Prasun Kumar
Chandrasekhar Kuppam *Editors*

Bioelectrochemical Systems

Vol.2 Current and Emerging Applications

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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.

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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, Electro-fermentation technology, Biofuels and biochemical production from waste biomass, valorizing lignocellulosic biowastes, and waste remediation technologies including bio-electrochemical 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
Photosynthetic Microbial Fuel Cells: From Fundamental to Potential Applications

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

Abstract In photosynthetic microbial fuel cell (MFC), algae and photosynthetic bacteria undergo photosynthesis to generate electricity by harnessing the solar energy. The microorganisms on absorbing solar energy initiate a series of reactions to generate protons (H^+ ions), electron, and oxygen through splitting of water. The energy from these reaction series is harnessed by placing photosynthetic organisms in anodic chamber separated from cathodic chamber by a semipermeable membrane selective for hydrogen ions. The electrons generated in an anodic chamber by photosynthetic activity of microbes travel through an outer circuit to the cathodic chamber, where they combine with protons and oxygen at the reductive electrode (cathode) to generate water. This technology has huge potential for converting solar energy into electrical energy and might also help to reduce the carbon footprint. The chapter discusses the concept, fundamentals, process design and operation of photosynthetic MFC. Furthermore, the role of photosynthetic organisms in MFC, various bottlenecks faced by MFC systems and their potential applications are also outlined in the chapter.

Keywords Algae · MFC · Photosynthesis · Photosynthetic microbial fuel cells · Solar energy · Water splitting

1.1 Introduction

Sun is the major and primitive source of energy. It supports directly and indirectly the existence of all forms of life on the planet earth. Solar energy, being an inexhaustible and renewable source of energy can play a vital role in the sustainable development through a minimum carbon footprint. Furthermore, on the scale, over the energy return on investment (EROI), solar energy possesses an advantage with

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4% EROI over the coal-fired power station having 11% EORI (Pehl et al. 2017). Studies show that earth's annual potential solar energy ranges from 1575 to 49837 exajoule (EJ) (UNDP 2000). Tapping the solar energy would solve the problems of energy demand that is showing a dramatic increase due to population explosion, industrialization, and urbanization. Overexploitation and human population explosion have laid a tremendous pressure and stress on natural resources mainly on energy and water. Henceforth, water and energy are the major concerns around the globe in recent times. Due to swift urbanization, industrialization and population explosion many scientific studies and economic reports convey that the energy demand of the world has been increasing annually since the late twentieth century (Chang and Brada 2006). Nearly 86% of the current energy demand are met through fossil fuels and thus resulting in its depletion (Chang 2001; Chang et al. 2004). Fossil fuel depletion and increasing energy demand have necessitated the need to explore alternate energy sources especially bioenergy. Throughout the globe these non-conventional energy resources are promoted through technology development for sustainable development. The search of new technology has resulted in the development of bioelectrochemical systems (BES) to harvest energy from the organic portion of wastewater to overcome the problems of energy crisis, water scarcity, and wastewater treatment.

Bioelectrochemical systems (BESs) are among the sustainable technologies that can generate energy through wastewater treatment. Among the different bioelectrochemical systems, MFCs are popularly explored for the past two decades. MFCs are dual chamber bioelectrical device that possess an anodic chamber with a bioanode (electrode with microorganisms) and anolyte (feed), a cathodic chamber comprising of an electrode and catholyte separated by proton exchange membrane (PEM) which is mostly "Nafion" (Hai et al. 2007; Fornero et al. 2008). Electrons generated through the microbial oxidation of the substrate are directed towards the cathode chamber via an electric circuit, while the protons pass through Nafion membrane into the cathode chamber to combine with electron and oxygen to form water (Huang and Logan 2008; Yoshizawa et al. 2014; Yong et al. 2014). Till date, MFCs are explored for the treatment of varied types of substrates and reported to show energy generation of 114 mW/m^2 to 4920 mW/m^3 (Durruty et al. 2012; Kracke et al. 2015) with an overall chemical oxygen demand (COD) reduction of 85% (Dong et al. 2015).

1.2 Microbial Fuel Cells

Traditionally MFCs were either H-type dual chambered reactor with PEM membrane or single chambered cubic/cylindrical reactors with an air cathode. These configurations were simple yet utilized expensive catalyst and chemical mediators (Liu and Logan 2004; Rabaey et al. 2004; Logan et al. 2006). The consistent research and innovations have led to the development of advanced types of MFCs to tackle with different shortcomings of the system. These MFCs, based on constructional

variations, are termed as flat plate MFC, continuous tubular packed bed MFC, membrane-less MFC, stacked MFC, photosynthetic/bio-solar MFC, origami star inspired fuel cell, and 3-D paper based MFCs. A brief discussion about each design is outlined in the following section.

In flat plate MFCs, the wastewater or the anolyte follows a serpentine path. In this reactor to reduce the internal resistance, membranes are positioned in between the electrodes. The geometric surface area of the membrane will increase when positioned vertically. Electrodes placed close to each other limits the growth of electrogenic bacteria on opposite side of the anode. In flat plate plant microbial cells, flat plate configuration increases the geometric planting surface area to facilitate increased planting. The flat plate MFC is weather independent, cost effective and it can be constructed at any place where plants can grow. However, the power output of this technology is very low (Helder et al. 2010).

As name suggests, continuous tubular packed bed MFCs are tubular reactor with innovative cathode and anode arrangement to increase the current and power density. They are operated under continuous mode. In most of the continuous tubular packed bed MFCs, ferricyanide and oxygen are used as catholyte. Studies show that ferricyanide shows better results when used along with reticulated vitreous carbon electrode in packed bed reactors formed out of granular activated carbon (He et al. 2006). Ferricyanide is generally not recommended for certain environmental reasons. Membrane-less MFCs were developed to bring down the overall cost by eliminating polyelectrolyte membrane (PEM) and expensive catalysts. It is designed in a way as to ensure effective separation of anode and cathode and transfer of electrons. Glass wool and glass beads are also used as an alternative to PEM (Narayanan et al. 2012). A membrane-less MFC using real wastewater was shown to achieve power density of around 10 mW/m^2 (Ghangrekar and Shinde 2007). Other low-cost options were also tried as an alternative for PEM.

Stacked MFCs comprised of a series of multiple cells assembled and stacked to collect enough energy to operate any gadget. The power output in an MFC reactor is much less compared to other popular power supply systems. But when multiple MFCs are stacked, the power output increases radically. A system comprising six MFCs operated with ferricyanide, stacked in series through copper wire could produce power density of 51 W/m^3 and when same reactors were assembled in parallel, the power density was recorded to be 59 W/m^3 (Aelterman et al. 2006). The major problem faced in this reactor is of voltage reversal arising while obtaining higher voltage output.

