen gas yield, whereas HER and HPR decrease with the variation of pH value rather than the optimal pH, from the time when the general potential difference increases as the pH of the solution rises because of decreases in anodic potential with a rise in the pH.

7.4.2 Temperature

The microbial activity and MECs is temperature sensitive and MEC performance is greatly influenced by temperature. Thus, temperature (K or °C) is the key factor that influences current density (I_V), hydrogen production, and overall power recovery of MECs.

Subsequently, the MEC system is operated in anaerobic fashion, that is, the anodic chamber is in completely anaerobic condition where the tangible substrate or nutrient degradation take place, and the microbial growth and activity primarily depend on the mesophilic temperature range 35 –40 °C as reported and described by

Khan et al. (2014). Also, MEC energy recovery and performance are greatly influenced by variation in operating temperatures, although the degree of fluctuations of the EAB is lesser than with methanogens (Hu et al. 2009). The variation of temperature in MEC performances was estimated by Lorenzo et al. through exploring the MEC at 20 °C, 25 °C, and 30 °C temperatures, observing that maximum COD degradation and highest C_E were found at 30 °C.

Some studies also reported MEC operation at lower temperatures ($\leq$20 °C) and compared with the methanogenic reactors (Pham et al. 2008). Therefore, the temperature variation over EAB performances can be reflected in four ways: the microorganism evolution of EAB (1), the transport rate of mass/electrons (2), the activity of the nutrient/feedstock with respect to EAB (3), and the overpotential of the electrode such as anode and cathode materials (4), respectively. Thus, optimum temperature has direct and significant effects in the Y_{H_2} in MECs.

7.4.3 Types and Concentrations of Substrate

Substrate or feedstock degradation is empirically converted in the yield of hydrogen and energy recovery of MECs. Thus, the substrate source is the performance governing factor that affects H_2 production and energy yield of MECs. Diverse types of feedstock or substrate vary in organic degradation/conversion rates and proton generation rates, resulting in variation in the hydrogen yield (Y_{H_2}).

Various types of substrate can be used for hydrogen generation in MECs, such as polysaccharides, monosaccharides of sugars, acetate, carboxylic acid, proteins, and alcohols, as well as complex waste materials such as municipal wastewater, biomass hydrolysate, animal wastes, industrial/domestic wastewater, and foodstuff or beverage wastewater (Cheng and Logan 2007; Kadier et al. 2014, 2016a, b). A brief classification of substrates that can be employed in practical MEC operation and research is presented in Fig. 7.4.

Diverse substrates give different rates of feedstock conversion and H^+ generation or release rates, and thus variation in HPR to a larger extent has been reported (as shown in Table 7.1). Sodium acetate or acetate is the most widely explored and reported feedstock in MECs, probably as it is a general effluent of dark fermentation (DF). Jeremiasse et al. (2010a) obtained the higher HPR of 50 m^3 H_2/m^3 day by using acetate as the feedstock at 1 V applied voltage in laboratory-scale MEC operations. Acetate as substrate in MECs is widely explored for high hydrogen recovery (r_{H_2}), as being almost the theoretical value of 4 mol H_2/mol acetate, and recently researchers have been working to maximize it (Call and Logan 2008). Furthermore, Cheng and Logan (2007) investigated various fermentable substrates such as glucose and cellulose as well as nonfermentable substrates such as VFA, butyric, lactic, acetic, and propionic acid in the double-chamber MEC operation at 0.6 V applied voltage, and observed 67–91% hydrogen recovery. A comparable study was also described by Yang et al. (2015), examining DF by-products such as

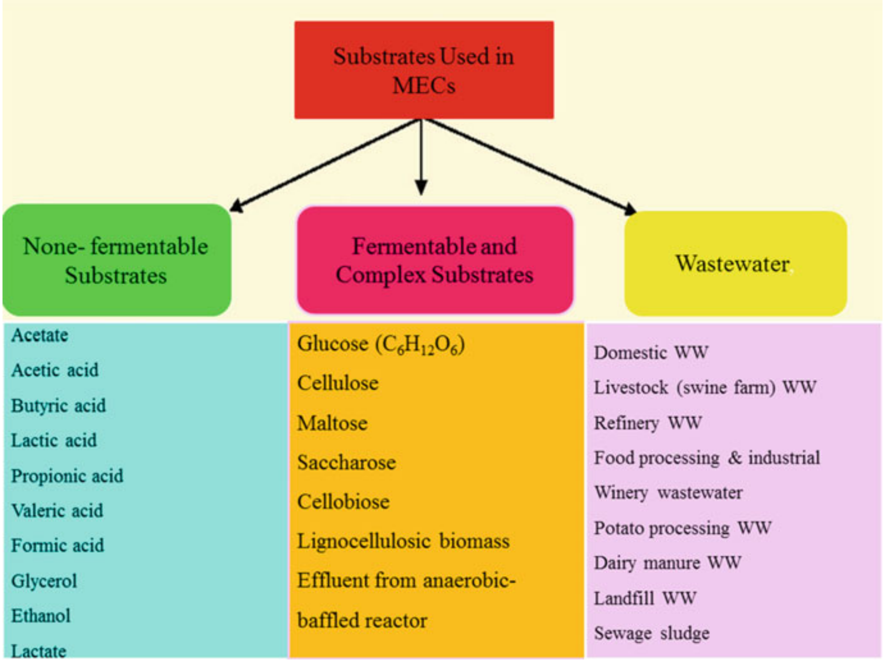

Fig. 7.4 Classification of various substrates explored in MEC literature

acetate, butyrate, and propionate as feedstock in practical MECs operation. He obtained the highest I_V for butyrate of 2.5 ± 0.06 A/m^2, acetate (6.0 ± 0.28 A/m^2), and propionate (1.6 ± 0.14 A/m^2). It was also shown that extra hydrogen can be generated by utilizing the effluent or end products of an ethanol DF fermenter (Lu et al. 2009), and from $C_6H_{12}O_6$ at lower temperatures of 4 °C in the practical operation of MEC (Liu et al. 2012b). In a similar study, very large variation in the results was obtained with some earlier MEC research work by Selembo et al. (2009b) (see Table 7.1).

Moreover, Sakai and Yagishita obtained the maximum Y_{H_2} of 0.77 mol H_2/mol glycerol with glycerol (B-glycerol) as substrate in a double-chamber MEC. Selembo et al. (2009b) gained a competitive HPR of 2.01 ± 0.41 m^3 H_2/m^3 day with ultrapure glycerol and 1.87 ± 0.30 m^3 H_2/m^3 day with glucose as substrate in MECs. Chookaew et al. (2014) successfully enhanced the H_2 production in MECs with glycerol by combining DF, MFC, and MEC. It was hypothesized that microorganisms such as bacteria produced higher rates of hydrogen by fermentation of carbohydrates in comparison to the fermentation of proteins. There are some reports that hydrogen production at HPR and yields can be obtained by proteins as substrate in MECs. A hydrogen production rate of 0.42 ± 0.07 m^3 H_2/m^3 day was obtained by Lu et al. (2010) by using protein-containing wastewater in the MECs, suggesting that a good rate of hydrogen generation can be achieved by using protein wastewater as substrate in an MEC. Similar research work by Nam et al. (2014) testified that obtaining hydrogen production from cellulosic fermentation of wastewater as substrate, which contains higher concentrations of proteins, alcohol, and volatile fatty acids (VFAs) in a continuous-feeding MEC system, required keeping protein content as the governing factor.

Protein content was completely degraded in the MEC at a high rate of applied voltage as $E_{ap} = 1.0$ or 1.2 V. A maximum HPR of 0.63 ± 0.02 m^3 H_2/m^3 day was achieved with synthetic wastewater containing merely acetate ($E_{ap} = 0.9$ V) as compared to the 0.49 ± 0.05 m^3 H_2/m^3 day with fermentation wastewater as the substrate.

Some recent reports suggested that lignocellulose as substrate in MECs for hydrogen production is a promising feedstock because of the abundance and renewability properties of lignocellulose materials, although lignocellulose materials cannot be directly used by microbes in MECs for hydrogen production and require pretreatment. Lignocellulose must first be converted into low molecular weight compounds such as monosaccharides. Such research also reported that integration of the multistage process of MFC/MEC with DF or the two-stage process of MEC and DF was explored to utilize lignocellulosic materials for hydrogen production (Lalaurette et al. 2009; Lu et al. 2009; Wang et al. 2011; Ullery and Logan 2015). In this regard, Catal (2015) demonstrated the direct production of hydrogen from lignocellulosic biomass and various carbohydrates as substrates in the MEC and achieved HPR in the range of 0.01 m^3 H_2/m^3 day (arabinose) to 0.09 m^3 H_2/m^3 day (cellobiose). Furthermore, Yuan et al. (2014) explored hemicellulose as substrate by *Moorella thermoacetica* in MEC for electricity and H_2 production in a two-chamber MFC/MEC following DF. Lewis et al. (2015) successfully demonstrated a novel approach for hydrogen generation from switchgrass by utilizing an integrated pyrolysis–MEC system, and achieved Y_{H_2} ranging from $50 \pm 3.2\%$ to $76 \pm 0.5\%$. Recently, Rivera et al. (2017) reported that cheese whey (CW) as substrate in the MEC system can recover hydrogen as well as energy and by-products from industrial wastewater. Interestingly, Khan et al. (2014) estimated by comparative study about variation in output power densities and C_E with the bacteriological species profile of double indistinguishable BESs in the MEC, where both MECs were operated with two different substrates, acid navy blue r (a naphthalenedisulfonate dye) and azo dye-reactive orange 16.

7.4.4 Applied Voltage (E_{ap})

From various previous studies (Liu et al. 2005a; Rozendal et al. 2006a, 2007; Ditzig et al. 2007), HER at the cathode of MECs is not a thermodynamically favorable reaction; therefore, a range of applied voltage as low as 0.11–0.23 V is needed for hydrogen gas production in the MEC system. However, some research groups reported that lowering the applied voltage E_{ap} of the MEC will reduce the hydrogen yield Y_{H_2} and organic matter removal. Specifically, in those cases in which E_{ap} was remaining at less than 0.3 V, the HPR was lowered (Tartakovsky et al. 2009).

The peak value of HPR was observed when E_{ap} varied from 0.8 to 1.0 V (Logan et al. 2008). In addition, acetate removal and HPRs were proportional to applied voltage (E_{ap}) in the acetate-fed single-chamber MEC operated at 0.4–1.2 V applied voltage (Tartakovsky et al. 2009). Similarly, studies conducted with various feedstocks demonstrated a similar dependency among E_{ap} and HPR and feedstock/nutrient degradation rates (Lu et al. 2009; Escapa et al. 2009). Furthermore, overall 96% r_{H_2} was recovered by utilizing an ethanol–H_2 coproducing fermentation in MECs at E_{ap} of 0.5–0.8 V; in contrast, 83% of the overall r_{H_2} was recovered by utilizing the effluent as the substrate in the MEC at applied voltage E_{ap} of 0.6 V (Lu et al. 2009). Estimation of the voltage variation for augmenting anode potential by Nam et al. (2011) indicated that overall hydrogen production at E_{ap} of 0.2 V of anode potential is higher in a single MEC, and this is higher than that for the same system under the same operating conditions at $E_{ap} = 0.6$ V (Nam et al. 2011). A higher Y_{H_2} and hydrogen production rate have been achieved with 0.2–0.8 V of E_{ap} in neutral pH at 25 °C in a typical MEC system, whereas reduction in anode energy loss was obtained with lower anode potential without current limitation (Lee et al. 2009). Cheng and Logan (2007) demonstrated that hydrogen production was achieved with $E_{ap} = 0.2$ V in MEC but that 0.3 V E_{ap} may reduce hydrogen production and cause low HPR with erratic system performance (Liu et al. 2005a; Rozendal et al. 2006a). Logan et al. (2008), Liu et al. (2010), and Kadier et al. (2015), among others, recommended the use of $E_{ap} > 1.2$ V because of the higher requirement of electrical energy in the MECs, turning it nearer to a water electrolysis process. Electron transfer at the MEC anode becomes restricted by the metabolic activity of EAB with $E_{ap} \geq 1.2$ V (Tartakovsky et al. 2009); further, numerous studies emphasized that the most commonly utilized E_{ap} value seems to be 0.3–1.2 V (Cheng and Logan 2007; Hu et al. 2008; Call and Logan 2008; Logan 2008; Lalaurette et al. 2009; Tenca et al. 2013). To obtain a high current density (I_V) and HPR in MECs, $E_{ap} \geq 0.7$ V has been selected because this range of applied voltage is acceptable for comparatively fast cycle times compared with individual findings achieved with low E_{ap} (Chae et al. 2008; Call and Logan 2008; Choi et al. 2019; Kadier et al. 2018a).

7.4.5 Solution Conductivity, Electrode Distance, and Electrolytes or Ionic Strength

Electrolyte or solution conductivity is a significant factor that alters hydrogen yields and HPR in the typical MEC operation, but generally these effects are not very significant. Higher electrolyte solution conductivity offers enhanced electron transfer or ion transfer, leading to enhanced energy recovery in the MEC: thus, solution or electrolyte conductivity is inversely proportional to the resistance R_{in} factor of the

MECs. Many reports, including Call and Logan (2008), indicate that effects of solution conductivity as increases of solution conductivity from 7.5 to 20 mS/cm result in increased HPR and hydrogen yield. Additionally, Logan et al. (2006) demonstrated that higher solution conductivity can reduce ohmic losses in the MEC system. On the other hand, such losses can be also be decreased by optimizing the electrode distance as distance between the anode and cathode, by incorporating a membrane of low resistivity and checking the proper connections of the MEC system (Logan et al. 2006). MECs fed with a low-conductivity solution show considerable reduction in R_{in} of the system when applying a small change in electrode distances. Higher-conductivity solutions have no remarkable effects on the R_{in} of the MEC with electrode distance (Rozendal et al. 2008b). Increasing the electrode position from 0.4 to 1.4 cm and reducing anolyte/electrolyte conductivity from 7.8 mS/cm to 1.8 mS/cm offers increases in the R_{in} of MECs (Hutchinson et al. 2011). The effects of various electrolyte solution conductivity, solution resistance, and HER of MECs are well described by Merrill and Logan (2009).

Merrill and Logan (2009) also demonstrated that MECs at lower pH or in acidic condition (pH 5) work more efficiently in phosphate and acetate electrolyte solutions, whereas carbonate electrolytes improved the overall MEC energy recovery in the pH range 5–9. Furthermore, Jeremiasse et al. (2009) reported that the use of biocompatible buffers led to reduction in concentration overpotential and enhanced the HPR. Moreover, Munoz et al. (2010) showed that the use of phosphate-buffered solution (PBS) at pH 8 can multiply the hydrogen yields by many fold; a maximum HPR of 4.9 $\mathrm{l/h/m^2}$ was achieved because phosphate ions can enhance I_V and H_2 production. A similar study reported by Yossan et al. (2013) examined four different catholytes: PBS, NaCl solution, acidified water, and water. The performance of MECs with a 134 mM NaCl solution (HPR, 0.171 ± 0.012 $\mathrm{m^3\ H_2/m^3}$ day) and acidified water (HPR, 0.171 ± 0.004 $\mathrm{m^3\ H_2/m^3}$ day) was competitive with that using a 100 mM PBS buffer (0.237 ± 0.031 $\mathrm{m^3\ H_2/m^3}$ day). Such results clearly indicate that the NaCl solution could be a potential alternative as MEC catholyte for reducing cost and chemical reuse or disposal.

7.4.6 MEC Reactor Configurations

The MEC reactor system geometry and configuration also have direct effects on the capital cost, current density, HPR, and also the energy efficiency of the MEC system (Kadier et al. 2016b, 2018b; Escapa et al. 2016; Varanasi et al. 2019). In the past decade, new reactor configurations and geometry have been explored, enhancing the performance of MECs from 0.0045 and 0.02 $\mathrm{m^3\ H_2/m^3}$ day (Logan 2008) to 17.8 $\mathrm{m^3\ H_2/m^3}$ day (Cheng and Logan 2011), an increase of 100- to 5000 fold. Various reactor designs of MEC have been studied, such as two-chamber, single-chamber, and stacked construction, by integrating MEC with other electrochemical technologies.

7.5 Conclusion

This chapter systematically summarizes the effects of crucial factors on the efficiency of the process of wastewater treatment with bio-H_2 in an MEC system. Electrochemically active bacteria (EAB) also affect the performance of MECs to a significant extent. In recent reports a diverse range of microorganisms (EAB) used in MECs are isolated and well characterized. However, very little research has been reported on the mechanisms of microbial extracellular electron transfer in MECs, and this has drawn recent research attention. Moreover, this literature review demonstrates the different substrates that have been explored in MECs so far, and also their effects on MEC energy recovery. In the initial phase (2005–2008) of MECs, simple feedstocks such as pure acetate and glucose were mostly used, whereas current research is additionally focused on utilizing more unconventional renewable substrates such as industrial wastewater, biomass, and treated wastewater with the aim of a sustainable environment. Furthermore, many good efforts are summarized for the development of various diverse types of effective and economical anodes or cathodes and their material and catalysts for hydrogen production in MECs. Some reported materials such as SS, Ni, and palladium (Pd) nanomaterials coating the cathode have potential for large-scale MEC operation with enhanced efficiency. Industrial or large-scale application of MECs must be optimized for a good balance among efficiency and availability of the cathode material to catalyze HER. The optimum pH and temperature of MECs can facilitate the aid to the microbial community and enhance MEC performance.

Furthermore, solution conductivity, ionic strength, and salinity are crucial factors for MEC operation. Despite the destructive impacts of salt concentration on microbial growth, conductivity, higher salinity, and ionic strength enhance the conductivity of electrolytes as well as overall MEC performance. Undoubtedly, the potential of MECs for wastewater treatment and bioenergy production requires us to be committed to its commercial application in the future.

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Chapter 8
Effective Cathode Catalysts for O_2 Reduction Reactions

Dang-Trang Nguyen and Kozo Taguchi

Abstract Microbial fuel cells (MFCs) have emerged as one of the promising technologies for energy-harvesting from domestic waste treatment. However, the slow cathodic reaction kinetics in MFCs causes a high overpotential of the oxygen reduction reaction (ORR) and leading to potential loss. Therefore, the development of effective cathode catalysts is required to facilitate the practical application of MFCs. Various studies have paid attention to improving the cathodic catalysis by developing various types of materials, which have properties such as active ORR catalytic activity, low-cost, durability, and scalability. In this chapter, the development status and fundamental principle of several catalysts used in the cathode of MFCs are discussed, which include electrocatalysts, photoelectrocatalysts, and biocatalysts.

Keywords Microbial fuel cell · Oxygen reduction reaction · Cathode catalyst · Electrocatalyst · Biocatalyst · Photoelectrocatalyst

8.1 Introduction

The development of microbial fuel cells (MFCs) offers a promising method for energy-efficient domestic waste treatment while generating electricity (Fig. 8.1). Currently, the low power output (Fig. 8.2) and high cost restrict the practical application of MFCs. The cathodic reaction is considered to be the major bottleneck in MFC development (Wang et al. 2014b; Yuan et al. 2016). Therefore, the development of stable and active cathode catalysts is necessary to facilitate electron transfer efficiency from the cathode to the terminal electron acceptor. Because of the inexhaustible availability of oxygen, it is considered as an ideal terminal electron acceptor. However, the oxygen reduction reaction (ORR) is sluggish due to the

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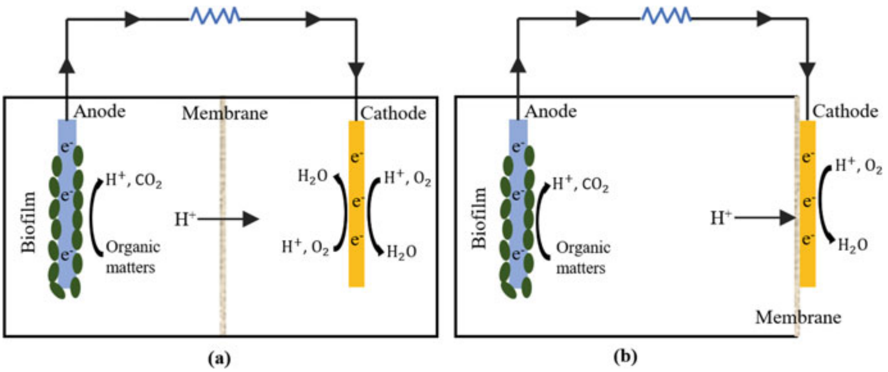

Fig. 8.1 Diagrams of (**a**) a two-chamber MFC and (**b**) a single-chamber MFC

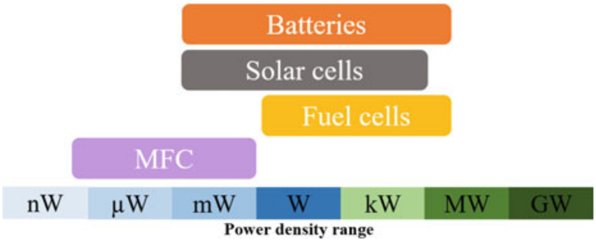

Fig. 8.2 Schematic diagrams of the present power generation capacity of MFC technology in comparison with other energy production technologies. Reprint with permission from Bullen et al. (2006). Copyright (2006) Elsevier

O=O bond which requires high energy to break, resulting in the potential loss at the cathode (Fan et al. 2008). Non-catalyzed cathodic materials are inefficient due to the high overpotential of ORR. Therefore, active catalysts are generally required to enhance the performance of MFCs.

High-performance cathode catalysts are required to have active ORR catalytic activity, low-cost, durability, and scalability. In general, three families of cathode catalysts are electrocatalysts, photoelectrocatalysts, and biocatalysts. Table 8.1 lists some cathode catalysts, which have been used in MFCs. The catalytic reaction in the cathode of MFCs significantly depends on electron transfer efficiency at the interface between the electrode and catalyst. Thus, the electrical and structural attributes of cathode catalysts have a substantial influence on overall cathodic performance.

Among various electrocatalysts investigated to date, platinum (Pt) shows the most active ORR catalytic activity (Santoro et al. 2017a). Nevertheless, the high-cost nature of Pt and its instability when working with wastewater hamper its scalability (Wang et al. 2014b). Thus, many attempts have been made to develop alternative electrocatalysts. Several scalable materials with active ORR catalytic activity have been investigated (Table 8.1).

Table 8.1 Representative cathode catalysts used in MFCs

Category	Material type	Catalyst	Reference
Electrocatalysts	Metal-based materials	Pt	Halakoo et al. (2015)
		Ni	Kadier et al. (2015)
		Ag	Noori et al. (2016)
		MnO_2	Majidi et al. (2019)
	Carbon-based materials	Carbon black	Song et al. (2019)
		Activated carbon	Bose et al. (2019)
		Carbon nanotubes	Bhowmick et al. (2019)
		Graphite	Chen et al. (2018)
		Graphene	Santoro et al. (2017b)
	Carbon-metal hybrids	MnO_2-activated carbon	Singh and Chandra (2013)
		Co_3O_4-carbon nanotube	Ge et al. (2015)
		Co/Fe-carbon nanotube	Türk et al. (2018)
Photoelectrocatalysts	Semiconductors	TiO_2	Mashkour et al. (2017)
		$CuInS_2$	Wang et al. (2014a)
		$LiTaO_3$	Benzaouak et al. (2017)
Biocatalysts	Enzymes	Laccase	Mani et al. (2017)
	Microorganisms	Mixed culture	Xia et al. (2013)

Photoelectrocatalysts take advantage of some semiconductors that can harvest light energy to facilitate cathodic reaction in MFCs. The semiconductors are required to have the capability of utilizing the redox potential gained from light energy to support ORR in the cathode. So far, only a few photoelectrocatalysts have been utilized to make cathode catalysts in MFCs such as TiO_2, $LiTaO_3$, and $CuInS_2$.

Different from abiotic electrocatalysts and photoelectrocatalysts, biocatalysts are based on enzymes and microorganisms. Enzymes with high redox activity have been utilized to improve the ORR in the cathode of MFCs, such as laccase and bilirubin oxidase could be immobilized on air-breathing cathodes to work as cathode catalysts (Santoro et al. 2016; Mani et al. 2017). In addition to enzymatic catalysts, some particular microorganisms can consume electrons from the cathode to enable ORR (Jiang et al. 2017), and thus can be used to facilitate the cathodic reaction. Fundamental research has indicated that microorganisms utilized in the biocathode have similar electron transfer pathways as exoelectrogens (Freguia et al. 2010). A variety of microorganisms, such as *Shewanella putrefaciens, Geobacter sulfurreducens, Acidithiobacillus ferrooxidans, and Chlamydomonas reinhardtii*, have been used to improve ORR (Liu et al. 2014).

In general, the most popular use case of MFCs is energy-harvesting from domestic waste treatment. Therefore the catalyst materials should possess some properties, including low-cost, simple synthesizing processes, high catalytic activity, durability, and stable operation in the wastewater environment. Those properties are also the challenges in developing high-performance cathode catalysts.

8.2 Cathode Catalyst and ORR Mechanisms

In the anode compartment of MFCs, microorganisms consume biofuels and produce electrons and protons. These electrons and protons migrate to the cathode compartment, where the reduction reaction takes place.

The rotating ring disk electrode (RRDE) is a technique usually used for studying the mechanisms of cathode catalysts during ORR (Schmidt and Gasteiger 2010). The RRDE technique enables studying the electrochemical reaction mechanisms on the surface of cathode catalysts (Jia 2014; Kodali et al. 2017). ORR catalyzed by abiotic catalysts follows two different pathways, which are four-electron and two-electron pathways (Yuan and He 2015). The four-electron pathway can be represented by Eq. (8.1).

$$4H^+ + 4e^- + O_2 \rightarrow 2H_2O \tag{8.1}$$

While Eqs. (8.2) and (8.3) represent the two-electron pathway.

$$2H^+ + 2e^- + O_2 \rightarrow H_2O_2 \tag{8.2}$$

$$2H^+ + 2e^- + H_2O_2 \rightarrow 2H_2O \tag{8.3}$$

The four-electron pathway is favorable because, for one step, two oxygen atoms can reduce four electrons, resulting in higher reduction potential. Meanwhile, the two-electron pathway takes two steps to reduce four electrons leading to energy loss. Moreover, the production of hydrogen peroxide (H_2O_2) in the second step (Eq. (8.3)) causes damages to the membrane and catalytic layer.

At neutral condition, Pt catalyst facilitates the four-electron pathway with negligible intermediate products. Meanwhile, carbon-based catalysts (carbon black and activated carbon) and other metal-free catalysts tend to have the two-electron pathway with more H_2O_2 production (Artyushkova et al. 2015; Merino-Jimenez et al. 2016).

Cathodic energy losses (also called cathode overpotential) due to the cathodic catalysis in MFCs can be categorized into kinetic losses and thermodynamic losses. Kinetic losses include activation, ohmic, and concentration losses. These overpotentials depend on the strength of the generated current density. Activation losses dominate when the low current density is generated. They are caused by a low electron transfer rate between the cathode catalyst and oxygen during ORR. Therefore, activation losses are expected to reduce when the surface area and the activity of the cathode catalyst are improved. In addition, the dominance of ohmic losses is in the middle current density region. To minimize ohmic losses, the electrical and ionic conductivity of the cathode and catholyte, respectively, must be maximized. Besides, concentration losses tend to become significant at the high current density region, where electron supply is abundant, leading to large concentration gradients in the cathode vicinity. By improving the diffusibility of oxygen into the cathode, concentration losses can be reduced.

Thermodynamic losses at the cathode of MFCs are caused by the potential difference between redox couples inside the cathode. There is an inverse relationship between the cathode potential and thermodynamic overpotential (Liu et al. 2014).

8.3 Electrocatalysts

Abiotic electrocatalysts based on metal and carbon are the most popularly used cathode catalysts in MFC studies. Electrocatalysts can be categorized into three major groups based on the based materials, which are metal-based, carbon-based, and carbon-metal hybrid electrocatalysts.

8.3.1 Metal-Based Electrocatalysts

Some pure metals exhibit excellent catalytic activity towards ORR. Precious and rare Pt is the most active ORR catalysts according to the theoretical calculation based on both O_2 binding energy (Fig. 8.3a) and O&OH binding energy (Fig. 8.3b) (Nørskov et al. 2004). Therefore, the performance of Pt-based cathodes is usually higher compared with other types of metal-based cathodes. The cathodic catalysis of Pt follows a multi-process mechanism. According to the density functional theory, the catalytic mechanism took placed at the Pt surface can be demonstrated by two different mechanisms depended on the current density level. At low current density, the dissociative mechanism dominates (Eqs. (8.4), (8.5), and (8.6))

$$^{\bullet} + \frac{1}{2}O_2 \rightarrow {}^{\bullet}O \tag{8.4}$$

$$^{\bullet}O + H^+ + e^- \rightarrow {}^{\bullet}OH \tag{8.5}$$

$$^{\bullet}OH + H^+ + e^- \rightarrow {}^{\bullet} + H_2O \tag{8.6}$$

while at high current density, the associative mechanism takes place (Eqs. (8.7), (8.8), (8.9), (8.10), and (8.11)) (Zhdanov and Kasemo 2006).

$$^{\bullet} + O_2 \rightarrow {}^{\bullet}O_2 \tag{8.7}$$

$$^{\bullet}O_2 + H^+ + e^- \rightarrow {}^{\bullet}HO_2 \tag{8.8}$$

$$^{\bullet}HO_2 + H^+ + e^- \rightarrow H_2O + {}^{\bullet}O \tag{8.9}$$

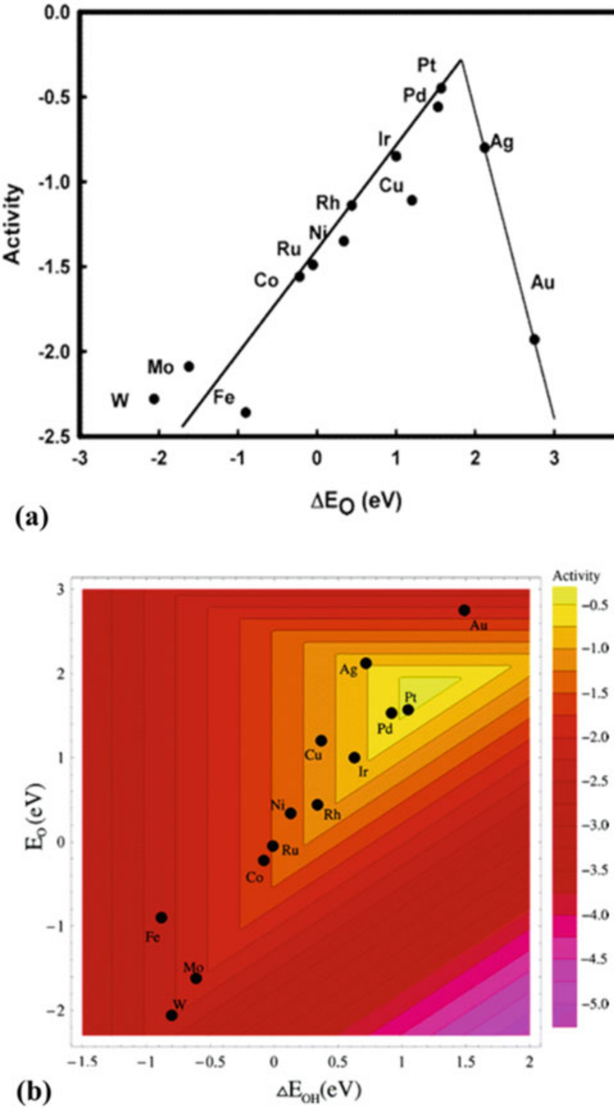

Fig. 8.3 The ORR catalytic activity of some pure metals evaluated in the cathode. Plots are based on O_2 binding energy (**a**) and O&OH binding energy (**b**). Reprint with permission from Nørskov et al. (2004). Copyright (2004) American Chemical Society

$$^{\bullet}O + H^{+} + e^{-} \rightarrow {}^{\bullet}OH \qquad (8.10)$$

$$^{\bullet}OH + H^{+} + e^{-} \rightarrow {}^{\bullet} + H_2O \qquad (8.11)$$

(In these equations ● indicates active sites on Pt surface).

Although Pt exhibits high catalytic performance, its real-world applications are impractical due to high cost and scarcity. Furthermore, the stability of Pt-based cathodes is easily affected by some organic ions commonly existing in wastewater, such as Cl^- and S^{2-}. With these shortcomings, Pt is hardly expected to use in large-scale MFCs.

To lower the cost without compromising the overall performance of Pt-based cathodes, many studies tried to reduce the amount of Pt. Some methods, such as coating Pt on carbon paper and alloying Pt with inexpensive transition metals, have been proposed. Pt alloys can reduce the oxygen-binding energies and further improve the ORR catalytic activity of pristine Pt. Also, other pure metals, such as Ag, Ni, and Co, can be used as alternatives for Pt in the MFC cathode with comparative catalytic performance.

Furthermore, the cathodic catalysis occurring in the MFC cathode is similar to that in conventional electrochemical systems (CESs). Since decades ago, manganese oxides (e.g., MnO_x) have been utilized in the cathode of CESs (Thackeray et al. 1993). Therefore, manganese oxides are also extensively applied in MFCs as an active cathode catalyst. It has been found out that the catalytic properties of MnO_x depend on its oxidation state (Stoerzinger et al. 2015). Also, the crystal structure and morphology of MnO_x can affect the catalytic activity and the ORR electron-transfer pathway(Liu et al. 2010). Most of the MnO_x-based cathodes used in MFCs exhibited only about 50% the power density of the Pt-based cathodes (Yuan et al. 2016).

8.3.2 Carbon-Based Electrocatalysts

Carbonaceous materials seem to be viable for the practical application of MFCs because of low-cost and stability. To understand the catalytic mechanism of carbon-based materials, a mechanism was proposed by Tammeveski et al. (2001)) as follows (Eqs. (8.12) and (8.13)).

$$Q + e^- \rightarrow Q^{*-} \tag{8.12}$$

$$Q^{*-} + O_2 \rightarrow {O_2}^{*-} + Q \tag{8.13}$$

Q, Q^{*-}, and ${O_2}^{*-}$ represent quinone groups, semiquinone radicals, and superoxide anion, respectively.

Various carbonaceous materials have been used to support ORR in the cathode of MFCs (Table 8.1). Most of the carbonaceous materials exhibit the two-electron pathway. However, the vertically aligned nitrogen-doped carbon nanotubes (CNTs) and nitrogen-doped graphene are considered to follow the four-electron pathway (Higgins et al. 2011). Generally, the specific surface area (SSA) of carbonaceous materials is considered very large, which also facilitates ORR.

Graphite and Graphene

Graphite is usually used for the anode electrode in MFCs. Pure graphite is not considered as an effective ORR catalyst due to the lack of active sites (Deval et al. 2017). However, the cathode based on graphite activated by nitric and phosphoric acids was proved to significantly improve MFC performance (Zhang et al. 2016a). Also, nitric acidic treatment of graphite could enhance its ORR catalysis due to the increase of SSA and the introduction of nitrogen functional groups (Li et al. 2018).

Graphene is a single-layer carbon nanosheet, which can be extracted from the graphite surface. Graphene with high electrical conductivity and large SSA is an active catalyst for cathodic catalysis (Yuan and He 2015). Nitrogen-doped graphene exhibits the four-electron pathway mechanism. Some studies using the nitrogen-doped graphene as cathodic catalysts even obtained slightly better catalytic performance than Pt (Liu et al. 2013b). A novel approach of implanting mesoporous graphitic carbon nitride (C_3N_4) into graphene could enable a high nitrogen/carbon ratio up to 19.7 %, which resulted in exhibiting the complete four-electron pathway towards ORR (Feng et al. 2013).

CNTs

CNTs are constructed by one or multi-layers of graphene sheets wrapped concentrically. There are two types of CNTs, which are single-walled and multiwalled. Single-walled CNTs are superior to multiwalled CNTs in most aspects (Luo et al. 2015). However, due to the cost of single-walled CNTs is much more expensive than multiwalled CNTs, multiwalled CNTs are more popularly used in MFCs (Nguyen and Taguchi 2019a, b).

ORR catalytic activity of CNTs usually follows the two-electron pathway. However, the porous nitrogen-doped CNTs (PNCN) were found to exhibit the four-electron pathway and significantly enhance its ORR catalytic activity. Ref. (Wen et al. 2014) has demonstrated that the performance of the PNCN-based cathode was significantly better than that of the Pt-based cathode. Moreover, the cathodic catalytic activity of CNTs could be improved by making the composite of CNTs and some polymers (Ghasemi et al. 2016).

Carbon black

A popular carbon-based material usually used as a support material in the cathode is carbon black (Song et al. 2019). Although natural carbon black is not considered an active ORR catalyst, conducting chemical treatments to introduce some functional groups into carbon black can exhibit moderate ORR catalytic activity (Zhang et al. 2016b). For instance, the power density generated by an MFC using the nitric acid-treated carbon black-based cathode has increased 3.3-fold compared with the pristine carbon black-based cathode (Duteanu et al. 2010).

Furthermore, by mixing carbon black with activated carbon to make the cathode of MFCs, the catalytic activity and stability of the cathode were significantly enhanced (Zhang et al. 2014b). Due to its abundance, carbon black is expected to be used as a scalable cathodic catalyst for MFCs.

Activated carbon

Another widely used carbon-based electrocatalyst is activated carbon, which has a highly porous structure, large SSA ($>1000\ m^2\ g^{-1}$), and high electrocatalytic activity (Nguyen and Taguchi 2019c). Activated carbon produced from different carbonaceous precursor materials will possess different porous structures and catalytic functional groups. For instance, bituminous coal-, peat-, coconut shell-, phenolic resin-, and hardwood-based activated carbon powders were used as cathode catalysts in MFCs and yielded varied ORR catalytic performance (Watson et al. 2013a). This study also revealed (1) the inverse relationship between the content of acid functional groups and the onset potential and (2) acidic functional groups on the surface cause poor cathodic performance.

The cathodic catalytic activity of activated carbon can be improved by treating activated carbon in acid or alkali solutions. Similar to other carbon-based materials, doping nitrogen into activated carbon is also an effective method to improve its ORR catalysis. Nitrogen atoms could be doped into activated carbon by treating activated carbon in nitrogen precursors such as ammonia gas at high temperature (Watson et al. 2013b) or solid-state cyanamide (Zhang et al. 2014a). By utilizing these methods, the catalytic performance of nitrogen-doped activated carbon could outperform Pt/C in MFCs.

8.3.3 Carbon-Metal Hybrid Electrocatalysts

Generally, the purpose of hybridizing carbon- and metal-based electrocatalysts is to take full advantage of both types of materials. Carbon-metal hybrids can be fabricated by mechanically mixing method or electrochemical deposition method. Many studies have shown superior ORR catalytic activity of carbon-metal hybrids compared with Pt/C.

Graphite/Graphene-Metal

The ORR catalytic activity of graphite is poor due to the lack of active sites. Therefore, some metals such as silver and iron were coated on the surface of graphite to create ORR active sites (Ma et al. 2015). Also, Fe_2O_3 and FeOOH were deposited on graphite to enable high ORR catalytic activity (Wang et al. 2013).

Graphene is usually used in combination with various metals to enable outstanding ORR catalytic activity. Graphene was hybridized with iron and silver to exhibit high cathodic catalysis (Lv et al. 2019). Moreover, graphene shows the high catalytic performance when combined with metal catalysts, such as Pt-Pd, Ni-Co, MnO_2, and SnO_2 (Ben Liew et al. 2014).