Origami star MFC is inspired by a Japanese technique of paper folding known as "origami." The reactor can be folded to form a star with an inlet at the center. When the solution containing bacteria is allowed through the inlet, the cell opens into Frisbee ring structure (Fraiwan et al. 2016). The ring consists of eight reactor modules, which are self-sufficient and work independently. Each cell comprised of an anode and cathode separated by PEM and the cathodes are exposed to the oxygen when the reactor is in open condition. The power output of this reactor is very low (in microwatts) but it can practically work as biosensors. The 3-D paper MFC is superior in designing and innovative in sustainability. Here capillary force is used to

direct the liquid through the MFC. This system does not require any external power source and it produced power density of nearly 25 W/m^3 (Hashemi et al. 2016).

Recently, photosynthetic microbes have been utilized in bioelectrochemical systems to develop a hybridized system which is self-sustaining in nature (Chaturvedi and Verma 2016). The microbes that form biofilm on the anode work in synergy with the photosynthetic community. The organic matter and the oxygen need of heterotrophic bacteria are fulfilled by the photosynthetic microbes, while the metabolic by-products (carbon dioxide and water molecules) of the heterotrophic bacteria are utilized by the photosynthetic community for the process of photosynthesis. The architecture of these kinds of reactors is such that the anode chamber is protruded and the electrode is sandwiched (Mohanakrishna et al. 2015). It has gained much popularity because of promising yield and cleaner energy. The upcoming sections will extensively portray mechanism and working principle of photosynthetic MFC, plant MFC, different microorganisms involved in the process and its biochemistry.

1.3 Photosynthetic Microbial Fuel Cells

Photosynthetic microbial fuel cells (PMFCs) are a type of microbial fuel cells (MFCs) that utilize solar energy for its operations. Living organisms like algae, plants, and certain bacteria possess natural potential to trap solar energy by the process of photosynthesis. When these living photoautotrophic organisms are inoculated into the PMFCs, they convert solar energy directly into electric energy (Deng et al. 2012). The PMFCs on their system assembly front are very similar to the conventional MFCs and consist of dual chamber assembly separated by the proton exchange membrane. The chambers possess their respective electrodes made up of similar or different materials. Carbon, graphite, platinum, nickel, germanium, titanium are some materials used as electrodes, due to cost-effectiveness and bio-friendly nature (Santoro et al. 2017). Carbon among all these materials is most favorable and used in various forms like carbon cloth, carbon fiber, carbon brush, carbon rods, and carbon plates. These chambers are connected with each other via an outer circuit by which electron flows from anodic chambers to the cathodic chamber where it combines with protons that comes across the PEM and completes the reaction. The main feature that differentiates PMFCs from MFCs is the use of photoautotrophic organisms. These organisms are preferably used in cathodic chamber where they release oxygen as a result of photosynthesis. Oxygen could further serve as the electron acceptors. The photoautotrophic organisms like *Cyanobacteria* and *Heliobacteria* are used in anodic chamber and they are easily compatible with the carbon electrodes without any modifications (Strik et al. 2011). Other conventional MFCs generally operate in a dark anaerobic condition which leads to the fermentation process in the anodic chamber. The redox reaction carried out by photosynthetic bacteria is the core working principle of MFCs. The extracellular electron transfer (EET) of bacteria is further carried out by two mechanisms. The first

method is indirect one where soluble redox mediators are used for EET between the exterior electrode and interior cell. These mediators are produced naturally by an organism or exogenously added into the system. A study conducted by Marsili and his associates showed that *Shewanella oneidensis* MR-1 secretes flavins during reduction of extracellular minerals and these flavins contribute to around 80% of EET (Marsili et al. 2008). Earlier, MFCs are very much reliable on synthetic mediators like naphthoquinone derivatives, viologens, and ferricyanide although these synthetic mediators are out of trend due to their substantial problems during MFCs scale-up and toxicity. In direct EET method the bacteria is hypothesized to use conductive appendages that have been mentioned in various studies as conductive pili or nanowire. Current studies reveal that the nanowire of *Shewanella* is not a pili but a periplasmic extension of the outer membrane. Moreover, *Shewanella* sp. also generates a pool of multiheme cytochromes to form interconnected multiple electron transfer pathways between the intercellular respiration and outer membrane (Shi et al. 2009).

On the other hand, PMFC requires light conditions for their operations. In PMFC, a wide range of photoautotrophic organisms like cyanobacteria and blue green algae showed the photocurrent on the electrode. The PMFC usually does not consume external mediators during current generation. The transfer of photosynthetically derived electrons is carried out by certain carriers between the chloroplast membrane and outer membrane. Figure 1.1 depicts the photosynthetic Z-scheme of photosynthetic electron transport system. From the figure, it is clearly evident that the water

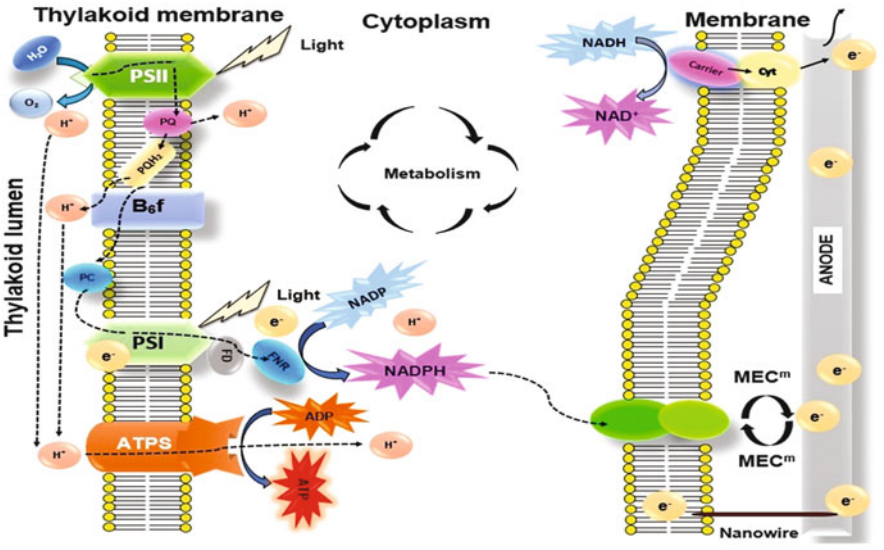

Fig. 1.1 Schematic diagram for conversion of light energy to electron by Z scheme and transfer of electron from microbe to anode. *Cyt* cytochrome, *FNR* ferredoxin-NADP reductase, *PSI* photosystem I, *PSII* photosystem II, *Fd* ferredoxin, *PC* plastocyanin, *PQ* plastoquinone, *ATPS* ATP synthase