CNT-Metal

High ORR catalytic metals can be coated on the surface of CNTs to improve further its ORR active sites. For example, Pt/CNTs hybrid was demonstrated to exhibit higher ORR catalytic activity than Pt while reducing the amount of used Pt (Halakoo

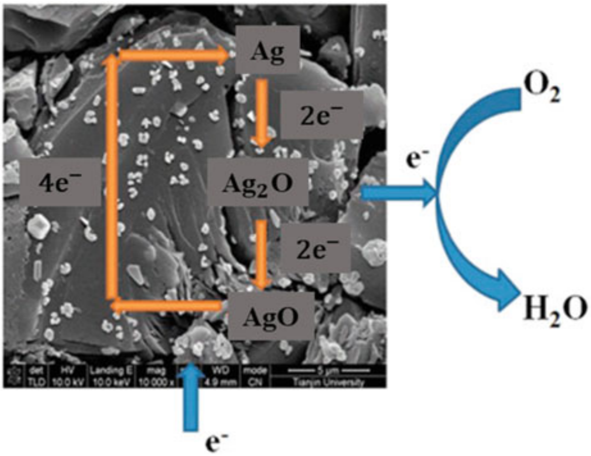

Fig. 8.4 SEM image of the activated carbon-Ag hybrid and the proposed charge transfer mechanism. Reprint with permission from Pu et al. (2014). Copyright (2014) Elsevier

et al. 2015). Compared with Pt/carbon cloth cathodes, Pt/CNTs cathodes could achieve 100% higher the power density despite using only one-fifth of Pt quantity (Ghasemi et al. 2013). Instead of using high-cost Pt, iron and cobalt could also be used to improve the cathodic performance of multiwalled CNTs in MFCs and to reduce the overall material cost (Iannaci et al. 2016; Türk et al. 2018).

As a non-precious metal oxide, MnO_2 was hybridized with CNTs to enhance the cathodic performance while maintaining cost-effectiveness (Lu et al. 2013). Moreover, cobalt oxide (Yang et al. 2019) and nickel oxide (Huang et al. 2015) were hybridized with CNTs to make low-cost and stable ORR catalysts.

Carbon Black/Activated Carbon-Metal

Both activated carbon and carbon black are effective base materials for metallic electrocatalysts to reduce the overall cost (Yuan et al. 2011; Pan et al. 2016). However, activated carbon is preferable recently due to its high ORR catalytic activity. By mechanically mixing activated carbon with Co_3O_4 or MnO_2, the charge transfer resistance was reduced while maintaining the high SSA. Thus, those cathodic materials showed significant performance improvement (Singh and Chandra 2013; Ge et al. 2015).

Electrochemical deposition of metal on carbon is an effective method to form closer interactions between metal and carbon materials. For instance, using this method to deposit silver on the activated carbon-based cathode, the resistance of the electrode was much reduced, and the cathodic ORR was altered to the four-electron pathway with Ag_2O and AgO as intermediate products (Fig. 8.4). As a result, the power density increased by about 70% compared with the pristine activated carbon air-cathode (Pu et al. 2014).

8.4 Photoelectrocatalysts

Semiconductors, such as TiO_2, $LiTaO_3$, and $CuInS_2$, have been investigated to be used as the photocathode in MFCs. Under light irradiation, such photosensitive materials become active catalysts towards ORR. Photoelectrocatalysts usually possess properties such as (1) ultraviolet/visible (UV/Vis) light absorption capacity, (2) electron–hole charge separation and transportation, and (3) generating a redox potential to facilitate the reactions to reduce electrons received from the anode (Benzaouak et al. 2017). The typical response of the output voltage of an MFC equipped with a photocathode under light and dark conditions is shown in Fig. 8.5.

TiO_2 was one of the first photoelectrocatalysts applied in the cathode of MFCs to drive ORR due to its low-cost, photostability, and chemical stability. Rutile TiO_2 was deposited on the graphite surface to make the photocathode of MFCs (Lu et al. 2010). The photocathode enhanced the power density by 1.57 times under light irradiation. However, TiO_2 suffers from (1) a large number of recombination of electron-hole and (2) a relatively wide energy band gap (3.0–3.2 eV) leading to ultraviolet-only responsiveness.

Lithium tantalate ($LiTaO_3$) semiconductor belongs to the group of tantalate photocatalysts. Its defects related to nonstoichiometry favor its photocatalytic activity towards water splitting and solar cell applications. Recently, $LiTaO_3$ has been proved to exhibit active ORR catalytic activity (Benzaouak et al. 2017). Its performance as the photocathode in the MFC showed three times enhancement in the power density. This type of material also suffers from the narrow light absorption band due to a wide energy band gap (4.8 eV).

Another photoelectrocatalyst material for use in MFCs is photocatalytic copper indium sulfide ($CuInS_2$) semiconductor. This material with flower-like nanostructures was examined as an active photoelectrocatalyst in the cathode in MFCs (Wang et al. 2014a). The performance of this cathode was just slightly lower than that of the controlled Pt/C cathode. This study also proposed a mechanism of cathodic ORR of the light-responsive $CuInS_2$ photoelectrocatalyst. Electron–hole

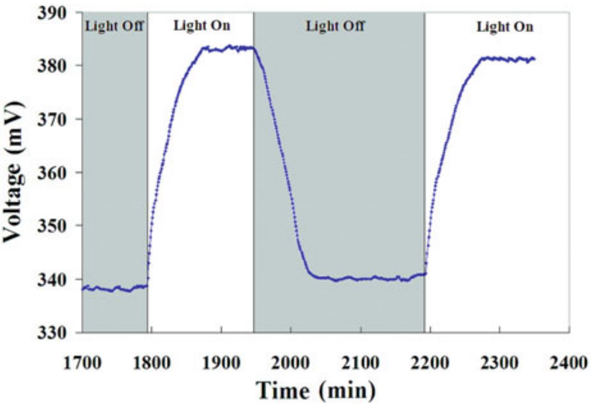

Fig. 8.5 The typical form of the output voltage of an MFC equipped with a photocathode (rutile TiO_2-coated graphite) under light and dark conditions. Reprint with permission from Lu et al. (2010). Copyright (2010) American Chemical Society

pairs are generated when $CuInS_2$ receives photon energy from the illuminated light. As a result, the four-electron pathway is triggered. The whole process that occurs at the photocathode can be described by Eqs. (8.14) and (8.15), where e_{cb}^- and h_{vb}^+ are the photogenerated electrons and holes, respectively. e_{cb}^- can reduce the terminal electron acceptor (O_2), while h_{vb}^+ recombines with the electrons come from the bioanode.

$$\mathrm{CuInS_2} + hv \rightarrow \mathrm{CuInS_2}\left(\mathrm{h_{vb}}^+ + \mathrm{e_{cb}}^-\right) \tag{8.14}$$

$$\mathrm{O_2} + 4\,\mathrm{H}^+ + 4\,\mathrm{e_{cb}}^- \rightarrow 2\,\mathrm{H_2O} \tag{8.15}$$

8.5 Biocatalysts

8.5.1 Enzymes

Enzymes with redox catalytic activity have been examined as biocatalysts in the biocathode of MFCs. Two main groups of enzymes that have been used for ORR catalysis are multicopper oxidases and redox enzymes. The former group includes laccase and bilirubin oxidase, and the latter group includes cytochrome oxidase. Laccase is preferable for the biocathode due to its redox potential is similar to the redox potential of the O_2/H_2O redox couple, leading to a low cathodic overpotential (Shleev et al. 2005). The ORR catalytic activity of laccase was described with the involvement of a copper redox center (Liu et al. 2014). Electrons can be transferred to the active sites of laccase through both direct and mediated electron transfer mechanisms.

It has been reported that the use of laccase in the biocathode of MFCs could generate multifold higher power density compared with the controlled Pt-based cathode (Schaetzle et al. 2009). Furthermore, bilirubin oxidase was immobilized in a CNT cathode for enhancing ORR (Strack et al. 2013). This enzymatic cathode showed sustained ORR catalytic activity for over one day in on-site tests.

Enzymes are inherently limited in electron conductivity and short lifetime. Besides, when cathodic pH is close to neutral, multicopper oxidases exhibit low catalytic activity because copper hydroxo complexes are formed. On the other hand, bilirubin oxidase performs better than laccase when cathodic pH is close to neutral (Liu et al. 2014).

There are some engineering methods to increase the performance of enzymatic biocathodes. Immobilizing enzymes on the cathode surface is the most common method to improve enzyme–electrode interaction and biocathode robustness. Using redox polymer for mediated electron transfer can improve the electron transfer efficiency between enzyme–enzyme and enzyme–cathode interfaces. Utilizing conducting binders to modify the cathode is another effective method to improve direct electron transfer efficiency (Vaz-Dominguez et al. 2008).

8.5.2 Microorganisms

During MFC operation, it is hard to avoid the formation of biofilms on the cathode due to the migration of microorganisms. Instead of trying to prevent this problem from occurring, many researchers have turned this issue into an advantage by utilizing microbes as natural biocatalysts to accept electrons from the cathode. Microbes used for ORR catalysis in the biocathode usually possess one or multiple electron transfer pathways commonly found in exoelectrogens, such as direct electron transfer through direct membrane contact or pili contact.

Electrons are transferred from outside to inside microbial cells through the electron transfer chains. Inside the cell, a part of the energy is used for cell growth. However, in autotrophic microorganisms, a small number of electrons can migrate to the active sites of microbial enzymes, where they are reduced by soluble electron acceptors, such as O_2, nitrate, and other oxidants (Liu et al. 2014).

A mixed culture of microorganisms on a carbon-based cathode of an MFC showed a significant decrease of cathodic overpotential, which was comparable to the controlled Pt catalyst (Rabaey et al. 2008). *Leptothrix discophora*, a manganese-oxidizing bacteria, was used in an aerobic biocathode to biomineralize manganese oxides deposited on the electrode and to facilitate cathodic catalysis (Allison et al. 2005). The current density obtained by this method was multifold higher than that obtained by an unmodified air-cathode. Some phototrophic bacteria were also used as cathode catalysts to drive ORR (Strik et al. 2011). For instance, green alga, *Chlamydomonas reinhardtii*, was found to mediate oxygen reduction directly under sunlight (Liu et al. 2013a).

One major challenge of microbial biocathodes is the inefficient electron transfer between the cathode and microbial cells, causing low ORR catalytic activity. Another issue is related to microbial community dynamics leading to cathodic process complexity. Also, long start-up time and long-term stability are two inherent limitations of microbial catalysis.

Growing biofilms on the biocathode can improve the direct electron transfer between the cathode and microorganisms. Bacteria cells enclosed in biofilms are also more robust and stable than suspended-growth cells. However, mass transfer is limited in biofilms because of the dense structure formed after a given operating period. Recently, modern metabolic engineering techniques have been expected to improve these issues (Liu et al. 2014).

8.6 Conclusions

The advances in research in electrochemistry, bioelectrochemistry, and photoelectrochemistry have enabled the development of various novel catalytic materials with desired properties. However, our knowledge about the ORR catalysis and engineering cathode catalysts in MFCs is still limited.

Although metal-based electrocatalysts usually exhibit higher ORR performance than carbon-based electrocatalysts, they are not preferable in terms of stability and cost. Carbonaceous electrocatalysts are promising cathode catalysts for practical large-scale applications because of cost-effectiveness and durability. The hybridization of metal and carbon can take advantage of both materials to improve overall catalytic activity and stability while significantly reduce the cost.

Photoelectrocatalysts are interesting because they can utilize solar energy to facilitate ORR in the cathode. According to the basic principles, many more semiconductors can be expected to exhibit the cathodic catalysis. However, a few semiconductors have been recognized with such capability so far. More efforts should be put into developing new materials with novel structure and morphology for high catalytic activity.

Enzymatic biocatalysts have demonstrated high catalytic activity towards ORR. Nevertheless, two major challenges of low electrochemical interaction with the cathode and short lifetime need to be solved before expecting their real-world applications. New advances in developing supporting materials, such as novel redox and immobilization polymers, are expected to be the solution for those shortcomings. Furthermore, the advantage of microorganism-based biocatalysts lies in the living nature of microorganisms. However, some issues, such as poor electron transfer efficiency and long start-up time, need to be improved to obtain sustainable and stable operation.

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Chapter 9
Effective Cathode Catalysts for Oxygen Reduction Reactions in Microbial Fuel Cell

Nishit Savla, Santimoy Khilari, Soumya Pandit, and Sokhee P. Jung

9.1 Introduction

Microbial fuel cells (MFCs) are bioelectrochemical systems that directly convert chemical energy contained in organic matters like wastewaters into electrical energy by utilizing the metabolic (catalytic) activity of microorganisms (Kim et al. 1999; Bond and Lovley 2003). In the anode chamber of MFC, the substrates are oxidized by electroactive bacteria to produce carbon dioxide with electrons (e^-) and protons (H^+) (Logan and Regan 2006). The generated e^- get transported to the cathode through the outside circuit, while H^+ will transfer through the electrolyte and reach the cathode. Additionally, the electrons and protons mix with the oxygen molecule and reduced to a water molecule on the cathode. The oxygen reduction reaction (ORR) at the cathode is considered a rate limiting step due to its heterogenic nature. An efficient catalyst is, therefore, required to reduce the cathodic overpotential (Chandrasekhar 2019). The costly noble metal dust platinum was used during the early phase of research; however, recently different types of nonmetal materials were applied such as metal-based catalysts, carbon-based catalysts, carbon–metal hybrid catalysts, and metal–nitrogen–carbon advanced catalysts for efficient ORR

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(Clauwaert et al. 2008). The performances of MFCs having different ORR catalysts were compared in terms of power output. In certain cases, biocathode was utilized for ORR. The present chapter broadly discussed the varied cathode catalysts used for the oxidation–reduction reactions in MFCs. The chapter included the synthesis procedure of ORR catalyst, nature, stability, and electrochemical performance as cathode catalysts. This chapter is expected to deliver an understanding of the applications of cathode catalysts in MFCs to boost method efficiency furthermore as to build the method economically viable.

9.2 Oxygen Reduction Reaction (ORR) in Fuel Cell

In electrochemistry and electrodialysis, the most fundamental process is oxygen reduction reaction (ORR) because of its limiting reaction to widespread commercialization of fuel cells (Khotseng 2018). There have been several studies proposed for understanding the mechanism of ORR; among them the study proposed by Wroblowa et al. is the most common one, which suggests that oxygen may be reduced through adsorption at the electrode via two pathways (Wroblowa et al. 1976): the direct pathway which requires 4 electrons and 4 protons for the formation of water which can be represented by the following reaction:

$$O_2 + 4e^- + 4H^+ \rightarrow 2H_2O, E^O = 1.23\ \text{V (vs.SHE)} \tag{9.1}$$

Another alternative pathway is the indirect pathway where water is formed along with hydrogen peroxide as an intermediate, which is gradually reduced further to form water. This pathway is also called oxygen partial reduction for hydrogen peroxide generation.

$$O_2 + 2H_2O + 2e^- \rightleftarrows HO_2{}^- + OH^- \tag{9.2}$$

$$HO_2{}^- + H_2O + 2e^- \rightleftarrows 3OH^- \tag{9.3}$$

After summing both reactions (9.2) and (9.3) it yields into reaction (9.1) which represents that the net reaction of both the pathways is the same.

It is always found that the ORR always requires more than the above-mentioned equilibrium potential ($E^0 = 1.23$ V). The extra potential needed to execute the ORR is known as overpotential (Gewirth et al. 2018). Electrocatalysts are used to minimize the overpotential of ORR. Thus, overpotential is an important factor to determine the efficacy of an ORR catalyst. The multielectronic ORR process is a combination of several discrete electrons coupled with or decoupled proton transfer elementary reactions. Fig. 9.1 depicts a well-established mechanism of complex ORR process on a catalyst surface. The reduction of molecular oxygen at the electrode surface is initiated by the diffusion of dissolved oxygen present in the solution (O_2*, where * signifies the active site on the catalyst surface). Further,

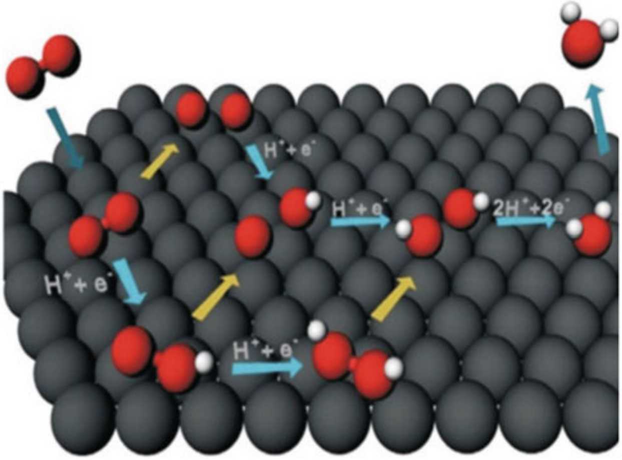


Fig. 9.1 Schematic of different molecular oxygen reduction pathways on a catalyst surface. Black, white/gray, and red balls represent the catalytic surface, hydrogen, and oxygen, respectively (adapted from (Khilari and Pradhan 2018)

the adsorbed O_2 experiences sequential bond cleavage and reduction. Three different pathways can be considered on the basis of the mode of bond cleavage to form O*. The first pathway is coined as a dissociative pathway. The dissociative pathway involves direct dissociation of O_2 to produce O*. Subsequently, the O* is reduced by the electron and proton to OH* and H_2O successively. The second pathway is denoted as the associative pathway where an O_2* generates OOH* species rather than O* as in the previous case. Further, the O-O bond of the intermediate OOH* is broken to form OH* and O* intermediates. The third pathway is termed as the peroxo pathway (also called as the second associative pathway), where O_2* is first reduced to OOH* and form HOOH*. All three pathways intercompetating in nature on a catalyst surface. Moreover, physicochemical characteristics of catalyst surface, as well as the surrounding environment, determine the reduction pathway which has to follow to reduce O_2. It is always preferable to exclude the third pathway for sustainable catalysis as it generates corrosive peroxide (H_2O_2) intermediate. The H_2O_2 formation not only hampers the catalytic process but also deteriorates the polymeric membrane by generating reactive radicals (Khilari et al. 2013). The ORR catalytic activity of an electrocatalyst also depends on thermodynamic parameters such as free energy change during the reduction process. Thus, the estimation of free energy change on the catalyst surface during ORR remains a key to develop better-performing electrocatalyst. Generally, density functional theory (DFT) is utilized to calculate the free energy change on a catalyst surface and suggests a possible ORR pathway. It is well established from the DFT study that dissociative mechanisms are predominate at low O_2 coverage, whereas high O_2 coverage offers associative pathway (Nørskov et al. 2004). Although the preference towards a feasible pathway mostly depends on the characteristics of the electrode material.

The ORR kinetics at an electrode surface exclusively depends on the adsorption of intermediates (O*, OH*, and OOH*) at the catalyst surface. So, it is important to experimentally probe these intermediates to determine the exact reaction mechanism. However, monitoring of the intermediates is not an easy task (Shao et al. 2016). In this context, computational study can be made to calculate the surface interaction energies with sufficient accuracy. A DFT study executed by Nørskov et al. refers that the adsorption of O_2 on the electrode is responsible for the origin of overpotential (Nørskov et al. 2004). It is observed that at high potential O_2 is strongly adsorbed on the electrode surface. This strong adsorption hinders the proton and electron transfer to the adsorbed O_2 molecule (Fig. 9.1). Moreover, switching the potential to a lower value weakens the stability of adsorbed O_2 and reaction becomes feasible. Moreover, the binding affinity toward various oxygenated species evolved during the course of ORR controls the catalytic activity of the catalyst. So, an optimum binding strength to the intermediates is an essential criterion for efficient ORR catalysis. A very weak binding of adsorbed O_2 or oxygenated species on the catalyst surface hampers the proton and electron transfer thereby ORR kinetics. However, the strong binding of O* and OH* limits the desorption of end product (H_2O) and makes the active sites inaccessible for further O_2 adsorption. Different catalyst has a different binding energy of intermediates. Mostly, the inherent binding characteristic of a catalyst depends on its electronic structure (Stephens et al. 2012). A high energy metal d-state relative to Fermi energy exhibits stronger interaction of the electrode surface to the intermediates (Song and Zhang 2008). Thus, the catalyst with an appropriate electronic structure can offer suitable intermediates binding energy and enhance ORR catalysis remarkably.

Non-oxygen Terminal Electron Acceptors

Various studies have been accustomed to finding an appropriate e^- acceptor for MFC. Potassium ferricyanide has been reported in numerous studies as an electron acceptor, due to its power generation (426 mV, 181 mW m^{-3}) than dissolved oxygen (DO) (150 mV, 22.5 mW m^{-3}) (Wen et al. 2012). Even though ferricyanide is cost-effective and a good electron acceptor, it may show side reactions at low pH producing toxic and hazardous HCN. There are various other non-oxygen terminal catholyte which can be used as electron acceptors; they are enlisted in Table 9.1.

In a study presented by Pandit et al. it was recorded that the performance of potassium persulfate based on power generation was slightly lower than that of potassium permanganate, but looking at the overall performance criteria based on its stability during the process. A different comparative study on the performance of persulfate and ferricyanide suggests that persulfate would produce high power after some cycles of the process, thus it could be suggested that persulfate is more likely to be considered for the time being. Moreover, research suggests that external aeration could improve the synergetic electron uptake of both oxygen and non-oxygen terminal electron acceptors thereby improving their performance (Pandit et al. 2011).

Table 9.1 List of various terminal electron acceptors and their half-cell reactions (Khilari and Pradhan 2018)

Electron acceptor	Cathodic reduction reaction	Reaction conditions	*E*	*E*^{0}
$Fe(CN)_6^{3-}$	$Fe(CN)\ _6^{3-} + e^- = Fe(CN)\ _6^{4-}$	$[Fe(CN)\ _6^{3-}] = [Fe\ (CN)\ _6^{4-}]$	0.36	0.36
MnO^{4-}	$MnO^{4-} + 3e^- + 2H^+ = MnO_2 + 2H_2O$	$[MnO4] = 5$ mM, pH = 7	1.1	1.7
O_2	$O_2 + 4e^- + 4H^+ = 2H_2O$	$pO_2 = 0.2$, pH = 7	0.8	1.23
O_2	$O_2 + 2e^- + 2H^+ = 2H_2O_2$	$pO_2 = 0.2$, $[H_2O_2] = 5$ mM, pH = 7	0.33	0.69
NO^{3-}	$2NO^{3-} + 10e^- + 12H^+ = N_2 + 6H_2O$	$[NO^{3-}] = 5$ mM, $pN_2 = 0.2$, pH = 0.7	0.73	1.25
NO^{3-}	$2NO^{3-} + 2e^- + 2H^+ = NO^{2-} + H_2O$	$[NO^{3-}] = [NO^{2-}]$, pH = 0.7	0.42	0.83
$S_2O_8^{2-}$	$S_2O_8^{2-} + 2e^- = 2SO_4^{2-}$	$[S2O_8^{2-}] = [SO_4^{2-}] = 5$ mM	1.96	1.96
ClO^{4-}	$ClO^{4-} + 8e^- + 8H^+ = Cl^- + 4H_2O$	$[ClO^{4-}] = [Cl^-]$, pH = 7	0.87	1.29
$Cr_2O_7^{2-}$	$Cr_2O_7^{2-} + 6e^- + 14H^+ = 2Cr^{3+} + 7H_2O$	$[Cr_2O_7^{2-}] = [Cr^{3+}] = 5$ mM, pH = 7	0.42	1.36
Cu^{2+}	$Cu^{2+} + 2e^- = Cu(s)$	$[Cu^{2+}] = 5$ mM	0.27	0.34
VO_2^{2+}	$VO_2^{2+} + 2e^- + 2H^+ = VO^{2+} + H_2O$	$[VO_2^{2+}] = [VO^{2+}]$, pH = 7	0.17	1.00
CO_2	$CO_{.2} + 12e^- + 12H^+ = C_6H_{12}O_6$ (bio mass) $+ 3O_2$	N.A.	N. A.	N. A.

9.3 Determination of Figure of Merit of ORR Catalyst: Electrochemical Protocol

The ORR catalytic activity is generally estimated by employing a catalyst on the cathode and comparing with the state-of-the-art ORR catalyst Pt under similar conditions (Anderson et al. 2005). Further, the ORR mechanism is analyzed from the half-cell reaction in a standard electrochemical cell. The standard half-cell analysis of an ORR catalyst is executed with a thin-film catalyst deposited on a rotating disk electrode (RDE) or rotating ring disk electrode (RRDE)(Shih et al. 2008). The cathodic polarization plot is taken by employing a linear sweep voltammetric technique in a steady state or with a known rotation of RDE or RRDE. A typical ORR polarization plot of a catalyst composed of three characteristic regions is shown in Fig. 9.2. The first region (Zone I) denoted as the kinetic-control region where the ORR rate is considerably slow and cathodic current density increases very slowly. The second region (Zone II) represents mixed kinetics and diffusion-controlled region where a significant increase of current density with the potential drop is observed. The third region (Zone III) corresponds to the diffusion-controlled region where the electrode's current density is decided by the diffusion rate of reactants reaching to the rotating electrode surface (Bocchetta et al. 2016). In addition to these three characteristic zones, two most important additional

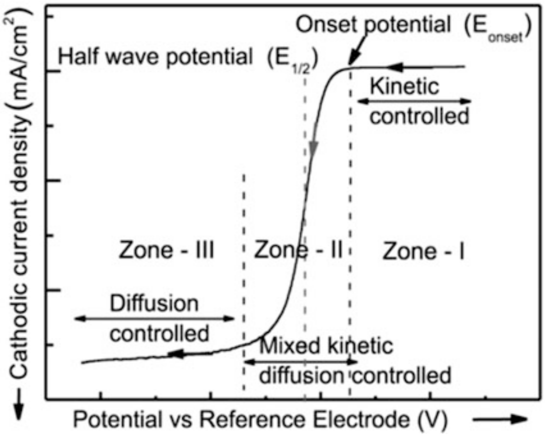


Fig. 9.2 Different characteristics region of ORR polarization plot of a catalyst

parameters generally used to evaluate catalyst performances are onset potential (E_{onset}) and half-wave potential ($E_{1/2}$), respectively. The more positive value of E_{onset} and $E_{1/2}$ suggests better catalytic activity of the catalyst. Although the definition of E_{onset} varies from article to article some articles report E_{onset} as the potential required to achieve 5% of the diffusion-limited current density (J_L) (Zhou et al. 2016). Another definition suggests that E_{onset} is the potential where the current density exceeds the threshold value of 0.1 mA cm^{-2} (Khotseng 2018). Thus, it is essential to mention the definition of E_{onset} used in the ORR study of a catalyst and should be compared to the state-of-the-art Pt catalyst at identical conditions. The ORR mechanism on the catalyst surface can be derived from the kinetic limiting current density (J_K). In this context, the mass-transport corrected polarization plot is used to find kinetics parameters with the help of Koutecky–Levich equation (Khilari et al. 2014).

$$1/J = 1/J_L + 1/J_K \tag{9.4}$$

where J is the measured current density, J_K and J_L denote the kinetic limiting and Levich current, respectively. The measured current density must be the catalytic current density which mostly interferes with the capacitive current density. Generally, the porous earth-abundant oxides and carbon materials form double layer by adsorption of ions on the catalyst surface which results in the interference between catalytic current and capacitive current. The capacitive interference can be minimized by employing a very slow scan rate during polarization study of the electrode and background correction. The elimination of background currents can be possible by subtracting the current density recorded in an N_2 saturated electrolyte experiment from the O_2 saturated measurement (Shao et al. 2016).

Two important parameters are extracted from the above-mentioned protocol, which is the average number of electron transfer (n) to each O_2 molecule and amount of H_2O_2 intermediate generation during ORR (Song and Zhang 2008). These two parameters are considered as the key point to figure out an efficient ORR catalyst. The following equations are utilized to calculate the “*n*” value of a catalyst:

$$B = 0.2\mathrm{nFC}_0(D_0)^{2/3}\,\upsilon^{-1/6} \tag{9.5}$$

$$4I_d = n(I_d + I_r/N) \tag{9.6}$$

where n, F, D_0, C_0, and υ are assigned as the number of electrons involved in the reaction, Faraday constant (96,485 C mol^{-1}), diffusion coefficient of O_2 in the electrolyte, bulk O_2 concentration, and kinematic viscosity of the electrolyte, respectively. Further, I_d, I_r, and N denote the disk current, ring current, and current collection efficiency of the Pt ring, respectively (Tang et al. 2018). The efficiency of an ORR catalyst can be determined from the H_2O_2 generation. The H_2O_2 was generated at the catalyst surface coated on a disk electrode diffused to the Pt ring and subsequently reduced to water or OH^-. Thus, the quantitative estimation of H_2O_2 generation during ORR can be done with the ring current measurement. The following equation is utilized to calculate the percentage of H_2O_2 intermediate generation on a disk electrode (Liu et al. 2017).

$$\%H_2O_2 = 200 \times (I_r/N)/(I_d + I_r/N) \tag{9.7}$$

The $4e^-$ pathway is considered as the efficient ORR pathway, whereas $2e^-$ pathway leads to the formation of H_2O_2 which hampers the performance as well as the stability of the cathode. Apart from the “*n*” value and % H_2O_2 production, the electron transfer coefficient is another important parameter that influences the catalyst performance. The electron transfer coefficient of ORR catalyst can be determined from the Tafel slope in a linear portion of the Tafel plot. Generally, the Tafel plot of a catalyst is plotted by considering the Tafel equation as follows (Jiang and Jiang 2014)

$$\eta = (\mathrm{RT}/2.303\mathrm{n}\alpha\mathrm{F})\ \log\ (i/i_0) \tag{9.8}$$

where η, T, R, and α represent overpotential, the temperature on an absolute scale, the universal gas constant, and electron transfer coefficient, respectively. Moreover, I and i_0 correspond to measured ORR current density and exchange current density, respectively. The electron transfer coefficient can be obtained from the Tafel slope (RT/2.303nαF) (Fig. 9.2). So, the smaller value of the Tafel slope reflects higher transfer coefficient reflecting the increment of current density resulting in a slower increase of overpotential. In order to obtain the Tafel plot from the RDE study, the mass-transport correction is essential on the current recorded polarization. The following equation (Eq. (9.9)) has been used to estimate the mass-transport corrected current of a polarization study (Wang et al. 2008)

$$J_k = J \times J_L/(J_L - J) \tag{9.9}$$

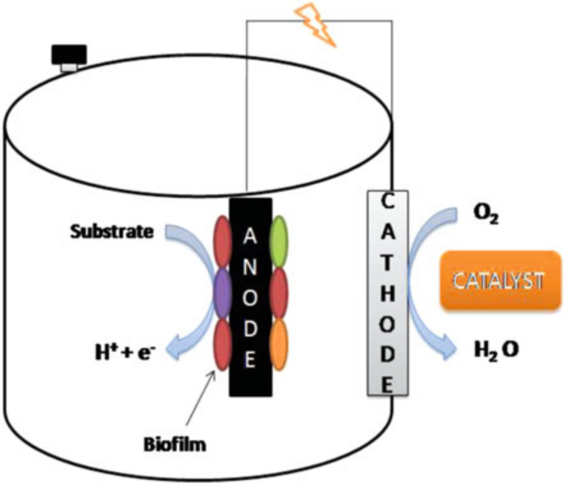
9.4 Catalyst Materials Used in MFCs

In a general catalyst-free MFC (Fig. 9.3), there is a limitation of oxygen reduction reaction kinetics at the cathode in neutral media with high overpotentials which is due to the low availability of H^+ and OH^- ions. To overcome these limitations, various catalysts, e.g., Platinum (Pt) is being used to speed up the oxygen reduction reactions (ORRs) (Yuan et al. 2016). In the last decade, usage of platinum as a cathode in methanol fuel cells was successfully conducted making Pt as the best choice as a cathode catalyst in MFCs. However, due to its high cost, it makes the MFC process not feasible. Many efforts are being made to develop an inexpensive catalyst which could give the results just like platinum but can also make the oxygen reduction reaction in MFCs viable (Fig. 9.4) (Rozendal et al. 2008).

9.4.1 *Carbon-Based Cathode Catalysts*

In recent years, there have been numerous types of carbon-supported materials which were successfully used as cathode catalysts in MFCs to enhance oxygen reduction reactions. These carbon-based materials have high surface areas and high electrical conductivity nature along with their cost which is comparatively low against other noble-metal oxygen reduction cathode catalysts, which make them an ideal candidate to be used as cathode catalyst or supporting materials in cathode

Fig. 9.3 General schematic of an MFC



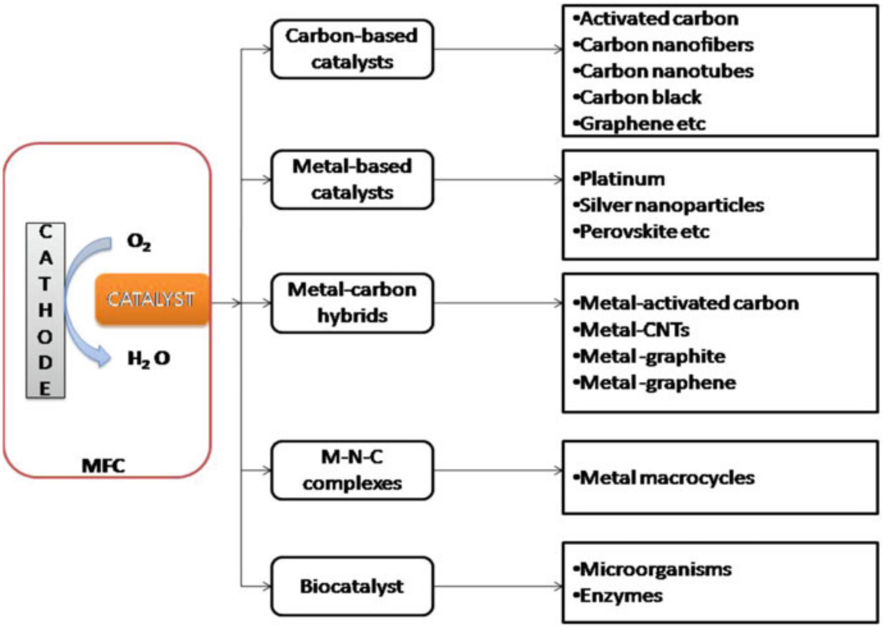


Fig. 9.4 A concept map representing various types of cathode catalyst with appropriate examples

preparation. The carbon-supported materials which are used in MFCs include activated carbon, carbon, and graphene.

9.4.1.1 Activated Carbon Catalyst

Activated carbon is additionally referred to as activated charcoal (it is also noted for its high degree of microporosity) (Koo et al. 2019), derived from the charcoal by thermal or chemical activation (Zhang et al. 2015). The activated carbon materials stuffed with air-filters are widely utilized in the air and gas refining industry to get rid of impurities (Harry et al. 2006). Recently, it is been widely used as a vigorous and cheap cathode catalyst in MFCs; encouraging outcomes are achieved with activated carbon powder primarily based air-cathode MFCs (Watson et al. 2013). Zhang et al. developed an affordable activated carbon cathode electrode for oxygen reduction reaction in MFCs. This extremely active electrode is ready by cold-pressing the activated carbon with a polytetrafluoroethylene (PTFE) binder around a nickel mesh. MFCs operated with activated carbon cathode yielded an extreme power density of 1.22 W/m^2 associated with 1.06 W/m^2 obtained with a platinum catalyst. The results of this study prompt that the activated carbon possesses several functional groups, pore size distribution, and active sites which can provide oxygen reduction catalysis in MFCs (Zhang et al. 2009). However, the most common heteroatom found in activated carbon functional group is oxygen. This oxygen functional group is also present in numerous chemical groups, together with acids, such as ketones and enteric rings. With the intention of understanding the aspects that are distressful the performance of activated carbon cathodes in MFCs, Watson et al. investigated nine diverse activated carbon materials made up of four totally different precursor materials. Among all, cathodes prepared with the coal-derived activated carbon yielded the highest power densities (1620 mW/m^2) in MFCs (Watson et al. 2013).

9.4.1.2 Carbon Nanotube (CNT) Catalysts

Recently, CNTs have to been generally thought of as a backing material for the catalyst reaction because of their excessive surface areas, noncorrosive and high catalytic nature, and ease of functionalization with various groups. Over the past few years, many conducting polymer-based CNT electrodes have been prepared in varied ways (Mikhaylova et al. 2011). The higher catalytic activity of CNTs is because of their excessive surface areas and conjointly the equal distribution of the catalytic units onto the electrode. Ghasemi et al. investigated CNT/Pt as an inexpensive cathode electrode with the aim of testing a cheap material such as a cathode in MFCs to yield the next power density (Ghasemi et al. 2013). They compared the power yielding effectiveness of carbon nanocomposites over different electrode materials and explicit that CNT/Pt composite electrodes would increase 8.7–2.2% power yielded in MFCs (Fig. 9.5).

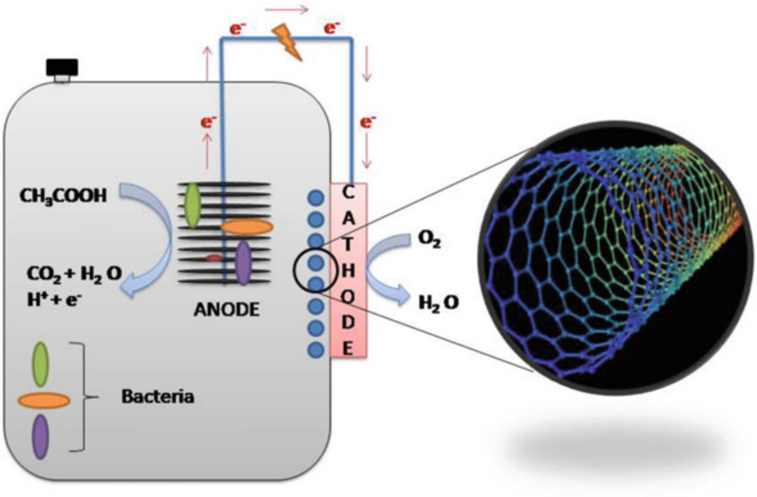


Fig. 9.5 Schematic of classic air-cathode MFC displaying cathode with catalyst (carbon nanotubes) layer and anode with biocatalyst (bacteria) at anode [adapted from (Chandrasekhar 2019)]

9.4.1.3 Carbon Black

Among the carbon-based cathode catalysts, carbon black is generally used because the support material for a metal catalyst as a result of its a high surface area to volume ratio, a wonderful electrical conductivity and high stability (Zhang et al. 2017). Carbon black could be a type of paracrystalline carbon which is mostly made by the thermal decomposition or partial combustion of petroleum hydrocarbons like tar, ethylene cracking tar, etc. It is widely used as a pigment and reinforcing filler in tires. Once the acid treatment, the surface oxidized carbon black shows substantial chemical action activity (Suryanto and Zhao 2016). The introduction of functional groups or doping heteroatoms (such as chemical element, sulfur, oxygen, etc.) will form active catalytic sites that build carbon black itself a vigorous catalyst. Carbon black as an oxygen reduction cathode catalyst demonstrates high economic practicability; as an example, Yuan et al. with success examined the chance of utilization of polypyrrole/carbon black as a catalyst in air-cathode MFC for oxygen reduction. MFCs operated with a polypyrrole/carbon black catalyst that yielded the most power density than the 70th of those with Pt/C. The polypyrrole/carbon black catalyst was 15 times additional economical (401.8 mW/m^2) than Pt/C (90.9 mW/m^2) once the yielded power density was normalized to the fabric price (Yuan et al. 2010). The primary aim of this study is to lower the value of the cathode MFCs by substitution of carbon-based metal catalysts (Pt/C) with cheap and extremely semiconductive polymer carbon composite materials. However, additional studies are needed to analyze the stability of those carbon black composite catalyst materials. In another study, Duteanu et al. investigated the potency of changed carbon powder as a catalyst for gas reduction reaction within the cathode of MFCs (Duteanu et al. 2010). During this study, carbon powder was with chemicals changed by acid treatment and therefore the chemical study was performed in a very solution at pH ranging from 6 to 7.5. MFCs operated with this nitric acid-treated carbon black catalyst yielded an extreme current density of 1115 mA/m^2 that is 78% more than those Pt supported carbon cathode MFCs. Such chemically altered carbon blacks are a cheap substitute for top worth Pt catalyst utilized in the cathode of MFCs for oxygen reduction reactions (Pandit et al. 2017).