oxidation occurred at PSII and reductants for other cellular processes are generated in PSI (Schröder 2007). The diffusing carrier's plastoquinone and the cytochrome b_6f complex act as a bridge between PSII and PSI. Other metabolic processes like respiration are also linked with the photosynthetic electron flow via soluble carriers. This increases the difficulty in determining the source of electrons. Previous studies showed that power output enhanced under the influence of light energy instead of dark reaction in PMFCs (Fischer 2018). This indicates that the release of excess electrons during the process of photosynthesis is the main reason for EET in photoautotrophs. Studies also showed that in cyanobacteria and algae, the electron transfer across the cytoplasmic membrane towards the outer cell membrane is facilitated by carriers like ferredoxins, plastoquinone, soluble cytochrome, and NADPH (Chandra et al. 2018).

Depending upon the type of photoautotrophic organism inoculated into PMFCs and configuration used in PMFCs they are of many types: microalgal MFC, photosynthetic bacterial PMFC, electrogenic microalgal MFC, tubular PMFC, and airlift type PMFC. The maximum power output recorded so far by an MFC is 8.98 W/m^2 (Pocaznoi et al. 2012). According to Fan et al. (2012), under laboratory condition such high output is generally not observed and it remains normally in the range from 1 W/m^2 to 3 W/m^2. In the last few decades, not much has been done to improve the low power density of MFCs and their scale-ups are also facing many hurdles because of its dependence on many factors like substrate, bacteria, electrode material, and so on. Hence huge prospects are available and are yet to be explored, especially the light condition. It is assumed that in PMFC, combination of light and microbes will show a synergistic effect on the enhancement of total power density and voltage output of the cell. Detailed study in this direction will open the doors for other prospects of the PMFCs like treatment of various industrial wastewater (Du et al. 2017), biofuel production (Shukla and Kumar 2018), chemical synthesis (Nevin et al. 2008), kitchen waste degradation (Pei et al. 2018), and biosensor applications.

1.3.1 Photosynthetic Bacteria Based MFC

Photosynthetic bacteria based microbial fuel cells are a type of PMFCs where the photosynthetic bacteria is used for harnessing the energy from solar radiation. In photosynthetic bacteria based MFCs, the photosynthetic bacteria is used both in anodic and cathodic chamber. Photosynthetic bacteria are categorized into two segments: anoxygenic and oxygenic photosynthetic bacteria (APB and OPB) (Gautam et al. 2017). Figure 1.2 outlines the categorization of different photosynthetic bacteria into anoxygenic and oxygenic phototrophs. From the figure, it is clearly evident that cyanobacteria is an oxygenic phototroph and is an important member of OPB (Fig. 1.2). It obtains carbon from atmosphere during the photosynthesis process. On the other hand, anoxygenic phototrophs consist of organisms like green bacteria, purple bacteria, and heliobacteria (Wang-Otomo 2016). This category of bacteria does not produce oxygen as a by-product during their metabolic

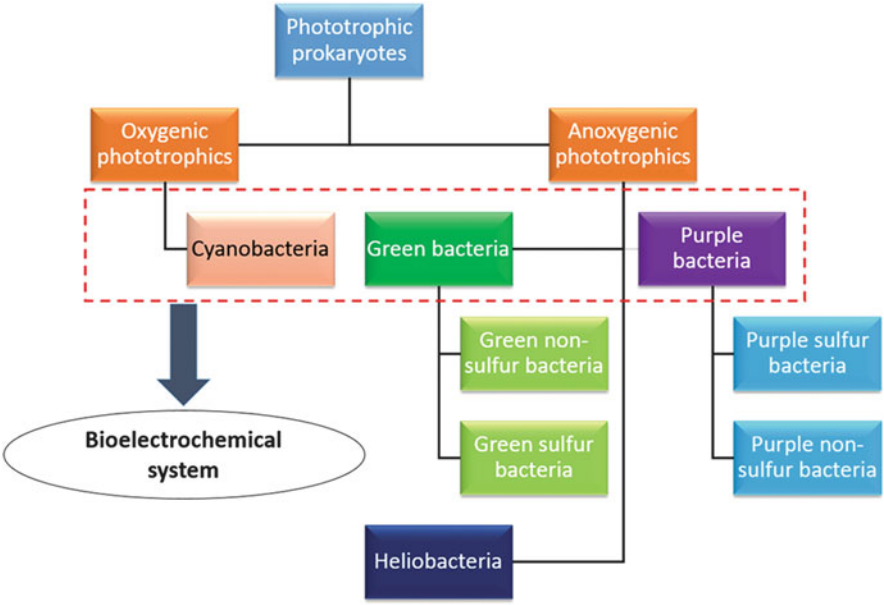

Fig. 1.2 Types of photosynthetic prokaryotes

processes. Recent studies explore that the photosynthetic bacteria shows more compatibility with carbon electrodes instead of inorganic electrodes which leads to reduction in the risk of stability and poisoning of the electrode surfaces.

PMFCs can be considered as one of the most promising energy developing system in the future. A very few studies have been reported so far with photosynthetic bacteria and Genus Cyanobacteria is more popularly used in MFC. Some of the commonly reported bacteria of Genus Cyanobacteria in photosynthetic bacteria MFC include *Spirulina platensis*, *Nostoc* sp., and *Synechocystis* sp. PCC-6803. Cyanobacteria based bioelectrochemical systems are known as biophotovoltaics (BPVs). In this process, bioelectricity is produced through the light driven water oxidation in oxygenic photosynthetic reaction center (Wei et al. 2016; Sawa et al. 2017). Literature review shows that various species of Cyanobacteria possess electrogenic activity. As compared to heterotrophic organisms, Cyanobacteria generate and release electrons towards the electron acceptor which are situated outside the membrane due to electrogenic activity in the presence of light (Pisciotta et al. 2010). It is still a matter of research that how cytochrome bd quinol oxidase or any other compounds produced in the series of other reactions helps in the transportation of electrons towards the acceptor present outside the membrane.

In a typical Cyanobacteria based microbial fuel cell, the biofilm of phototrophs developed on the anode (carbon electrode) shows the electrogenic activity under the light condition. Photophosphorylation process occurs in an anodic chamber and releases electrons and protons to 3-membrane bound protein complexes, namely, the photosystem I (PSI), photosystem II (PSII), and a cytochrome bf complex (Gajda

Table 1.1 Photosynthetic bacteria used in plant microbial fuel cell (PMFC)

S. No.	Photosynthetic bacteria		Reference
1.	*Rhodopseudomonas palustris*		Berk and Canfield (1964)
2.	*Rhodopseudomonas sphaeroides*		Janzen and Seibert (1980)
3.	*Heliobacteria*		Gupta (2005)
4.	*Geobacter sulfurreducens*		Xing et al. (2008)
5.	Cyanobacteria	*Synechocystis* PCC-6803	Fu (2009)
		Synechocystis sp.	Liu et al. (2017)
		Spirulina platensis	Figueredo et al. (2015)
		Nostoc sp.	Lee and Choi (2015)

et al. 2015). The transportation of e^- across the large protein system is performed by portable compounds such as plastocyanin (PC) and plastoquinone (PQ) (Fig. 1.1). In a study conducted by Roberts et al. (2004), it was observed that plastoquinone being a lipophilic carrier molecule plays an important role in the electrogenic pathway of Cyanobacteria (Roberts et al. 2004). These molecules widely carry electrons across the pathway that comprises of PSII, PSI and across the outer membrane of the Cyanobacteria, which play important role in the photosynthetic energy conversion. Water photolysis by PSII and photosynthetic electron transport chain (ETC) are the primary source of extracellular e^- released under light condition by the Cyanobacteria species. Moreover, after photolysis of water molecule, the released e^- are transferred towards PSI by PQ where the system acquires chemical energy by the transformation of light energy. From the above discussion, it is clear that photosynthetic microbial fuel cell captures electrons at the cellular level from the electrogenic activity of the cyanobacteria by using outer electrode (anode). The transport of electrons towards the cathode chamber occurs via external metallic wire channels, whereas protons move through the proton exchange membrane (PEM) fixed in between the anode and cathode chambers (Ivashin et al. 1998; Chandra et al. 2012).

Apart from the basic mechanism of the photosynthetic bacteria, the light condition and type of feed in an anodic chamber perform a vital role in the total output of the power generation. In one such study, Xing et al. (2009) observed that when glucose was used as feed in an illuminated anodic chamber, around 8–10% increase in power density was obtained as compared to dark reaction. An increased power density (i.e.) around 34% was observed when glucose was replaced with acetate in the same MFC configuration. Currently, researchers are exploring the synergistic effect and the mechanism involved in the photosynthetic microbial fuel cells operated with widely prevalent (Heliobacteria and Cyanobacteria) and unreported photosynthetic bacteria. Table 1.1 summarizes the species of photosynthetic bacteria explored so far in harnessing the solar energy.

1.3.2 Photosynthetic Algal Microbial Fuel Cells-PAMFCs

Microalgae or microphytes are another type of photosynthetic microorganisms used in PMFCs. Photosynthetic algal microbial fuel cell (PAMFC) has gained an alarming attention in recent times due to the potential of microalgae to derive electric energy from light and biochemical energy. The idea for generating electricity from the biological route emerged in the latter half of the eighteenth century. The attempt to harness the biogeochemical energy from the biological pathways was potentially studied from the beginning of twentieth century. The efficiency of utilization and conversion of the solar irradiance by all the photosynthetic organisms vary from organism to organism. Photosynthetic algae lead the other photosynthetic organisms by providing maximum output of around 9% followed by C_4 and C_3 plants with 6% and 4.6% conversion efficiency, respectively (Wang et al. 2012). Properties like high photosynthetic efficiency, rapid rate of reproduction, and neutral lipid content make the microalgae eco-friendly and one of the good sources of fuel. Moreover, the magnitude of carbon sequestration by microalgae is two orders higher than terrestrial biomass. Similar to cyanobacteria, microalgae used carbon dioxide and solar radiations to produce oxygen through the process of photosynthesis. Two major dimensions are being explored by researchers for using algae in MFCs. The first one is to use algae as the important source of substrate in anodic chamber of MFCs for dark reaction with electrogenic microorganisms and secondly to utilize them as biocatalyst in a cathodic chamber of the MFC (Shukla and Kumar 2018).

In microalgae photoreactor, microalgae can either be attached to the cathode or used as suspension (catholyte). Attached microalgae (biocathode) through photosynthesis evolve oxygen and release it into the catholyte (Mohan et al. 2014). However, microalgae in suspension will play the role of electron acceptor. It has also been proved that microalgae help in reducing overpotential on the surface of cathode area (McCormick et al. 2011). Studies with *Desmodesmus* sp. have shown that the microalgae have potential to reduce the cathodic resistance through increased oxygen concentration, released during the photosynthesis process (Wu et al. 2014). Furthermore, this will eliminate the need of mechanical aeration within the system. The concept of "zero carbon discharge" has been proposed by utilizing live microalgae grown on cathode while using dead microalgal cells as substrate for anodic biofilm. Biocathode comprising *C. vulgaris* gave better efficiency with intermittent light energy, while continuous light availability decreased the efficiency through shortening of the life span of microalgae (Wu et al. 2013).

Energy consumption can be reduced by integrating algal photosynthesis with MFCs in a sensible fashion. In an attempt, Lee et al. (2015) explained self-sustaining nature of microalgae MFC. Microalgae MFC is self-reliant and sustainable as it converts CO_2 to biomass and other alkanes. These biomass and alkanes (organic fraction) are ultimately reused as substrate in the same MFCs. In the integrated system, algal photosynthesis releases oxygen inside the cathode compartment. This will not only facilitate better working of MFCs, but also favor utilization of produced biomass in various applications. Baicha et al. (2016) showed working mechanism of