9.4.1.4 Graphene/Graphite Catalyst

Graphene is an allotrope sort of carbon. Because of its uncommon properties like high strength, high electrical conductivity, and nearly clear nature, it gains a lot of attention because it is the best cathode catalyst for MFCs to boost oxygen reduction reactions (Koo et al. 2019; Ghasemi et al. 2013). It is an undeniable fact that the catalyst action and mechanism dissent with the sort of carbon materials. Santoro et al. investigated three-dimensional graphene nanosheets as a cathode catalyst for MFCs in operation under neutral conditions. MFCs operated with three-dimensional graphene nanosheets as a cathode catalyst yielded the next power density (2.05 W/

m^2) compared to activated carbon (1.01 W/m^2) (Santoro et al. 2017). The three-dimensional graphene nanosheets were prepared to employ a kill templating strategy with success, operating as a catalyst for MFCs. Among different carbon-based catalyst materials, reduced graphene oxide becomes a lot of prevailing, thanks to its high electrical conductivity over linear dispersion of electrons, succeeding in exceptional support for a catalyst to enhance the reaction rate (Liu et al. 2016). Li et al. investigated nano-flower-shaped graphene oxide hybridized mineral nanocomposite as a catalyst for each electrogenesis and effluent treatment in MFCs. Initially, the oxide catalyst is effectively guaranteed to the surface of graphene oxide for nanocomposite catalyst (Li et al. 2017). MFC operated with graphene oxide/magnesium oxide composite catalyst yielded a higher power density of 755.6 mW/m^2 that was corresponding to 86.5% of the Pt/C catalyst (870.7 mW/m^2)

9.4.1.5 Carbon Catalysts made from Sustainable Originators

Additionally to the precise categories, numerous additional carbon materials are prepared with a range of viable precursors, comprising biochar made of the sewage sludge (Yuan et al. 2015), the cellulose ensuing nitrogen and phosphorus dual-doped carbon (Liu et al. 2015), chitin, and petroleum coke. The MFCs operated with these carbon catalysts have achieved higher e^- transport dynamics and equivalent or higher catalytic activity to Pt/C. As an example, three cellulose derived catalysts yielded most power densities reaching from 1041 to a pair of 0.2 W/m^2 due to the various MFC patterns, on the opposite hand, all crushed their control MFCs operated with Pt/C (Liu et al. 2014).

9.4.2 Metal-Based Cathode Catalysts

9.4.2.1 Metals and Alloy Catalysts

Among many pure cathode catalysts, Pt has been extensively used for oxygen reduction reactions in MFCs, because it can decrease the cathodic reaction activation energy and enhance the reaction rate (Jung and Pandit 2019). Even so, its high worth, restricted quantity, and interactions with contaminants have driven efforts within the direction of a much more robust and cheap catalyst. Consequently, preparing the binary metal catalysts (e.g., PTFE, PteMn, and PteCu) (Mallika and Easton 2013), multicomponent Pt-based catalysts (Santoro et al. 2015), and even nonPt catalysts, for instance, Fe, Mn, Co, or nickel, is an efficient line of attack to create the MFC technology economically viable. However, the stability of the metal catalysts is taken into account jointly of the key problems that impede their viable applications (Yan et al. 2014). Yan et al. prepared PteFe alloy utilizing glycerine as a good stabilizer and solvent with a straightforward two-step technique at room temperature.

PteFE alloy catalyst loaded with MFCs yielded 1680 mW/m^2 of most power density that 18% higher (1422 mW/m^2) than that of Pt/C. The activity of metallic iron occurred for a PteFe alloy, even though its most power density yield in MFCs beneath neutral environments was extremely stable than that with Pt/C (Yan et al. 2014). Researchers also use Pt and metal current collector. They found that the metal current collector act as an oxygen catalyst. It was evidenced by impedance analysis (Nam et al. 2017).

9.4.2.2 Metal Oxide Catalysts

Manganese, copper and vanadium metal oxides, MnOx, CuOx, and VOx are cheap, good electrical conductors and additionally profusely accessible materials (Khilari et al. 2015). These metals and metal oxides were effectively used as cathode catalysts in fuel cells for several years (Khilari et al. 2013). Vital efforts are made to extend the oxygen reduction reaction in MFCs by combining MnO_2 as a catalyst with the dissimilar support of carbon. Similarly, vanadium is an unrestricted metal that is extravagantly present within the earth's crust. Several researchers investigated the potential chemical process applications of V_2O_5 as a catalyst for lithium-ion batteries. Conversely, only a few researchers investigated V_2O_5 as a catalyst in MFCs. Noori et al. operated single-chambered MFCs withV_2O_5 micro flowers as the catalyst on the cathode and also the outcomes were connected with MnO_2 nanotubes. MFCs operated with V_2O_5 yielded 31% higher current density than MnO_2 nanotubes. In another study, Noori et al. prepared a stainless steel mesh cathode using V_2O_5 nanorods as a catalyst by utilizing reduced graphene compound as a support material and also the performance was evaluated as compared with V_2O_5/Vulcan XC. The MFCs operated with V_2O_5 nanorods yielded a better power density (533 mW/m^2) compared to V_2O_5/Vulcan XC (384 mW/m^2). Authors additionally calculated the electrode fabrication price, where cathode fabrication price using V_2O_5/reduced graphene catalyst was found terribly cheaper than Pt/C (Noori et al. 2017). Varied extra metal oxides, such as perovskite oxide, cobalt oxide, lead oxide and zirconium oxide, are investigated as oxygen reduction cathode catalysts in MFCs.

9.4.2.3 Metal-Activated Carbon

Due to its superior electrical properties as supporting material for the metal catalyst, activated charcoal has been used more and more as a substitute to carbon black (Khilari et al. 2014). Ge et al. constructed spinel nano-cobalt (II, III) oxide (nano-Co_3O_4) by a hydrothermal technique. The mixing of cobalt (II, III) oxide (Co_3O_4) with activated charcoal showed increment within the power density yield (Ge et al. 2015). The MFC operated with Co_3O_4 yielded an extreme power density of 1500 mW/m^2; moreover, the total resistance of the electrode was principally reduced. In another study, Ge et al. invented a nano urchin-like nickel cobaltite

($NiCo_2O_4$) using a hydrothermal technique. Here, conjointly, the blending of $NiCo_2O_4$ with activated charcoal showed increment within the power density yield (Ge et al. 2016). Recently, Huang et al. planned an auspicious approach to synthesize N-doped activated charcoal supported cobalt (II) oxide (CoO) nanosheets as oxygen reduction reaction catalysts in MFCs. CoO nanosheets were with success generated on N-doped activated charcoal using an in-situ synthesis technique. The prepared catalyst was with success used as a cathode catalyst in MFCs, achieved an extreme power density of 1650 mW/m^2, which is 122.5% above that of the control function (Huang et al. 2017).

9.4.2.4 Metal CNTs

MnO_2 could be a capable cheap metal catalyst; it is been combined with CNTs in varied studies. During this method, Zhang et al. developed an active and cheap cathode catalyst. The CNTs were coated with MnO_2 by an in-situ hydrothermal method (Zhang et al. 2011). The in-situ MnO_2/CNTs expressively increase the performance of MFCs. The proper diffusion of MnO_2 over the surface of CNTs allows e transfer for oxygen reduction reaction in the MFC. Lu et al. made a completely unique manganese–polypyrrole–carbon nanotube (Mn-PPy-CNT) composite (synthesized by solvothermal method) as a cathode catalyst for oxygen reduction reaction in MFCs. Moreover, the Mn-PPy-CNT catalyst loaded MFCs yielded 213 mW/m^2 at a loading of 2 mg/cm^2 and long-run stability (Lu et al. 2013).

9.4.2.5 Metal Graphene/Graphite

Graphene and carbon are well established as active support materials for the metal catalyst (Pandit and Das 2018). Wen et al. prepared the MnO_2–graphene nanosheets hybrid with microwave irradiation technique as an affordable cathode catalyst for MFC (Wen et al. 2012). The MFC operated with MnO_2–graphene nanosheets bestowed good catalytic activity, yielded an extreme power density of 2.08 W/m^2 that is larger than that of MFCs operated with the pure MnO_2 catalyst. In another study, Khilari et al. synthesized nanotubular shaped MnO_2/graphene oxide nanocomposite using an affordable, easy and time economical hydrothermal technique. The MFCs operated with MnO_2nanotube/graphene oxide nanocomposite-modified electrode yielded an extreme power density of 6.45 W/m^3 that is significantly above control function (unmodified electrode) (Khilari et al. 2013). Moreover, this material takes less startup time and shows smart stability throughout the course of the MFC operation.

9.4.3 Metal–Nitrogen–Carbon (M–N–C) Complex

9.4.3.1 Metal Macrocycles

The M–N–C complexes refer to an intensive diversity of resources with the metal–nitrogen (M–N) functional groups in carbonic backgrounds. Metal macrocycles are a class of M–N–C complex whose effectiveness has been studied for many years (Chandrasekhar 2019). These metal macrocycles were widely investigated as cathode catalysts to boost oxygen reduction reactions and found that they are having stability-related problems below acid conditions. Further studies are needed to beat these limitations (Yuan et al. 2016). The pyrolyzed M–N–C complexes are thought to be the foremost capable oxygen reduction catalyst because of their high chemical action activity and stability with reference to that of the Pt/C catalyst. Even supposing huge developments have been earned in this area of investigation, there are few limitations in each of their oxygen reduction chemical action activity and strength of those metal macrocycles. Besides, pyrolyzed FePc was verified to be sturdy in long-standing MFC experimentations (Birry et al. 2011). However, few other metal macrocycles like cobalt–nitrogen–carbon (Co–N–C) and iron–nitrogen–carbon (Fe–N–C) and their oxygen reduction chemical action efficiencies are wide investigated worldwide and their attainable use as a reasonable catalyst for the cathode in MFCs has been studied in recent times (Aelterman et al. 2009).

9.4.3.2 M–N–C Complex Made Up of Alternative Precursor

Recently, researchers have investigated precursors aside from metal macrocycles used for M–N–C complexes like polymers, metal-based organic frameworks, and organic materials (Chandrasekhar 2019). Generally, the preparation of polymer-based M–N–C complexes follows a series of steps, beginning with polymerization of monomers followed by a corporation step with metal salts and also the final pyrolysis step (Cao et al. 2016). For example, poly (2,6-diaminopyridine) was infused with Co and Fe salts and finally pyrolyzed at 700 °C within the presence of ammonium ion (Zhao et al. 2012). The MFCs operated with the final product yielded an extreme power density of 1.2 W/m^2 that is 2.2 times larger than that of commercially offered Pt/C catalyst.

9.4.4 Biocatalyst

In this section, we will discuss microorganisms and enzymes discharged by microorganisms as a reasonable oxygen reduction reaction catalyst in MFCs. As we mentioned earlier, the oxygen reduction at the cathode is one among the key challenges of MFCs, many chemical catalysts are studied to beat this issue (Jung

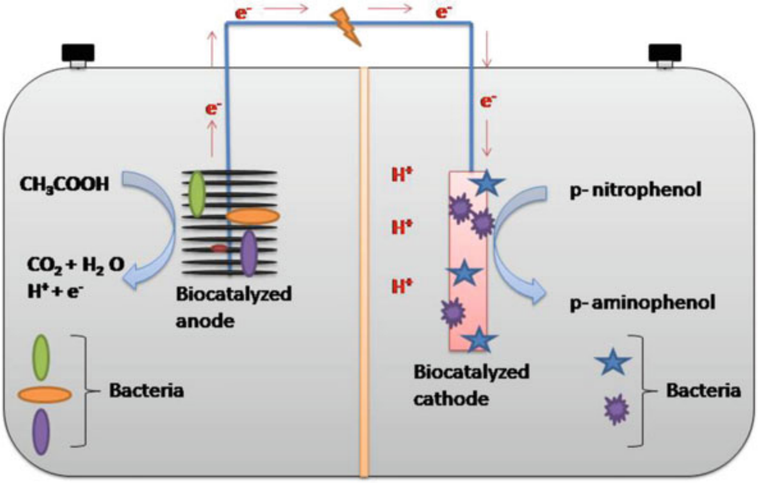


Fig. 9.6 Schematic representation of the bioelectrochemical reduction of p-nitrophenols to p-aminophenols in the biocathode MFC

and Pandit 2019). Besides, the biocatalyst (microorganisms and/or enzymes) will act as an oxygen reduction catalyst by accepting e from the cathode to reduce the oxygen (Chandrasekhar 2019). It is verified that biocatalyst also can be accustomed to remove the leftover pollutants/organic matter from the anode effluent, therefore manufacturing good-quality of water and further increasing the efficiency of MFCs (Pandit et al. 2014). It was further found that algae can be utilized for efficient ORR as it can produce oxygen (Kakarla et al. 2017). Bacteria at the cathode are useful in ORR, although the process is sluggish in nature (Roy and Pandit 2019). Zhang et al. conducted a scientific comparison on the biocathode material (graphite felt, carbon paper, and stainless steel mesh) and evaluated it in terms of power density. MFCs operated with graphite felt biocathode yielded the highest power density of 109.5 mW/m^2 followed by carbon biocathode (32.7 mW/m^2) and stainless steel mesh biocathode (3.1 mW/m^2) (Zhang et al. 2012). Besides, from the electrochemical analysis, it has been concluded that the biocathode was the main limiting issue for the three MFCs. Zhang et al. investigated the biotransformation of p-nitrophenol to p-aminophenol within the cathode of MFCs. Under the optimum initial p-nitrophenol concentration, MFC reached a 100% reduction in efficiency (Zhang et al. 2012). It has been concluded that the microorganisms may play a key role in p-nitrophenol degradation within the biocathode MFC (Fig. 9.6). Savizi et al. investigated the performance of biocathode of dual-chamber MFCs for the enzymatic decolorization of reactive blue 221 (Savizi et al. 2012). The laccase enzyme was immobilized on the surface of the changed graphite and used as a conductor within the cathode compartment of MFC. In this experiment, it has been verified that the laccase protein might act as a catalyst in MFCs for gas reduction also as a group action of reactive blue 221 (Savizi et al. 2012). However, for the enzyme-based

MFCs, the restoration of the catalytic activity of the catalyst is a lot of robust related to abiotic catalysts. Therefore the additional investigation is required to overcome these limitations.

9.5 Challenges in MFCs During ORR

Several method parameters; for instance, system pH, alkalinity, substrate nature, and concentration play a key role in MFC performance (Nam et al. 2017). It is necessary to keep up optimum conditions to reinforce MFC performance in terms of each bioelectricity generation and waste remediation (Gurung et al. 2012). As we mentioned earlier, the cathode catalyst is additionally very essential for better oxygen reduction reaction dynamics (Khilari and Pradhan 2018). Cheap and stable cathode catalyst materials are preferred, particularly when MFCs are operational with complex wastewater effluent as a substrate (Scott and Yu 2015). Besides, method optimization is an important criterion to cut back the losses initiated by activation, ohmic, and concentration overpotentials. Moreover, it is necessary to provide optimum conditions within the anode chamber to extend the anodophilic microbic population density to realize a higher power density and additionally to reinforce the method efficiency (Rozendal et al. 2008). Continued efforts are being made to make increased e transfer mechanisms between the conductor and the electrochemically active microorganisms by utilizing applicable catalyst coating on the surface of the conductor.

9.6 Conclusions

The main purpose of the MFC technology is to succeed in cost-efficient wastewater treatment, hence the cathode catalyst ought to be extremely active, stable, cheap, and easy to prepare. Based on the pH circumstances, the overall equation of oxygen reduction reaction will either be H^+ consuming or OH^- producing. During this regard, carbon-based catalysts like the nitrogen-doped carbon materials (carbon nanotubes [CNTs], CNT cups, carbon nanocapsules, etc.) are synthesized via chemical vapor deposition ways, and might be the most effective capable materials for real commercial applications. Further studies are needed to know the components of activated charcoal precursors for mass production. Metal catalysts (both pure and composite metals) are not abundantly enticing at the moment compared to carbon-based materials in terms of stability and value. The M–N–C complexes have gotten a lot of attention as oxygen reduction catalysts in MFCs because of the presence of active metals or functional groups. However, additional study is required to overcome a few limitations like catalyst stability in acid medium. It is expected that the oxygen reduction cathode catalysts for MFCs can play an important role in cheap and eco-friendly wastewater treatment technology in the future, and there will be a growing demand for recognizing their appropriate application niches.

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Chapter 10
Enhancement of Electrochemical Activity in Bioelectrochemical Systems by Using Bacterial Anodes: An Overview

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Abstract Bioelectrochemical systems (BESs) have been extensively investigated in the past decade, because of their high potential for use in wastewater treatment and energy recovery applications. BESs can be broadly classified either as a microbial electrolysis cell (MEC) or a microbial fuel cell (MFC). An MFC operates under resistance, while an MEC operates under an applied voltage. These new technologies are still in their infant stages, and intensive efforts are underway to increase their energy output. The complete performance of the MEC/MFC relies upon the different parameters like the anode, cathode, substrate, reactor type, and design; but the principal part of the MFC/MEC system is the anode. The performance in an MFC/MEC strongly relies on its anode's activity and efficacy, which is considered the limiting element. The significant parameters influencing the anode's bacterial activity are the electrode material and the exoelectrogenic biofilm on the anode. This chapter talks about the current advances in the anodes used in MEC/MFC systems, especially anode amendment, and microbes immobilization techniques to raise the energy output, robustness, and anode biocompatibility, resulting in higher performance with fewer limitations.

Keywords BES · MEC · MFC · Anode · Bacteria · Immobilization · Surface coatings

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10.1 Introduction

Conventional energy sources are being depleted at a faster rate than in the past; hence, the integration of new energy sources is highly essential for sustainable development (Gaurav et al. 2017). In addition to this, waste generation is increasing tremendously, because of quick industrialization and populace development (Gandu 2012; Anupoju et al. 2015; Gandu et al. 2016). The effective disposal of domestic and industrial wastes is vital, as is resource recovery through sustainable and inexpensive processes (Hoornweg et al. 2013; Taiwo 2011; Friman et al. 2013; Gangagni Rao et al. 2012; Gandu et al. 2012, 2015). Thermal, chemical, and biological techniques are available to treat organic wastes and to generate desirable products like syngas, biodiesel, biogas, hydrogen, etc.; the natural biological approaches are particularly promising and sustainable (Chandrasekhar and Venkata Mohan 2014a, a, b; Naik et al. 2010; Rao et al. n.d.; Gandu et al. 2013; Deval et al. 2017). Over the last few decades, considerable attempts have been made to establish a viable bio-based economy, to replace fossil fuels and chemicals with new sustainable biological methods like bioelectrochemical systems (BESs), which use microorganisms (Wang et al. 2015a, b; Zhang et al. 2019). BESs have been thoroughly investigated worldwide over a recent couple of years for the concurrent goals of energy production and control of waste and waste-based byproducts (Mohan and Chandrasekhar 2011a; Chandrasekhar and Venkata Mohan 2012).

Whenever microorganisms are associated with oxidation or reduction, the structure is classed as a microbial or bioelectrochemical system, or in general terms, a BES (Harnisch et al. 2009). In BES, the driving force for electron transfer is an oxidation response at the anode and cathode responsible for reduction response, which together generates a potential distinction (Patil et al. 2011). BES construction involves multiple disciplines, including microbiology, biotechnology, electrochemistry, materials science, and environmental science. The BESs that have been developed vary in their reactor configuration and species of microorganisms. The BES expression strongly depends on the activity and efficacy of the bacterial anode, which is considered the limiting factor (Pandey et al. 2018; Santoro et al. 2017; Schechter et al. 2014). In this case, the present chapter provides an overview of BESs in (1) their types; (2) their operational and design factors; and (3) recent developments in BES anodes, especially anode modifications and bacteria immobilization strategies to raise the anode's energy yield, durability, and biocompatibility.

10.2 Types of Bioelectrochemical Systems

BESs can be broadly classified either as a microbial fuel cell (MFC) or a microbial electrolysis cell (MEC) (Fig. 10.1a, b). In the anode chamber of the BES, a substrate is oxidized by the bacteria, ensuing in the generation of protons and electrons that transfer towards the cathode (Lovley 2006; Logan et al. 2007). The MFC operates

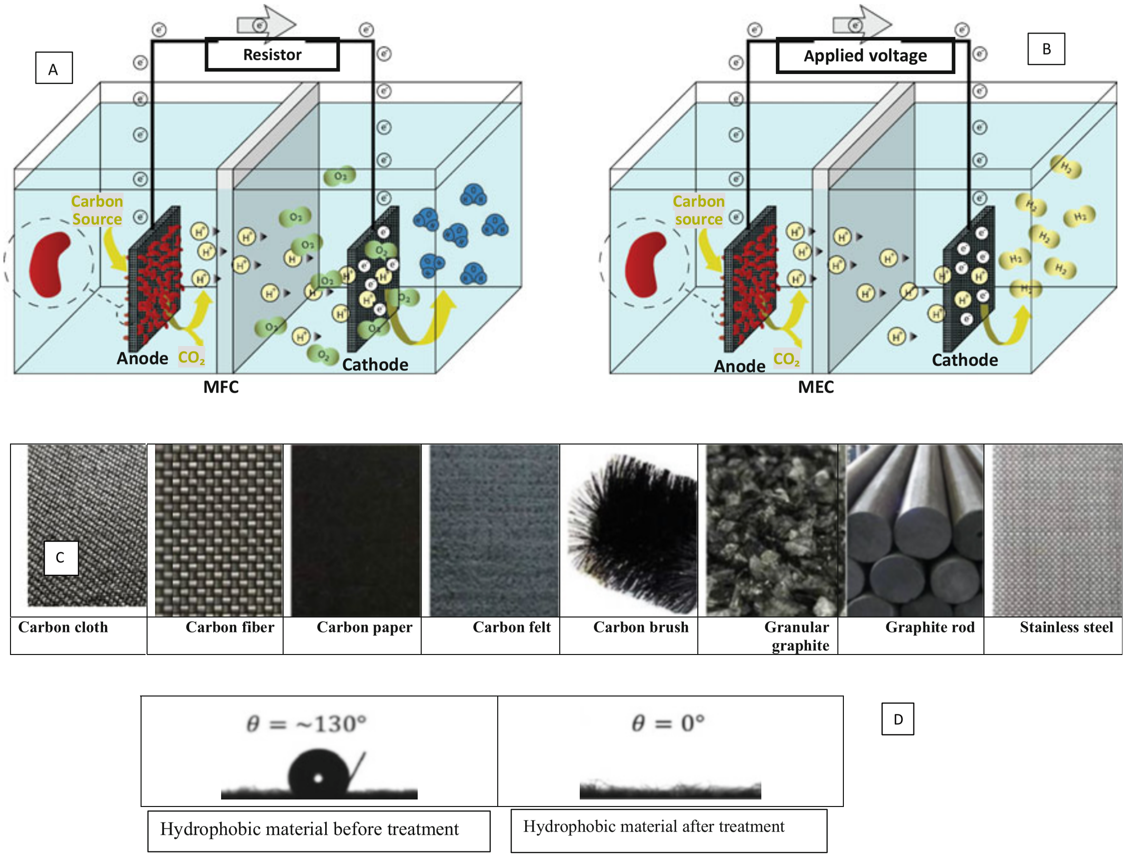

Fig. 10.1 (**a**) MFC (**b**) MEC (**c**) different types of anodes (**d**) Contact angle before and after treatment of anodes

under resistance, while the MEC operates under an applied voltage. Specifically, oxygen reduction in the MFC occurs under aerobic conditions to produce H_2O at the cathode, leading to electricity generation. In the MEC, anaerobic conditions permit hydrogen gas generation obtained by the reduction of H^+ (Kadier et al. 2016b; Sun et al. 2019). MFCs and MECs are further characterized and subdivided as follows.

10.2.1 The Microbial Fuel Cell (MFC)

In a dual compartment, MFC system generates energy through bacteria by oxidizing of the organic substrate, which is driven by the metabolic activity. The traditional MFC comprises a cathode and an anode, alienated through an ion exchange or proton permeable membrane as well as joined to an outer electrical circuit. The MFC configurations are either a double chamber or single chamber. The dual-chamber MFC is the one utmost regularly used technique since this one has a selective exchange membrane that enables protons (but not oxygen) to diffuse between the two chambers. Over time, a single-chamber MFC was developed from the double-chamber model in order to remove the membrane; these reactors may eventually show the most promising results (Pandit et al. 2017; Mohan and Chandrasekhar 2011b; Chandrasekhar et al. 2017). The MFC's anode surface helps promote attachment of microbial and oxidation of organic material, consequently producing electrons, which are concurrently moved towards the cathode half-cell through an outer circuit with a resistance range from 1 Ω to 10 kΩ. Thus, the MFC directly captures the energy as a form of electricity. In confirmation, Feng et al. (2014) observed effective MFC degradation of higher chemical oxygen demand (COD), noted as >79%, with electricity generation of 0.47 W/m^3 (Feng et al. 2014). Another recent study (Wu et al. 2016) revealed that higher power density nearly 50.9 W/m^3 with membrane MFC (Wu et al. 2016). The substrate type, the potential of anode, materials utilized for construction, design, and configuration all impact the MFC's performance; while the electrolyte affects the microbial activity and electron transfer rate (Fan et al. 2007; Pant et al. 2010; Sharma et al. 2015).

10.2.2 The Microbial Electrolysis Cell (MEC)

Like the MFC, a basic MEC design also comprises a cathode chamber and an anode chamber. These remain alienated through a selective membrane in a dual-chamber configuration or lacking a membrane in a single-chamber MEC. The MEC mostly intended towards generate hydrogen gas (H_2) at the cathode (Liu et al. 2014a, b, c; Rozendal et al. 2008a; Rozenfeld et al. 2018, 2019; Kadier et al. 2016a, 2018; Chandrasekhar et al. 2015). The process is five times more cost-effective compared to H_2 production through water electrolysis (Jeremiasse et al. 2010). The MEC cathode operates to assist hydrogen production under anaerobic conditions. However, an applied potential of 0.2 V is also compulsory for the proton reduction, resulting in a specified voltage that permits for highest MEC hydrogen production. Rozenfeld et al. (2018) observed that increasing the higher currents in 28-fold increments obtained the highest hydrogen production rate: 0.0736 m^3/day/m^2 at 0.8 V, amply compensating for the 0.2 V applied voltage (Rozenfeld et al. 2018).

10.3 Operational and Design Parameters of the BES

The BES anodic chamber produces electrons via biodegradation of organic matter, while the cathodic chamber usually serves as an electron acceptor that reduces the protons (with or without oxygen). Several optimization factors may further improve the overall performance of a BES, such as temperature, pH, conductivity, species of microorganisms, and choice of anode/cathode design parameters. These are now discussed in detail.

10.3.1 Temperature

Temperature is an important condition for bacteria growth and bacterial activity. The optimum temperature for the relevant microorganisms is around 25–40 °C for growth, enzyme activity, stable biofilm creation, substrate degradation rate, and electricity production. Many studies have observed that increasing the temperature will improve the power output of the BES. Behera et al. (2011) operated two-chambered MFCs at temperatures between 20 and 55 °C and observed a higher power density of 34.38 mW/ m^2 at 40 °C (Behera et al. 2011). Tang et al. (2012) increased the operational temperature in an MFC from 11 to 35 °C; the highest power density was 193.8 mW/ m^3, obtained at 30 °C (Tang et al. 2012). Li et al. (2013) operated an MFC between 10 and 55 °C, producing a higher power density of 7.89 W/ m^3 at room temperature around 37 °C (Li et al. 2013). Chen et al. (2014) and Lu et al. (2012) found a significant linear relationship from 4 to 30 °C, and performance improved with temperature (Cheng et al. 2011; Lu et al. 2012). However, while the temperature reaches a certain level, the activity and structure of the microbial enzyme are damaged, reflected in falling BES performance (Liu et al. 2005c; Adelaja et al. 2015). Kyazze et al. (2010) studied the relation between temperature and MEC hydrogen production: at 23 °C the average rate was 42.2 ml/ day, at 30 °C it increased to 56.5 ml/ day—but at 52 °C it drastically decreased (Kyazze et al. 2010).

10.3.2 pH

A suitable pH range is required for the ideal development of microorganisms. For example, Sun et al. (2019) considered the significance of different pH ranges on anodic biofilm formation in a single-chamber MEC. This research observed at pH 8.0 generated a higher power density of 2.73 mA/cm^2, which was 56% and 23% better compared to pH 7.0 and 9.0, respectively (Sun et al. 2019). Cui et al. (2019) also observed 85–90% more hydrogen production and current density (83.7 A/m^3) under alkaline conditions (Cui et al. 2019). So, microbial activities are greatly suppressed if the pH is outside of the appropriate range. Moreover, the variation of the pH can cause charge changes in biological macromolecules such as proteins and nucleic acids; these permanently disturb their biological activity, since the electrical charge of their cell membranes affects their ability to absorb nutrients (Rousk et al. 2010). In contrast, Kyazze et al. (2010), who studied the influence of catholyte pH on hydrogen production in a double-chamber tubular MEC, the most elevated hydrogen generation rate observed at 0.85 V and at pH 5 (Kyazze et al. 2010). This demonstrates that BES microorganisms adjust to different pH conditions, making it vital to identify the suitable pH values for exoelectrogenic microorganisms nor does the pH level remain uniform within the BES. During the process, bacteria oxidized the carbon-based material to generate protons and electrons. The electrons are moved to the cathode through an external circuit, whereas the protons migrate to the cathode in the electrolyte solution. Simultaneously, the cathodic reaction consumes protons and electrons. But sometimes massive internal resistance can hamper the transmission of protons and reduce the proton transfer rate far below the anodic output and the cathodic consumption, thus producing a pH gradient from anode to cathode (Gil et al. 2003). In short, proton accumulation occurs close to the BES anode, forming an acid-to-alkaline transition zone from the anode to the cathode (Lu et al. 2012).

10.3.3 Conductivity

Conductivity is an essential component of a BES (Fan et al. 2007; Jung et al. 2007). The best electrolyte should have the good conductive ability and be able to maintain microbial activity. However, the actual conductivity of organic wastewater ranges from 0.1 to 50 ms/cm (Santoro et al. 2018; Stefanova et al. 2018), and large-scale experimental situations have reported weak current outputs (0.005–0.306 mA/cm^2) (Pant et al. 2010). Past investigations have demonstrated that a considerable power increase can be achieved by adjusting the wastewater conductivity. For example, Heilmann and Logan (2006) reported a 33% power increase by supplementing the anolyte with 300 mg/L NaCl (Heilmann and Logan 2006). Liu et al. (2005a, b, c) stated MFC power output increasing around 80% by adding 300 mM NaCl to the wastewater (Liu et al. 2005c). Besides, Luo et al. (2012) reported a microbial desalination cell with power output four times greater compared to the control

MFC, achieved by increasing the conductivity 2.5 times over that of the control (Luo et al. 2012). The level of electrolyte conductivity is fundamental in power generation (Abrevaya et al. 2011; Li et al. 2016).

10.3.4 Microorganisms

Microorganisms can be used as catalysts in BES cells, but organisms also produce multiple enzymes that can degrade various substrates (Bullen et al. 2006). A BES could be inoculated with an isolated or mixed microbial culture. The *Shewanella* and *Geobacter* species were initially identified as the exoelectrogenic bacteria of choice, and they have been well studied. Later, several other microorganisms were discovered to produce electrons.

Ishii et al. (2008) studied air-cathode MFCs with *Geobacter sulfurreducens*, which generated a lower power density of 461 mW/m^2 compared to mixed microbial cultures (576 mW/m^2) (Ishii et al. 2008). Nevin et al. (2008) described a higher power density of 1.9 W/m^2 with *G. sulfurreducens*, compared to 1.6 W/m^2 with a diverse microbial consortium (Nevin et al. 2008). Call et al. (2009a, b) examined *G. sulfurreducens* through a mixed consortium efficiency at 0.7 V potential, and the results showed 1.9 m^3 H_2/m^3/day in both cases—which might have been due to the predominant presence of the *G. sulfurreducens* community (72%) in the mixed culture (Call et al. 2009b).

Thus, the desired high-power densities were often detected with the anode in the appearance of the *Geobacteraceae* community (Kiely et al. 2011; Logan 2009). Alternatively, mixed cultures were found to be more beneficial than pure cultures if wastewater was a substrate (Logan et al. 2008; Biffinger et al. 2007; Lin et al. 2013). Moreover, the pure-culture BES requires strict operating conditions and a sterile environment, resulting in high costs (Sun et al. 2019).

10.3.5 Substrate

Various substrates used in BESs vary from low molecular weight carbon-based compounds to higher molecular weight carbon-based compounds. Numerous experimental studies showed pure substrates like glucose, acetate, butyrate, lactate, proteins, cellulose, or glycerol were used (Cheng and Logan 2007a, b; Logan et al. 2008; Rozenfeld et al. 2017; Cui et al. 2019; Futamata et al. 2013; Chen et al. 2014; Liu et al. 2005a). Jeremiasse et al. (2010) reported that maximal hydrogen production rates of 50 m^3 H_2/m^3/day obtained at 1 V applied voltage with acetate in a double-chamber MEC (Jeremiasse et al. 2010). Selembo et al. (2009a, b) detected an H_2 generation rate of 0.83 m^3 H_2/m^3/day in a single-chamber MEC reactor at a lower temperature (4 °C) with glucose (Selembo et al. 2009b). Others reported electricity generation directly from complex organic wastewater obtained from a wide range of sources: municipalities, dairies, swine farms, slaughterhouses, tanneries, molasses processors, refineries, wineries, breweries, and various industries discarding wastewater rich in sulfides and other chemicals (Liu et al. 2014a, b, c; Chandrasekhar et al. 2017; Venkata Mohan et al. 2010; Katuri et al. 2012; Behera et al. 2010; Mathuriya 2013; Kaewkannetra et al. 2011; Zhang et al. 2009, 2014a, b; Mshoperi et al. 2014; Pepe Sciarria et al. 2015; Venkata Mohan et al. 2008a, b; Velvizhi et al. 2014; Chandrasekhar and Ahn 2017).

Jiang and Li (2009) reported that increased power output 1.2 W/m^3 with the BES substrate concentrations of 850 mg/L (Jiang and Li 2009). However, the increase leveled off, with the changes stopping at extreme concentrations of 1000–1500 mg/L (Venkidusamy et al. 2016). Wagner et al. (2009) studied diluted swine wastewater in an MEC using a graphite anode; they reported that hydrogen gas was produced at 0.9–1.0 m^3 H_2/m^3/day. In another experiment, 0.74 m^3 H_2/m^3/day and 4.5 mA of electrical current generated with potato wastewater as a carbon source at an applied voltage of 0.9 V in a MEC (Kiely et al. 2011).

10.3.6 Cathode Design

The cathode construction is the main task in assembling an MFC/MEC. The chemical reaction that happens near the cathode is crucial due to its triple-phase nature involving electrons, protons, and oxygen reacting with a catalyst. The process must take place on a conductive surface. If the cathode surface area is significantly improved, it has the potential to accomplish higher power or hydrogen production. The effectiveness of a catalyst is regularly evaluated by observing at its current or hydrogen generation with the activity of plain carbon electrodes on the surface area. The oxygen reduction reaction (ORR) and hydrogen evaluation reaction (HER) in a working BES should have a neutral pH and ambient operating temperature. The cathode materials, therefore, can significantly influence the performance of a BES to the degree that they possess high redox potential to receive the electrons.

The utmost extensively described catalyst material in a BES cathode is platinum (Pt) (Liu et al. 2005b; Ditzig et al. 2007; Kadier et al. 2016b). Yet this element carries some disadvantages like it is expensive, Pt has higher sensitivity to poisoning by adsorption of numerous inorganic as well as organic molecules, which change the cathodic potential to increasingly negative values and consequence to reduced HER (De Silva Muñoz et al. 2010). Possible alternatives are nickel and stainless steel, stainless steel brush, SS mesh, NiW and NiMo on a carbon woven textile, and Ni froth, among others (Selembo et al. 2009a; Call et al. 2009a; Zhang et al. 2010; Hu et al. 2009; Jeremiasse et al. 2010; Rozenfeld et al. 2017; Chandrasekhar 2019). Rozenfeld et al. (2018) studied a MEC cathode made with molybdenum disulfide. The experiment involved electrodes made with Pt, with exfoliated MoS_2-EF, and with pristine MoS_2; these exhibited respective current densities of 17.46, 12.67, and 3.09 mA/ cm^2, respectively; and HER rates of 0.106, 0.133, and 0.083 m^3/day/m^3, respectively (Rozenfeld et al. 2018).

Recently, researchers (starting with Zhao et al. 2008) have been working on biocathodes with mixed microbial consortia (Jeremiasse et al. 2012). Biocathodes are drawing interest because of their numerous advantages over abiotic cathodes (He and Angenent 2006), including cost-effective fabrication and easy task. Biocathodes are useful in the denitrification of effluents, and their microbial metabolism might be harnessed to either provide valuable products or expel undesirable compounds (Zhang et al. 2014a, b; Cai and Zheng 2013). Rozendal et al. (2008a, b) observed a current density of 3.3 A/m^2 with biocathodes in a MEC at an applied voltage of 0.8 V (Rozendal et al. 2008b)·

10.4 Anode Materials

The BES anode can be defined as the electrode where oxidation and electron transfers occur simultaneously. The anode electrodes must have numerous properties in order to improve the interfaces among the anode and the bacteria. The significant features are electrical conductivity, surface area, corrosion resistance, biocompatibility, mechanical strength, environmental safety, and economical (Rinaldi et al. 2008; Guo et al. 2017; Mustakeem 2015). Generally, anodes that are made with carbon or metal-based materials have most of the features as mentioned earlier (Rimboud et al. 2014). Among the carbon-based materials used in anode electrodes are carbon textile, carbon felt, carbon brush, carbon mesh, carbon rod, carbon paper, granular graphite, granular activated carbon, and graphite plate. The metal-based complexes include stainless steel mesh, stainless steel plate, stainless steel scrubber, nickel sheet, copper sheet, silver sheet, gold sheet, and titanium plates (Baudler et al. 2015; Zhou et al. 2016) (Fig. 10.1c).

In BES design, *carbon cloth* is used very frequently as an anode material (Guerrini et al. 2014; Santoro et al. 2011, 2017; Zhao et al. 2008). It usually has greater porosity, larger surface area, maximal conductivity, robustness, and flexibility, but it is not economical. Rozenfeld et al. (2019) studied the ability of plasma-pretreated carbon cloth to improve single-chamber MECs; they reported high currents of 11.66 A/m^2 at an applied voltage of 0.6 V (Rozenfeld et al. 2019). *Carbon fibers* have been explored, with *warped titanium* used as a carbon brush (Feng et al. 2010b; Liao et al. 2015; Cheng and Logan 2007a, b); this produced a high surface area and volume-to-area ratio, but it increased the material price. *Carbon brushes* are anodes, and continuing studies are seeking to reduce the cost of material (Hutchinson et al. 2011; Rossi et al. 2019). *Carbon rods* are mostly utilized as current antennas because of their low surface area (Liu et al. 2004; Jiang and Li 2009) and the price is relatively reasonable for BES studies.

Another anode material is *carbon mesh*, relatively economical but suffering from low electrical conductivity and lower robustness (Wu et al. 2017; Wang et al. 2009). Another inexpensive material is *Carbon veil*, which has moderately more excellent conductivity and porosity (Boghani et al. 2014; Winfield et al. 2014; Gajda et al. 2016, 2018; You et al. 2016). As a monolayer, carbon veil is relatively delicate, flexible, and porous (Ieropoulos et al. 2008; Artyushkova et al. 2016). *Carbon paper* also has a porous, planar structure, but as a costly and delicate material, it is mainly used in lab-scale experiments only (Santoro et al. 2014; Srikanth et al. 2008). Min et al. (2005) studied carbon paper as an anode in a double-chamber MFC, with acetate in the presence of *Geobacter metallireducens*, which produced a power density of 38 mW/m^2 (Min et al. 2005).

Carbon felt is another commonly used anode and features greater porosity, higher surface area, and exceptional conductivity. Carbon felt has the big pores to permit microorganisms and inhabit the bacteria inside of the anode. The material cost is comparatively low and robust depending on the material thickness (Calignano et al. 2013; Lv et al. 2012; Zhu et al. 2011). Deng et al. (2010) studied a carbon felt anode by using anaerobic slurry as a bacterial source and glucose as the MFC carbon source, reporting a power density of 784 mW/m^2 (Deng et al. 2010).