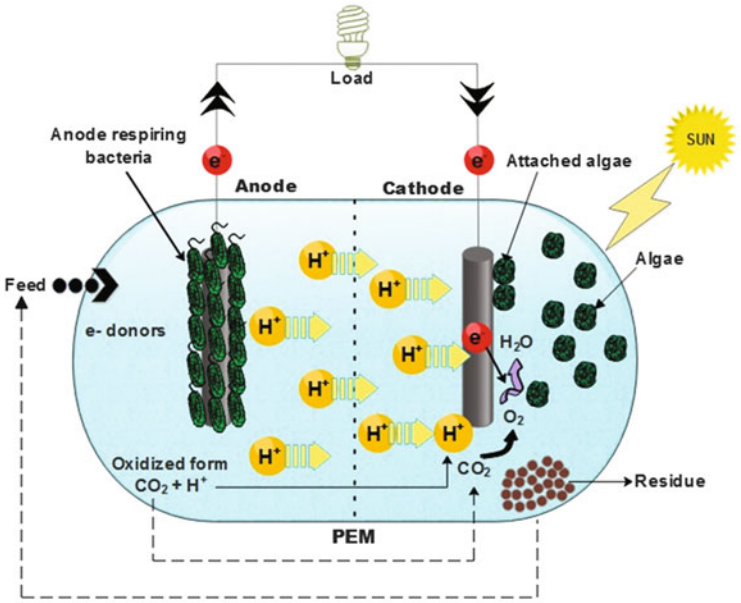

Fig. 1.3 Microalgae–microbial fuel cell. Reuse of cathodic residue in anodic chamber as feed which makes the system cyclic for unlimited time period but demands continuous supply of H_2O, CO_2, and light for the generation of power and chemicals (Lee et al. 2015)

microalgae in MFCs. Figure 1.3 depicts the configuration of MFC where anodic and cathodic chamber join in a way as to form cyclic microbial fuel cell. The production of oxygen, biomass, and other value-added by-products by microalgae are also illustrated in the figure. However, the primary motive behind the use of microalgae in MFCs is to generate the metabolic oxygen in catholyte which would act as electron sink and generate electricity. As mentioned earlier, this will also reduce the need of mechanical aeration within the cathode chamber (Xie et al. 2011).

A very interesting aspect regarding the development of microalgae-MFCs is that this system has the potential to provide low-cost carbon dioxide. The anodic chamber of microalgae-MFC is a rich source of CO_2. The CO_2 produced during organic matter (feed) oxidation is transferred to the cathodic chamber used for the growth of microalgae. Unquestionably, carbon dioxide is available in our atmosphere but at very low quantity (0.04%) and commercial extraction of it from air is not a cost-effective process. A study conducted by Powell et al. (2011) revealed that in a bioethanol production plant CO_2 was captured along with microalgae. The CO_2 generated in anodic chamber was channelized towards the cathodic chamber containing microalgae where it is reduced to release oxygen with anodic proton and electrons. But there is always an uncertainty over the success of this process because the carbon dioxide concentration usually exceeds with respect to the generation of electrons in anodic chamber in yeast driven MFCs. Ethanol production acts as yield factor for the low production of electron in anodic chamber. It was

experimentally observed that when ethanol was formed in the MFC system the electrons are not generated through the side reactions and are conserved by a process known as Crabtree effect. Crabtree effect is a process in which the glycolytic activity acted as a check point on the respiration power of the cells (Yeast). The inhibition of respiration during the process of high rate of glycolysis emerged as Crabtree effect.

Although the technology of PAMFC is advantageous over conventional MFCs, still there are several aspects that hinder the smooth functioning and efficiency of the system. It is known that microalgae are capable of growing in various conditions but the sequestration of carbon dioxide requires autotrophic algal growth and the rate of cell cultivation is light dependent. Hence light becomes a limiting factor. The PAMFC therefore should have larger cathodic area for receiving sufficient light. Moreover, the harvesting of the algal cells and their subsequent processing are an expensive process. Similarly, at anode the steadiness of the performance and the columbic efficiency for substrate consumption are relatively low (Sivakumar et al. 2018). Lower surface density of cells by the factor of $1/10^{th}$, resistance of anodic biofilm for electron transfer by same factor of $1/10^{th}$, and buildup of extracellular and intracellular mass transfer gradient in anodic biofilm are some of the reasons behind low columbic efficiency and substrate consumption (Zhang et al. 2015).

These limiting factors suppress overall performance of the system. Sometimes local buildup of protons affects the rate of extracellular electron transfer and causes high current loading. High current load may cause cell failure and create power overshoot. This results in cell voltage and current decrease. Therefore, R&D encourages every endeavor to enhance the quantum efficiency of the microalgae cultivation, which in turn would help in increasing the power output in PAMFC.

1.3.2.1 Applications of Microalgae MFC

Microalgae supported MFCs are used as different devices to trap light energy and convert it into electric current with the help of biological pathways. Microalgae are reported to be highly efficient in solar energy conversion. They are also widely used as a substrate for production of biomass and valuable products. Microalgae by using the end products of bacterial metabolism help in the recovery of nitrates and phosphates and provide oxygen to the bacteria to thrive. The nutrients recovered can be used as fertilizers in agriculture, which in turn would eliminate the problem of eutrophication (Gajda et al. 2015). Algae assisted cathode during the initial stages of acclimatization demonstrated high polarization resistance and power density of 13.5 mW/m^2 (Del Campo et al. 2013). In one of the studies, anodic off gas was introduced into algae supported cathode, which worked as carbon capture cells. The system could efficiently capture CO_2 and simultaneously generate 5.6 W/m^3 of power density (Wang et al. 2010).

1.3.3 Plant Microbial Fuel Cells (PMFCs)

In this system, plants and bacteria are used to generate green electricity through solar energy conversion. PMFC comes under mediator-free category in which they derive energy directly from the plants. The various plant species which possess this kind of potential include grasses such as cod and reed sweetgrass, rice, algae, lupines, and tomatoes. The main benefit of such kind of MFC is that it generates power from living plants via in situ-energy production. About 70% of carbon deposited by photosynthesis process is translocated into the soil as rhizodeposition and almost 60% of it can be recovered as energy by using PMFC technology. According to Strik et al. (2011), theoretically a PMFC can generate a power density up to 3.2 W/m^2 which can be estimated to be around 280 MWh/ha year. The PMFC comes under open-loop biosystem category and comprises of two segments: biocontrol and bioprocess unit. In the biocontrol unit, plants act as a receiver of external solar energy to obtain voltage/power as a result of photosynthesis process (Strik et al. 2008). At bioprocess unit, root exudates are available as substrate on which rhizosphere microbial organisms can act as catalyst for the uptake of substrate. A series of redox reactions occur which in turn results in the generation of voltage/power output. The PMFC also offers scope for various other multidisciplinary areas such as study of plants and microorganisms, their interactions and electrochemistry. Engineering discipline plays a vital role in designing and upscaling the PMFC process at commercial level.