Furthermore *Granular activated carbon* (GAC) used as part of the anode electrode, because of its biocompatibility, porosity, and lower price; but these are offset by low conductivity (Zhao et al. 2016; Yasri and Nakhla 2017), which downgrades its primary use to packing material instead of the actual anode. Additionally, *GAC is joined with carbon rods* as a current collector (Jiang et al. 2011) because of its essential characteristics like the greater surface area.

Granular graphite has properties like GAC, except for lower surface area and higher electrical conductivity (Rabaey et al. 2005; Feng et al. 2010a); besides, it is used as bedding material somewhat the independent electrode. Rabaey et al. (2005) studied an anode of granular graphite in a tube-shaped MFC, noting a power density of 90 W/m^3 in the presence of acetate (Rabaey et al. 2005).

Graphite sheet serves as an exceptionally straightforward anode that ensures more excellent electrical conductivity and a relatively lower price. Its small surface region and surface/volume proportion brings about lower yield levels than porous materials (Dewan et al. 2008; ter Heijne et al. 2008), but it is regularly utilized as help for altered structures because of its robust nature. *Reticulated vitreous carbon* has distinctive features, extremely high conductivity with more significant porosity that allows the bacteria to enter and colonize the total electrode. Unfortunately, the material is very delicate and costly for BES use (Lepage et al. 2012).

Other carbon compounds like *activated carbon nanofibers*, *electrospun carbon fibers*, *and carbonized plant stem* are used as electrodes (Karra et al. 2013; Santoro et al. 2017). A few metallic anodes have been utilized as an electrode in BESs. *Stainless steel* (plate, foam, mesh) is very conductive, robust, and cheap (Santoro et al. 2017; Guo et al. 2016; Ledezma et al. 2015). Erable and Bergel (2009) studied *stainless steel grids* as anodes cleaned with 2% HF/0.5M HNO_3 solution in an MFC; this led to an active bacterial anode that delivered a current density of 8 A/m^2 at -100 mV vs. SCE (Erable and Bergel 2009). Guo et al. (2017) studied *steel felt coated with iron-oxide nanoparticles*, which made a better current density of 27 mA/cm^3 at -0.2 V, 16.5 times greater than the untreated stainless steel felt (Guo et al. 2014a, b). Rozenfeld et al. (2019) studied an anode of *plasma-pre-treated carbon cloth with stainless steel* for improvement of single-chamber MEC activity; this resulted in high currents of 16.36 A/m^2 and 0.0736 m^3/day/m^2 of hydrogen production, compared to a stand-alone plasma-pre-treated carbon cloth anode (11.66 A/m^2) (Rozenfeld et al. 2019). Recent research studies described, other metals such as *copper*, *nickel*, *silver*, *gold*, *and titanium* were likewise effectively examined as anode electrode (Baudler et al. 2017; Zhou et al. 2016).

10.4.1 Anode Modifications

The modification of the material on the anode is the primary strategy for increasing the BES power production rates. This is accomplished by (a) surface treatments, (b) surface coatings, and/or (c) surface coatings of immobilized bacteria. All these techniques have their benefits and disadvantages. Surface treatment and surface coating methods should be eco-friendly, cost-effective, and biocompatible (Tsai et al. 2015; Kumar et al. 2013). Anode biocompatibility was improved by changing the anode surface science: increasing the positive charge, hydrophilic, adding functional groups like nitrogen or oxygen, or adding immobilized bacteria on the surface of anode (Du et al. 2017; Santoro et al. 2017) (Fig. 10.1d).

10.4.1.1 Surface Treatments

The surface of anode materials is altered by different methods, including treatments with ammonia, heat, acid, and plasma. These surface modifications enhance BES performance.

The objective of ammonia *treatment* is always to enhance the attachment of bacteria germs into the anode. Bacteria accumulation is primarily dependent upon the positive interactions of the anodes since the bacteria are negatively charged. The treatment creates accentuating positively charged functional groups spread across the anode surface. Any increment in the microorganism adhesion increases the transport of electrons to the anodes. Previously Cheng and Logan et al. (2007) reported a continuous stream of ammonia vapor over carbon cloth anodes significantly decreased the BES startup period after treatment (Cheng and Logan 2007a, b). The ammonia-treated carbon material revealed an average power density of 1970 mW/m^2; it is more significant than compared to an untreated anode (1330 mW/m^2) (Cheng and Logan 2007a, b). New improvisation with this strategy, by the surface treatment for graphite-fiber brush anodes, also given a higher power density of 2400 mW/m^2, credited to the anode area and little resistance of these graphite fibers (Cheng and Logan 2007a, b). Xie et al. (2012) used the *Rhodopseudomonas palustris* filamentous bacteria with the graphite-fiber brush in addition to copper anodes, resulting in an extreme power density of 2720 mW/m^2 (Zuo et al. 2008). Xie et al. (2012) used the *Rhodopseudomonas palustris* filamentous bacteria with ammonia gas treated graphite-fiber resulted in an incremental in power density of 2720 mW/m^2 (Zuo et al. 2008).

Although higher power generation values are guaranteed by ammonia treatment, the need for sophisticated environments, equipment, and strategies reduces the potential scale-up of these operations to commercial levels.

Electrode surface modifications with *heat treatment* are also cost-efficient. Carbon mesh anodes with thermal treatment make out of a 3% more power density in the MFC (Wang et al. 2009) by facilitating the cohesion and inoculation of the bacteria on the anodes. Carbon fiber brush anodes undergoing heat treatment demonstrated that higher growth in power density more than 15%, in contrast to control anodes (Feng et al. 2010b).

Wang et al. (2009) described a sustainable method for increasing the maximum MFC power density by 3%. It included a carbon mesh heating in a furnace at temperature of 450 °C for 30 min; the MFC reached a higher power density of 922 mW/m^2 at an approximate potential of 0.6 V, correlated to an MFC employing untreated carbon mesh which yielded only 811 mW/m^2 (Wang et al. 2009).

Acid *treatment* is just another way to alter the surface of electrodes, achieved by treating them into various kinds of acids. It enhances the natural anode surface area and assists in the functional group's protonation. For example, nitric acid pre-treated graphite-felt anodes demonstrated that a twofold increase in power density (Scott et al. 2007).

The *thermal and acid treatment* combinations also shown increases in MFC power. For example, Feng et al. (2010a, b) experimented with surface oxidation of carbon fiber soaked in acid (H_2SO_4) and subjected to heating, which improved power density up to 1370 mW/m^2, 34% higher than the control anode (1020 mW/m^2). Moreover, this power density was 25% higher compared with just acid treatment (1100 mW/m^2), and 7% greater than compared with simple heat treatment (1280 mW/m^2) using carbon fiber brush anodes in air-cathode microbial fuel cells (Feng et al. 2010a). Scott et al. (2007) investigated anode surface modification by nitric acid and heat treatment, thereby achieving a power density of around 28.4 mW/m^2 (Scott et al. 2007). The acidic modification of the anode carbon cloth material increases the ratio of saturated-to-unsaturated carbon on the surface, leading to a decrease in electrode resistance and a shorter startup period. Scott et al. (2007) also observed that graphite anode surface activation by nitric acid, continued by heat treatment, enhanced the power density about threefold (Scott et al. 2007).

Plasma treatment is an environmentally friendly and very recent method to enhance the hydrophilicity of an anode. Eliezer and Eliezer (2001) reported on *cold nitrogen* plasma's ability to enhance a carbon felt anode's hydrophilicity and biofilm formation (Eliezer and Eliezer 2001). Cold nitrogen plasma treated carbon cloth receives a modified chemical surface with increased micro-porosity. Adding nitrogen doping atoms additionally creates the carbon hydrophilic nature and more accessible to microorganism's attachment (Kaplan and Rose 1991). Plasma with nitrogen gas treatment has been described to make nitrogen-based molecules like amines, pyridines, and pyrroles on electrode surfaces. Anode surface treatment by oxygen plasma results in a formation of –C–OH, –COOH, also C=O functional groups (Mujin et al. 1989; Kogelschatz 2003). Yick et al. (2015) revealed that using argon plasma treatment for carbon nanotubes instigated bacteria improvement, for example, Gram-positive bacteria and Gram-negative bacteria (Yick et al. 2015). Rozenfeld et al. (2019) analyzed MEC action while employing stainless steel and plasma-pretreated carbon cloth anodes; the outcomes confirmed a greater current density of 16.36 A/m^2, in contrast to control anodes (11.66 A/m^2) (Rozenfeld et al. 2019).

10.4.1.2 Surface Coatings

The anode surface characteristics could be improved by coatings with diversified synthetic and natural compounds or materials containing carbon nanotubes, ferric oxides, Au nanoparticles, goethite nano-whiskers, chitosan, agarose, and more. The still newer introduction of other nanomaterials to melamine sponges, Berl saddles, carbon nanotubes, and high-capacitance electrode materials has also produced successful coatings for BES anodes (Kumar et al. 2013, 2018).

Advanced carbon nanostructures like graphene, carbon-based nanostructures (Nanotubes and nanofibers), used as anode-coating materials to improve BES surface area and conductivity. The carbon nanostructures are most easily paired with carbon paper, nickel foam, and stainless steel mesh (Guo et al. 2014a, b; Hou et al. 2014). Sun et al. (2019) studied with *carbon nanotubes* by using *carbon paper in an MFC cell result in 20% more* power density (Kumar et al. 2013). Interestingly, Liang et al. (2011) stated that the addition of carbon nanotubes to an MFC could also increase total output voltage and shorten the MFC startup time (Liang et al. 2011). Some novel carbon-nanotube anode coatings have shown unique biofilm morphology, with better performance than plain gold electrodes (Ren et al. 2015).

The modification of anodes with nanocomposite materials tends to enhance BES microbial activity. Combinations of *carbon with stainless steel* have resulted in a high surface anode and have shown high efficiency in wastewater treatment (Sonawane et al. 2014). The nanocomposites *graphene oxide and tin oxide added to carbon nanotubes* were tried as anode coatings in MFCs, and the results showed higher power densities of 1421 mW/m^2 and 699 mW/m^2, respectively, compared to an uncoated anode (457 mW/m^2) (Mehdinia et al. 2014). This improved MFC performance was credited to the higher surface area of the graphene in the nanotubes, whereas the tin oxide caused synergic effects, for instance, more anode area and higher conductivity; all of which significantly enhanced the biofilm formation and increased the electron transfer (Mehdinia et al. 2014).

Other conductive polymers like *polyaniline*, *polypyrrole*, *polyacetylene*, *and polythiophene* have been widely investigated as coatings in fuel cells (Wessling 2010). These were shown to enhance the power output when compared to untreated carbon cloth anodes (Fan and Maier 2006; Schröder et al. 2003). They have proven useful for increasing the surface areas, biocompatibility, enhancing electron transfer, and saving costs (Zhang et al. 2017; Wang et al. 2013). Scott et al. (2007) observed graphite anodes modified with PANI/C, PANI 900, KjB, PANI tubes, and graphite, reporting maximum power densities of 26.5 mW/m^2, 26.1 mW/m^2, 20.1 mW/m^2, 15.2 mW/m^2, and 9.5 mW/m^2, respectively (Scott et al. 2007). The low power density of the last type (the unmodified graphite-felt anode) due to of very smaller surface area needed to improve bioelectrochemical transfers with microorganisms or the lower anode charges on the anode.

The investigated conductive polymers have thermal stability, electrical conductivity, corrosion resistance, acid–base properties, and an affordable process for polymerization. They hold promise for the enhancement of surface areas, conductivity, and charge transfer rate while providing a rough surface for biofilm generation and a super-capacitive role to keep charges (Kumar et al. 2013; Hindatu et al. 2017).

Another compound, *anthraquinone-1,6-disulfonic acid* (*AQDS*) coating on *graphite*, comprehensive a higher power density of 98 mW/m^2 at 0.24 V cell voltage, compared to a control graphite anode (20 mW/m^2) (Lowy et al. 2006). *A graphite plate coated with an* Mn^{2+} *and* Ni^{2+} *anode* reached a power density of 105 mW/m^2 at 0.35 V (Lowy et al. 2006). Furthermore, kinetic activity was increased 0.9-fold by coating *graphite nanocomposites with the Sb(V) hexadecyl pyridinium bis complex* (Lowy and Tender 2008).

Melamine sponges layered with graphene and carbon nanotubes have provided a large conductive electrical surface for *Escherichia coli* growth, along with high porosity for efficient mass transport and electron movement in an MFC. These coated sponge anodes aremore robust compared to conventional carbon and metal-based anodes in an MFC (Chou et al. 2014). Similar studies conducted by Xie et al. (2012) examined *graphene-coated sponges* resulting in a higher power density of 1.57 W/m^2, or 394 W/m^3(Xie et al. 2012).

Carbon-coated Berl saddles have become an innovative low-cost anode material that favors optimal bacteria adhesion and efficiently recovers the electrons released by the bacteria metabolism. The coating increases the surface area with an abundant void bed while also showing a maximum power density of about 2–3 times more than that obtained with commercial anode materials (Hidalgo et al. 2014).

Liu et al. (2014a, b, c) considered *carbon nanoparticles* and *chitosan* as a coating material on anodes, reporting a higher current density and coulombic efficiency (500 mA/m^2, 32%, respectively), compared with raw carbon paper (150 mA/m^2, 19%, respectively) (Liu et al. 2014a, b, c). This fabricated electrode turned into to increase in MFC overall performance. The anode performance was enhanced by injecting it in poly-N-isopropyl acrylamide. In this research, maximal current and power densities were observed, due to the higher surface area, the wide variety of dynamic positions, and the enhanced interaction closeness among the electrolyte and catalyst (Kumar et al. 2014).

10.4.1.3 Anode Surfaces Coated with Immobilized Bacteria

The various types of anode, as mentioned earlier surface treatments and coatings were not sufficient to protect exoelectrogenic bacteria in harsh wastewater conditions, or to prevent the attachment of non-desired bacteria. For these reasons, a new coating strategy has evolved to include immobilization of the bacteria with polymers.

This method has been explored over the past two decades; it is currently applied to drug delivery, the food industry, and environmental remediation (Rabanel et al. 2009; Burgain et al. 2011; Moslemy et al. 2004). Immobilization of bacteria in a polymer allows bidirectional transmission of molecules: namely, oxygen inflow, growth elements, and nutrients vital for bacterial metabolic rate; and also the diffusion of waste products to outward.

In nature, many microorganisms possess the ability to adhere to and survive on divergent surface varieties, causing bacteria to grow within natural conditions.

To this end, the anode material used in a BES must have physical, chemical, and biological consistency, along with mechanical strength, easy availability, nontoxicity, and cost-efficiency. Other physical characteristics like swelling, porosity, and particle behavior should be favorable, in addition to compatibility with bacterial development, biodegradability, solubility, and application specificity. The ideal immobilizing material provides microbial cells with protection from physicochemical variations which include pH and temperature; enables higher bacterial masses, higher yield and efficacy; higher substrate consumption improvement; and decreases the risks of bacterial contamination (Saucier and Poo 2006; Nedovic et al. 2011). Extensively diversified polymers are used for the immobilization of microbial species, like pectin, alginate, agar, gelatin (bioorganic), silica gels, and polyvinyl alcohol (inorganic) (Bayat et al. 2015). However, natural organic varieties have higher biocompatibility and lower costs than artificial polymers; organic polymers are also more easily formed into gels suitable for immobilization (Duarte et al. 2013). Natural polymers endorsed for good cytocompatibility, allowing solute dispersion and electron interchange. However, they may be at risk of swelling and to chemical/biotic degradation (Srikanth et al. 2008; Le Ouay et al. 2013; Estevez-Canales et al. 2018). On the other hand, silica gel (inorganic polymers) also permits both solute dispersion and electron swap (Le Ouay et al. 2013; Estevez-Canales et al. 2018); and they offer better optical and mechanical properties, which makes their gels extra durable and more straightforward to regulate than natural polymer materials (Depagne et al. 2012; Wang et al. 2015a, b).

Although immobilization of biomolecules have been studied for quite a long years (Bjerketorp et al. 2006; Karimzadeh et al. 2018; Ahuja et al. 2007), research with electroactive bacteria appropriate for BES technology, such as *Shewanella*, is very recent (Yu et al. 2011; Luckarift et al. 2012; Sizemore et al. 2013). Luo et al. (2016) studied the effect of in-situ immobilization on anode performance in an MFC with sodium acetate. The immobilized anode generated higher power densities of 610 mW/m^2 compared to the control MFC (343 mW/m^2) (Luo et al. 2016). Yong et al. (2013) studied *Shewanella oneidensis* immobilized in an MFC with graphite and alginate granules, reporting coulombic efficiency 0.8–1.7 times higher than a normal MFC (Yong et al. 2013). In addition, the bacteria-immobilized BES demonstrated significantly higher resistance to the stun of high salt concentration than did the BES with suspended bacterial cells. Luckarift et al. (2010) standardized the silica-based immobilization of *S. oneidensis* in MFC anodes. In this study, one-step vapor deposition of silica was used to immobilize *S. oneidensis* to form an aqueous sol–gel in the MFC anode, which improved the greater power density of the MFC (Luckarift et al. 2010).

10.5 Conclusions

Today, waste management practices are increasing rapidly in all sectors, due to requirements of hygiene and the search for renewable energy sources. Different variants of the biological methods available for the management of organic waste materials and for the generation of energy are currently the focus of many laboratory studies—especially BES technology, which answers both needs simultaneously. Therefore, the BES must be robust enough to function in the real environments surrounding bioremediation, wastewater treatment plants, and simultaneous energy generation. BES performance has been extraordinarily improved in the course of the most recent decade by adjusting the engineering and individual segments of the reactors. The activity in the anode compartment is considered to be the key to a successful BES, since only it decides electron generation and transportation. The electrode-anode materials and alteration techniques are thus the pre-prominent main factors that oversee the overall performance of BESs. In this book chapter, we outlined the categories of bioelectrochemical systems, reviewed the important factors affecting their performance, and focused on the best anode materials and enhancements, including the new strategies for immobilization of exoelectrogenic microorganisms.

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Chapter 11
Biocatalysts in Electrofermentation Systems

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Abstract In electrofermentation biosynthesis reactions, cells or enzymes are electrically stimulated by electron input where the biocatalyst grows or is immobilized, increasing the yield of the target product. Biocatalysts play a crucial role—they can substitute the usually expensive metal-based catalysts in electrosynthesis, not to mention that they operate at mild temperature and pressure. The ample diversity of enzymes and microorganisms allows for the use of several types of feedstock, thereby preserving specificity. After the technology is scaled up, production costs should continuously decrease, which is not true for metallic catalysts. The key to achieving high efficiency of enzyme- and microbe-catalyzed cells lies within electron transference effectiveness from the electrode to the biocatalyst. This chapter presents the most accepted means of electron transference between electrode and biocatalyst. In addition, it addresses the most recent applications of pure culture and microbial consortia as well as synthetic biology approaches.

11.1 Introduction

The demonstration that microbe-electrode electron transfer is possible has incited great potential for application of a technology bridges fundamental research in microbiology, biochemistry, engineering, materials science, and electrochemistry. This has culminated in the creation of bioelectrochemical systems (BESs), which paves the way for various high-impact applications of BES-based technologies in electrical, biochemical, and chemical feedstock production. However, these technologies require considerable improvement before they are deemed viable (Kumar et al. 2018).

BESs can be summarized as an electron transport between a biotic and an abiotic component; the microorganism or biomacromolecule acts as the catalyst, which

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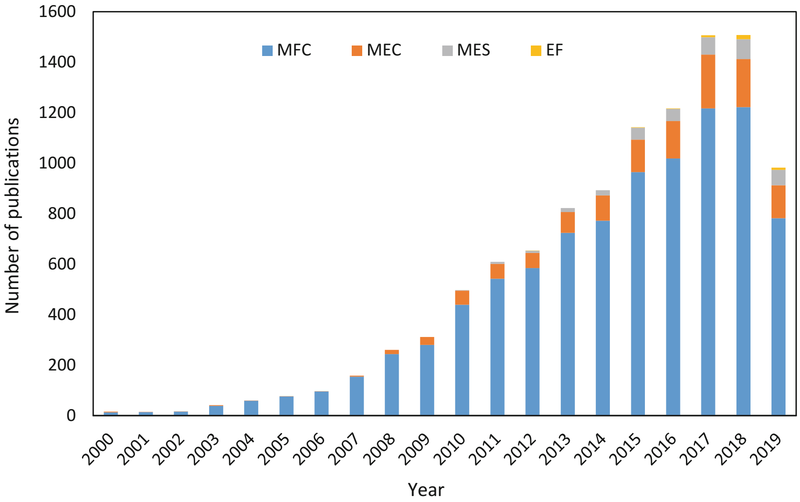


Fig. 11.1 Data of the Web of Science™ database for microbial fuel cell (MFC), microbial electrosynthesis (MES), microbial electrolysis cell (MEC), and electrofermentation (EF) (18th August 2019)

facilitates the electron exchange procedure (Kumar et al. 2018). Depending on the electron flow direction, two BES categories exist. In microbial fuel cells (MFCs), a biocatalyst that can perform extracellular electron transfer drives substrate oxidation and electrical energy generation in the anode. In microbial electrosynthesis (MES), electron transfer occurs in the opposite direction—the biocatalyst receives electrons from a cathode and produces biochemical compounds (Choi and Sang 2016). MFCs and MES are complementary. In combination, they can deliver and receive electrons, respectively, thus mimicking biochemical energy conservation processes.

Currently, the typical MES application is almost restricted to CO_2 use as a substrate, which generates limited low-value products, mostly acetate or methane (Jiang and Jianxiong Zeng 2018). Electrofermentation (EF) has emerged from the need to expand the spectrum as the of higher-value chemical production.

Scientific production in this area can be critically evaluated through the Web of Science™ database from 2000 until today (18th August 2019). A compilation of the search for bioelectrochemical methodologies, such as microbial fuel cells (MFCs), microbial electrosynthesis (MES), microbial electrolysis cells (MECs), and electrofermentation (EF), shows the rising importance of these topics in the last years (Fig. 11.1). In particular, MFCs have received expressive attention with ca. 1200 publications in 2018. Indeed, the possibility of concomitant wastewater treatment and energy generation has made MFCs the most studied bioelectrochemical approach, which has brought them the nearest to a real scale application. Interest in MECs and MES arose as a relatively new topic after 2005 and 2010, respectively, and grew exponentially thereafter, but at much lower rates than the interest in MFCs. Finally, the term electrofermentation (EF) started to appear consistently in the literature only after 2015, but EF is also a kind of MES.

11.2 General Aspects of Electrofermentation (EF)

Although "classic" electrocatalysis has long been known and employed in industrial processes like galvanoplasty, the use of microorganisms as catalysts allows complex reactions to be conducted, especially reactions concerning organic compounds. As a matter of fact, microorganisms possess a wide array of truly catalytic enzymes that are already fit to work together in metabolic reactions (Schievano et al. 2016). Even though microbes cannot be denominated as catalysts because they consume a small fraction of energy for cell maintenance and reproduction, these costs can be minimized and do not represent a sufficiently significant portion of the energy involved in the target process (Schievano et al. 2016).

Electrofermentation (EF), also called bioelectrochemical fermentation or electricity-driven biosynthesis, can be considered as a BES that controls self-driven fermentation. EF encompasses carbohydrate or alcohol fermentation, using cathodes as additional electron sources or sinks. When the biochemical reaction is mainly oxidative, the electrode acts as an anode to help dispel the superfluous electrons. Conversely, when the reaction is mostly reduction-based, the working-electrode deliveries electrons as a cathode (Moscoviz et al. 2016). Be it as it may, the energy derived from these energy sources is often transported by reductive equivalents like NADH and ATP, which participate in simpler redox reactions to form the product of interest. Therefore, electron supply by an electrode may force changes in NADH/NAD+ balance, which can subsequently impact the overall biological regulation and the products of fermentation (Schievano et al. 2016). In contrast with other BESs, EF operates with much lower current densities (Moscoviz et al. 2016). EF in integrated BES allows one to address two basic traditional fermentation issues: the electron surplus, which can be balanced by fermentation at the anode, and the shortage of reductive equivalents, which can be balanced at the cathode and increase the redox power by twofold. Whereas MFCs work as the anode and serve as an electron sink that facilitates complex oxidations, MES works mostly as the cathode and supports the electron donation and energy supply for complex reductions, generating current at the anode and consuming it at the cathode. Although the use of MFCs for electricity production has attracted much attention, the low-power densities achieved with these cells so far and the low potential revenue make their integration with EF an interesting strategy to produce added-value products of great interest.

EF is advantageous over traditional fermentation, which is hampered by substrate purification costs and sustainability concerns relative to the dedicated land use and agroindustrial transformations, the emission of greenhouse gases and toxic byproducts, and the financial and environmental burden of additives, for example. Additionally, EF can help to overcome the issue of the frequent redox imbalance of fermentative reactions, many of which can only become spontaneous if they happen under specific temperature, pressure, and gas composition conditions that raise the availability of certain electron donors as well as the pH and ion balance. Furthermore, product refining remains a problem of traditional fermentation and sometimes accounts for up to 80% of total costs of the process (Schievano et al. 2016). Lastly, the different potential values required by biochemical reactions mean that the use of EF could steer the metabolism toward a desired product through control of the potential that is available at the electrode.

Other benefits of EF compared to the traditional approach include: (1) more favorable biomass-to-product ratios since anodes provide less thermodynamic gain relative to oxygen, favoring product formation rather than cell growth; (2) better control over metabolic pathways through control of the potential value, thereby changing or reducing the product spectrum; and (3) better distribution of the electrons generated in situ, which enhances the process kinetics and even drives thermodynamically less favorable reactions, as opposed to sparged H_2 gas, for instance, which needs to dissolve (Schievano et al. 2016).

Given the shift of a petrochemical-based economy to a bioproduct-based economy, EF emerges in a moment of great economic opportunity. Its advantages over traditional fermentation allow room for substantial growth in the coming years (Harnisch et al. 2015). Harnisch et al. (2015) and Rabaey et al. (2011) showed that the costs of feeding a reactor with electricity from the grid or sugar are basically the same, which allows for competition side by side.

An obstacle that still has to be faced in EF for alcohol and volatile fatty acid production (which easily occurs in BESs) is that these products present as ions in neutral pH, so they can permeate through the ion interchange membranes that are used in these approaches. This culminates in lower efficiency due to product leakage (Gildemyn et al. 2015). In addition, the scope of products that can be electrofermented is limited by three main factors: energy, redox power, and materials (Harnisch et al. 2015).

Fermentations electrically driven by pure cultures can well target high-added value products, including chemicals (Rago et al. 2019). EF can also be employed to direct fermentation catalyzed by microbial consortia and brings substantial benefits for the use of agrofood waste toward bioproducts (solvents, polymers, electrofuels, and biomolecules) (Rago et al. 2019).

11.3 Biocatalysts in Electrofermentation

Biocatalysts play a crucial role in BESs—at mild temperature and pressure, they favor reactions that are thermodynamically less favorable under these conditions. Biocatalysts can be employed several times if the correct conditions are maintained. Moreover, biocatalysts can use numerous renewable feedstocks because of the broad diversity and specificity of enzymes and microorganisms. Besides that, scaling up this technology foresees lower production costs, which cannot be achieved by inorganic catalysts (Aquino Neto and De Andrade 2013).

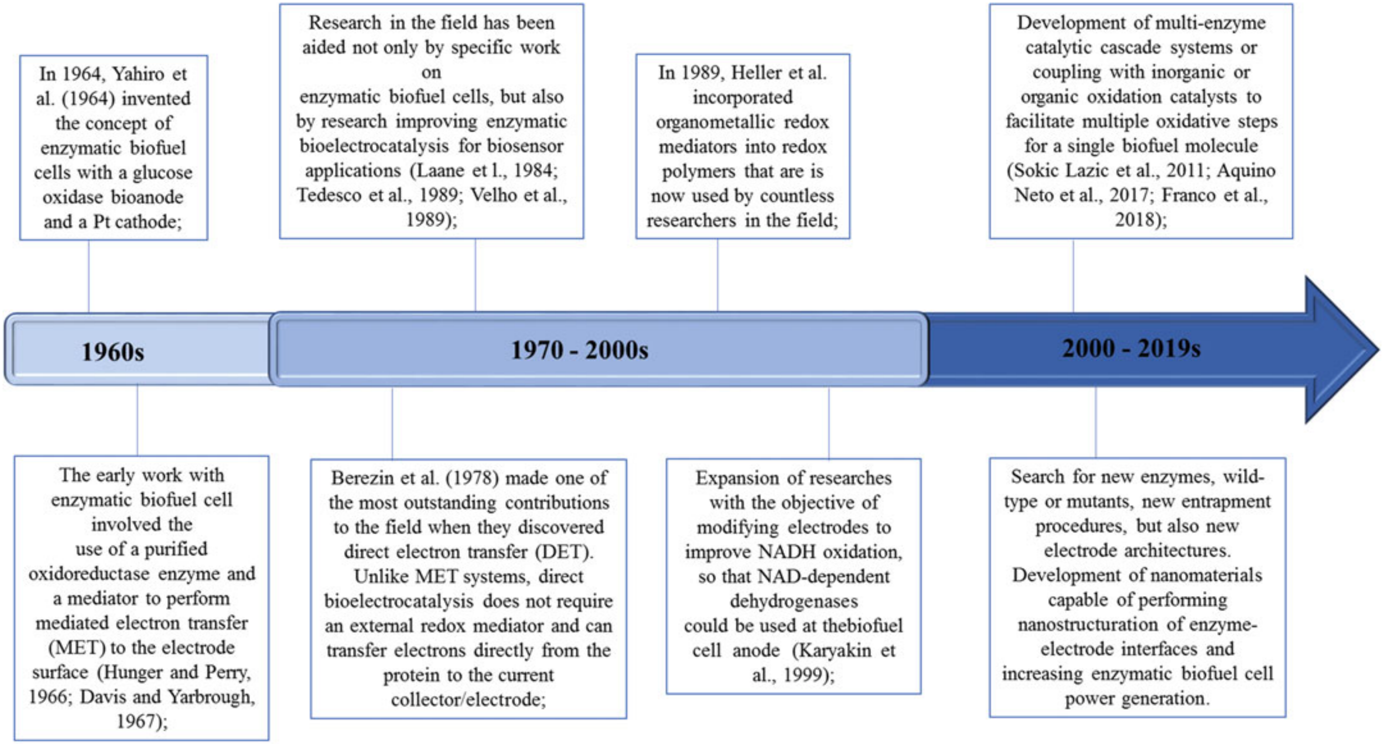
Contrarily to conventional electrochemical systems based on metallic catalysts, bioelectrochemical synthesis can be conducted with the whole microorganism or enzymes as biocatalysts (Aquino Neto and De Andrade 2013; Kumar et al. 2018). The two biocatalysts, the enzyme and the whole microorganism, are intimately linked because the reactions taking place on the electrode surface rely on membrane-bound enzymes, some of which work as oxidoreductases (Lapinsonnière et al. 2012). Even though both catalysts are of biological origin, microbes are active, can self-duplicate, so they provide microbial electrodes with the longest lifetime. Contrary to microbes, enzymes alone cannot replicate and have to be produced by microorganisms before being purified and used (Lapinsonnière et al. 2012). They are mostly substrate specific, but can transfer a maximum of two electrons. Furthermore, bacteria are able to form a natural biofilm on the electrode surface as well as appendages and/or molecules responsible for electron transfer (Lapinsonnière et al. 2012).

Electron transport made by enzymes is one of the first BES models. Figure 11.2 shows the evolution of scientific research into enzymatic bioelectrocatalysis in the last decades.

The development of scientific research into enzymatic biocatalysis began with the first paper published by Yahiro et al. (1964), who defined the idea of enzymatic biofuel cells with a glucose oxidase anode and platinum in the cathode. Two years later, Hunger (1966) developed an enzymatic fuel cell by using an oxidoreductase, and they defined the concept of electron transfer by mediator (MET) to the electrode. In the 1970s, Berezin et al. (1978) discovered direct electron transfer (DET) and made one of the most important contributions in the field. The authors found out that, contrary to mediated electron transfer, direct bioelectrocatalysis does not require an external redox mediator and that electrons can be directly transferred from protein to the electrode.

In the 1980s, researchers published the first studies on enzymatic bioelectrocatalysis for biosensor applications (Laane et al. 1984; Tedesco et al. 1989; Velho et al. 1988). Nowadays, enzymatic biosensors constitute valuable bioanalytical devices for qualitative and quantitative assays of myriad elements for medical diagnoses (Abolhasan et al. 2019), biological (Zappi et al. 2019) and biomedical research (Hsu et al. 2016), food safety (Chan et al. 2015), and environmental monitoring (Mousty et al. 2001; Pundir and Chauhan 2012; Shahar et al. 2019). By providing its high selectivity, the enzymes are the most essential components enzymatic biosensors, and, add to the many other advantages of bioelectrochemical detection, low cost, and simple instrumentation (Zhao et al. 2017).

In the beginning of the twenty-first century, great advances were made in enzymatic bioanodes for fuel cells. However, the biggest advances in this area lay in the search for new materials capable of increasing the efficiency of these systems. The key to highly efficient enzymatic cells has been related to the rate of electron transference from active site of the enzyme to the surface of the electrode (Yahiro et al. 1964; Berezin et al. 1978; Aquino Neto and De Andrade 2013). Because enzyme active sites typically contain a coenzyme molecule that is tightly bound and



In 1964, Yahiro et al. (1964) invented the concept of enzymatic biofuel cells with a glucose oxidase bioanode and a Pt cathode;

The early work with enzymatic biofuel cell involved the use of a purified oxidoreductase enzyme and a mediator to perform mediated electron transfer (MET) to the electrode surface (Hunger and Perry, 1966; Davis and Yarbrough, 1967);

Research in the field has been aided not only by specific work on enzymatic biofuel cells, but also by research improving enzymatic bioelectrocatalysis for biosensor applications (Laane et l., 1984; Tedesco et al., 1989; Velho et al., 1989);

Berezin et al. (1978) made one of the most outstanding contributions to the field when they discovered direct electron transfer (DET). Unlike MET systems, direct bioelectrocatalysis does not require an external redox mediator and can transfer electrons directly from the protein to the current collector/electrode;

In 1989, Heller et al. incorporated organometallic redox mediators into redox polymers that are is now used by countless researchers in the field;

Expansion of researches with the objective of modifying electrodes to improve NADH oxidation, so that NAD-dependent dehydrogenases could be used at thebiofuel cell anode (Karyakin et al., 1999);

Development of multi-enzyme catalytic cascade systems or coupling with inorganic or organic oxidation catalysts to facilitate multiple oxidative steps for a single biofuel molecule (Sokic Lazic et al., 2011; Aquino Neto et al., 2017; Franco et al., 2018);

Search for new enzymes, wild-type or mutants, new entrapment procedures, but also new electrode architectures. Development of nanomaterials capable of performing nanostructuration of enzyme-electrode interfaces and increasing enzymatic biofuel cell power generation.

Fig. 11.2 Evolution of scientific research into enzymatic biocatalysis in the last decades buried inside the enzyme, the distance between the enzyme active site and the electrode surface is too large for effective electron transfer to take place (Rasmussen and Minteer 2016; Huang et al. 2018).

Nevertheless, the expansion of enzymatic bio cells still faces quite a few limitations. Firstly, power density remains quite low in most cases and is not enough to drive devices. Moreover, the systems' long-term-stability still poses a challenge and requires highly effective and stable immobilization of the enzyme at the electrode. In several cases, electrode amendment with conductive micro- or nanomaterials has proven a valuable tool to enhance enzymatic bioelectrode performance (Poulpiquet et al. 2014).

For example, various conductive nanoparticles (Shakeel et al. 2019), organic polymers (Bonfin et al. 2019), and carbon nanomaterials (Kang et al. 2019; Pankratov et al. 2019) are being developed as electrical conductors between enzymes and the electrode surface. Available conductive nanomaterials including carbon nanomaterials, such as carbon nanotubes and graphene (Bollella et al. 2018; Perveen et al. 2018) have been gaining substantial space in the fields of materials science research due to their particular properties. In general, carbon nanomaterials are promising to improve the bioanode overall performance, which is desirable for efficient enzymatic fuel cells.

As to their functionality, enzymatic reactions are applicable for both oxidation and reduction reactions in the anode and in the cathode, respectively. Enzymatic biofuel cells are the most widely studied system: they constitute efficient devices for the conversion of biofuels' chemical energy into electricity (Kim et al. 2014). The enzyme in the cathodic compartment executes the reduction reaction aided by the electrons inflow from anodic oxidation (Bandapati et al. 2017). The electrons are transferred through the exterior circuit linking anode and cathode, while the protons flow through the electrolyte and ion exchange membrane/bridge.

These reactions must operate at the same pH between chambers. Just as in conventional systems, the rate and stability of enzyme-catalyzed reactions are also directly influenced by temperature, which can show an optimal activity profile ranging from temperatures as low as 3–5 °C, or as high as 50–80 °C, depending on the biological origin (Cosnier et al. 2018).

How the enzyme is deposited on the electrode is extremely important for system development. Regarding physical methods, deposition can happen in two manners: either physical adsorption or through entrapment. Limitations of these methods include the ease of enzyme denaturation because of their weak binding with the supporting material. Therefore, chemical immobilization approaches such as entrapment, encapsulation, covalent bonding, and cross-linking are presently preferred Chung et al. 2017).

Because it is usual that a sole enzyme anode partially oxidizes a biofuel via a 2-electron process, multi-enzyme catalytic cascades have been developed to maximize the biofuel cell energy density. Modeling of immobilized enzymatic cascades involving different reactions is well established for steady-state conditions, both in well-mixed solutions and more complex systems (Arechederra and Minteer 2008; Idan and Hess 2013).

These types of fuel cells are rapidly gaining research attention because the catalysts are ubiquitous, the design is simple, and hydrocarbon fuels like sugars and alcohols are promptly available (Lau et al. 2015). These cells oxidize high-energy-density biofuels and are capable of harvesting more electrons per substrate molecule, constituting a strategy to be employed in the oxidation cascade. The main idea, therefore, behind this study of enzymatic cascades, lies within "metabolic channeling," which is the uninterrupted transfer of intermediates between enzymes without mediators molecules being liberated into the reaction solution (Macazo and Minteer 2017).

Franco et al. (2019) obtained complete ethanol electrooxidation through the use of carboxylated multi-walled carbon nanotubes (capable of collecting up to twelve mol electrons per mol alcohol), using a bifunctional enzymatic/organic electrocatalyst based on modified linear poly(ethylenimine) and oxalate oxidase. Compared to previous systems, this new hybrid system proved to be a good choice for its excellent selectivity and the achievement of both high electrochemical oxidation rates and high carbon dioxide yields as the final product. These systems also compose valuable multifunctional hybrid-catalysts with promising potential in the design of low-power electronic devices, such as pacemakers and sensors, based on hybrid bioanode architectures for energy conversion/storage, and to participate in multistep reaction cascade systems.

Enzyme-catalyzed cathodes, especially those based on metal-oxidases like, bilirubin oxidase, peroxidases, and high redox potential fungal laccases (~0.58 V vs Ag/AgCl), are highly relevant in literature for playing the role of metal catalysts even more efficiently, but their role as biocathodes is limited by hydroxyl-ion (and to a low extent, chloride) inhibition (Soukharev et al. 2004; Dominguez-Benetton et al. 2013).

Currently, the potential of enzymes as catalysts in electrosynthesis has also been explored for value-added product synthesis. Addo et al. (2011) studied methanol production from CO_2 with the help of carbonic anhydrase. Other models of redox enzymes with good performance in direct electron transfer to electrodes product recovery potential include carbon monoxide dehydrogenase (CODH) for CO and CO_2 interconversion, fumarate reductase for fumarate and succinate interconversion, and ethanol dehydrogenase for ethanol evolution (Reeve et al. 2017).

Although made very attractive by their regio- and stereoselectivity, advancements and widespread application of these enzyme catalysts are hampered by the expensive recycling of their reduced cofactors. In this context, we draw attention to the potential of NiFe hydrogenases in enzymatic electrosynthesis, seeing as their pass on chain of iron–sulfur clusters allows for rapid electron transference between the electrode and the catalytic site and the ability to carry out the NAD^+/NADH reaction with non-detectable overpotential. At more negative potentials, the enzyme accepts electrons from the electrode to produce H_2 and negative current from a reduction reaction with the dissolved protons, while at more positive potentials, the enzyme reverses itself to strip electrons from H_2 and generate protons and a positive current at the electrode. This is mostly important from a catalytic viewpoint because electrocatalytic NADH oxidation or NAD^+ reduction is difficult to achieve with standard electrodes without huge overpotential supplies (Reeve et al. 2017).

11.4 Microbial External Electron Transfer Mechanisms in Biocathodes

The characteristics that are generally required for a microorganism to be an electrogen are common to anode microorganisms in MFCs in the energy generation mode and to cathodes as energy acceptors with reduced product. Biocathode investigations have shown that bioelectrodes electrochemically "trained" to act as bioanodes may be converted into biocathodes by changing BES operating conditions (Rozendal et al. 2008). Electrogens are normally good biofilm builders and bear an enzyme complex with redox characteristics, e.g., cytochrome c, Fe-S proteins, and ferredoxins, in the outer membrane. They can also produce exogenous mediators. Even if the microorganism does have these characteristics, it alone may not be an electrogen as it might need another microorganism as electron acceptor.

The key to achieving high efficiency for enzyme- as well as microbe-catalyzed cells is related to the efficiency of the electron transference from the catalyst to the electrode surface or in the opposite direction. The means through which microorganisms deliver electrons to the anode is much better elucidated as compared to the cathode. Until now, it is known that the electron-accepting mechanisms can constitute, but not necessarily be, the inverse way through which the cell delivers electrons to the electrode (Rosenbaum et al. 2011). The ability to use electrons from electrodes is related to transfer of electrons between insoluble substances and microorganisms, a process known as extracellular electron transfer (EET). EET at electrodes allows interfacing of microbial metabolisms with an external circuit. Consequently, these microbial electrocatalysts can facilitate chemical reactions that cannot be achieved by traditional electrocatalysis. The EET mechanisms in cathodes are supposed to resemble the procedures at the bioanode, but the constituents operate at changed redox potentials (Rosenbaum et al. 2011). EET approaches can be direct (DET) or mediated electron transfer (MET) (Fig. 11.3).

Direct electron transfer is accomplished by direct contact between the microbes and the solid-state electron acceptor, be them the electrode or other cells. The main conductive structures are probably bacterial nanowire and naturally occurring outer membrane c-type cytochromes (Shi et al. 2009). In contrast, MET uses redox-active compounds to transport electrons between the electrode and the microorganism. Microbial cells can secrete electron shuttles, or they can be added exogenously (Wu et al. 2013). Microbial consortium primary metabolites or other intermediates can achieve EET. Among these primary metabolites/intermediates, H_2 and formate stand out as important for their role mediating syntrophy between electron-donating and electron-accepting species and have thus been considered diffusible electron shuttles (Baek et al. 2018). The comprehension of these electron transfer

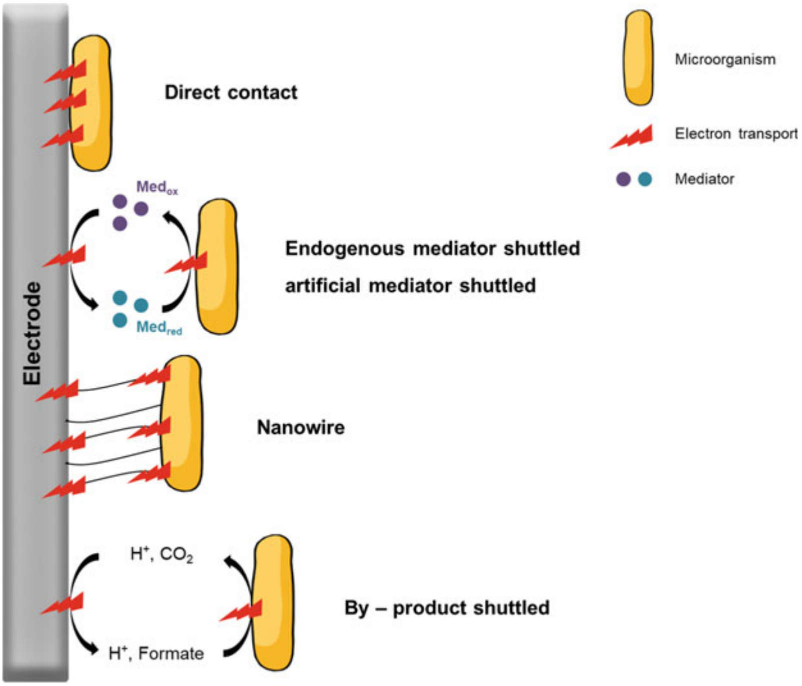


Fig. 11.3 Main mechanisms of EET performed by microbes in electrodes (modified from Choi and Sang 2016)

mechanisms (Fig. 11.3) is important for their optimization on a biological and electrochemical level and better direct microbe-electrode based systems.

11.4.1 Direct Electron Transfer

The DET is greatly reliant on membrane-bound redox proteins, such as cytochromes, to connect the internal metabolism with extracellular ion transport. Most studies have reported a correlation between the capacity of DET and c-type cytochrome-containing microorganisms. These cytochromes generally require direct contact between the cell membrane and electron acceptors, such as electrodes. However, given the variety of oxidoreductases in nature, it is expected that other membrane redox proteins besides the c-type cytochromes can also contribute to electron uptake from a cathode (Rosenbaum et al. 2011). Choi and Sang (2016) suggested that redox proteins such as hydrogenase, ferredoxin, rubredoxin, and Rnf complexes participate in EET. These proteins are mostly metalloproteins and can assume different redox potentials (Fig. 11.4), which are supposed to be part of an electron wiring.

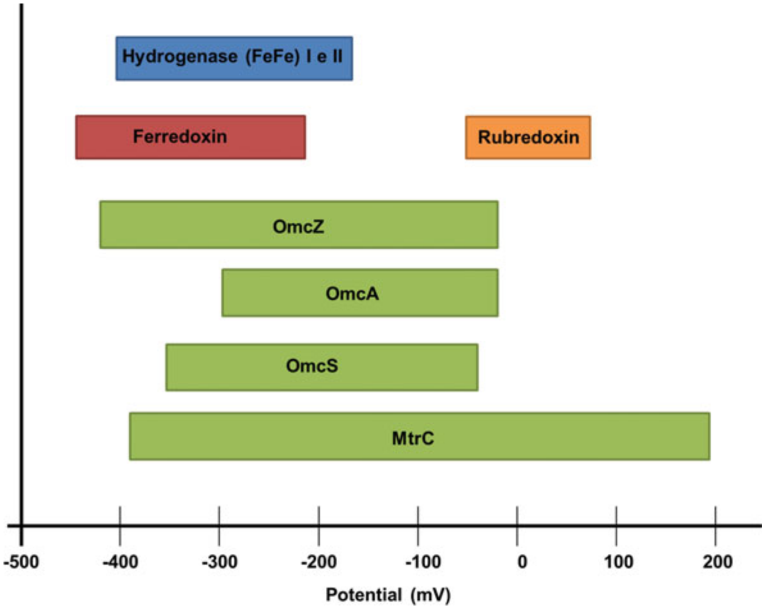


Fig. 11.4 Redox potential range of some of the proteins and other biomolecules taking part in direct electron transfer. Cytochrome c-types MtrC and OmcA for EET in *Shewanella oneidensis* and OmcZ and OmcS for EET in *Geobacter sulfurreducens* (Choi and Sang 2016). FeFe-hydrogenase of *Clostridium pasteurianum* (Peters et al. 2015); ferredoxin and rubredoxin (Hosseinzadeh and Lu 2017) participate in electron wiring

The comprehension of these phenomena can be greatly explored through natural nutrient cycling mechanisms, such as dissimilatory metal reduction (DMR) in sediment inhabiting cells such as *Shewanella* and *Geobacter*. This generally couples organic matter oxidation with reduction of insoluble metals in the lack of other electron acceptors, associated with energy conservation (as opposed to other reductive equivalent neutralization strategies such as fermentation). Insoluble oxides, especially Fe(III) and Mn(IV) oxides, are non-soluble in water at pH near neutrality, so bacteria inhabiting sediments rich in these can perform DMR to sink electrons from the bacterial membrane directly to the surface of external insoluble metal oxides (Shi et al. 2009; Lovley 2012). Microorganisms that carry out DMR are considered potential exoelectrogens (Lovley 2012).

Geobacter and *Shewanella* are also recognized for another mechanism for electron transfer through distances larger than that allowed by direct electron transfer, but shorter than as to require mediated electron transfer. Conductive pili or pilus-like structures, usually referred to as nanowires act as alternative electron ways extending the direct EET space and maximizing the electron transport effectiveness (Fig. 11.5a) (Kumar et al. 2018). *Geobacter* is known for high transfer efficiencies through its nanowires to solid electron acceptors such as Fe (III) oxides or such as electrodes (Lovley 2012). While *Geobacter sulfurreducens* does this through

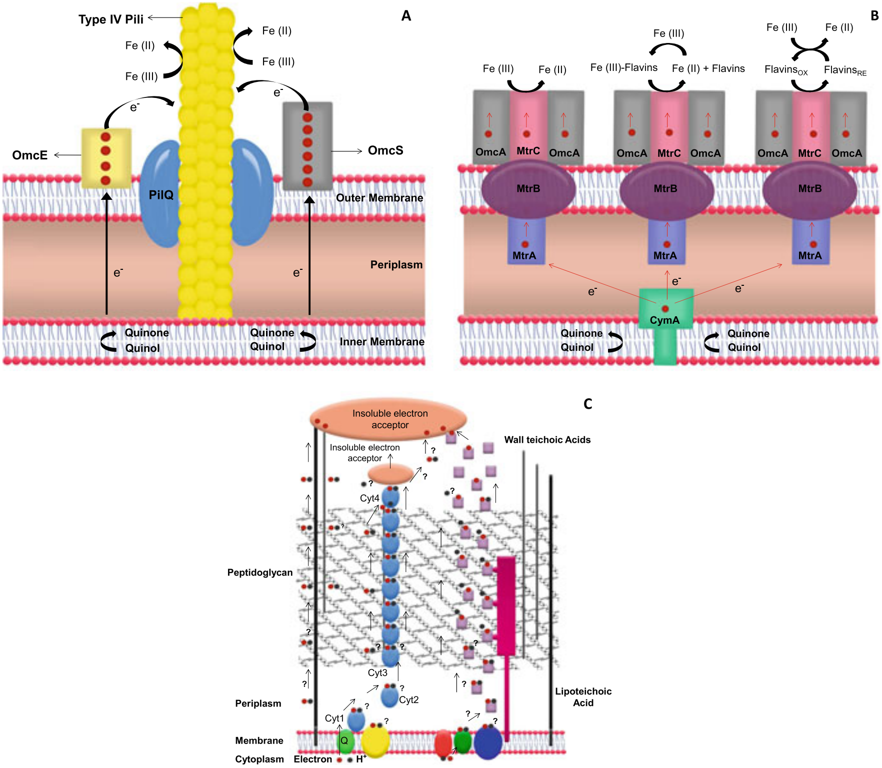


Fig. 11.5 Proposed extracellular electron transfer mechanisms for Gram-negative (**a** and **b**) and Gram-positive (**c**) bacteria. (**a**) represents the proposed PilA pathway in *G. sulfurreducens* using outer membrane cytochrome c-type proteins OmcE and OmcS and conductive nanowires to perform direct long-range EET (Lovley 2012). (**b**) represents the cytochrome c-type Mtr and OmcA pathway(s) for EET in *S. oneidensis* using a redox active protein series capable of either binding directly to solid electron acceptor or reducing extracellular electron shuttles such as flavins (Shi et al. 2009). (**c**) represents the proposed *T. potens* MHC pathway, based on direct contact to an extracellular electron acceptor through multiheme cytochromes (Cyts) anchored in the peptidoglycan layer or linked to teichoic acids. The exact path of protons and electrons is still for the most part unclear (Carlson et al. 2012; Lusk 2019)

pilin-protein-rich pili connected to the outer membrane cytochromes OmcE and OmcS, *Shewanella oneidensis* MR-1's nanowires are not true pili and are rather evaginations of its outer membrane, rich in decaheme cytochromes MtrC and OmcA (Fig. 11.5b) (Shi et al. 2009). These c-cytochromes mediate electron transference from the quinone/quinol pool in the bacterial inner membrane to the outer membrane where they can find a new electron acceptor through direct EET, indirect, or both (Fig. 11.5a, b) (Shi et al. 2009; Rosenbaum et al. 2011).

Most c-type cytochromes proteins, including MtrC from *S. oneidensis*, exhibit wide operating potential ranges (from 200 to 400 mV—Fig. 11.4), which thermodynamically enables direct electron transfer not only to Fe (III) oxides, but also to more accessible and diffusible extracellular substances with different redox potentials, like flavins (Shi et al. 2009). The spectrum of c-type cytochromes is most likely chosen as the terminal electron acceptor availability and potential, as *G. sulfurreducens* seems to adjust its redox activity to the available electron acceptor potential (Rosenbaum et al. 2011). Indeed, cytochromes have very flexible redox potential varying from −50 mV to +0.560 V. Purified Cyc2 has the highest redox potential for a c-type cytochrome, i.e., +0.560 V, which explains its importance in mechanisms of electron transfer to O_2 (Yarzábal et al. 2002).

The cytochromes link the extracellular electron source to more electro-positive electron acceptors within the periplasm and inner membrane, for the electrode to function as a cathode. During this process, energy is conserved, but occurrence without energy conservation is also possible if the potential difference between electron donor and acceptor is not enough for ATP synthesis (Rosenbaum et al. 2011).

Although not so well known as for Gram-negative bacteria, multiheme c-type cytochromes also have a crucial role in insoluble metal reduction of Gram-positive bacteria. The occurrence of a thick peptidoglycan layer makes many metal reducing Gram-positive bacteria conduct EET via proteins that are packed into the cell wall and attached to the peptidoglycan, or situated along teichoic acids, involved as metal binding sites for the cells as represented in Fig. 11.5c (Ehrlich 2008; Carlson et al. 2012). While genetic studies of electron transfer mechanisms have traced in Gram-positive thermophilic bacteria to very ancient ancestral origins within bacterial evolution, EET in Gram-negative mesophilic bacteria seems to be evolutionarily distinct from that occurring in Gram-positive bacteria, having appeared relatively recently (Lusk 2019). *Thermincola potens* strain JR, a thermophilic Gram-positive bacterium with unusually high richness of multiheme c-type cytochromes, combines acetate oxidation to Fe(III)oxide or anthraquinone-2,6-disulfonate (AQDS) reduction, but cannot reduce soluble iron forms such as ferric citrate or ferric nitrilotriacetic acid. This distinction between obligate use of insoluble iron instead of soluble forms as an electron acceptor is still a great driver of mystery (Carlson et al. 2012).

Since natural electron uptake involves c-type cytochromes so much, these are also likely to play an essential role in external electron transfer in biocathodes.

Besides cytochromes, periplasmic metalloproteins play an important role in bacterial electron transfer. The combination of periplasmic enzymes and c-type cytochrome likely provides the electrical wiring that is necessary for microorganisms to accept electrons. Several membrane-bound metalloproteins such as hydrogenase (Lubitz et al. 2014—Fig. 11.3), formate dehydrogenase (da Silva et al. 2012), and Rnf complexes (a membrane-bound NADH: ferredoxin oxidoreductase) lead to bioelectrochemical reduction.

Indeed, various hydrogenase-containing microorganisms can apparently use electrons from polarized electrodes to catalyze the reduction of their natural electron acceptors or to produce H_2. For the *Desulfovibrio* genus, c-type cytochromes seem to be essential for electron transference by involving hydrogenases (Rosenbaum et al. 2011).

Three major categories of hydrogenase capable of catalyzing reversible H_2 oxidation to protons and electrons exist in microbes. These categories are distinct by their active site metal content: [NiFe]-, [FeFe], and [Fe]-hydrogenases covering a broad spectrum of redox potentials (Fig. 11.4). Some bacteria, especially Firmicutes and sulfate-reducing bacteria, can possess both [NiFe]- and [FeFe]-hydrogenases, and [NiFe]-hydrogenases have been associated with earliest metabolisms employing H_2 as the elemental reducing source on Earth (Wu et al. 2013). Some hydrogenase-containing microorganisms depend on the presence of another membrane-bound complex, the Ferredoxin (Fd):Rnf complex (a membrane-bound NADH:ferredoxin oxidoreductase). The complex Rnf is a respiratory enzyme catalyzing ferredoxin oxidation by reducing NAD+; the change in the negative free energy of this reaction is used to create an ion transmembrane gradient that contributes to ATP synthesis (energy conservation) (Westphal et al. 2018). Thanks to coupling of their energetic properties through electron bifurcation, the accumulation of reduced Fd and NADH promotes efficient H_2 production. Besides substrate phosphorylation and electron transport phosphorylation, this third mechanism of energy preservation uses exergonic proton reduction by Fd-derived electrons to allow endergonic proton reduction by electrons from NADH (Peters et al. 2015). Rnf complexes such as these occur in acetogens like *Clostridium ljungdahlii* and *Acetobacterium woodii* and are key to CO_2 capture by these microorganisms (Westphal et al. 2018).

In these microorganisms, Fd functions as electron shuttle and harbors iron–sulfur clusters that participate in oxido-reductive pathways. However, under stress conditions in cells grown in low-iron medium, small proteins containing a flavin mononucleotide (FMN) as redox active component called flavodoxins can replace Fd, directing to $NADP^+$ and N_2 reduction (Ludwig et al. 1997).

Rubredoxin (Rub) is another electron transfer protein, whose a Fe-S cluster makes it the electron transfer assets of sulfate-reducing bacteria (SRB). Rub has been found in *Clostridium pasteurianum* and *Clostridium fornicoaceticum* and is also able to substitute Fd in a few oxidation–reduction reactions. The reactions usually occur at a very low rate in this case because the Rub redox potential is approximately 400 mV (Fig. 11.4) higher than the Fd redox potential. Rub is a superior electron acceptor in the CO dehydrogenase reaction in acetogenic bacteria (Ragsdale and Ljungdahl 1984).

A Mo-containing formate dehydrogenase can also accomplish direct electron transfer from cathodes. This happens via formate and CO_2 cycling by a moiety in a reaction that holds promise for CO_2 fixation and consequent generation of fuels or useful chemical building blocks (Reeve et al. 2017). Sulfate-reducing organisms such as *Desulfovibrio* spp. are described by periplasmic hydrogenases and formate dehydrogenases (FDHs). Contrary to most bacterial enzymes, they transfer electrons to soluble cytochromes c instead of reducing the quinone pool directly (da Silva et al. 2012). The electrical wiring is probably provided, in this case, by the combination of periplasmic enzymes (e.g., hydrogenases and formate dehydrogenases) and a c-type cytochrome (Choi and Sang 2016).

11.4.2 Indirect Electron Transfer

While some bacteria transfer their internal electrons by direct contact, some microorganisms do not need physical connection between the cell membrane and the surface of the electrode for electron transfer to happen. In fact, such microorganisms can self-excrete or take advantage of an externally added redox-active compound for indirect electron transfer (IET) (Fig. 11.3). In this sense, mediators are self-produced or added chemical compounds with appropriate redox potentials to coordinate electron shuttling between the biocatalyst and the electrode (Wang and Jia 2007). In a microbial consortia MFC bioanode, Rabaey et al. (2004) observed that the community consisted mostly of facultative anaerobic bacteria and *Pseudomonas* species and attributed its electrochemical activity to the excretion redox molecules, such as pyocyanin and phenazine-1-carboxamide by *Pseudomonas aeruginosa* (and sometimes other groups) (Rabaey et al. 2004). In addition to direct electron transfer by cytochrome c, many *Shewanella* species can excrete flavin-derived molecules or menaquinone-related molecules as redox mediators to facilitate exocellular electron transfer (Rosenbaum et al. 2011; Cheng et al. 2019). The *S. oneidensis* flavin-mediated EET is reversible (Fig. 11.5b) and allows the bidirectional EET process to occur. The enzyme-catalyzed reduction of free flavins should be very slow because such a huge energy barrier occurs between the flavin and the OM c-Cyt ($Ep = +50$ mV), and the direct ET process happens through a one-electron reaction of flavins ($Ep = -145$ mV) linked with the flavin-binding site in OM c-Cyt (MtrC protein). An interaction between flavin and MtrC enables a one-electron redox reaction via semiquinone. The flavin Ep shifts positively from -260 to -145 mV, resulting in a much faster electron transfer rate as compared to the two-electron EET process (Okamoto et al. 2013).

External dosing of artificial mediators is required in some situations because numerous microorganisms cannot produce redox mediators (Cheng et al. 2019). Although low-molecular-weight redox species can help shuttle electrons, it is important to keep in mind a couple of important requirements to afford effective electron transference from/to the bacterium to an electrode: (a) The mediator should easily enter through the microorganism membrane, to achieve the goal inside the bacterium. (b) The potential of the mediator should lie within the range of the anode potentials. The use of molecules as mediator will enable and hasten internal electron transference. (c) The mediator oxidation states should not interfere in other metabolic processes; that is, other metabolic processes should not be inhibited by none mediator oxidation states. (d) The mediator should be able to leave cell through the bacterial membrane easily. (e) The mediator should have chemically stable oxidation states and be soluble in the electrolyte solution while not adsorbing onto the bacterial cells. (f) The electrochemical kinetics of the redox process at the electrode should be fast and reversible (Wang and Jia 2007). Methyl viologen-mediated electron transfer from cathodes to hydrogenase during H_2 production has been suggested as electron transfer mode.

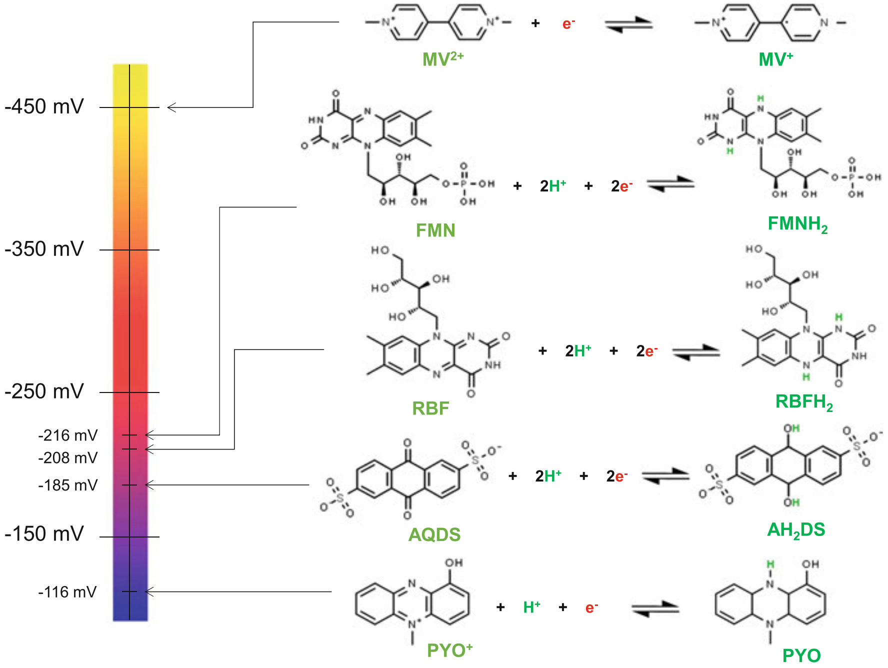


Fig. 11.6 Redox potential of some self-produced or external mediators in indirect EET: methyl viologen (MV), flavin mononucleotide (FMN), riboflavin (RBF), anthraquinone-2,6-disulphonate (AQDS), pyocyanin or 1-hydroxy-5-methylphenazine (PYO) (Choi and Sang 2016; Alatraktchi et al. 2016; Liu et al. 2018)

The effectiveness of the mediation of electron transport from the internal bacterial byproducts to MFC anodes has been assayed with the combination of microorganism to many different organic and organometallic compounds. For example, 2,6-anthraquinone disulfonate or methyl viologen (MV) addition is a prerequisite for electron shuttle from a BES cathode to the microorganism for reductive dichlorination and perchlorate reduction, respectively, and phenoxazine, phenothiazine, phenazine, indophenol, bipyridinium derivatives, thionine, and 2-hydroxy-1,4-naphthoquinone can efficiently maintain high cell voltage output when current is drawn from the biofuel cell (Delaney et al. 1984). However, some dyes are not as effective mediators because microorganisms cannot reduce them rapidly, or they lack sufficient negative potential (Fig. 11.6).

11.4.3 Interspecies Electron Transfer (IET)

Interspecies electron transfer (IET) is essential for diverse anaerobic microbial communities to function properly. In anodic MFCs, electroactive bacteria that can perform anodic respiration can be associated with fermentative partners in anodic biofilms, so that the substrate can be converted into metabolites that are usable by the electroactive bacteria. The fermentative bacterium role might be to provide substrate to the electroactive bacterium, which in turn makes the fermentation thermodynamically more advantageous by eliminating its byproducts (Moscoviz et al. 2016). IET networks are essential constituents for fermenter–exoelectrogen interactions and can be either indirect and dependent on diffusible electron carriers like hydrogen gas, formate, or other metabolites or direct and involve the use of conductive pili, membrane-to-membrane contacts, or conductive support surfaces for biofilm growth (Baek et al. 2018).

The best studied approach for electron exchange consists of interspecies H_2 transfer. Here, while the electron-donating partner produces H_2 from proton-reduction, the electron-accepting partner oxidizes it to reduce another electron acceptor. Similarly, formate can also assist as the electron carrier instead of H_2 in formate IET, and organic compounds bearing quinone moieties and sulfur compounds can serve as interspecies electron shuttles in laboratory cultures (Smith et al. 2015).

Recently, a polarity reversion process promoted bidirectional IET between *G. sulfurreducens* and a pure *Thiobacillus denitrificans* culture, taking advantage of bacteria to act as “electron bridges” to deliver electrons to and from denitrifiers, thanks to their bidirectional electron exchange capacity (Liang et al. 2019).

Since it is so greatly dependent on structures or compounds electrically connecting metabolisms of separate bacteria, biofilms that favor contact and interaction between microorganisms promote IET and can be a useful tool for designing and controlling these interfaces. Being a still young research field, the relative importance of each transfer mechanism is still not fully established, but it seems that circumstances can favor one over others. Moreover, these biofilms present typically stratified structural organizations, where electrogens are abundant close to the electrode surface, and fermenters dominate the upper part of the biofilm. Thus, biofilm thickness can be an important factor acting these interactions—the thicker the biofilm, the lower the diffusivity in the biofilm, which results in gradient conditions within the biofilm and limits IET (Moscoviz et al. 2016).

11.5 Microorganisms as Biocathode Catalysts

During EF, anaerobic cathodes allow the use of different electron acceptors, such as CO_2 and organic molecules, which can create potential opportunities for microbe-catalyzed electric current conversion into various value-added products (Huang et al. 2011). This can be accomplished by employing pure or mixed consortia. While pure cultures present the advantage of better predictability and control, they are less resilient and resistant to environmental variations in substrate composition and more susceptible to contamination.

11.5.1 Pure Cultures

The first report on cathodic electrosynthesis approaches involving microorganisms was published by Hongo and Iwahara (1979) where they described the increase in glutamic acid production during glucose fermentation, thanks to cathodic current supply to *Corynebacterium glutamicum* (formerly *Brevibacterium flavum* 2247) through different electron carriers or mediators. Currently, several groups have been working on microbial electrosynthesis from CO_2 in consortia as well as pure cultures, producing mainly acetate (May et al. 2016), ethanol (Ammam et al. 2016), methane (Jiang et al. 2013; Villano et al. 2010), or even higher alcohols and carboxylic acids (Liu et al. 2018).

Numerous acetogenic microorganisms including *Serratia Marcescens* (Nevin et al. 2010) and *Pseudomonas delhiensis* (Hou et al. 2019) carry out acetate electrosynthesis from CO_2 by maintaining a graphite cathode at −400 mV vs SHE. Changing the electrode material and/or lowering the cathode potential can influence the production rate (Rojas M del et al. 2018). Because they use carbon dioxide as final electron acceptor, acetogens seem to be the preferred biocatalysts for microbial electrosynthesis, as well as gas fermentation (Aryal et al. 2017). Most works have also employed *Sporomusa* sp. (Aryal et al. 2017), *Acetoanaerobium* spp. (Xafenias and Mapelli 2014), *Acetobacterium woodii* (Straub et al. 2014), *Clostridium ljungdahlii*, *Clostridium aceticum*, or *Moorella thermoacetica* (Nevin et al. 2011) for acetate production.

In another study, Hou et al. (2019) demonstrated sustainable microbial acetate electrosynthesis from bicarbonate and consequent H_2 evolution and Cd (II) elimination in multifunctional microbial electrolysis with electrochemically active bacteria. The authors conducted studies with *Ochrobactrum* sp., *Pseudomonas* sp., *Pseudomonas delhiensis*, or *Ochrobactrum anthropic* and applied a constant cathodic potential of −700 mV to the reactors. On the basis of the data, *P. delhiensis* favors acetate production, whereas *O. anthropi* favors H_2 production. The Cd (II) elimination rate by all EAB (1.20–1.32 mg/L/h), as well as acetate production rate by *P. delhiensis* (29.4 mg/L/day), and H_2 evolution by *O. anthropi* (0.0187 m^3/m^3/day) rise in the existence of a circuital current. Such known-how permits directed manipulation of the microbial electrolysis conditions, to help acetate production from carbon dioxide with simultaneous H_2 generation and Cd(II) removal from metal-polluted waters. Nowadays, biofuels or biogases are the main bioenergy power generation alternative to the conventionally used power source (fossil fuel), and the use of MFCs is useful for sustainable bioenergy synthesis via, for example, sugars lignocellullosic biomass and CO_2 EF (Srivastava 2019). Therefore, MEC devices employ the microorganism electrocatalytic activity to supplement energy for electrolysis, thereby producing H_2 and methane (Rathinam et al. 2019).

Methanogenesis is the main industrial process involving methanogens that is the only group known to produce methane as their major metabolism from a limited source of substrates like H_2 + CO_2, formate, methanol, and acetate (Beese-Vasbender et al. 2015). Mayer et al. (2019) tested different archaea strains and quantitatively compared the final methane concentration and yield in terms of CO_2 conversion, productivity, and Coulombic efficiency in order to identify suitable organisms for efficient microbial electrolysis. They found that *Methanococcus vannielii*, *Methanococcus maripaludis*, *Methanolacinia petrolearia*, *Methanobacterium congolense*, and *Methanoculleus submarinus* produce methane via microbial electrosynthesis at −700 mV vs SHE. In addition to methane production, the authors detected biological H_2 production during the EF process, probably because hydrogenases are involved. On the basis of the study data, *M. maripaludis* is the most effective methane producer in microbial electrosynthesis concerning methane productivity (8.81 ± 0.51 mmol m^{-2} day^{-1}) and Coulomb efficiency ($58.9 \pm 0.8\%$) and most likely employs a direct electron transfer pathway.

Ammam et al. (2016) optimized in *Sporomusa ovata* growth medium composition to improve MES and gas fermentation. The authors verified that supplementing tungstate increases ethanol production by *S. ovata* by 2.9-fold during H_2:CO_2-dependent growth, improves acetate production by 4.4-fold in a *S. ovate*-driven MES reactor, and enhances propionate and butyrate conversion to their corresponding alcohols, 1-propanol and 1-butanol, respectively, during gas fermentation. Gene expression analysis suggested that the acetate, ethanol, 1-propanol, and 1-butanol biosynthesis were improved, thanks to tungsten-containing aldehyde ferredoxin oxidoreductases (AORs) and a tungsten-containing formate dehydrogenase (FDH), which, respectively, contribute to the organic acid re-assimilation and to the Wood–Ljungdahl pathways.

In another study, Liu et al. (2018) investigated the acetogenic bacterium *Clostridium scatologenes*, which, during H_2 fermentation, may sequester CO_2 as acetate, butyrate, and ethanol. At a potential of −0.6 V, the authors obtained the highest acetate and butyrate production in the cathode (0.03 and 0.01 g/L, respectively), as well as maximum total Coulomb efficiency of 84%. An increase in the system redox potential to −1.2 V produced ethanol.

Microbial electrosynthesis can also generate acetate, H_2, and methane as well as multi-carbon products including butyric acid. Butyrate is a valuable substance with application in numerous fields such as the food and feed additive, pharmaceutical, cosmetic, and textile industries; its annual global market is currently greater than 80,000 tons (Wang et al. 2015). *Clostridium tyrobutyricum* can simultaneously metabolize sugar and glycerol in the presence of acetic acid; under these conditions, butyric acid biosynthesis can occur. Butyric acid production via microbial electrosynthesis using sugar, glycerol, and acetate affords useful understandings into the development of an effective and economical butyric acid production process based on residual renewable resources (Lee et al. 2015). For example, cathodes can increase butanol production by *C. pasteurianum* when solventogenesis is favored. Mostafazadeh et al. (2016) used MEC with *C. pasteurianum* for butanol generation. By varying four main factors, electrode material, glucose (substrate) concentration, temperature, and voltage, the authors achieved the optimum condition for the highest butanol production by *C. pasteurianum* via MES using a cathode as electron donor. The use of optimum conditions maximized the global production during three days of operation. More specifically, by optimizing the substrate amount in the fermentation broth and using graphite felt as electrode, the authors obtained 13.31 g/L butanol at 33.51 °C, 1.32 V, pH 6.7, and 150 rpm. Table 11.1 shows recent works on MES and the respective products.

11.5.2 Microbial Communities

EF can potentially minimize discrepancies between pure and mixed culture productions and aid understanding of more complex, mixed cultures, which is fundamental to formulate the base behind this technology.

Mixed cultures, co-cultures, and synthetic consortia present advantages: one microorganism may produce or remove compounds, such as growth factors and inhibitors, or create environmental conditions that are advantageous to another microorganism and which allow for more complex reactions that would be impossible to occur if just one microorganism was present in the cell. Conversely, disadvantages exist, including the difficulty in controlling constancy, byproduct formation, and metabolic selectivity. Here, EF represents a tool to drive microbiota metabolism to control product formation and to boost metabolic routes, especially when the substrate is a waste stream and the purpose is to refine it (Thrash and Coates 2008).

For microorganisms that employ carbohydrate as carbon as well energy source to produce more reduced compounds, one part of the carbohydrate always has to be oxidized to CO_2, for example, to deliver the necessary redox power. Consequently, a significant part of the substrate is missing, which diminishes the final carbon yield. When provided with additional electron donors such as an electron stream that replaces the carbon as energy source, the amount of oxidized sugar as an energy source diminishes, decreasing the amount of side products and raising both purity and productivity (Harnisch et al. 2015). Similarly, microorganisms that employ organic molecules as final electron acceptors, as seen in traditional fermentation, form fewer byproducts.

Metabolic Steering

By supplying surplus energy, biocathodes influence various metabolic reactions inside the cells that could otherwise be limited by energy availability and hence affect yields. Biocathodes also produce otherwise scarce or unavailable compounds. The electricity effect on the resulting products can be direct or indirect and can help to steer metabolisms and generate new products. Moreover, electron supply through a cathode increases the production of reduced compounds (Kracke and Krömer 2014; Steinbusch et al. 2010).

Electricity has long been used to catalyze abiotic reactions, including water electrolysis. The electricity flow in a biocathode means that many of these reactions will keep happening, independent of the microbiota. In other words, abiotic electrochemical reactions occur simultaneously with biocatalysis in these systems, to generate O_2 and H^+ in the anode, and H_2 in the cathode, which impacts the electrode

Table 11.1 Recent works on MES, biocatalysts involved, process conditions, and the respective products

Metabolism type	Microorganism	Substrate	Target product	MES setup and conditions		References
Acetogenic	*Ochrobactrum anthropi*; *Pseudomonas delhiensis* X5	Bicarbonate and CO_2	Acetate	Dual chamber reactors. Cylindrical chambers separated by cation-exchange membranes	Three-dimensional graphite felts act as cathodes and carbon rods for the anode. Cathodic potential maintained at −70 mV	Hou et al. (2019)
Acetogenic	*Sporomusa ovata*	Acetate	Ethanol	Dual chamber H-type MES reactor operating at 25 °C. Three electrode system with *S. ovata* in the cathodic chamber	Graphite rods used for both anode and cathode. Cathode potential set at −690 mV. The graphite cathode was constantly set to 0.4 V vs SHE	Ammam et al. (2016)
Methanogenic	*Methanobacterium maripaludis*	N_2-CO_2 gas	Methane	Bioelectrochemical cell with a three-electrode setup	Graphite rod working electrode and three-electrode setup (graphite rod length = 100 mm, diameter = 5 mm, immersed surface area = 1 cm^2). The applied potential was −700 mV vs. SHE	Beese-Vasbender et al. (2015)
Methanogenic	*Methanococcus vannielii*, *Methanococcus maripaludis*, *Methanolacinia petrolearia* *Methanobacterium*	N_2-CO_2 gas	Methane, H_2	Dual chamber H-separated by a Nafion 117 proton exchange membrane	Graphite rods used for both anode and cathode The applied potential was −700 mV vs. SHE	Mayer et al. (2019)

(continued)

Table 11.1 (continued)

Metabolism type	Microorganism	Substrate	Target product	MES setup and conditions		References
	congolense and *Methanoculleus submarinus*					
Acidogenic and solventogenic	*Clostridium beijerinckii*; *Clostridium tyrobutyricum*	Lignocellulosic biomass	Butanol and butyric acid	Single-chamber glass reactor (500 mL)	Graphite carbon felt used for both working and counter electrode Reference electrode is Ag/AgCl. Oxidation potential was constant at +1.15 V vs. Ag/AgCl	Lee et al. (2015)
Acetogenic	*Clostridium scatologenes*	Acetate	Acetic acid, butyric acid, and ethanol	Glass MES reactor (280 mL) in both anode and cathode chamber, separated by a proton exchange membrane	Carbon felt in the anode. The cathode is poised with potentiostat settings of −0.6, −0.8, −1.05, and −1.2 V	Liu et al. (2018)
Solventogenic	*Clostridium pasteurianum*	Glucose	Butanol	Dual chamber H-type reactor (300-mL each) separated with a Nafion 117 cation-exchange membrane	Graphite felt for both electrodes (precursor material = polyacrylonitrile fiber (PAN)); individual fiber; applied potential = −500 mV vs. SHE	Mostafazadeh et al. (2016)

vicinity pH (a very important factor affecting cell growth) in a non-buffered system. At ca. 2 V (vs SHE), reductive water electrolysis in the cathode is possible, so that OH^- ions are also added besides H_2. Microorganisms make use of H_2 and O_2 as electron donor and acceptor, respectively, which can be supplied via electrolysis. Actually, some of the first reports on the application of electricity in reactors include Sadoff et al. (1956), who applied current to provide O_2 to the aerobic *Pseudomonas fluorescens* via water electrolysis. Likewise, electrolysis can supply H_2 to microorganisms that cannot assimilate electric current directly, but which can use H_2 as an electron giver, to simulate denitrification, for instance. Liu et al. (2018) used the abiotic electrochemical reaction that fixes CO_2 as formate (which is a valuable intermediate substrate that degrades easily), which *Ralstonia eutropha* rapidly assimilates and then metabolizes to higher alcohols for biofuel production. Additionally, the cathode can abiotically participate in simply reducing electron shuttles (Thrash and Coates 2008). H_2 generation at lower cathode potentials is being contemplated as a strategy to enhance production rates with strains that are not naturally efficient in taking up electrons from cathode, e.g., *Clostridium ljungdahlii*. H_2 then can mediate electron transference between the electrode and the bacteria. This strategy would let the utilization of the whole reactor volume for the bioproduction: as an alternative of being restricted to the two-dimensional electrode, the suspended bacterial cells would have full access to dissolved H_2, so they do not require biofilm formation (Rosenbaum and Henrich 2014).