1.3.3.1 Working Principle of Plant Microbial Fuel Cell (PMFC)

Green plants perform one of the important phenomena on earth known as photosynthesis. They utilize solar energy to trap carbon dioxide and convert into energy rich organic compound, namely the carbohydrate. Based on the age, type, and ecological conditions of plant species, it is reported that around 60% of the net carbon that is fixed by the plants are transferred to the roots from their leaves. The schematic and working mechanism of the PMFC is clearly outlined in Fig. 1.4. The PMFC is broadly divided into two main units: the first one is a biocontrol unit in which the process of photosynthesis is carried out by plant in the presence of light and atmospheric carbon (Liljeroth et al. 1994).

The fixed carbon is translocated to the bioprocess segment of PMFC, the second unit. In the bioprocess unit, the rhizosphere region shows the presence of a number of rhizodeposits such as sugar, organic acids as roots release exudates; carbohydrates and enzymes as polymeric secretions. Certain gases such as ethylene and CO_2 are also present in the rhizosphere region of roots along with the dead cell materials. These rhizodeposits are used as a source of carbon by the microorganisms that are in mutualistic association with the plants. The microorganisms in turn protect the plant by forming a protective biofilm or releasing antibiotics.

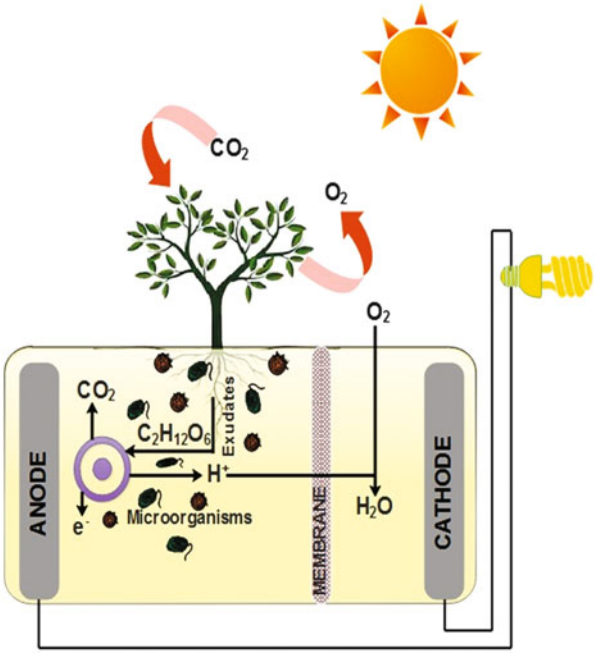

Fig. 1.4 Schematic representation of the PMFC

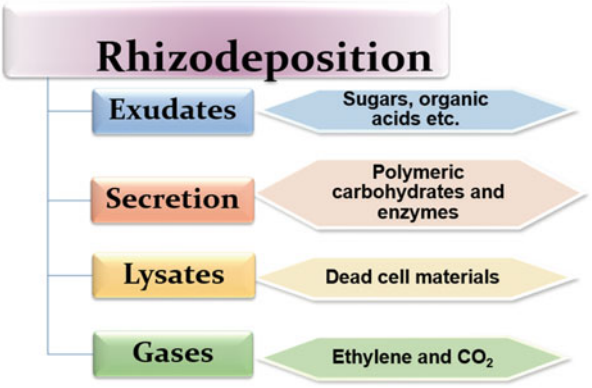

Fig. 1.5 Rhizodeposits of plants

Figure 1.5 depicts the various rhizodeposits released by plants in soil. The rhizosphere provides significant amount of substrate in the form of carbon for the growth of rhizobacterial populations. The microbiome of the rhizosphere plays a vital role in the development of the soil microenvironment. The exudates stimulate chemotaxis which eventually increases the population of soil microorganism in the rhizosphere. Amino acids, flavonoids, aromatic and dicarboxylic acids are examples of chemoattractant that are produced in the rhizosphere region. These chemoattractants attract rhizobia (gram-negative soil bacteria) to root hairs in the rhizosphere (Bais et al. 2006). These exudates and chemoattractants are consumed

by microorganisms as electron acceptor at the anode for acquisition of metabolic energy. A potential difference develops between the two electrodes. Due to this potential difference, electrons move through an electrical circuit from anode to cathode accompanied with an external resistor. Likewise, to remain electroneutral and to form water, the protons move across the selective membrane to combine oxygen and electrons.

Studies report that plant root system possessed around 40% of the photosynthetic productivity in form of rhizodeposits (Lynch and Whipps 1990). As mentioned previously, microorganisms present in the rhizosphere region show mutual beneficiary interaction with the plants through consumption of carbon present in the rhizodeposits. They also provide protection to plant roots against pathogens by forming protective biofilms or releasing antibiotics.

The success of a PMFC depends on the selection of a suitable and efficient plant species. Selecting an appropriate species will favor better rhizodeposition development and maximum power generation. The photosynthetic pathways of plants may facilitate the selection of the efficient plant species for the PMFC. As we all know, the plants are broadly classified according to the photosynthetic pathway as C3, C4 and Crassulacean acid metabolism (CAM) plants. Studies show that C4 plants are suitable for PMFC as they convert carbon dioxide to a four-carbon molecule before entering the Benson–Calvin cycle. They possess high photosynthetic efficiency and hence releases enormous amount of rhizodeposits as compared to other C3 and CAM plants. The rhizodeposits would serve as a substrate for many microorganisms thereby resulting in enhanced energy generation. Henceforth, the ability of C4 plants to produce more energy and adapt to hot and dry climates makes it attractive for use in PMFC. C4 plants can achieve high solar energy conversion ratio and henceforth suggested by various researches in PMFC applications for bioelectricity generation.

According to Wang et al. (2012), the C4 pathway of carbon fixation can be observed in almost 3% of the terrestrial plant species. The C4 pathway is a common phenomenon of the monocots like the grass species. The adventitious root system of the monocots/grass favors microbiome proliferation through increased rhizosphere surface area. There are two models in which PMFCs are configured. The tubular model PMFCs are the most studied by the researchers. A typical tubular PMFC model consists of a tube-shaped anode with a proton exchange membrane attached at the bottom to separate out the cathode positioned below it. Materials like glass tubes/beakers, plastic containers, and polyvinyl chloride (PVC) are used commonly to fabricate the PMFCs. In a tubular PMFC, the electrochemically active bacteria (EAB) present in the rhizosphere generate electrons which reach the anode initially and then the cathode to combine with the protons.

The second type of configuration is a flat plate model PMFC which is comparatively less popular and is reported by only few researchers (Helder et al. 2012). However, they provide better performance when compared to the tubular PMFC due to better internal resistance. The flat plate configuration is designed uniquely to lower the electron transport with high membrane resistance. A flat plate PMFC consists of a proton exchange membrane sandwich between the anode and cathode and placed vertically in the reactor.