However, owing to thermodynamic limitations, this process is best employed when the abiotic electrocatalysis product is needed at lower concentrations or extreme purity, or when the product does not require more electricity than the amount that was previously calculated for the microorganism. Furthermore, an economical evaluation must be made for each case.

Conversely, if uncontrolled, these reactions can be detrimental to the microbiota because O_2 is also able to react in the cathode, to produce H_2O_2 at low pH in reactors biocatalyzing acidophilic iron oxidation, for instance. Cl^- ions (which are abundant in seawater) are able to be oxidized at the anode, to form Cl_2, which easily reacts with water by forming hypochlorous acid (HOCl), a toxic substance. The rate at which each reaction occurs depends on the system conditions and must be carefully considered (Thrash and Coates 2008).

The simple existence of free electron flow also shifts metabolism, and controlling the electron and carbon flow can help to determine the final product distribution (Choi and Sang 2016). Because each potential in each medium influences microorganisms in different manners, these metabolic shifts can be modeled and reconstructed to predict opportunities for product stimulation in bioelectrochemical systems (Marshall et al. 2017; Pandit and Mahadevan 2011).

Keeping in mind that nature uses mixed cultures for internal nutrient cycling, the products and substrates of different metabolisms are very closely related, so that accumulation of a specific product implies in eliminating the consumers of the target compound through enrichment. Establishing a universal core community is hard because it varies considerably depending on the initial substrate and on the intended final product.

Biomass Yield

Anaerobiosis limits the amount of reactions that can yield ATP to four main ones: those catalyzed by acetate kinase, pyruvate kinase, phosphoglycerate kinase, and ATP synthase. ATP formation relies on the generated proton gradient, which means that direct electron influx from a cathode to the cytoplasm also brings protons back into the cytoplasm to maintain charge neutrality. By counterbalancing proton accumulation in the periplasm and redirecting protons to the cytoplasm, the chemical balance shifts, to facilitate ATP formation. For each four moles of protons transferred into the cell, ATP synthase delivers one mole of ATP. Consequently, electron supplement can promptly improve ATP production by reversing ATP synthase from ATP utilization (proton efflux) to ATP formation (proton influx). This leads to greater ATP generation inside the cells, so that the same amount of electrons from a mole of sugar provided for energy use through an electrode can generate a larger quantity of ATP. This will translate into more energy for other cellular processes (Pandit and Mahadevan 2011).

EF has been suggested to increase biomass yields because both ATP generation and reductive power (which hangs on electrode current supply and reduction degree of the substrate) augment. Therefore, one can deduce that rises in cell mass are greater for oxidized substrates than for more reduced ones. This has been shown in silica (Pandit and Mahadevan 2011) and experimentally demonstrated by Park and Zeikus (1999), who discovered that electrical improvement with *A. succinogenes* can lead proton translocation and boost ATP synthesis, besides to consuming additional glucose and forming more succinate. This is interesting because it allows the use of strains that have been rejected from traditional fermentations owing to their minimal growth rates.

In contrast to the lower value of most oxidation products, several high-value chemicals can originate from the reduction of cheaper chemicals with smaller molecules during EF targeting mainly cathodic reactions. Either a power grid or an anode chamber (in a double chamber MFC) can provide the necessary energy to sustain these cathodic reactions; the latter is more feasible. These anodic reactions must be taken into account to balance out the anodic and cathodic chambers. These reactions may be conducted by bioelectrocatalytic oxidation of low-cost compounds such as methanol to CO_2.

Hydrogen-Evolving Biocathode

The simplest biocathode is the H_2-evolving one. Most reports on these biocathodes in a MES reactor have been conducted with microbial consortia (Jafary et al. 2015). H_2 can be obtained in bioelectrochemical systems through both a biocathode that produces H_2 via metabolic reactions (as seen in *Clostridium*) or a bioanode (fed with waste stream, for example) that generates electricity and promotes abiotic water electrolysis at the cathode (in a double chambered MFC acting as MEC). Albeit less productive as of now, the latter process is attractive for H_2 generation because it does not require synthetic substrates and can use wastewater with a known composition, for which some aspects can be controlled through by loading strength, pH, and conductivity.

Given the present shift in the economy and resource availability and preference, many researchers have focused on perfecting H_2 production from sources that are otherwise wasted, especially wastewater. While very high efficiencies can be achieved by using simple carbon sources in a laboratory, wastewater complexity and variability are much harder to work with. However, systems fed with wastewater are still more efficient than systems that do not use EF. Electro H_2-production efficiency can be improved if provided electron recycling, electro CH_4-production, and deposition of non-conductive materials on the cathode surface are efficiently avoided.

In contrast to any other well-known fuel, H_2 has the highest gravimetric density. It is also secure and trustworthy with respect to electrochemical and combustion practices for energy exchange. Compared to conventional H_2 production (mainly via electrolysis or dark fermentation), bioelectrochemical systems require lesser employed voltage (0.2–1.0 V) (Kadier et al. 2015; Kadier et al. 2016) and can stoichiometrically transform substrates into H_2, in contrast to no more than 33% obtained by dark fermentation (Abreu et al. 2016).

Metataxonomic analyses of numerous studies have shown that the production of compounds that frequently act as intermediate substrates in nature and nutrient cycling, like H_2, hardly achieves high efficiencies in undefined mixed cultures because another microbe rapidly consumes the H_2 produced by a microorganism (for instance, in methanogenesis). Even for other metabolic pathways, when the undefined mixed culture allows one group to catalyze an oxidation reaction, if another group performs the reverse reaction (or a reduction of this compound) in the same chamber, the redox balance is thrown off as if some sort of “short circuit” were to happen, and the Coulombic efficiency diminishes as the boundaries between cathodic and anodic reactions become blurred. Please look at examples in Zhan et al. (2014). Hydrogenotrophic methanogens, especially archaea, face a very common obstacle because they consume the produced H_2 to form methane, a less valuable chemical than H_2. Decreasing the environment pH, exposing the biocathode to oxygen, and boiling the bioanodes prior to incorporation into the MECs can prevent methanogens development (Kadier et al. 2015).

Several metabolic pathways and methods can produce H_2, so inoculum can be employed in various enhancement approaches. For instance, an acclimatized microbial community was employed to synthesize H_2 from ammonia, as a carbon-neutral anode substrate; the anode produces current for cathodic water electrolysis (Zhan et al. 2014). The authors observed that both ammonia-oxidizing bacteria, such as *Nitrosomonas europaea* and ammonia-assimilating (*Stenotrophomonas* sp.) bacteria and their syntrophic system lower the hydrogen yield and the Coulombic efficiency by “short-circuiting” the cathodic and anodic reactions, while higher efficiencies may be related to a syntrophic mechanism for the oxidation of ammonia by *Nitrosomonas*, *Comamonas*, and *Paracoccus* (Zhan et al. 2014). Identifying the syntrophic relationships that promote the one-way metabolism of a substrate and the groups that counter this process are important to plan the enrichment process.

Methane-Evolving Biocathode

Although methanogenesis is undesirable in hydrogen evolution biocathodes (because of hydrogenotrophic methanogenesis and the lower caloric power of methane as fuel), this process is very interesting if coupled with CO_2 reduction.

Direct IET is attractive both from the ecological aspect, which improves understanding of the inner workings of the consortia and increases control over existing consortia and design and from the viewpoint of co-culture design, which provides better yields than any pure culture by creating interspecies links that expand metabolic routes toward a desired product. For instance, increased methane production in bioelectrochemical systems has been observed; 50% of methane resulting from an unknown pathway suggests that direct IET between *Geobacter* and *Methanosaeta* is a different way to the statement that H_2 or formate is the primary interspecies electron carrier during organic matter conversion into methane (Zhao et al. 2015). This explains the larger yields verified in many EFs.

DET supports direct CH_4 formation from electrons and protons without electron mediators, while indirect transfer involves H_2 (abiotic or biotically generated) production for methanogenic autotrophs or acetic acid production for acetoclastic methanogens. H_2 is then used to reduce CO_2 to CH_4 via the general hydrogenotrophic pathway (Baek et al. 2018); acetate (which may be added to the medium or can be fixed from CO_2 through homoacetogenesis) is used by acetoclastic methanogens. IET occurs between *Pelotomaculum thermopropionicum* and *Methanothermobacter thermautotrophicus* through flagellum-like appendages in the form of aggregates and can be implemented in both EF and conventional fermentation (Ishii et al. 2005).

Methane production can be more easily achieved through undefined mixed cultures because it can happen through the homoacetoclastic, hydrogenotrophic, or other metabolic pathways and needs no strict enrichment. Methane generation resembles biogas production in biodigestors, as shown in countless examples, like the undefined mixed culture originated from brewery wastewater generating CH_4, acetate, and H_2 (the two latter compounds can be converted into methane) from a biocathode operating at −590 mV (vs SHE) and CO_2 as substrate (Marshall et al. 2012).

Compounds with more than one carbon, such as acetate, have also been synthesized on cathodes by exploiting distinct homoacetogens (Nevin et al. 2010, 2011; Deutzmann et al. 2015). Limited electron transfer from the cathode to the microbes is usually considered to restrict the practicability of this process on a large scale. Likewise, potentials of 4200 mV have to be continually applied for considerable electron transfer rates (Villano et al. 2010). At these potentials, H_2, CO, or formate, as potential electron carriers at the cathode, can be formed and further metabolized by methanogens and homoacetogens (Villano et al. 2010; Deutzmann et al. 2015).

Autotrophic Bioproduction from Syngas

CO_2 serves as a carbon source for autotrophs, but it is already too oxidized to function as an energy source. This means that energy originates from other sources including light, syngas, hydrogen, or electrodes. Carbon fixation has aroused great interest in recent years and is greatly favored by EF—this technique allows for enhanced CO_2 fixation, and less carbon is released as metabolites like formate.

Although the electron transfer mechanism is not currently understood, several homoacetogenic and photosynthetic bacteria have been shown not only to generate sugars such as acetate from CO_2 (electron acceptor and carbon source) and H_2 (electron donor), but also to be capable of substituting H_2 for the cathode as electron donor in a process that mimics natural photosynthesis. These bacteria include *Sporomusa* spp., *Clostridium* spp., and *Moorella* spp. (Nevin et al. 2011).

Carbon fixation is very attractive to generate bioproducts for application as both biofuels and solvents. This process is divided into different platforms. To date, the best understood platforms for bioproduct conversion from common waste are the sugar platform, whereby five- and six-carbon sugars (for example, xylose obtained from lignocellulosic residue through enzymatic digestion) are used as substrate in traditional and alternative fermentations; the syngas platform, whereby thermochemical processes convert biomass into synthesis-gas (AKA syngas, containing H_2, CO_2, and CO), which is then used as feedstock that is further converted to fuels; and the more recent carboxylate platform, whereby organic feedstock often resulting from industrial and agricultural activities is converted to short-chain carbon compounds (such as acetate) as transitional chemicals by hydrolysis followed by fermentation with microbial consortia in engineered systems under anaerobic conditions (Agler et al. 2011).

Carbon fixation through electrosynthesis provides a shortcut where the syngas platform meets the carboxylate platform, allowing pure or mixed cultures in low organic carbon medium to assimilate syngas more easily and to generate. In turn, acetate and other carboxylates act as substrate to generate more complex chemicals through chain elongation or other reactions. The Coulombic efficiency achieved with the syngas platform is quite high (80–90%); i.e., it is more effective than photosynthesis. Moreover, this platform is highly specific to generate the desired products depending on the culture. Even though reduction rates are still comparably slow (Tremblay and Zhang 2015) because the field is new, acetate production is already being perfected and can later be integrated with the carboxylate platform, described in Synthetic Consortia (Tremblay and Zhang 2015; Choi and Sang 2016).

Heterotrophic Bioproduction

Chain elongation can be achieved by various methods that exhaust fermentable substrates and stimulate reverse β oxidation. Examples include propionate (C3) conversion to valerate (C5) in a system fed with glycerol (Dennis et al. 2013); acetate (C2) reduction to caproate (C6) and caprylate (C8) by using a *Clostridium kluyveri*-enriched mixed culture at 0.9 V (vs. SHE cathode potential) with H_2 generated *in situ* (indirect abiotic metabolic steering) (Eerten-Jensen et al. 2013); and acetate (C2) and butyrate (C4) reduction to alcohols (C1–C4), acetone (C2), and caproate (C6) in a SRB containing a mixed culture at 0.85 V (vs. Ag/AgCl) (Sharma et al. 2014). This platform for enhanced production of valuable molecules from carboxylates can be applied in undefined mixed culture (enriched through simple methanogen repression) feeding from syngas or sugar (Agler et al. 2011) or in medium that is already rich in carboxylates (such as landfill leachate containing acetate, propionate, and butyrate as the main substances) (Agler et al. 2011; Lozecznik et al. 2012) to upgrade waste to value-added biofuels; e.g., glycerol conversion into 1,3-propandiol (Sharma et al. 2014; Zhou et al. 2013), acetate conversion into butanol, and glucose conversion into polyhydroxyalkanoates (PHA) (Sharma et al. 2014). Substrates abiotically generated by the cathode like H_2 and formate can also be used for bioproduction through indirect metabolic steering in cultures (described in metabolic steering).

11.5.3 Synthetic Cultures and Consortia

Microbial communities represent the next research frontier, and the combination of systems biology with synthetic biology allows the creation of robust consortia that can execute extremely complex tasks while maintaining the predictability, reproducibility, and control of a pure culture. To engineer microbial consortia, there is great complexity to consider—not only a species–species view is necessary, but also an account of the organism–environment interaction and of ecological and evolutionary feedback dynamics. Thus, simply changing particular species populations in a community (by way of culture enrichment or inhibition) might elicit species-environment feedback that eventually tends to turn the microbial population to its initial state. In this method, the emphasis is on applying precise environmental conditions on the population of microorganisms. These may produce a superior thermodynamic in the system, to which the community composition and function then acclimate. If correctly planned, the resulting consortium may achieve ecological stability, simulating the states of intricate communities. Growth thermodynamics and redox biochemistry can guide the design of the exact nature of the environmental imposed conditions; i.e., the design of the consortium member, whereas electrodes are still used as an energy input or output resource to facilitate reactions with spatiotemporal control even further (Zerfaβ et al. 2018).

Microbial community engineering has been suggested both as a top-down method, to control metabolic developments with a view to stabilizing complex communities, and as a bottom-up method, to create specified, synthetic communities with the required functionality. Consortium design can happen on many different levels of complexity. It can range from simple co-cultures, where a strain is used for simple control like anaerobiosis maintenance (Qu et al. 2012), to complex metabolic coupling of pathways, for layered or segmented bioproduction and environmental regulation. Although EF employing a solid electrode operating at a set potential is promising, electron-independent electroenhancement provides similar effects. Various researchers have created co-cultures consisting of a “producer” microorganism, which forms the product of interest, and a “helper” exoelectrogenic microorganism, which transfers electrons from another substrate directly to the producer (Deutzmann and Spormann 2017; Ishii et al. 2005).

11.5.4 Synthetic Biology Approaches to Biocatalyst Optimization

Bioelectrocatalyst optimization is approached from two different directions: either by optimizing the electron transfer ability in less electroactive microorganisms or by introducing desirable metabolic pathways in already electroactive microorganisms for bioproduction. The first direction can happen through numerous strategies, including expression of electron shuttles that facilitate electron transport (e.g., through the heterologous pyocyanin production) or enhancement of their production pathway in *Pseudomonas* (Wang et al. 2013). The template organism *E. coli* has already been used to express mtrCAB from *S. oneidensis*, which has proven successful. Yet, there is still room for improvement in the coming years given that electron transport is very complex, and that tailoring other proteins less directly, but still intrinsically related to this phenomenon, permits finer tuning (Jensen et al. 2010). Other extracellular electron transport structures have also been heterologously expressed to enhance electron transfer in pure cultures such as *Pseudomonas*. These structures have been contemplated with commercialization as a raw resource for bioelectronics production, which can potentially gain market value and demand through application of cytochromes and nanowires in man-made electronic circuits (Liu et al. 2018). Electron transfer optimization can occur on any level of the five main steps involved in this phenomenon: (1) electron giver oxidation; (2) electron transfer from metabolism to electron transfer devices; (3) through cytochrome complexes; (4) through electron shuttles; and (5) cell attachment to surfaces over conductive biofilms (Teravest and Ajo-franklin 2016). Nevertheless, the novelty of this phenomenon means that this approach to generate strains that are fit for industrial implementation cannot be applied yet, but it is sure to be employed before long.

Currently, *C. ljungdahlii* is being intensively studied and contemplated as a potential host for powerful bioelectrocatalysis by using the syngas platform. One of the primary hurdles faced in the engineering of such autotrophs for bioelectrochemical systems is that the model organisms for extracellular electron transfer are mainly *Geobacter* and *Shewanella*, which are Gram-negative bacteria, while *Clostridium* is a Gram-positive organism. Therefore, the electron transfer mechanisms from one type cannot be successfully implemented in the other due to membrane differences. This has recently been seeing advances, especially thanks to new genetic tools such as CrispR (Teravest and Ajo-franklin 2016).

In contrast to expressing more efficient electron transfer systems in other organisms, expressing heterologous metabolic pathways to generate specific products in already electroactive microorganisms has the advantage that most desirable metabolic pathways are much better understood and have been sequenced, and challenges of this approach are more similar to the challenges of any heterologous expression, like adjusting codon preference, CG content, and chaperone and cofactor existence (Rosenbaum and Henrich 2014; Schuergers et al. 2017).

In addition to engineering electroactive microorganisms for reduction at cathodes, other studies have aimed to alter the redox balance in anodic cultures to produce reductive equivalent excess and induce energy generation instead of consumption while enabling previously unbalanced fermentations. A genetically modified *S. oneidensis* has been employed to express glycerol use and ethanol production genes heterologously while combining fermentation to the current discharge in the anode to eliminate redox constraint (Flynn et al. 2010).

At the same time that engineering microbes that can better interact with the energy supplied in bioelectrochemical systems can contribute to product and biocatalyst spectrum, some of the same obstacles faced by conventional fermentation systems must be tackled in bioelectrocatalysts, including improving resistance to acid to increase final carboxylate production titers. Similarly, because reductive equivalents such as NADH are intrinsic to metabolic function, optimizing the cellular redox balance is an important step in metabolic engineering. This makes various bioprocesses viable and has been shown to improve product yields in traditional fermentation (Fasan et al. 2011). By supplying direct redox power through an electrode, this redox metabolism is also manipulated in a different manner. However, genetic manipulation of reductive equivalent availability is not excluded because genetics can still be tweaked to serve bioelectrocatalysis better. Nowadays, pathway engineering aiming to lower the activation overpotential for desired bioelectrochemical reactions is suggested as a primary task for advancing microbial electrosynthesis to greater feasibility (Rosenbaum and Henrich 2014).

11.6 Conclusions

Electrofermentation (EF) or microbial electrosynthesis (MES) is a modern bioelectrochemical technology that has been proposed to generate chemicals. The external microbial electron transfer to the electrode has been elucidated, and some mechanisms have been proposed along the years. In this way, the use of pure enzymes as catalysts has helped to understand the phenomena in a less complex system. While electron transfer procedures to the cathode are still being investigated, evolving approaches based on biological systems including bacteria interacting with cathodes are supposed to contribute to amended processes. Besides CO_2 other substrates have been investigating for the future manufacture of higher-value chemicals. Finally, further researches are urged to assess the performances and commercial effectiveness of these combined procedures to make EF more competitive.

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Chapter 12
Microbial Electrosynthesis for Harnessing Value-Added Product via Carbon Dioxide Sequestering

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Abstract The increasing levels of carbon dioxide due to the burning of fossil fuels and industrial emissions are a threat to the ecosystem and hence an efficient technique is required to fix this damage. Recently, bioelectrochemical systems (BES) emerged to decrease CO_2 emissions and produce biofuel as a renewable source of energy. The microbial electrosynthesis (MES) is a new introduction to BES which uses microorganisms as a catalyst for utilizing CO_2 as their electron donor and reduce it to produce gaseous fuels like methane or liquid fuels like acetate, butyrate, ethanol, etc. The various compounds are produced depending majorly on the microorganism used, their metabolic pathway, and the magnitude of the applied external voltage. Microbial electrosynthesis process takes place in the biocathode by reducing CO_2 with electrons and protons generated during water oxidation at the abiotic anode. In the case of a biotic anode, oxidation could be performed at the anode chamber via electroactive bacteria (EAB) to convert biodegradable wastes to electrons and protons. The protons percolate through the ion exchange separators while an electron from anode reaches to cathode surface via external resistance to reduce the $CO_{2.}$ The electron mediators were used in cases to improve the kinetics of bioelectrochemical reduction of CO_2. The present chapter explains the principles of MES; it include the advantages of MES, biochemistry of electron transfer processes in biotic electrodes and microbes involved in cathode for

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reduction of CO_2. Further, this chapter discusses the recent developments in MES, illustrates the biochemical pathway involved in producing the different end products in the cathode chamber and possible downstream processes involved in the recovery of biofuel production. This chapter highlights major physicochemical parameters affecting microbial electrosynthesis processes and challenges. The chapter helps the reader to gain basic knowledge on MES, which has the potential to become the upcoming transformative, feasible and alternate technology to reduce the repercussions of excessive carbon dioxide in the atmosphere and save energy as well.

12.1 Introduction

The world energy requirements are currently fulfilled by the burning of fossil fuels and few inexhaustible resources like solar energy and wind energy. But, both of them have their own drawbacks. Burning of fuels lead to a large production of CO_2 and other harmful gases. CO_2 even though being non-toxic affect the environment by acting as a greenhouse gas (GHG). Increasing level of CO_2 in atmosphere due to anthropogenic activities is threatening various ecosystems (Sala et al. 2000) in the world by causing ocean acidification (Doney et al. 2009) other than global warming. Also negative changes in the earth climate have been experienced as per NASA (The relentless rise carbon dioxide—Climate Change—NASA. www.climate.nasa.gov/climateresourse/24). The use of solar and wind energy is extensively practiced but the uneven energy generation due to factors like clouds, night time, storms, still winds, etc. has prevented it to become a primary source of energy.

Hydrogen fuel was also considered to be a solution for the energy crisis. Electrolysis of water is a simple process for generating pure O_2 and H_2 which could be used as automobile fuel. Unfortunately, this requires very high cost electrodes other than being an energy intensive process (Borole 2015) and use of hydrogen confers technical difficulties such as low compressibility hence hard to store and high reactivity making it tough to transport.

On the other hand, practical solutions to reduce carbon footprint are being researched in the form of CO_2 sequestering such as carbon capture and storage to decrease daily emissions from power plants and industrial processes (Fig. 12.1). Though the practice is functional, it is not economically viable. One attractive method is to translate CO_2 into multi-carbon organics which can be used to manufacture organic compounds or liquefied shipping fuels of interest. The basic establishments for a real-world apparatus to fix CO_2 via this method include: (1) Ability to use inexpensive source of electrons like water by being physically immobilized in electrode (Centi and Perathoner 2009); (2) Economical and durable catalysts (Cole and Bocarsly 2010).

Electrochemical reduction of carbon dioxide in abiotic conditions is not feasible due to: non-specificity of products produced, poor stability of cathodes over long term and cost, slow process speed, competition with hydrogen production. This chapter focuses on the general knowledge of the MES biosystems which we hope

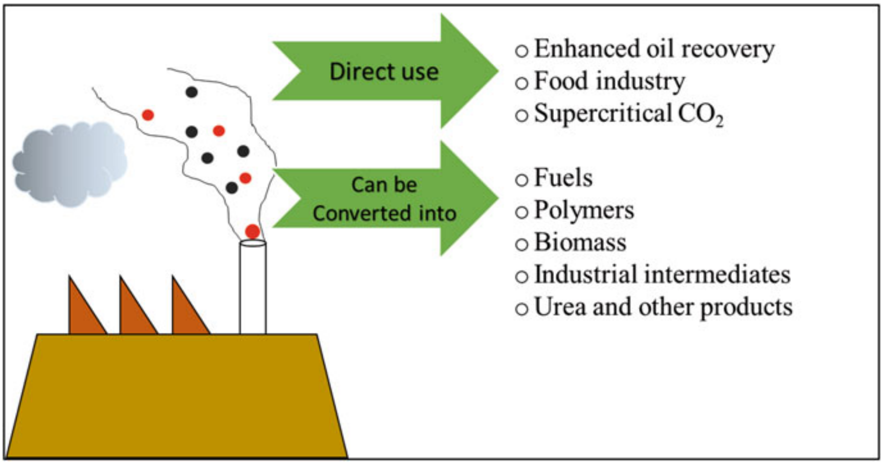


Fig. 12.1 Array of products which could be produced from CO_2 via direct use, biological or chemical conversion

could increase the understanding of basic concepts followed in this field of microbial electrosynthesis technology.

12.1.1 Competition with Hydrogen Production

For electrosynthesis, suitable electrocatalysts are needed with high specificity and adaptability compared to existing inorganic catalysts. Biocatalysts can be enzymes, organelles, and even whole cells. Although the first two can give high specificity and control in the reaction process, the use of complete microbes in bioelectrosynthesis has many benefits, including auto-regeneration capacity of catalyst, flexible use of substrate, and versatile product formation or transformation pathways. Some important factors and strategies to design better bioelectrochemical systems are reviewed by (Chandrasekhar and Mohan 2012, 2014b).

Carbon fixing microbes include algae, cyanobacteria, archaea, clostridia, and β-proteobacteria which can use diverse metabolic pathways to convert CO_2 into reduced organic compounds (Jajesniak et al. 2014). Two of the primeval pathways are photosynthetic pathway and Wood–Ljungdahl pathway (Figs. 12.2 and 12.3). Latter is seen mostly in acetogenic bacteria also called as reductive acetyl-coA pathway (Martin 2012). Photosynthesis is now largely used to produce biofuels from algae as its potential has been recognized (Chisti 2007). Another progress is development of bioelectrochemical system which could apply acetogenic bacteria energized by renewable electricity to yield carbon-neutral supplies and oils. Microbial electrosynthesis (MES) is now a famous term which was introduced by Nevin et al. (2010). MES uses the capacity of the bacteria to interchange electrons with the

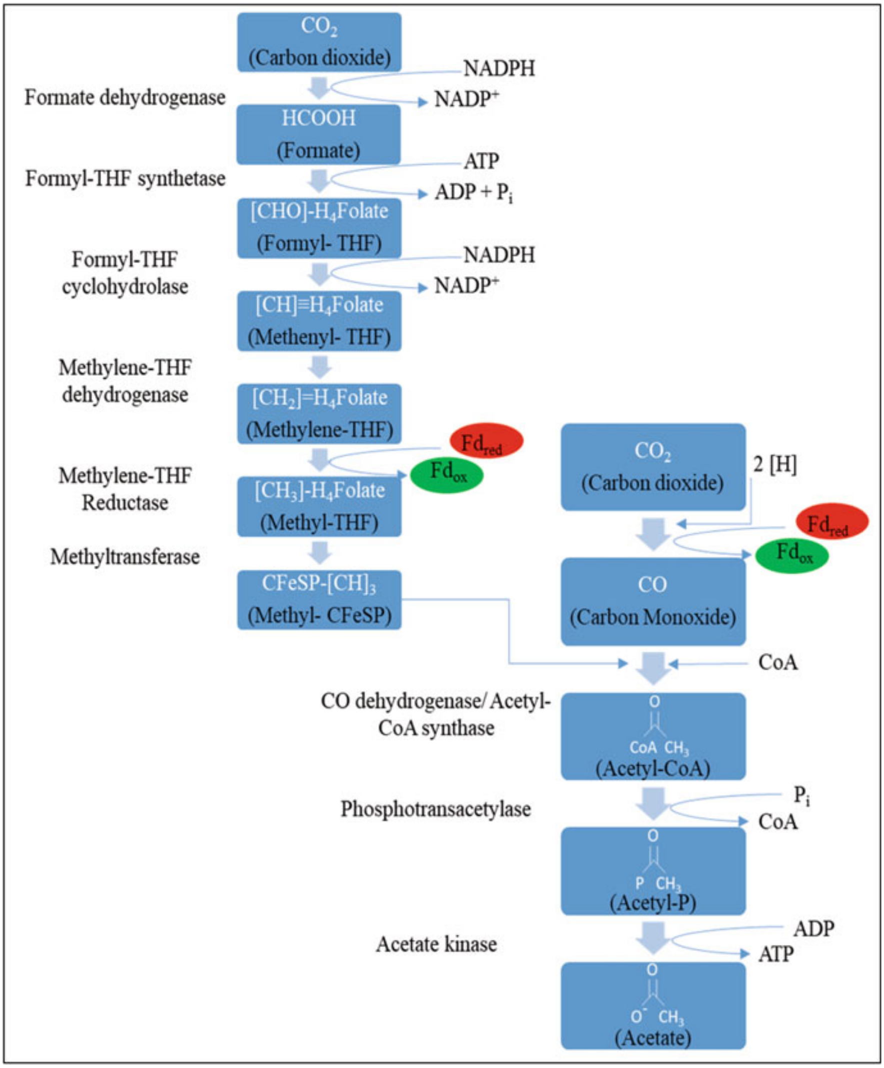


Fig. 12.2 Wood–Ljungdahl pathway redrawn from Fast and Papoutsakis (2012) and Schuchmann and Müller (2016) Left side is methylic branch and right side is carboxylic. *THF* Tetrahydrofolate, *CFeSP* corrinoid iron-sulphur protein, Fd_{red} ferredoxin in reduced form, Fd_{ox} ferredoxin in oxidized form

electrodes to produce value-added products. This ability of the bacteria is known as exoelectrogenicity and the name given to the species is exoelectrogens. The mechanisms of this will be briefed in this chapter along with modern achievements in this area and consider the limitations that prevent practical application of these technologies for large scale.

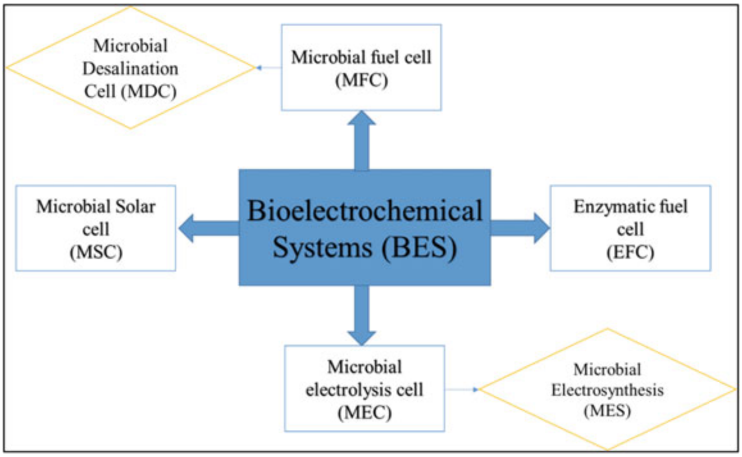


Fig. 12.3 Classification of BESs

12.1.2 *Types of Cells*

To make the concept simpler we recall the two types of chemical cells, i.e. electrolytic and galvanic. We know that galvanic cells produce electricity from stored chemical energy which are commonly used in small electronic appliances like remotes and watches. Electrolytic cells are those which use electricity to run a chemical reaction. This phenomenon is taken advantage by scientists to create what is known as rechargeable batteries run in two modes: electrolytic and galvanic. Always the reaction at cathode is reduction and at anode is oxidation. However, anode can be negatively charged as seen in galvanic cell and positively charged in electrolytic cell, whereas cathode is vice versa (Kipnis 2003).

The discovery of microbes causing change in potential difference has been a very important discovery in environmental science (Potter 1910). This knowledge is now advanced to a stage where microorganisms are used to produce electricity via designing microbial fuel cells (MFCs) or use their electron transfer properties to prepare chemicals (Chandrasekhar and Ahn 2017). The closed circuit of MFC contains a cathode and an anode connected by an external wire and separated by an ion selective membrane. When organic compounds (fuel) enter the anodic chamber, bacteria work to produce CO_2, protons, and electrons as by-products (Mohan and Chandrasekhar 2011a). The anode accepts the electrons produced and they pass to the cathode whereas the H^+ ions move into the cathodic chamber via the membrane freely. Then oxygen can bind here with hydrogen and electrons to form water (Bennetto 1984; Logan et al. 2006). Replacing the wire with an electrical device can harness the power developed by the microbes. All the systems using this phenomenon of exoelectrogenicity to either produce electricity or use it to create biochemical are called bioelectrochemical systems (BES).

12.2 Microbial Electrosynthesis (MES) Is Different from Microbial Electrolysis Cells (MECs)

MECs employ the assets of exoelectrogens by converting their chemical energy in to electrical form and this allows the electrolytic disintegration of H_2O (Wang et al. 2015). Exterior power source amplifies the flow of electrons towards the cathode from anode which stimulates the H_2 production at the cathode (Bowien and Kusian 2002). Greater flow of electrons is observable in MECs, than that of MFCs, which can be related to the supplementary voltage applied externally to overcome the cathode limitations (Dohnalkova et al. 2011). Since the energy needed for MEC to operate is less, it would be innovative to use a separate MFC itself as an energy source (Yu et al. 2013). The hydrogen created in MECs have opened routes to biochemically produce other molecules (Rozendal et al. 2008). Some reduction reactions occurring at the cathode surface are proton to hydrogen, oxygen to hydrogen peroxide, and carbon dioxide to methane and acetate (Green et al. 2015; McDonald 2003). However, the specific manufacture of useful compounds and biofuels like alcohols or organic acids via BESs is now largely due to microbial electrosynthesis (Grousseau et al. 2014; Torella et al. 2015).

Divergently, MES is a relatively new branch of BES which is a biocathode based approach that uses microbes to produce hydrogen, methane, acetate, and ethanol (Karthikeyan et al. 2019; Qian et al. 2019). Here electricity is fed deliberately to microbes to form products; electrons fed by them play role in reducing carbon dioxide or terminal electron acceptor. Recent attempts have even shown that larger carbon compounds like butyrate is possible through chain elongation reactions (Batlle-Vilanova et al. 2017). Biocathodes use the reducing power generated from the oxidation happening at the anode. The MES process can be made specialized to produce desirable reduced compounds. This can be achieved by using a specific biocatalyst used in the cathodic half reaction and the terminal electron acceptor. The shuttle molecules could also be altered to change the degree of reduction so that we could get product of interest.

To summarize, the principle of the process mainly lies in its extracellular electron transfer (EET) discussed in Section 12.4. The outward EET leads to the success of MFC and MEC while inward leads to the process of MES as detailed in the section electron transfer mechanism. The solid biocathode acts as the only electron donor to reduce carbon dioxide or reducible chemicals (like volatile fatty acids) into organic products. The anode can be applied as a catalyst for abiotic O_2 production and/or biotic decomposition of polluting agents (Jiang and Zeng 2018). Since the MES redox reactions are non-spontaneous, an outer power source is essential to ‘overcome the thermodynamic barrier’ to produce chemicals using the technology (Rozendal et al. 2008). The process of chemical production could be designed in batch, continuous mode or both in an alternating manner (Bajracharya et al. 2017) (Figs. 12.4 and 12.5).

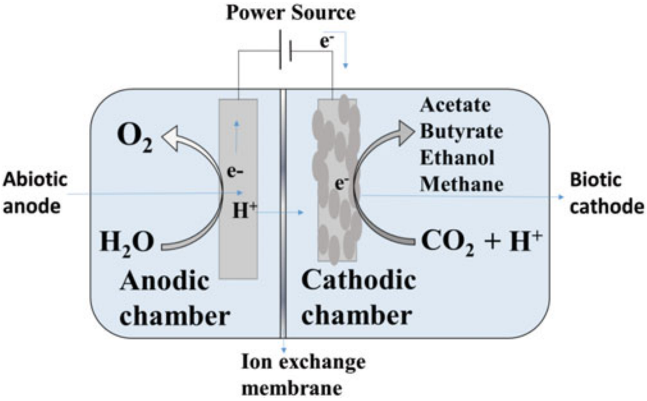


Fig. 12.4 Simplified schematic representation of microbial electrosynthesis process

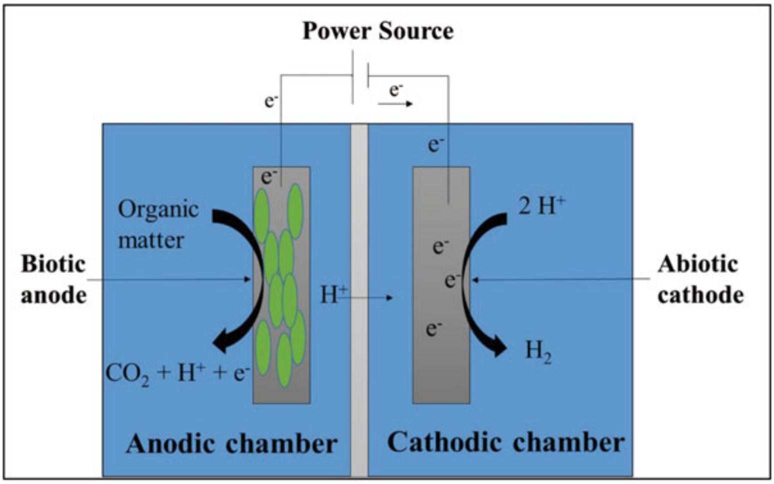
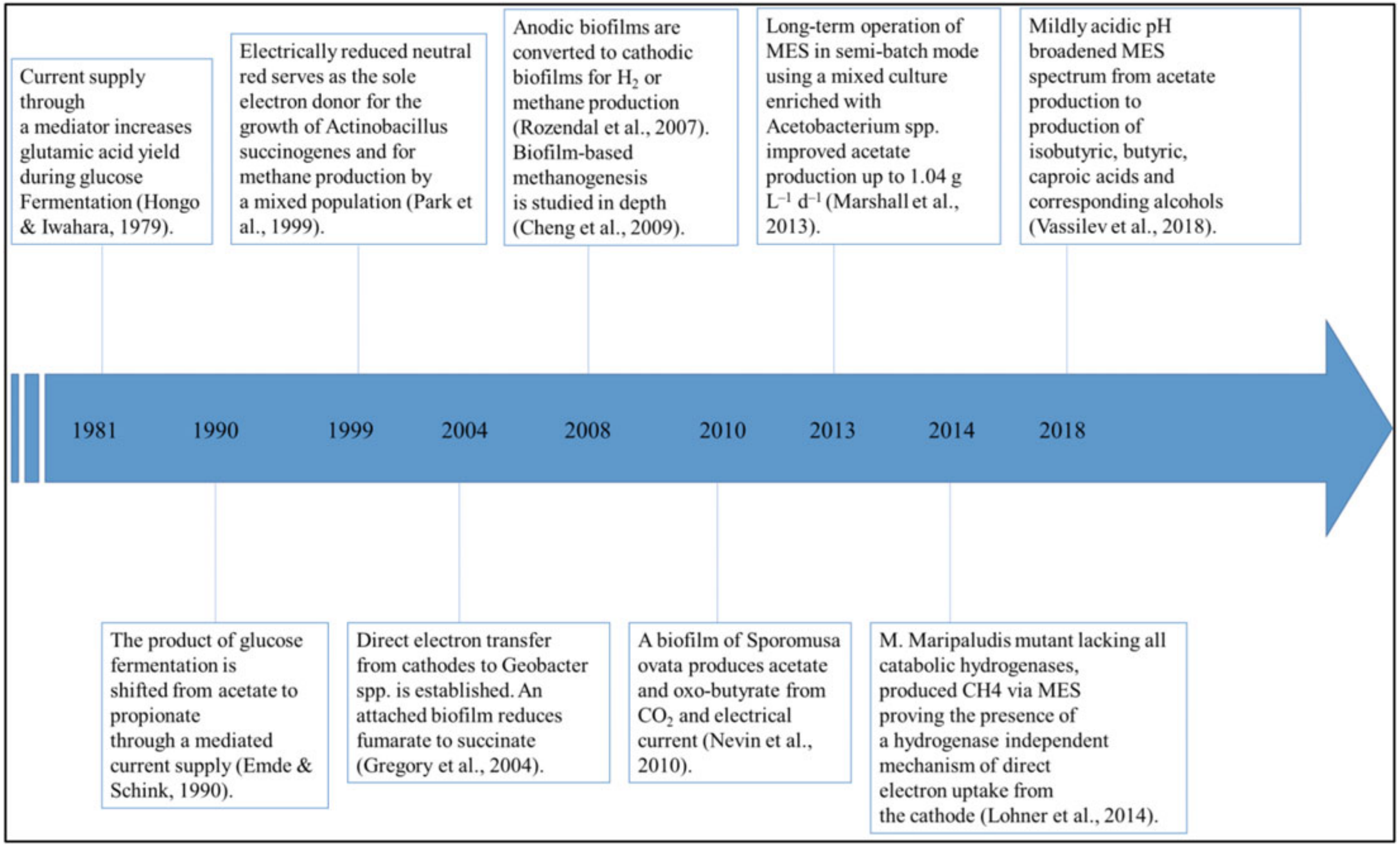


Fig. 12.5 Simplified schematic representation of microbial electrolysis cell

12.3 Microbial Species

Not all microbial species are efficient in producing electrons or reducing CO_2, they need to possess a special characteristic seen in some soil microorganisms called EET. Bacteria capable of conducting EET can be called electrogens. Certain Gram negative species of *Shewanella* and *Geobacter* are the most studied, however, some Gram positive species of *Clostridium*, as well as archaea and some microalgae can also perform EET (Sydow et al. 2014) (Fig. 12.6).