Researchers have upscaled the PMFC technology to the field using flow through systems (FTS) which consists of two components, namely ecologically engineered systems (EESs) and constructed wetland systems. As we know, wetlands possess an inbuilt natural mechanism to clean and treat the wastewater, they are potentially integrated with PMFC with an idea of wastewater treatment and power generation (Mohan et al. 2010). The first upscaling was tried in a roof top system by Netherlands Institute of Ecology. Large scale electricity production can be done through a tubular system proposed by Timmers et al. (2012). They proposed a model of tubular PMFC for large scale electricity generation. Installation of similar tube in the existing wetlands or natural area can facilitate electricity generation on a large scale. The above-mentioned upscale initiatives showed huge prospects in the PMFC development to meet the future demands of energy and waste management. The technology would also ensure the sustainable development without affecting our ecological balance.

1.4 Conclusion

Photosynthetic microbial fuel cell technology is attracting attention of the scientific community due to its eco-friendly nature of generating energy using photosynthetic organisms such as plants, algae, and bacteria. The technology is still in infancy and requires more detailed research for its widespread utilization. Selection of plants, algae, and bacteria, better understanding of the mechanism involved, root exudates evaluation, cathode and anode material, and process configuration and operation are some of the areas where detailed research is required. The PMFC technology has a lot of challenges to overcome to increase the bioelectricity production potential. Keeping aside these factors, PMFC is a sustainable technology with great potential to combat future energy crises.

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Chapter 2
Application Niche of Microbial Fuel Cell as a Bio-energy Source for Sustainable Development

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Abstract Water-energy crisis and wastewater treatment (WWT) issues can be addressed simultaneously in microbial fuel cell (MFC). Being a microbial electrochemical technology, it provides flexible platform for both aerobic and anoxic treatment processes and hence provides efficient WWT solution. Simple substrate to complex industrial wastewater can be effectively treated in such system. Over the advancement in research, MFC is capable to harvest electricity from nW to kW/m^3 with use of high redox catalysts and novel electrodes. The output electrical energy is sufficient to operate the different electronic appliances. With biostimulation approach, MFC can be a good option as biosensor to detect the concentration of heavy metals, COD dose, pH. Additionally, MFC attracts attention for by-product recovery during WWT. Valuable products and resources such as struvite from urine, manure, H_2O_2, NaOH, H_2 and methane gas, and other chemicals can be recovered during electrochemical reactions. According to various applications, MFC can be used for carbon capture and sequestration (in microbial carbon capture cells), for desalination of saline water (in microbial desalination cells), for biohydrogen production (in MFC-electrolysis coupled cell), for utilizing sediment as

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carbon source (in benthic MFC), for sanitation (in bioelectric toilet), and so on. In advance WWT system, MFC can be pre-treatment or post-treatment obtain for efficient WWT. Thus, it can be solution for biological oxidation process and as a tertiary treatment for disinfection, denitrification, aeration to make effluent suitable for discharge. Thus, MFC provides efficient and effective solution for WWT along with electricity and by-product recovery for sustainable development.

Keywords Bio-energy · Bio-sensors · Electricity generation · Microbial fuel cell · Redox reactions · Pollutant removal

2.1 Introduction

Water crisis, wastewater treatment (WWT), and depletion of conventional energy sources are major concerns present population facing in the present era. Nationwide WWT plants require about 0.1–0.3% of total energy consumption and are often considered as the largest consumer of energy (Water Environment Federation: Alexandria 1997). Moreover, such energy demand is likely to increase with stringent effluent discharge limits, increase in population density and overexpansion of urbanization, which continue to drive the advancement in wastewater treatment technologies and their waste-management framework. With advancement in technology, such energy demand is reduced by 30% with the application of energy efficient systems and modifications in treatment processes (Stillwell et al. 2010).

Considering high cost associated with conventional WWT plants, several process modifications and waste to energy techniques have been implemented to make WWT plant energy efficient. Nowadays wastewater is looking forward as a resource rather than waste, to recover valuable energy. Hence, current WWT plant design paradigm shifted towards maximization of energy and resource recovery. The hidden energy in the organic matter of wastewater can be a substrate for microbial oxidation processes. The upcoming bioelectrochemical system for waste to energy recovery can be capable to utilize this chemical energy to convert into electrical output through various electrochemical redox reactions by using microbes as a biocatalyst.

2.2 Microbial Fuel Cell

A microbial fuel cell (MFC) is waste fed oxic-anoxic duo reactor integrated with electrochemical system that allows to convert chemical energy present in organic compounds of wastewater to electrical output through biocatalytic reactions of electrogenic bacteria (Du et al. 2007; Jadhav et al. 2015). It consists of anaerobic anodic compartment and aerobic cathodic chamber. Bacteria degrade the organics from wastewater in anodic chamber and release protons and electrons. The protons