Acetogenic bacteria have the capability to reduce CO_2 to acetic acid and other multi-carbon products extracellularly with hydrogen as electron donor (Drake et al. 2008). *Anaeromyxobacter* species have been seen to reduce fumarate to succinate,



Current supply through a mediator increases glutamic acid yield during glucose Fermentation (Hongo & Iwahara, 1979).

The product of glucose fermentation is shifted from acetate to propionate through a mediated current supply (Emde & Schink, 1990).

Electrically reduced neutral red serves as the sole electron donor for the growth of Actinobacillus succinogenes and for methane production by a mixed population (Park et al., 1999).

Direct electron transfer from cathodes to Geobacter spp. is established. An attached biofilm reduces fumarate to succinate (Gregory et al., 2004).

Anodic biofilms are converted to cathodic biofilms for H_2 or methane production (Rozendal et al., 2007). Biofilm-based methanogenesis is studied in depth (Cheng et al., 2009).

A biofilm of Sporomusa ovata produces acetate and oxo-butyrate from CO_2 and electrical current (Nevin et al., 2010).

Long-term operation of MES in semi-batch mode using a mixed culture enriched with Acetobacterium spp. improved acetate production up to 1.04 g L^{-1} d^{-1} (Marshall et al., 2013).

M. Maripaludis mutant lacking all catabolic hydrogenases, produced CH4 via MES proving the presence of a hydrogenase independent mechanism of direct electron uptake from the cathode (Lohner et al., 2014).

Mildly acidic pH broadened MES spectrum from acetate production to production of isobutyric, butyric, caproic acids and corresponding alcohols (Vassilev et al., 2018).

Fig. 12.6 Some important achievements towards the development of microbial electrosynthesis

Table 12.1 Electrogenic bacteria and its potential application

Microorganism/mixed culture	Application/product	Reference
Geobacter sulfurreducens	MFC, MEC	Kiely et al. (2011b)
Pelobacter propionicus	MEC, MFC	Parameswaran et al. (2010) and Kiely et al. (2011a)
Methanobacterium palustre	Electromethanogenesis	Cheng et al. (2009)
Methanococcus maripaludis (electromethanogenic archaeon)	MES	Lohner et al. (2014)
Sporomusa ovata	MES/acetate, 2-oxobutyrate	Nevin et al. (2010)
Moorella thermoacetica	MES	Faraghiparapari and Zengler (2017)
Acetobacterium sp.		Xafenias and Mapelli (2014)
Rhodopseudomonas palustris TIE-1	MES	Ranaivoarisoa et al. (2017)
Megasphaera sp.	MES	Batlle Vilanova (2016)
Desulfitobacterium sp.	MES	Villano et al. (2011)
Desulfovibrio vulgaris Hildenborough	MFC/H_2	Pohorelic et al. (2002)
Anaerobic sludge	MEC/ethanol	Steinbusch et al. (2009)
Hydrogenophilic dechlorinating culture	MEC/cis-DCE, ethane	Aulenta et al. (2010)
Actinobacillus succinogenes	MEC/succinate	Park and Zeikus (1999)
Geobacter metallireducens	MEC/succinate	Gregory et al. (2004)
Geobacter sulfurreducens	MEC/succinate	Gregory et al. (2004)
Geobacter lovleyi	MEC/cis-DCE	Strycharz et al. (2008)
Dechloromonas agitata	MEC/Cl^-	Thrash et al. (2007)
Acinetobacter johsonii	MEC/ H_2O	Erable et al. (2010b)
Phototrophic mixed culture	MES/ biomass	Cao et al. (2009)
Anaerobic digester effluent	MFC/ Cr(III)	Tandukar et al. (2009)
Clostridium ljungdahlii	MES/acetate, 2-oxobutyrate	Nevin et al. (2011)
Ralstonia eutropha	MES/biofuels	Li et al. (2012)
Freshwater bog sediments	MES/1-butanol, propionate, butyrate	Zaybak et al. (2013)
Brewery wastewater	MES/H_2, formate, acetate	LaBelle et al. (2014)

nitrate to nitrite (Gregory et al. 2004). Many methanogenic bacteria can produce acetate other than the good amounts of methane via CO_2 reduction (Cheng et al. 2009; Kobayashi et al. 2013; Siegert et al. 2014). See Table 12.1 for more applications of methanogens to produce organic chemicals.

12.4 Electron Transfer Mechanism

Till the time of writing this chapter, four methods of extracellular electron transfer are known to happen in biocathodes as shown in Fig. 12.7. Microbes are capable of mediating electrons via largely two methods: direct and indirect. Direct mechanisms include biofilm formation and nanowires or pili mediated electron exchange. Indirect methods often include regenerative shuttles and interspecies electron transfer. Cathodic microorganisms use membrane protein machinery like c-type cytochromes and hydrogenases which help in direct type electron transfer (Rosenbaum et al. 2011), moreover the exchange via other cellular structures was reported by (Gorby et al. 2006). Direct and indirect intermicrobe cross feeding (syntropy) of electrons are also established among many species as demonstrated by Rotaru et al. (2014).

Shuttle mediated electron transfer mechanisms consist of the use of intermediary solubilized redox compounds which can be artificially provided or produced by the microbes. They easily interact with all the electrodes (Schröder 2007). Important examples include flavins and phenanzine compounds which are highly successful as soluble mediators (Marsili et al. 2008). Hydrogen is usually produced by pure electrochemical method or via biological pathway (Kadier et al. 2018). The resultant H_2 is produced on the surface of electrode which is used as reducing power by microbes.

The interspecies direct and indirect electron transfers are categorized into syntrophic relationships as per microbiology. This is more pronounced when applications of mixed cultures is practiced. Also energy is conserved, since electrons are bypassing the formation of intermediate molecules. This ensures the exchange of electrons directly between the microorganisms (Stams and Plugge 2009). Based on ΔG (Gibbs free energy) of different pathways, different redox materials and degradation of organic carbons via three methanogenic pathways (Gu et al. 2019) have

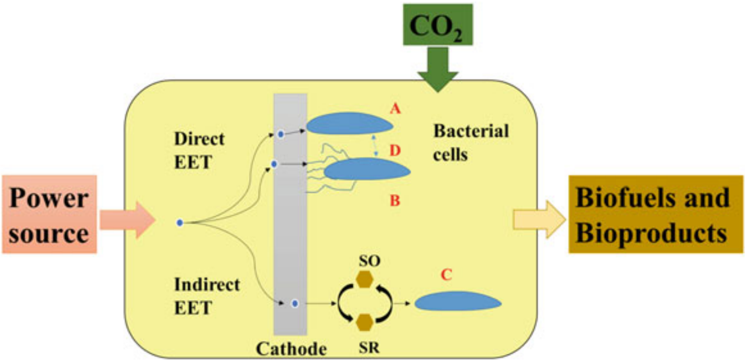


Fig. 12.7 Cartoon version of four types of EET. (a) Direct contact or biofilm mediated, (b) Pili or nanowire mediated, (c) Indirect or electron shuttle mediated and (d) Direct interspecies electron transfer. *SO* electron shuttle in oxidized state, *SR* electron shuttle in reduced state, ● electron

proposed a mechanism affecting direct interspecies electron transfer (DIET). That is the degradation of organic compounds improves transition towards DIET and enhances them.

The electrons first need to reach from the cytoplasm to the external plasma membrane in order to reach outside the cells. This is achieved with the help of electron transport chain in absence of oxygen as a terminal electron acceptor. The electrons are perceived to travel via polyhemic cytochromes (cytochrome with multiple heme) in cytoplasmic membrane (Debabov 2017).

12.4.1 Shuttle Mediated

In anaerobic environment, electrons can be transported from the cytoplasm to the anode via low molecular weight compounds capable of reversible redox reactions. It serves as the terminal electron acceptor and, once reduced, can itself transfer electrons to electrodes whereupon it becomes oxidized again. These compounds even though known for a long time realized its potential in EET only around 15–20 years back. They are called 'shuttles' in this age. These shuttle molecules can be intrinsically produced by the microbes itself or added externally in the soil/ medium. Examples for microbially synthesized molecules include phenazines by *Pseudomonas* (Rabaey et al. 2005) and flavins (riboflavin and flavin mononucleotide) by *Shewanella* (Marsili et al. 2008). Artificial shuttles include neutral red (Harrington et al. 2015), anthraquinone 2,6-disulfate (Thrash et al. 2007), and methyl viologen (Peguin and Soucaille 1996).

This method of electron transfer is preferred because the planktonic cells need not to be directly attached to the electrode or be present in its vicinity, thereby increasing the scope of exoelectrogenicity. This use of mediator also encourages separate-stage designs and that can conduct temporal and spatial decoupling of energy capture and bioproduction. In this approach, electrons are added to the shuttle molecules when the electrode loses them. After that they get oxidized by the microbial cells and the cycle of electrolytic regeneration continues (Godoy et al. 2017). Other inorganic compounds used in chemoautotrophy such as NH_3, NO_2^-, Fe^{2+}, and H_2 can also be electrolytically regenerated. They can be used as smart alternatives in this field as they simplify the construction of organic multi-C compounds from CO_2 using natural carbon fixation pathways (Khunjar et al. 2012).

12.4.2 Nanowire/Pili Mediated

Since (Reguera et al. 2005), scientists have been observant in the role of pilin in the transfer of electrons. Later other organisms including *Shewanella* were seen to be producing nanowires for the electron transfer (Gorby et al. 2006). It was shown that they necessarily may not be pili, extracellular polymeric matrix can also be the causative agent (Dohnalkova et al. 2011). There is no clear conclusion about the participation of pili and the mechanism still remains elusive, however, it is known that cytochromes play a big role in the travel of electrons along the thread like structures.

12.4.3 Direct Contact/Biofilms

To transfer electrons to electrodes without the use of low molecular shuttles, electron-conductive bacterial cells must physically contact with the electrode surface. Such bacteria usually form biofilms on electrodes.

Biofilms of the *Shewanella* and *Geobacter* genera are the best studied. Their thickness can reach 100 μM (in *Geobacter*) or less (in *Shewanella* cultures). The biofilms of both types possess considerable electric conductivity, which is exclusively realized by direct EET in the case of *Geobacter* and is provided by both direct and flavin-mediated electron transport in *Shewanella*. It is worth recalling that the *Shewanella* mutations stopping flavin synthesis also reduce electron transport in biofilms by 70–75% (Rosenbaum et al. 2011).

For *G. sulfurreducens* biofilms, the direct transport of electrons between cells was shown by cyclic voltammetry, and this transport was provided by the accumulation of reduced cytochrome forms (Liu and Bond 2012). The occurrence of such transport ensures that electrons from cell layers not directly adjacent to the electrode are still able to reach the electrode. It was shown that the current density in MFCs grows with increasing film thickness in *G. sulfurreduceus* (Jana et al. 2014).

Thus, the biofilm formation and properties have a definite influence on the processes in BES systems. Electron transfer between biofilm and electrodes is an example of the interaction between live organisms and inorganic material and is already being used in biosensors (Erable et al. 2010a).

12.5 Mechanism of Product Formation in MES

12.5.1 Methane

Production of biogas (CH_4) by the use of anaerobic digester is well known and long practiced even in villages. The use of anaerobic digestion gives other value-added products such as bio-manure or even bio-char. Since there is a continuous availability of organic wastes such as crop residues, vegetable peels, etc. this process is renewable (Chandrasekhar et al. 2015; Inyang et al. 2010). To make the generation of biogas more sustainable various BESs can be modified to increase the generation of methane, also the MES process can occur in a lower temperature than anaerobic digestion. This pathway of producing CH_4 via electrochemical reduction is known as electromethanogenesis. Even though multiple investigations of methane production via microbial electrosynthesis of carbon dioxide are available the electron transfer mechanism is not clear. However, most scientists agree upon the hydrogenotrophic methane production mechanism. This electrochemical method of CH_4 production is achieved via the Wolfe cycle (Thauer 2012). This mechanism was summarized by Costa and Leigh (2014). It was also found that electron bifurcation methods were used by the methanogens to couple the heterodisulfide reduction step back to the first step of carbon reduction either using hydrogen, formate or electrons as reducing agents (Kaster et al. 2011).When MECs are used for electromethanogenesis the advantage is that COD of water is reduced and concomitantly methane is yielded. Hence this method is an eco-friendlier way with regard to high CH_4 production and clearance of effluents from the digestion process (Wagner et al. 2009; Clauwaert and Verstraete 2009). In a two-chambered BES (Cheng et al. 2009) attained nearly 4.5 l/day m^2 of CH_4 yield with around 96% current efficiency, by maintaining the cathode potential at −0.8 V vs Standard Hydrogen Electrode (SHE). Unlike the conventional methanogenesis pathway, advantage of BES systems is the spatial separation of the oxidation of organic waste from methane production, which in turn protects the methanogenic bacteria against the toxic compounds which possibly may form the waste stream. Deval et al. (2017) has studied an BES mediated technique to reduce waste production by remediation process.

12.5.2 Acetate

MES can also be utilized to fix carbon dioxide in to multi-carbon compounds such as biofuels or value-added chemicals through reduction reactions which can be powered by electricity to overcome the thermodynamic barrier. This application is speculated as a futuristic technology for the reserving of electrochemical energy inside the C-C bonds. In the seminal studies conducted by Nevin et al. (2011), electron recovered in the harvest was nearly 85% of the electrons sent to the electrodes. Following experiments verified the procedure of carbon dioxide reduction using microbial electrosynthesis with even a larger range of microorganisms. These studies showed the CO_2 reducing capability of other acetogenic bacteria, including *S. sphaeroides, S. silvacetica, C. aceticum, Clostridium ljungdahlii,* and *Moorella thermoacetica* to formate acetate and 2-oxobutyrate. Similar recovery profile in acetate and 2-oxobutyrate were reported: 84% in *S. silvacetica*, approximately 48% in *S. sphaeroides*, around 82% in *C. ljungdahlii* and nearly 53% in *C. aceticum* correspondingly. Gong et al. (2012) presented that sulphide can also be exploited as a reducing compound at the anode for MES. Their experiment applied a sulphur oxidizing microbe, *Desulfobulbus propionicus*, at anode to catalyse the conversion of elemental sulphur to sulphate via oxidation. The reducing power generated in this biotic anode was used for carbon dioxide reduction in to acetate while *S. ovata* was growing on graphite based cathode. Marshall et al. (2013a) demonstrated the improved amount of acetate manufacture through reduction of carbon dioxide with the help of mixed microbial populations. Jiang et al. (2013)

worked on a biocathode of diverse microbial culture which received electrons from the anodic half-cell and produced hydrogen at abiotic conditions to convert carbon dioxide into methane and CH_3COO^-. Marshall et al. (2013b) showed the enhanced performance of MES for the production of acetic acid at the rate of 1 g/L day using autotrophic mixed cultures when functioned continuously for long periods. Additionally, the long-term application resulted into a stable biocathode. The environmental settings of the MES procedure should be such that the optimum metabolism of the microbe or biocatalyst must occur which is present in the biocathode. An appropriate terminal electron acceptor is a prerequisite for any reduction reaction. Adding to that, the cathodic potential which can overcome the activation energy barrier of that bio-reaction must be provided for the reaction to proceed in microbial electrosynthesis. Homoacetogens can competently translate carbon dioxide to acetic acid, which is a pivotal intermediary molecule for the manufacturing of value-added biochemicals (Batlle-Vilanova et al. 2016).

12.5.3 The Mechanism for Alcohol and Volatile Fatty Acids

Acetogens or acetogenic bacteria use Wood–Ljungdahl pathway for converting CO_2 to acetate and other compounds such as ethanol, butanol, butyrate, etc. Some of the important acetogens used for this process include *Acetobacterium*, *Clostridium,* and *Sporomusa* genus (Bajracharya et al. 2017). Through Wood–Ljungdahl pathway, CO_2 is converted into an intermediate metabolite Acetyl-CoA. ATP for this process is generated through transmembrane proton gradient and energy is conserved through Rnf complex. The intermediate metabolite, Acetyl-CoA can be converted to acetate and ethanol (2-carbon compound) on moderate reduction and butyryl-CoA under excess of reduction. Butyryl-CoA can then be used to produce butyrate and butanol(4-carbon compounds) or even hexanoate and hexanol(6-carbon compound).

The ability to produce different carbon compounds relies on the reducing ability of microorganisms. Another factor that plays an important role is the pH of the growth medium, a lower pH (acidic medium) produces alcohols which is due to the following reasons:

1. Excessive activity of the Rnf complex which leads to more energy production.
2. Presence of undisassociated organic acids, which appear toxic to microorganisms and hence force solventogenesis of volatile fatty acids to form alcohols to avoid further reduction of pH.

Combination of molecules excluding acetate and ethanol can be generated by chain elongation reactions. For example, butyrate could be produced by combining one acetate molecule and one ethanol molecule by the reverse β-oxidation process.

Hydrogen Oxidizing Bacteria

Hydrogen oxidizing bacteria use energy obtained from oxidation of hydrogen to reduce carbon dioxide and hence are anaerobic. An important hydrogen oxidizing bacteria used in experiments is *Cupriavidus necator* and most experiments are performed using this strain. They use Kelvin cycle for Carbon dioxide fixation. Bacterial growth and energy efficiency of hydrogen utilization for biomass production are dependent on the $H_2/CO_2/O_2$ ratio. It was shown experimentally that in presence of excess hydrogen and limited carbon dioxide the rate of consumption of gases increases with the increase in oxygen concentration (Low concentration) CO_2 + (excess) H_2 + (not defined)O_2 = increased rate of consumption of gases with an increase in oxygen concentration. Though this affects the energy efficiency of carbon dioxide and decreases reduction. An important feature of *C. necator* is their ability to accumulate large amounts of biodegradable biopolymers like polyhydroxyalkanoates. In a study performed in 2014, genetic engineering was performed on bacteria to enable them to synthesize isopropanol which serves as a valuable oxidizing additive to motor fuel. In the mutant strain, the Acetyl-CoA pathway was interrupted. Whereas the pathway used for conversion of the compound to acetoacetate and acetone along with a further reduction of acetone into isopropanol was activated (Bajracharya et al. 2017). Researchers at Harvard created a system where solar energy provided power for water electrolysis, coupled with the growth of *C. necator* for isopropanol synthesizing bacteria. To avoid overpotential, electrodes that were able to work in a neutral environment and were biocompatible were selected. Potential used for this process was 2.7 V because a lower potential resulted in the formation of toxic compounds and higher potential resulted in low efficiency.

12.6 Challenges

MES is still in its beginnings, fundamental and applied research needs to be done in the important steps of the process and performance such as (1) Ohmic losses, (2) Electrode-microbe interaction, (3) Microbe-substrate interaction, (4) Mass transfer, (5) Product extraction and purification, and (6) Reactor design (Batlle Vilanova 2016). Other than that extracellular electron transfer is a very poorly known molecular mechanism.

The role of electron transport system in the microbial outer membranes and outer membrane proteins (OMPs) needs to be explored more. The electrode materials can be manipulated to make it durable and high performing at minimum possible costs so that microbial electrosynthesis is profitable. In this regard strategies used in other BESs can be very useful like development of high surface area by using highly porous cathodes which are biocompatible. Carbon nanotubes CNTs are an attractive cathodic substance (Xie et al. 2012). So most of the improvements need to be targeted towards biofilm formation and maintenance. Surface chemistry of the electrodes can be enhanced by the immobilization of charged molecules, as they can improve the binding interaction of the bacterial cell envelope with electrode surfaces, but it is still a less inspected tactic (Kumar et al. 2017). Higher current density can provide more electron supply thereby increasing the reducing power.

One method can be to decrease the overpotentials caused in cathodes by switching to metal or graphene-based amalgamated electrodes. Hence for maintaining the redox potentials the thickness and layers of biofilms need to be optimized by the use of new field ‘biofilm engineering’ (Schrott et al. 2014; Steidl et al. 2016).

Other optimizations could help the product forming efficiency such as providing continuous flow of nutrients and the recovery of value-added compounds. The CO_2 solubility in the electrolyte is an another rate limiting factor. Improved diffusion and mass transfer of total carbon dioxide content is desirable for the real-world application. Gas diffusion electrodes may prove worthy in this case (Bajracharya et al. 2016). Anodic half-cell is a primary requisite for the MES process as it provides electrons and protons usually by water oxidation. Further studies in search of other advantageous oxidation reactions will make the whole system fruitful.

The use of cutting edge researches such as metabolic engineering, synthetic biology tools with recombinant DNA principles shall augment in the chain elongation and the product development other than ethanol, acetate, methane, and hydrogen (Chandrasekhar and Mohan 2014a). Mixed culture approach can increase the metabolome and reactome capacity which henceforth facilitates in multiple products manufacturing.

12.7 Conclusion

Electrobiosynthesis is a very new research area with great potential. The realization of this potential will require numerous fundamental studies on the EET, formation, and functioning of cathode biofilms and the transport of protons that compensate electron charges. A search for new, as well as the optimization of known, electrogens is required. Reconstruction of the genome is needed to activate electron transport or to change the cellular metabolism in order to broaden the range of obtained products. Bacteria *Clostridium ljungdahlii*, which are able to synthesize butyrate while growing on carbon dioxide, have already been constructed (Ganigué et al. 2015). The best-studied bacterium, *E. coli*, has been transformed to an electrogen as a result of 10 year efforts of numerous researchers (TerAvest et al. 2014).

In a nutshell, MES is ready to create a platform technology within various disciplines but not limited to biorefineries, renewable electricity generation, electrofermentation (Kumar et al. 2018), bioremediation, and electrosynthesis plants. Various organic acids used in industrial and residential purposes such as formate, acetate, propionate, and butyrate can be produced by using CO_2 whose high concentrations in air are now damaging ecosystems and climate. Further developments in system architecture (electrodes, exchange membrane, power source) and mechanisms of EETs could firmly establish the microbial electrosynthesis process industrially (Mohan and Chandrasekhar 2011b; Hongo and Iwahara 1979; Park et al. 1999; Emde and Schink 1990; Rozendal et al. 2007; Vassilev et al. 2018).

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Chapter 13

Electrotrophs and Electricigens; Key Players in Microbial Electrophysiology

Obinna M. Ajunwa, J. O. Audu, Prasun Kumar, E. Marsili, and A. A. Onilude

Abstract A wide range of microbes belonging to all three domains of life is known to generate electrical current and transfer electrons to anodes within a bioelectrochemical system. Typically these exoelectrogens are iron-reducing bacteria (e.g., *Geobacter sulfurreducens*) that are capable of producing high power density at moderate temperatures. Under nutrient sufficient conditions, other microbes ranging from extremophiles to yeasts can also produce high current densities. On the other hand, electrotrophic microbes grow on electrons derived from the cathode, but such microbes are less diverse and have uncommon traits. Electrotrophs shows low current densities (well below representative exoelectrogens) and utilizes several terminal electron acceptors for cell respiration. Thus, there is a vast diversity of electroactive microbes and their cultivation conditions that opens-up a new avenue for electrochemical devices particularly for H and CH production. The microbial fuel cell has been considered as an eco-friendly technology to harvest electricity harvesting from a variety of carbonaceous substrates. Here, microorganisms can be used as biocatalysts. This chapter provides an introduction to the currently identified electricigens, their

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taxonomical groups, and electricity-producing abilities. The mechanism of electron transfer from electricigens to electrodes is also discussed.

13.1 Brief Insights Into Microbial Electrophysiology

With the expansion of the realm of the life sciences based on deeper understanding of the workings of living organisms, newer disciplines and study focuses have emerged. The quest for energy by microbial species has led to intense physiological underplays relating to their interactions with physical entities and inanimate units that tend to affect their form and structure. A main form of these inanimate units occurs as electrons. Reguera (2011) opined that physical factors like sound, light, and electrical charge (electrons in motion) could act as viable means of conveyance of metabolic information among microbial species within a giving space and medium. Electrical communication further intertwines with the classical understanding of electron mobility within the cell membrane as well as the possible exportation of the electrons extracellularly (Reguera 2011). The influence of magnetic and electromagnetic flux as a physical factor interacting with ionic flow of electrons through the cell membranes has also been generously captured in the term *bioelectromagnetism* as documented by Hunt et al. (2009). Conceptually speaking, all of the above could be classified under the knowledge and discipline of microbial electrophysiology; this, however, does not discount the fact that the field has been earlier characterized by spheroplast-based single bacterium studies of electron and proton mobility through the cell membrane. This involved the detection of electrical oscillations in the form of ionic flow which were closely measured using techniques like patch clamp assay and voltammetry (Delcour 2013). In many cases, the microbial cells have to be converted into giant cell wall deficient cytoplasmic enclosures to enable direct poking of single cells with measuring devices for adequate measurements of ionic flow. This helps in determination of ion channels within microbial cell membranes (Berrier et al. 1989). It has also led to the determination of new ion-flow channels as well as unique systems amenable to mechanical perturbations like the mechanosensitive channels found within bacterial cell systems (Kung et al. 2010). Mechanosensitve channels can be either large or small and the characterization of their biophysical properties in terms of a unique gating mechanism has led to clearer insights on the methods microbial cells employ in permissible entrance of specific molecules or the outright closure of ion pores when charged with the required amount of mechano-chemical pressure (Booth and Blount 2012). The difficulty in single-celled microbial measurements of ionic electrical flow and impulses, coupled with the apparent scarcely present single planktonic units (without the dependence on cell clusters) has shown that the focus should rather be on the existence and functionality of microbial ion flow and electrical impulses from a conglomeration of cells, whether homogenous or heterogenous (Logan 2008). This therefore led to insights into community-based flow systems of microbial electrical currents, ably crystallizing into the second part of the field of microbial electrophysiology. Based

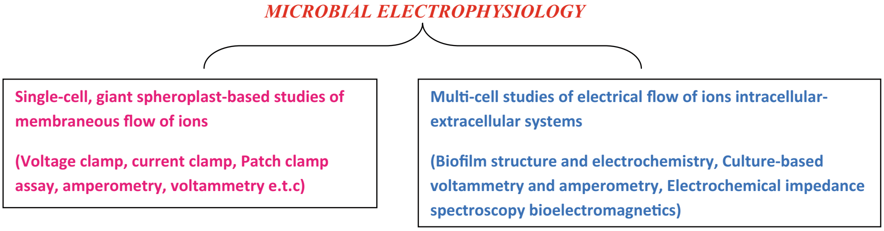

Fig. 13.1 Broad-based classification of microbial electrophysiology studies based on single-cell and multi-cell assays

on these, microbial electrical interactions were characterized to be both intracellular and extracellular as well; as recently discovered (Kracke et al. 2015). Regardless of the seeming variability in single-celled spheroplast-based ionic flow studies and multi-celled electrical ionic flow system, there is still a major similarity in their study aims. Microbial electrophysiology therefore seeks to inter-relate these functional routes of cellular interactions with electrons, flowing through microbial membranes inter-twined with membrane biochemistry and biophysics.

Ideally, cellular electron flow properties were thoroughly defined as membrane-bound transfer chain systems thereby leading to the proper characterizations of respiratory mechanisms within microbes. However, the discovery of extracellular electron transfer (EET) systems led to a change in narrative as this redefined aspects of microbial electrophysiology, bringing to the fore unique biomechanisms of microbial processes of electron yield and transfer (Biffinger et al. 2009; Feng et al. 2014). With the advent of studies on EET, a number of terminologies were developed to describe the actual properties or activities exhibited by the key microbial species to conform with. Terms like electrogenesis, exoelectrogenesis, electroactivity, electrochemical activity, electricigenicity, and so on were used to describe EET systems. Consequently, microbes with the unique properties of EET became known as electrochemically active microbes, electroactive microorganisms, exoelectrogens/electrogens, electric bacteria, electrotrophs, and electricigens (Logan 2008; Marsili et al. 2008b). These unique physiological variants of microbial cells capable of EET form a pivotal part of microbial electrophysiological studies and research at the moment (Fig. 13.1).

13.2 Classification of Electricigenic and Electrotrophic Activities

Based on the introduction of concepts of microbial electrophysiology, it is clearer that electricigens and electrotrophs play vital roles in cellular function and response with respect to EET. The discovery of exocellular electroactivity as made by M.C. Potter in the early twentieth century (around 1911) led to the inquisition into the first manifestations of laboratory-based microbially generated electricity (Potter 1911). The experiments performed at that time were carried out with the aid of chemosynthetic electronophores that aided in extracellular electron seepages from the cell envelope in an electrochemical-coupling manner (Logan 2008). These went on until further research works determined the specific functionality of biosynthetic electronophores self-produced by the electroactive microbes to aid in electron shuttling from intracellular to extracellular spaces. Most of these efforts were, however, focused in an electrobiotechnological fashion on bioelectricity generation from electroactive microbes (Rahimnejad et al. 2015).

The workability of a bioelectricity system integrates the ability of microorganisms to disintegrate organic matter in a bid to generate electrons flowing through a series of proteins utilized in respiratory mechanisms and enzymes available within the cell membrane, subsequently increasing the availability of adenosine triphosphate (ATP) (Kumar et al. 2016). There is a bioenergetic coupling of this process to the electrons released unto terminal/end-chain electron acceptors. These electron acceptors in most cases exist as soluble nitrates, sulphates, oxygen, etc. possessing the ability to cross the cell envelope barrier from the exterior and translocate intracellularly. The entry of these acceptors triggers chains of reactions leading to bonding with available electrons, forming products that are reversibly diffusible outside the cell (Newman and Kolter 2000). However, in the case of microbial electricity systems, electrons can be transferred by bacteria exogenously unto a solid electron acceptor like a metal or metal oxide (Iron or Iron oxide) (He et al. 2015). In fairness to the process, these bacteria with such qualities form the crux of the description of electroactivity in microbes. For the process of electrogenesis to be more efficient, a reactor system that allows maximum metabolic activity needs to be put into consideration. In most cases, a two-chambered reactor with a membrane-based ionic separation is usually adopted (Logan 2008). This is classically called a microbial electrochemical cell (MEC). The half-cell reactions taking place in each of the chambers (anodic and or cathodic) clearly defines the kind of microorganisms involved (Logan et al. 2019). In the case of the anodic chamber, electrons are released from the cell unto the electrode, thereby generating an electron pool within the system that eventually flows through external circuits and loads attached to the electrode causing an electric current. Such electricity producing organisms are referred to as *electricigens* (Doyle and Marsili 2018). The electrons produced then enter into the cathodic chamber after passing through the external electrical load of the MEC leading to an increased electron presence on the cathode. In the event that there are microorganisms present within the cathode or the cathode chamber in general, there is the ability of these microbes to utilize the electrons in an energy dependent fashion for their metabolic processes thereby leading to the synthesis of important compounds. The ability for an organism to assimilate externally produced electrons makes the organism an *electrotroph* (Ishii et al. 2015).

Based on research works, as well as scientific opinions, it is not too expedient to declare an organism an electricigen against its status as an electrotroph and vice versa because redox activities, that define physiological properties of these kind of organisms are usually cycles with a back and forth reaction, meaning that it is possible for an electricigen that donates electrons to the exterior environment to also accept electrons from the exterior environment dependent on the electrochemical status it is faced with in its immediate environment (Kracke et al. 2015). Due to this fact, it is pertinent to note that the species named as electricigens and/or electrotrophs are usually the same, however, the specificity of their activities at a given time as well as their applications in electrobiotechnology create the appropriateness of naming the organisms. For ease of concept, they are all regarded as electrochemically-active, electroactive, or electric microbes (Doyle and Marsili 2015; Logan et al. 2019).

With respect to the above descriptions, it is important to realize that the specific biomechanisms guiding electrotrophy and electricigenicity are species-specific and electrochemistry-specific. As determined by the above constructed systems of studying electrotrophs and electricigens (the MEC), it has been identified that these electroactive species utilize two main biomechanistic platforms for electroactivity as they interface with the electrode. The first is the direct electron transfer (DET), which makes use of cytochromes (associated with the cell membrane) with an outer-cellular orientation, and microbial nanowires which are appendages protruding from the cell membrane unto the external environment utilized in exocellular electron exchanges. The second is the mediated electron transfer (MET) that makes use of electron shuttles biosynthetically produced by the electroactive microbe in a bid to ferry electrons outside the cell in a pick-and-drop fashion (Kumar et al. 2016). It has also been demonstrated that despite the descriptive factor of characterizing electroactive microbes by their ability to transfer electron unto non-diffusible external surfaces (metals and electrodes), electroactive species have as well been identified by their potentials to receive electrons from the solid non-diffusible electron donor, thereby using it as a donor of electrons (Choi et al. 2014). This is the major basis for the physiological variation between electricigens and electrotrophs as shown in Fig. 13.2. Chief applications of electricigenicity are in

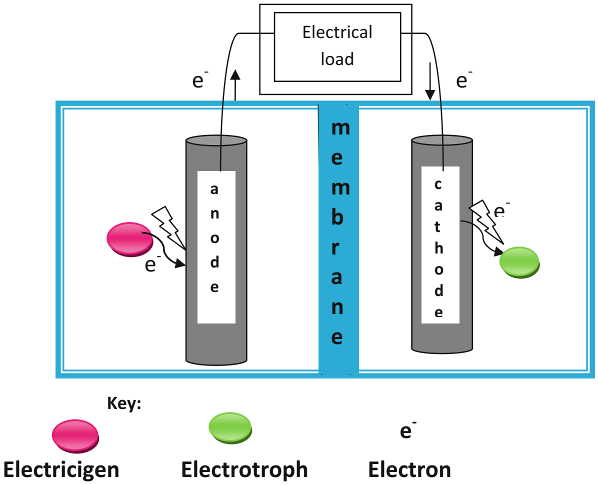

Fig. 13.2 Schematics of a microbial electrochemical cell (MEC) showing the basic concepts of cellular electron export and import defining electricigens and electrotrophs, respectively

bioelectricity generation, bioremediation, and biosensor design, while electrotrophy has been majorly applied in bioelectrosynthesis where electrons obtained from the cathode are used to biosynthesise useful industrial compounds classically referred to as electrobiocommodities (Lovley and Nevin 2013; Doyle and Marsili 2015; Kadier et al. 2017; Kumar et al. 2018). Electrochemically speaking, the MEC can produce oxidative and reductive currents. In producing oxidative currents, electricigenic interactions with the anode (which is an electrode of positive potentials) are employed in the MEC as the electroactive microbes oxidize the nutrients within the anodic chamber, subsequently producing an oxidative current and the anode becomes the electron acceptor that ends the electron flow chain. In the opposite (cathodic) chamber, electrotrophic interactions occur due to the negative potentials of the cathode, thereby making the electrode act as an electron-donating system producing a reductive current (Doyle and Marsili 2015).

13.3 Possible Physiological Factors Utilized in Microbial Electricigenicity and Electrotrophy

Based on research, electroactive microbes indulge in transport of electrons onto an external surface with the aid of two mechanisms: natural production of electromediatory metabolites, and produced cell surface associated systems like nanowires (Kumar et al. 2016). Studying the electrophysiology of *Shewanella* spp, it became obvious that the reduction of ferric ion involves membrane-attached electron ferrying agents (Pirbadian et al. 2014). Following mutagenic and biochemical studies, a variety of proteins located within the membranes (both outer and inner membranes) have been identified as responsible for dissimilatory mineral reduction (which is a form of electrogenesis) (Lower et al. 2001). This process of transferring electrons is not enough to give the ultimate explanation of the biomechanistic interactions involved in electricigenicity and how the processes intertwine in a synergistic pattern to yield optimum activity. This is attributed to the fact that many of the mechanisms remain unclear.

13.3.1 Nanowires

A structural formation of a nanosized pili-like protrusion on the cell surface of both of the most studied electroactive microbes; *Geobacter* and *Shewanella* species was reported by Gorby and Beveridge (2005). This appendage was conductive in nature and was classically referred to as a nanowire. They went further in their studies, and with the aid of conductive scanning tunneling microscopy (STM), the conductivity of the appendages was researched upon. In description of the experimental process, the samples were loaded onto a flat and conductive pyrolytic graphite surface, and also arranged in an orderly fashion. A conductive Platinum based tip was subsequently driven across the sample as the imaging conditions were under the influence of constant current. A current–voltage curve was then generated, and this demonstrated the conductive portions in relation to the scanned surface. As the tip was raspered across the surface of the sample, the conductivity of the appendage was shown as the current rose, indicating electrical conductivity in the ‘z-plane’ (a term which connotes the area from the tip to the surface).

By close observations, it was determined that these appendages were conductive, especially after showing similar conductivity along the ‘x-y plane’ (connoting the area lying in between test surface and the cell), and thus could function as nanowires ferrying electrons from the cell to a surface (Gorby et al. 2006). Tests carried out on mutants of *Shewanella* species also showed that mutants defective in the key cytochromes involved in respiration (mtrC and omcA) had the ability to produce non-conductive appendages, thus lacking the ability for electroactivity. It was also observed by Reguera et al. (2005) that *G. sulfurreducens* also has conductive appendages, albeit a little different from the nanowires of *Shewanella* species, as the nanowires of *Geobacter* were thinner when compared with the ones of *Shewanella* species. It appeared that the thickness of the *Shewanella* nanowires might have been as a result of an aggregation of a mass of smaller wires bundled together. There is also the possibility of production of nanowires by phototrophic oxygenic microorganisms like the cyanobacteria—*Synechocystis*, which are quite different from the iron-reducing bacteria (Gorby and Beveridge 2005). Subsequently, after examining these cultures in an MEC, the cells of the photosynthetic organism were able to produce electricity under carbon dioxide limited conditions, and in the presence of sunlight; but, however, not in the dark (Logan 2008).

There is also growing evidence of the potential for nanowire-mediated electron transfer between species. Gorby et al. (2006) experimentally determined this fact using scanning tunneling microscopy to monitor the quantum effects of electron transfer from *Pelotomaculum thermopropionicum* (via the thick pili-like conductive appendage it produces) unto a methanogenic bacteria *Methanothermobacter thermoautotrophicus.* In this form of association, the fermentative *P. thermopropionicum* releases electrons in order to regenerate intracellular NADH. These electrons are released in the form of hydrogen gas, and are transferred in an interspecies fashion from a nanowire producing organism to a nanowire non-producing organism. It is evident though that more information and research on this subject matter is needed. The figure below gives a representation of the use of nanowires by electroactive microbes (Fig. 13.3).

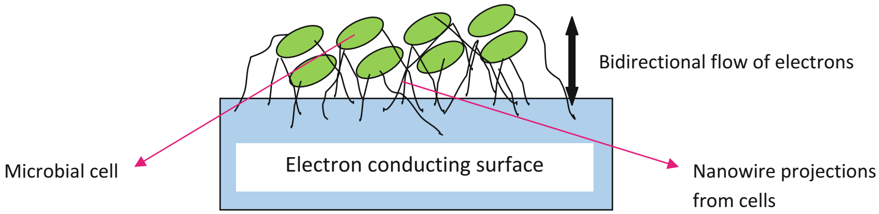

Fig. 13.3 Representation of conductive nanowire production by electroactive microbes

13.3.2 Cell Surface Electron Conveyance

Despite the possession of nanowires with conductive ability within electroactive species, there is still the possibility of electron transfer by direct cell-surface-electrode liaison without the activity of long wire-like conductors (Gorby et al. 2006; Logan 2008). Research conducted by Gorby et al. (2006) showed that at closer observations of micrographs showing *Shewanella oneidensis* cells attached unto an anode, there exist surface protrusions which could be points of conductivity.

This, however, still calls for further research as it is still not clear the protein based mechanisms the cells were using in their conductive activity (Gorby et al. 2006). Lower et al. (2001) had previously examined the nature of adhesiveness of anaerobically cultured *Shewanella oneidensis* unto an iron (goethite) surface in comparison with the aerobically grown cells. They observed that the anaerobically grown cells were two to five times more adhesive unto the iron surface than the aerobically grown cells, thus yielding the hypothesis that strains with more adhesive force can lead to a higher exocellular electron transfer because of the closer contact required by cell envelope bound cytochromes. Figure 13.4 below gives a hypothetical view to the route of direct cell electron transfer.

13.3.3 Mediators

In earlier researches on electroactivity, chemical mediators which served as electron shuttles were added into the reaction chambers so as to facilitate electron transfer by bacteria and even yeast (Kardi et al. 2016). Hernandez-Fernandez et al. (2015) had explained that several types of chemicals can be used as mediating substances to facilitate exocellular electron movement from the cell unto the electrodes exterior of the cell especially in non-electroactive or weakly electroactive species. Mediating

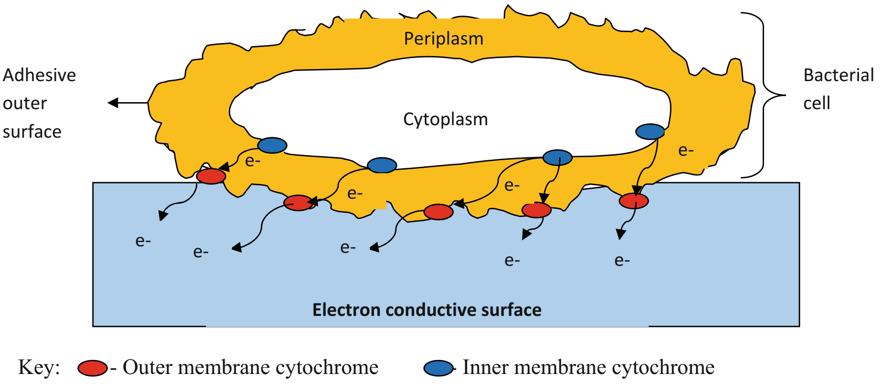

Fig. 13.4 Schematics of possible route of direct cell surface extracellular electron conveyance

chemicals that have been used include, for instance, anthraquinone-2-6, disulfonate (AQDS), potassium permanganate, neutral red, thionin, potassium ferricyanide, and methyl viologen, among others. Research conducted by Rabaey et al. (2004) had shown that cells can also produce their own endogenous mediators as evidenced by *Pseudomonas aeruginosa* which reportedly secreted electron mediating substances (phenazines) that enhanced its electrogenicity; however, this process as well as the mechanisms involved has not been fully understood as it is still uncertain if these mediating compounds are secreted for exocellular electron transfer or other purposes. Queries established by Hernandez et al. (2004) and Voggu et al. (2006) seem to be in line with the idea which stated that these compounds have been determined to have antibiotic properties, and thus, a key reason for their secretion may be to function in respiratory inhibition of competing microorganisms.

On a rather controversial note, Myers and Myers (2004); Newman and Kolter (2000) and Lies et al. (2005) had all suggested mediator production as a main route of *Shewanella* electron transfer, however, with the subsequent finding that *S. oneidensis* produces nanowires, doubts about the exogenously secreted mediator electron transfer system have been raised by Gorby and co-workers (Gorby et al. 2006); but they were quick to note that cellular damage could lead to the seepage of electron mediating compounds from the cell, but that it was a minute systematic route for electron transfer.

13.3.4 Electroactive Biofilms

In many cases, electroactive microbes employ specific proteins intracellularly located that link up with extracellular physiological organelles and metabolites in biomechanistic processes that favour electron seepage onto desired external surfaces so as to balance the electrochemistry of the cells (Astorga et al. 2019). It is obvious that for direct electron transfer (DET) to occur, there has to be sufficient cell contact with the conductive solid surface using a number of physiological organelles. This clearly distinguishes the process from mediated electron transfer (MET). Recent knowledge, however, claim to disrupt this fact thereby blurring the line between the defined roles of DET and MET mechanisms. An intermediary between DET and MET processes which involves the surface adsorption of MET redox substances like flavins on to the DET appendages, organelles, and cellular matrixes was observed (Edwards et al. 2017) thereby contributing overall to measured electroactivities. This therefore opened the discussion on the exact degree of contributions these physiological structures can confer to electroactivity of certain unique microbial species. In fact, there is the major uncertainty of specific definition of which electroactive mechanism is utilized by cells processing these important organelles. As evidenced by previous transcriptomics studies, the mere presence of these organelles does not directly imply that it is maximally utilized at the given time as the route for EET (Babauta et al. 2012; Ishii et al. 2015).

With the close associations and observed synergistic interplays between DET and MET mechanisms it is quite obvious that there needs to be adequate cellular contact and attachment on to the conductive surface for good electrochemical potentials to be observed. This void is comfortably filled by the classical understanding of the enormous roles electroactive biofilms have played in anodophilicity of specific microbial species. Microbial systems enmeshed in a complex web of exocellular matrixes attached unto living or inanimate surfaces have been referred to as biofilms (Kumar et al. 2016). Data from EET driven systems have drawn links to the understanding that electroactive biofilm yield can be directly proportional to the biofilm biomass (Parameswaran et al. 2013). Microbial fuel cells and other bioelectrochemical set-ups are positively charged and enable a good formation of electroactive biofilms thereby leading to an improved electron harvesting process. Reports are, however, quick to add that approaching certain levels of biofilm thickness, there can be a limitation in electron flow from the producing cells unto the electro-conductive surfaces thereby underscoring the need for a balance in biomass quantities and electroactivities for best optimal yields (Deval et al. 2017a, b; Logan et al. 2019).

Some works have directed their focuses on the roles played by the conductive surfaces as they affect the structure and form of biofilms growing on them. Properties like electrode polarization potentials, electrode surface area, electrode type, current limitation on electrodes, external resistors applied through the conductive surfaces among others, whether under electricigenic or electrotrophic conditions are important in determining the roles of the conducting surface (Babauta et al. 2012; Chandrasekhar and Mohan 2014; Liu et al. 2016). From the angle of the biotic components of biofilm, a number of factors have been linked to electroactive biofilm studies and the levels of elctroactivity measured within certain biofilms. These include microbial species type, electrcigenic or electrotrophic metabolic conditions, growth phase, and oxygen demand by the responsible organisms. The roles of extracellular polymeric substances (EPS) in electroactive biofilms are curently being researched upon. Li and Li (2014) demonstrates that EPS from *Shewanella oneidensis* and *Pseudomonas putida*—two important electrigenic bacteria were redox active. The EPS played a prominent role in the species-specific electroactivity observed in the two organisms. Using electrochemical, spectroelectrochemical, and proteomics approaches, they proved that the extracted EPS from the strains possessed heme-binding proteins which confered on the species their electroactive nature. They concluded that heme-binding proteins were majorly involved in the redox property exhibited by these species due to their ability to transport electrons between electroactive bacteria and external acceptors of electrons. They, however, surmised that only further experimentations will give clearer elucidation into the molecular as well as the biomechanisms of the roles played by EPS in electron transfer.

Tan et al. (2019) in a more recent work investigated the mechanistic basis for electron transfer features exhibited by EPS produced by electroactive bacteria using a combination of nanobiotechnoogy and surface-enhanced Raman spectroscopic technique. Their work was of value because of the dearth in adequate information

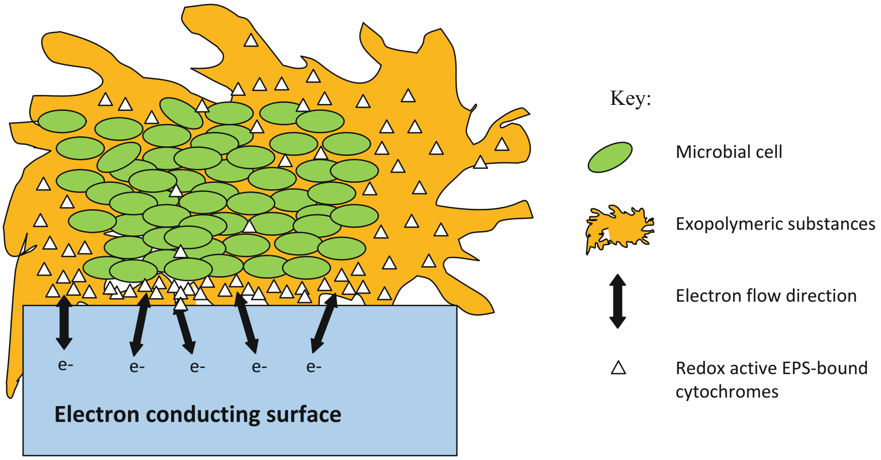

Fig. 13.5 Schematic representation of an electroactive biofilm showing EPS-bound cytochrome based redox activity

on electron transfer mechanisms taking place within EPS owing to its structural complexity and the lack of clarity on defined routes for species-specific EET processes. With the aid of Nickel and Silver nanoparticles, Tan et al. (2019) were able to magnify the ability for electron transfer between the EPS and the metal nanoparticles leading to detectable alterations in Raman signals thereby demonstrating EPS electroactivity in a redox manner. With further comparative electrochemical analyses, there were proven differences in redox capabilities between non-electroactive and electroactive strains. Biomechanistically it was concluded that the pophyrin present in cytochromes c functioned as the pioneer redox molecule within the EPS of electroactive bacteria (Fig. 13.5). Futher investigations are, however, sought to give clearer pictures of this sublime phenomena.

13.4 Bioenergetics and Electrophysiological Biomechanisms of Electricigenicity and Electrotrophy

Electrotrophy and electricigenicity as the case may be are naturally occurring processes within specific ecosystems, and despite the fact that they may seem to be thermodynamically opposite, they are quite similar in functionality. There is a major link between anaerobiasis/electicigenicity on the anodes and lithotrophy/electrotrophy on the cathodes as regards electricigenic and electrotrophic microbial systems, respectively (Logan 2008; Kumar et al. 2016). For increased functionality of electroactivity, the electrode potentials are involved in an important role in driving cellular energetic responses elicited. Kumar et al. (2016) indicated that the electrode potential of a certain bacterium is a direct indication of the property of statutory protein or enzyme or any other cellular component responsible for electron transfer. This, however, does not stand in all cases as depending on the electrode potential; electroactive microbes have abilities to switch their physiologies and respiratory pathways. The variability clearly means that a bacterium may possess more than a single route for extracellular electron interactions through its membrane and will not hesitate to employ different routes if given the corresponding stimulus in the form of electrode potential (Zhu et al. 2012).

13.4.1 Biomechanisms of Electricigenicity

The generally known electricigenic mechanisms are inherent in the most studied organisms—*Geobacter* and *Shewanella* species. Elucidated biomechanistic process of exocellular electron exportation involves the use of direct cell-extracellular surface contact as well as mediated transfer using electron shuttle molecules (Marsili et al. 2008a, b). The presences of redox active compounds bound within the cells envelope (used in direct electron transfer) or secreted extracellularly (used in mediated electron transfer) are the key responsible factors that convey electrons unto the external acceptors. Kumar et al. (2016) explained that these compounds and molecules act by utilizing the available difference in energy between electron acceptor and donor thereby causing ionic gradient formation across the cell membrane, which subsequently propels the generation of energy by converting the electrical potential difference into ATP. According to Liu et al. (2014), working with *Geobacter sulfurreducens* PCA led to the identification of the probable mediatory roles of a set of proteins in extracellular electron transfer using the cell membrane as a conduit. These proteins form a co-functional aggregated unit with is referred to as trans-outer membrane porin-cytochrome complex (Pcc). The Pcc complex is made up of the main cytochrome (OmcB) which sits on the outer membrane and is most pivotal in the interactions with the other component units of electron transfer protein system. The Pcc interacts with other cytochromes—a periplasmic c-type cytochrome, an outer membrane-based c-type cytochrome, an outer membrane protein, and a transcriptional factor all working in unison for an effective extracellular electron transfer (Liu et al. 2014).

One common fact in *Geobacter* species electricigenicity, however, is the consistent utilization of c-type cytochromes, as a good number of these cytochromes have been employed. The challenge of deciphering the actual electrophysiological functions and roles of some of the c-type cytochrome still persist, as only OmcB and OmcZ among the plethora of possibly utilized cytochromes have clearly elucidated functionalities with the role of others still largely unknown (Kumar et al. 2016). It was also noted that with respect to direct electron transfer in *Geobacter*, there exists interplay between cytochromes and cell surface pili used in exocellular electron exportation within a biofilm structure. This is usually dependent on the distance between the electricigenic cells and the electrode or electron acceptor within the biofilm layers, as the cells near the electrode use only the cytochromes while the farther cells utilize a fusion of activities between the pili and the cytochromes to reach the electrodes (Logan et al. 2019). The role of cytochromes in electricigenic species like *Desulfovibrio alaskensis* and *Thermincola potens* have also been x-rayed with multiheme cytochromes and transmembrane complexes responsible for periplasmic, and subsequent exocellular electron release (Wrighton et al. 2011; Keller et al. 2014).

Other physiological properties like the flagellin protein encoded for by the *fliC* gene and the type IV pili encoded for by the *pilA* gene functioned in Fe (III) oxide reduction and electricigenicity as proven by gene deletion analyses in *G. metallireducens* (Tremblay et al. 2011). Surface charge modification especially by *G. sulfurreducens* was mediatory for the proper binding of the cells unto the electrodes with the requisite anchoring mechanism needed for cytochrome activity thereby leading to effective electricigenicity. This was made possible by the action of genes *xapA* and *xapK* encoding extracellular polysaccharides necessary for the cytochrome to bind effectively to the electrodes (Rollefson et al. 2011). Nanowires have also been proven to be responsible for electricigenic phenotypes observed in specific bacteria. Marsili et al. (2008a, b) explained that direct electron transfer is as a result of the presence of surface extending nanowires and physical connection with the electrode by biofilm formation. There is, however, a basic difference between *Geobacter* nanowires and *Shewanella* nanowires, as *Geobacter* nanowires are regarded as type IV pili, while *Shewanella* nanowires have similar structure with outer membrane vesicles as they can be observed as protruding parts of the outer membrane and periplasm (Pirbadian et al. 2014).

The presences of more than one type of electricigenic microorganism in mixed cultures of *G. sulfurreducens* and *G. metallireducens* show that there have been synergies in their electron transfer systems, as they exhibited interspecies electrical interactions with the use of pili within their cell mixtures (Chen et al. 2014). Electrical connections have also been observed between mixed cultures of *Methanosaeta harundinacea* and *G. metallireducens* and in a number of other cell–cell based interactions, as this was utilized in extracellular electron exportation based on unique physiological demands (Rotaru et al. 2014) (Fig. 13.6).

13.4.2 Biomechanisms of Electrotrophy

With respect to electrotrophy, it was determined that there was a direct link between the ability for microorganisms to obtain electrons and carbon from inorganic sources, a process contrasting from phototrophic processes of certain microorganisms that allow them to harness energy from sunlight in the synthesis of organic molecules (Kracke et al. 2015).

With the understanding that there is usually a bioenergetics-based coupled reaction for redox potentials of spatially segregated individual components of the redox pair, the extracellular interactions of electrons with microbial cells and solid inorganics or metals with conductive and semi-conductive properties, have led to the

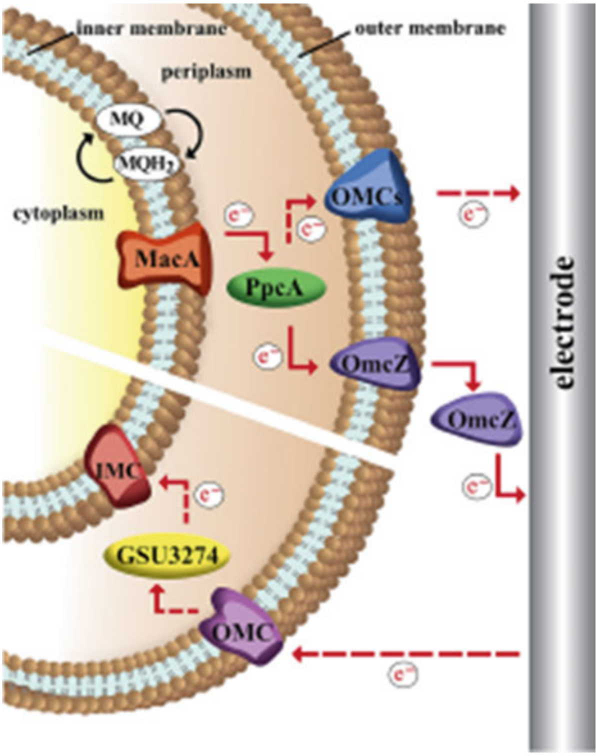

Fig. 13.6 Schematic representation showing the inner membrane cytochromes (IMC) and outer membrane cytochromes (OMCs) utilized in modulated forms during defined electricigenic and possible electrotrophic modes of *Geobacter* sp. electrophysiology. Electron flow can be modulated by the defined interactions between menaquinone (MQ) and the cytochromes. Hypothetical mechanistic flow is represented by dashed arrows while experimentally proven electron flow is in solid arrows (Source: Kracke et al. 2015)

determination of electrolithotrophy as a unique microbial nutrition form (Kim and Gadd 2008). Using a Gram negative iron (II)-oxidizing bacteria; *Acidithiobacillus ferrooxidans* as an example, the direct assimilation of electrons from a solid electron source was demonstrated by Ishii et al. (2015). *A. ferrooxidans* possesses a startling physiology as it is one of the rare microorganisms with the ability to acquire energy by oxidizing ferrous iron present within low pH environments. With the aid of the extreme acidic pH the bacteria has evolved a system of reversing electron flow from Fe(II) to NADH thereby according to its chemolithoautotrophic and electrolithotrophic status (Brasseur et al. 2004a, b; Bruscella et al. 2007; Ishii et al. 2015). Reports by Ishii et al. (2015) explained that it was observed that the organism had a switch mechanism that allows it to change the source of external electron importation from diffusible Fe^{2+} to solid inert conductive surfaces. In fact *A. ferrooxidans* was able to thrive in the presence of electric currents as the only source of energy and electrons; these electrons were used in series of activities leading to generation of proton motive force, and the functional fixation of CO_2 under stringent bioenergetic conditions of electrotrophy.

Elucidating mechanisms of electron assimilation by *A. ferrooxidans* showed the probable involvement of biosynthesized redox molecules, however, the assimilation was dependent on electrode potential applied, as the cells' growth were reduced under an open circuit condition—this proved that the endergonic form of electron transfer reaction was responsible for proton motive force generation through reduction of intracellular oxygen, as well as CO_2 fixation (Ishii et al. 2015). There are stark similarities between this mechanism and the activities of self-synthesized flavins acting as attached cofactors coupled to outer membrane c-type cytochromes in *Shewanella oneidensis* and *Geobacter sulfurreducens* (Okamoto et al. 2013, 2014). A further analyses of the *A. ferrooxidans* system revealed that reductive effects of the species on Fe (II) was linked to the electrophysiological functionalities of the Type IV pili which possessed two functional pilin protein parts—adhesin domain PilV and major domain PilW; and they worked in synergy with C-cytochromes to aid in the mobility of the electrons (Li and Li 2014). From a superficial view, it may be seen that this technique resembles a mediated electron assimilation process rather than a direct form of electrotrophy due to the use of flavins cofactors; however, a closer observation notes that cell-based activities were made possible because of the fused interactions between the surface pili and flavins rather than the diffusible mode of flavins as secreted mediators.

Another electrotrophic mechanism was studied in the Gram negative bacteria *Shewanella oneidensis* which utilized the versatility of c-type cytochromes and the Mtr pathway (much similar to the Omc system in *Geobacter* species) creating a proton gradient. A particular use of this system was observed when *S. oneidensis* acted in reducing fumarate to succinate with the aid of directly attached electrotrophic biofilms (Ross et al. 2011). Studies done by Nevin et al. (2010) characterized the electrotrophic systems of two Gram negative bacteria *Sporomusa ovata* as they determined its utilization potentials of electrode-supplied electrons in the reduction of CO_2 to acetate and butyrate. The electrode-attached cellular activities led to a coupled ATPase-driven bioenergetic process of H+ gradient generation and subsequent electrolithotrophic assimilation of electrons supplied with the aid of membrane-bound cytochromes *b*, *c*, *d* and quinones to aid in the bioelectrosynthesis of target compounds. With respect to Gram positive bacterial electrotrophic systems, Sasaki et al. (2014) targeted *Corynebacterium glutamicum* process of lactic acid production with electrotrophic assimilation occurring at the cathodic chamber of the microbial electrochemical cell, thereby exposing interactions between membrane-bound cytochromes *a*, *b*, *c*, *d*, and bound flavins as well as quinones in the generation of ATP using the H^+-ATPase activity as well as dehydrogenase.

Further expounding the Gram positive bacterial electrotrophic system with *Clostridium ljungdahlii* biomechanistic elucidations as an example, an electron bifurcation process was shown. This process was aided by ferredoxin reduction linked to H^+ gradient generation using membrane-bound Ferredoxin: NAD^+-oxidoreductase complex leading to the generation of ATP. With this mechanism, there was a significant electrotrophic consumption of electrode-supplied electrons for CO_2 reduction to acetate (Logan 2009). It was, however, worthy of note that cytochromes and quinones were excluded in this process, thereby making it quite unique. Another Gram positive-based cytochrome-excluded electrotrophic system was observed in production of acetate and hydrogen from CO_2 by a community of bacteria dominated by *Acetobacterium woodii*. The electron bifurcating process of ferredoxin reduction was reported in this case with the aid of sodium ion gradients through membrane-

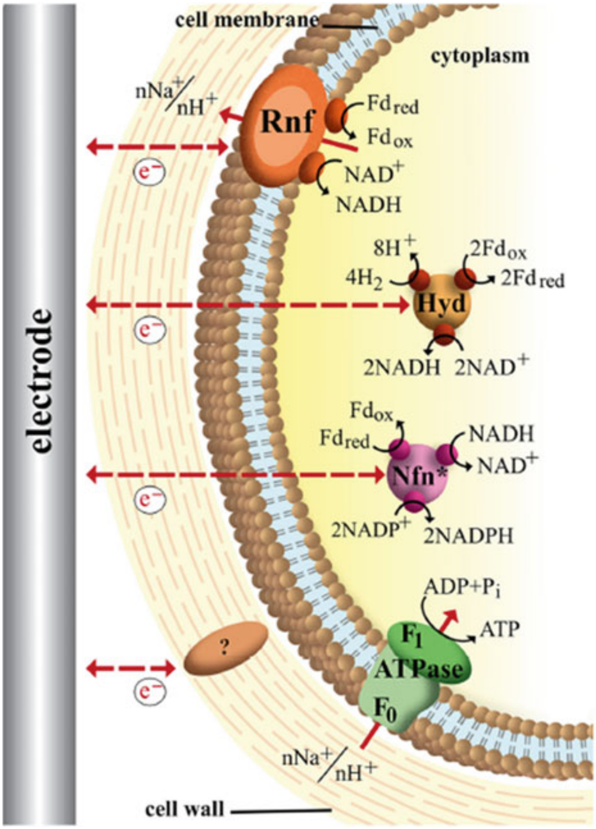

Fig. 13.7 Schematic representation of biomechanistic flow of electrons under electrotrophic modes in *Clostridium ljungdahlii* and *Acetobacterium woodii*, showing complexes of Ferredoxin-oxidoreductase (Rnf-complex) and soluble complexes capable of electron bifurcation. *C. ljungdahlii* electron transport was in the form of H^+ while *A. woodii* utilized Na^+ in electron transfer. (Source: Kracke et al. 2015)

bound ferredoxin-oxidoreductase complexes thereby generating ATP (Marshall et al. 2012) (Fig. 13.7).

13.5 Bioprospecting for Electroactive Microbial Species with Respect to Ecological Niches

Considering the vast majority of microbial species that exist within the potentiality of these determined physiological properties, it is rather baffling to note that only two species of microbes (especially bacteria) have been extensively studied as electroactive species—*Geobacter* spp and *Shewanella* spp. Doyle and Marsili (2018) clearly stated the lopsidedness in research focus on these two organisms as against the potential presence of a vast array of electroactive species. So far, *Geobacter* and *Shewanella* species' electroactivity are the strongest ever discovered in nature, hence the plethora of works that focused on them. Extensive research has focused on identification of electroactive species from anaerobic subsurface environments majorly because they are characterized by reduced redox potentials and an inadequacy in nutrient concentration levels. This subsequently drives the birthing of metabolic pathways within these species highly specialized in metal-reduction as the main activity. Despite this, there is increasing experimental evidence that the term electricigens or electrotrophs is not ascribed to any special taxonomic group of microorganisms, but can be easily spread over many microbial groups/families (Kracke et al. 2015). This automatically brings to the fore the fact that the diverse presences of these organisms within a variety of environments can show that electroactive microbial species can dwell as a taxonomically differentiated species with various environmental preferences. Environments like wastewaters have been implicated as target ecological sites harbouring electroactive species because of the original technological demand of energy generation from waste matter using the processes of applied bioelectrochemistry of electroactive species (Logan 2008). Doyle and Marsili (2018) corroborated recent research exploits which demonstrated that diversity of electroactive species may be much more robust than what has been discovered till date. In fact they proposed that many common microbial species quite non-obvious to experimenters due to our earlier methods of probing microbial physiology may be utilizing extracellular electron transfer in a bid to survive under challenging and difficult environmental conditions. It was, however, added that the relevance of some of the organisms have not been fully deciphered and characterized. The propinquity to discover more electroactive microbes thereby increasing the pool of possibly applied electroactive species in electrobiotechnology, lies in the incorporation of more physiological factors in the biopropecting design (Kumar et al. 2016). Factors like pH, temperature, salinity, and atmospheric pressure should be considered when selecting ecological niches for bioprospecting for electroactive species. Ecological sites with extreme values for the above-mentioned physiological parameters when searched, may lead to the discovery of novel electroactive species, novel mechanisms as well as novel redox compounds from microbes that can be applied in bioprocesses (Doyle and Marsili 2015). It was further explained that above all, non-wastewater environmental samples like marine sediments, soils, and freshwater samples have potentials to serve as good points for electroactive microbial species.

Extensive microbial community profiling of such sites has the potentials to uncover hitherto unknown electroactive species. Such community analyses in the form of metagenomics and metatranscriptomics have been proposed to augment the current culture—based techniques in searching for novel strains (Ishii et al. 2013; Wong et al. 2014; Varrone et al. 2014). In depth analyses with specific activities like metabolomics and proteomics will also aid in the discovery of novel biomechanistic systems the electroactive microbes will utilize in exocellular electron importation and exportation (Ishii et al. 2013). Potentiostatic conditioning of electrodes in a defined bioelectrochemical set-up has been defined as a means of providing a selective pressure for the electroactive microbes to colonize the electrode so as to induce the growth of more of the electroactive microbes among the pool of microbes in an environmental sample (Doyle and Marsili 2015). This idea was earlier proposed by Liu et al. (2008) with the focus on cultivating electroactive biofilms of bacteria on an electrode in an enrichment format. Using a potentiostat and special arrangements of electrodes, specific potentials were applied unto the electrodes and the requisite biofilm based on the potentials applied were formed (Bard and Faulkner 2001). This clarified the fact that microbes possessed surface charges and specific mechanisms for exocellular electron mobility and would equally respond to a surface charge similar to the native surface charge thereby inducing their direct development as a clog of biofilm on surfaces (Pierra et al. 2015).

Effective bioprospecting for electroactive species involves the use of electroanalytical techniques like voltammetry, electrochemical impedance chronoamperometry, and chronocoulometry among others as viable techniques needed for defined analyses of enriched or naturally occurring electroactive species within samples from any environmental site. This is so because they are directly utilized in: monitoring electroactive biofilm conductivity, determining current generation monitoring, specific detection of novel electrochemical mechanisms utilized by electroactive microbes, and characterization of electroactive microbial species and communities (Yuan et al. 2011; Carmona-Martínez et al. 2013).

13.6 Specific Electrophysiological Techniques Adopted in Studying Electrotrophic and Electricigenic Behaviour in Microbial Species

The mechanistic determination of electrode reactivity underpins redox reactions that lead to better insights on the electroactive potentials of microorganisms. This can be achieved using a range of electrochemical techniques.

13.6.1 Cyclic Voltammetry (CV)

This is one of the most frequently used and direct means of electrophysiological characterization of electroactive species. Cyclic voltammetry utilizes a triple-electrode system/configuration of working electrode, counter electrode, and reference electrodes targeted at obtaining maximum and efficient redox read-outs. These are usually placed in an electrochemical chamber and connected to a potentiostat/electrochemical work station. Cyclic voltammetry can be employed to decipher the functionality of mediating substances as well as to define the oxidation-reduction potentials of important active components (Rabaey et al. 2005). Further value of this technique can lie in its importance in the examination of the electrochemistry of microbial strains in single cells or consortium (Niessen et al. 2004), and also for the characterization of the properties of test electrode materials (Zhao et al. 2005).

In the normal operation of a cyclic voltammetry system a potentiostat is used in poising the potentials to be applied through the electrodes. During the conduction of experiments, the reference electrode is positioned next to the working electrode (anode for electricigens; cathode for electrotrophs) and appropriate voltage scans (in V/s) are applied bi-directionally (forward/backward). By this method of scanning, cyclic voltammetry can aid in determining the functional presence of microbially synthesized electron mediators (Rabaey et al. 2004).

CV analysis of the bioelectrochemical set-up is meant to be conducted to analytically ascertain the level of production of mediating substance by microbes in broth/suspension, and the redox potentials of the mediators. Logan (2008) proposed that cyclic voltammetric analyses should be carried out on: (1) the substrate in the reactor before microbial activity; (2) the utilized substrate after complete assimilation by microorganisms; (3) centrifuged microbial and washed culture, resuspended in physiological solution devoid of substrate; and (4) suspension lacking cells drawn from the supernatant after centrifugation. It was further explained observation of redox peaks in any of the first three cases could indicate the presence of a cell membrane associated mediator, while the peak in the substrate alone shows that the substrate or something within the medium is responsible for the electrogenicity as it is acting as a mediator. In execution of CV analyses, it is important to use a sterile mineral salt medium with no extra addition of redox indicators. This is because the presence of redox indicators could act as mediators and therefore should exclude from the media as they may cause interference in the voltammetric readings. L-cysteine can be utilized as a dissolved oxygen scavenger. There are also reports of its identification as a possible electron shuttle/vector under certain defined parameters (Doong and Schink 2002). However, Logan et al. (2005) remarked that the biodegradability of cysteine can make it thus serve as a nutrient source and electron donor to be utilized by some unique bacteria. Iron can also be used as a mediator as it undergoes a redox cycle from Fe(III) to Fe(II) state by reductive bacterial activity, but then re-oxidizes at the cathodic electrode (or membrane) via the reaction with oxygen, and in some cases with Potassium ferricyanide employed as a mediator (Bond et al. 2002; Emde et al. 1989).

Biosynthetic/naturally produced mediators are a major source of shuttle-dependent electroactivity, however, there is a major speculation about the roles and activities of these mediators, creating the need for additional work in enhancing our knowledge of electron mobility and mediator transport within microbial electrochemical reactor set-ups (Fig. 13.8).

13.6.2 Differential Pulse Voltammetry (DPV)

The DPV technique can be employed in the assessment of enhanced discrimination of Faradaic currents which are mostly currents accruing from the transfer of electrons back and forth an electrode. In this case, the potential variability consists of a wave like superimposition of small pulses. According to Marsili et al. (2008a, b)

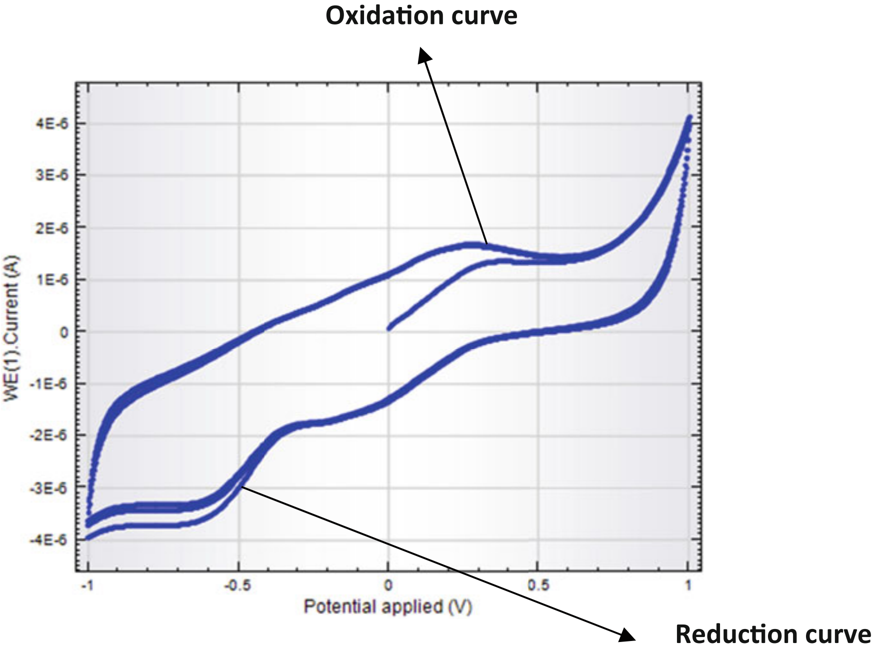

Fig. 13.8 A typical cyclic votammogram of an electroactive biofilm

differential pulse voltammetry measurements using carbon electrodes colonized by *Shewanella* sp in a growth medium, showed an adsorption of flavins on the electrodes with them functioning as acceptors of external electron transfer. Compared to CV, DPV is preferred because of its ease of interpretation and ability to detect lower concentrations of electroactive compounds by optimizing capacitive and faradaic currents, thus making it easy to study weak redox peaks (Zhang et al. 2017).

13.6.3 Chronoamperometry (CA)

CA is another technique for studying microbial electrophysiology, and it involves the control of the potential of electrode as it is held in a constant value while the current produced is measured with respect to time. A work by Bond and Lovley (2003) involved this technique while working with *Geobacter sulfurreducens* and acetate as the substrate. The electrode (graphite) was held at an oxidizing potential of +200 mV versus Silver/Silver chloride reference electrode. They inoculated a bioelectrochemical chamber possessing acetate as the electron donor, with an electrode kept poised at an oxidizing potential of +200 mV versus Ag/AgCl. It was observed at the end of the experiment that the mechanism involved was a direct electron transfer from the cells on the electrode. This was ascertained by a replacement of the medium with a buffer that lacks nutrients. Another important fact is that anode potentials can be used to regulate microbial growth rate and anodic biofilm formation. A study was done with three microbial electrochemical reactors fed continually with acetate and a poising of their anode potentials at 0, −200, and −400 mV versus standard Silver/Silver Chloride electrode. It was determined that the optimal anode potential for microbial growth was at −200 mV (Aelterman et al. 2008).

13.6.4 Chronopotentiometry (CP)

In the case of CP, the current at the electrode is poised at a constant value, while the potential generated is measured in relation to time. This is a direct opposite of the Chronoamperometry. Cheng et al. (2006) conducted a study on this technique where they investigated the effect of polytetrafluoroethylene cathodes on an air cathode microbial electrochemical reactor. A constant current was applied and the electrode potential was measured, which were then plotted to ascertain the functionality of the electrode.

13.6.5 Combined Electrochemistry–Spectroscopy

Working with *Geobacter sulfurreducens* on gold electrodes, Busalmen et al. (2008) researched into the use of spectroelectrochemical techniques to enhance the knowledge of microbe-electrode interactions, molecular structures as well as electron transfer mechanisms in vivo. The direct application of subtractive Fourier transform Infrared (IR) spectroscopy and enhanced infrared absorption spectroscopy gives a better perspective on in vivo understanding of molecular structures utilized in mechanisms of electron transfer, and the interactions between microbes and electrodes. From the IR spectrum, the experiment showed the functionality of cell wall attached proteins (most probably extracellular cytochromes) in the transfer of electrons unto the electrode. There are also reports on the application of Raman spectroscopy as an analytical technique to monitor EET. Raman spectroscopy has proved to be non-destructive and is regarded to have good reliability and feasibility in EET studies (Janissen et al. 2015; Tan et al. 2019).

13.6.6 Electron Microscopy

The use of indirect techniques like high resolution microscopy (scanning electron/tunneling microscopy) has been utilized in the study of electrophysiological interactions between microorganisms and the electrodes (Logan 2008). Despite the fact that they are not directly electrochemical techniques, they have provided very complementary information on microbe-electrode interactions. The techniques have also helped in elucidating the functions of extracellular appendages on the cell wall to electron generation. Reguera et al. (2005) used this method to detect electro-conductive pili also regarded as 'nanowires' in *Geobacter* species, and this heralded the convictions of long-range direct electron transfer between bacteria and electrodes. This knowledge of nanowire-mediated electron transfer is still in its infancy as the mechanisms of interaction between nanowire-associated proteins and the electrode surface as they make contact is still not well understood. Xie et al. (2010) earlier developed a method of combining microscopy with electrochemical experimentation for the study of proton exchange channels, and this can be adapted into the microbial electrochemical cell systems for bioelectricity generation as shown by a lot of recent research works on electricigenicity.

13.6.7 Simple Time-Based Current–Voltage Monitoring and Measurements

Simplistically speaking, the use of basic voltage/current versus time measurements has been used to get time-based values on voltage profiles, acclimation times, effects of electrolytes, effects of bacterial growth on electrogenicity, effects of substrate addition or exhaustion and subsequent electricity generation (Kim et al. 2005; Ajunwa et al. 2018). This technique does not necessarily need the poising of either current or voltage, and can be easily adapted in laboratory settings, using simple bioelectrochemical reactors and multimeters for measurement.

13.7 General Outlook

With more defined physiology of electricigens and electrotrophs, more and more applications of these species are springing forth. The basic concepts of understanding of the principles and biomechanisms governing the processes are, however, the focal points in recent research. For instance, there are new evidences of electricigenic properties within the human gut microbiota (Naradasu et al. 2019) as well as proofs of electroactivity of potentially pathogenic species like *Klebsiella pneumonia* (Deng et al. 2010) and *Listeria monocytogenes* (Light et al. 2018), thus indicating the possible implications of microbial electroactivity in some infectious diseases. Clearer understanding of electrophysiological mechanisms of such systems can shed light on the possible correlations between virulence and antibiotic resistance of such species.

Further applications of microbial electricigenicity and electrotrophy can be tailored into areas such as electrophysiological elucidation of microbial infection pathogenesis, biomining, bioelectronics, biosensors, and electrofermentative production of novel microbially synthesized biopolymers and commodity chemicals. Potentials of microbial electrophysiology; with respect to electricigenic and electrotrophic species seem to be expansive and can be harnessed with deeper research exploits.

13.8 Conclusion

It is evident from research that microbial species are involved in a plethora of activities; with electricigenicity and electrotrophy being highly specialized and unique examples. The ecological significances of electricigenic and electrotrophic species are still being understudied. Applications of these electrophysiological concepts, however, range from energy generation in the form of bioelectricity to bioremediation, electrofermentation, bioelectrosynthesis, and biodetection of cells and molecules. Newer concepts of application, however, are being sought and developed. It is therefore pertinent to involve a more radical approach in the electrophysiological characterization of novel electrotrophic and electricigenic microbial species.

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