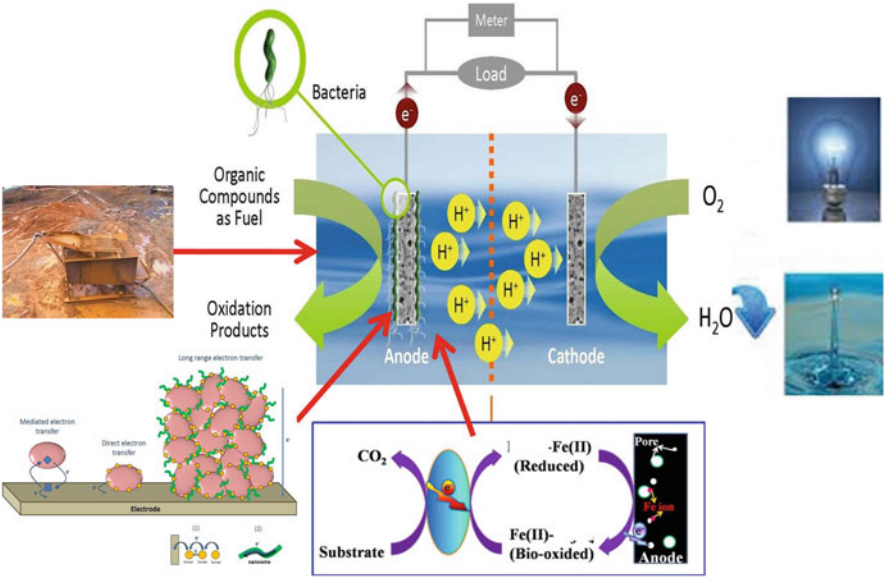

Fig. 2.1 Factors contributing for performance of microbial fuel cell

transported to cathodic reduction site through ion exchange membrane. Further, electrons are transferred through external electric circuit to harvest the electricity. Thus, electricity generation along with efficient wastewater treatment can be achieved in MFC (Jadhav et al 2014). Performance of MFC is dependent on design aspects, electrode properties, operating conditions, wastewater characteristics, bacterial conditions, and other factors (Fig. 2.1). Such system provides flexible platform for both oxidation and reduction reactions and hence suitable for treatment of various contaminants from wastewater (Venkata and Chandrasekhar 2011; Chandrasekhar and Young-Ho 2017). Additionally, it can be used for operating the different electronic appliances, charging mobile phone battery, operating sensors, and LED bulbs. With biostimulation approach, MFC can be a good option as biosensor to detect the concentration of heavy metals, chemical oxygen demand (COD) dose, pH, biological oxygen demand (BOD). Additionally, MFC attracts attention for by-product recovery during WWT. Valuable products and resources such as struvite from urine, manure, H_2O_2, NaOH, H_2 and methane gas, and other chemicals can be recovered during electrochemical reactions.

2.3 Applications of MFC

2.3.1 Electricity Generation

Electricity production is the principal identification and initial motive of the MFCs. MFC has been recorded for bioelectricity generation for well over a century (Potter 1910; Allen and Bennetto 1993; Mathuriya and Sharma 2009; Mathuriya and Pant 2019). Reports have shown that any chemical that can be oxidized by microbiota can further transform into electrical energy (Jadhav et al. 2018; Pant et al. 2010). MFC offers several benefits compared to existing renewable technologies, viz. (a) MFCs recommend decent efficient conversion of chemical energy of the substrate to energy. (b) The MFCs demonstrate attractive technology for safe and soundless performance. (c) MFCs prove efficient energy conversion compared to existing power plants operated from fossil fuels (Rabaey and Verstraete 2005; Mathuriya 2014). (d) Even at ambient temperature MFC performs efficiently. (e) Obtained bioelectricity from MFCs is sustainable. Nearly every MFC research article over 100 years has shaded light on MFC's electricity generation capabilities. In MFC research, some research groups have recently reported attractive improvement in the power output (Table 2.1).

2.3.2 Wastewater Treatment

Waste is the reject generated through various human activities and industrialization, which creates problem when disposed with water. Wastewater holds a significant amount of complex pollutants that cause several environmental issues due to their complex degradation mechanism. Further wastewater is resource of enumerable bacterial communities that can sustain even in severe environmental conditions (Jadhav et al. 2019). MFCs utilize these microbial communities to generate bioelectricity from the effluent wastewater (Mathuriya 2014). MFC offers various advantages including: (a) less energy consumption as compared to activated sludge process (ASP) and do not need regulated and controlled distribution systems such as those required by other fuel cells (Watanabe 2008). (b) MFCs can treat effluent streams those are not suitable for anaerobic digestion, e.g., wastewater containing low COD, volatile fatty acids. (c) Electricity harvested by MFCs can potentially reduce half of electricity cost required for aeration in activated sludge process (Rittmann 2008; Watanabe 2008). (d) MFCs generate 50–90% less sludge (Du et al. 2007). This is the only kind of alternative system capable of directly converting organic waste into electricity (Fig. 2.2).

It was first suggested in 1911 that MFCs could be potential solution for wastewater treatment (Habermann and Pommer 1991). The existing energy-consuming bioreactors could be replaced by an MFC (e.g., ASP), along with recovering the energy (3.8 kWh/kg COD) and valuable by-products from wastewater without

Table 2.1 Performance of MFC at different microbial inoculums

MFC type	Inoculum	HRT/ operating time	Power density	Reference
Dual chamber MFC	Mix activated sludge	120 h	1.77 W/m^2	Kaewkannetra et al. (2011)
Dual chamber MFC	*Lactobacillus* genus	3 m	1800 ± 120 W/m^2	Kassongo and Togo (2011)
Single-chamber MFC	domestic wastewater	23 h	4200 mW/m^3	Sharma and Li (2010)
Up-flow anaerobic sludge blanket (UASB) reactor-MFC-biological aerated filter	Mixed active sludge	60 day	1.41 W/m^2	Zhang et al. (2009)
Single-chamber MFC	Domestic wastewater	–	2.11 W/m^2	Feng et al. (2014)
Dual chamber MFC	Anaerobic sludge	25 h	1600 mW/m^2	Liu et al. (2019)
Single-chamber MFC	Anaerobic activated sludge	–	6.8 W/m^3	You et al. (2006)
Three MFCs hydraulically connected in series	–	96 h	1.82 W/m^2	Galvez et al. (2009)
Single-chamber MFC	Mixed inoculum	48 h, 72 h	2.9 W/m^2	Catal et al. (2009)
Single-chamber MFC	*Golenkinia* sp. SDEC-16		6255 mW/m^3	Hou et al. (2016)
Membrane MFC	–	5 day	621.13 mW/m^2	Mansoorian et al. (2016)
Constructed wetlands combined with microbial fuel cell	Mixed microbial flora	–	3714.08 mW/m^2	Xu et al. (2018)
Cylindrical single-chamber	*P. aeruginosa*	–	3322 ± 38 mW/m^2	Zhang et al. (2019)

aeration cost (which is equivalent to 1 kWh/kg. COD in conventional aeration system). Moreover, MFC produced less sludge (0.02–0.22 g biomass-COD/g substrate-COD) than conventional aerobic treatment (0.53 g biomass-COD/g substrate-COD) (Clauwaert et al. 2007).

MFCs can treat diverse wastewater from mono-sugars to composite industrial effluents. Some specific mentions are as follows: Human excreta (Gajda et al. 2018; Kretzschmar et al. 2016), cassava mill wastewater (Adekunle et al. 2016), processing effluent (Jayashree et al. 2016), dairy wastewater (Mathuriya and Sharma 2009; Faria et al. 2017), paper wastewater (Mathuriya 2014), molasses (Hassan et al. 2019), brewery wastewater (Mathuriya and Sharma 2009); landfill leachate (Sonawane et al. 2017); oilfield wastewater (Sheikhyousefi et al. 2017); oil sands tailings (Jiang et al. 2013); terephthalic acid (Marashi and Kariminia 2015), refinery waste (Srikanth et al. 2016), tannery wastewater (Mathuriya 2014) (Table 2.2), many

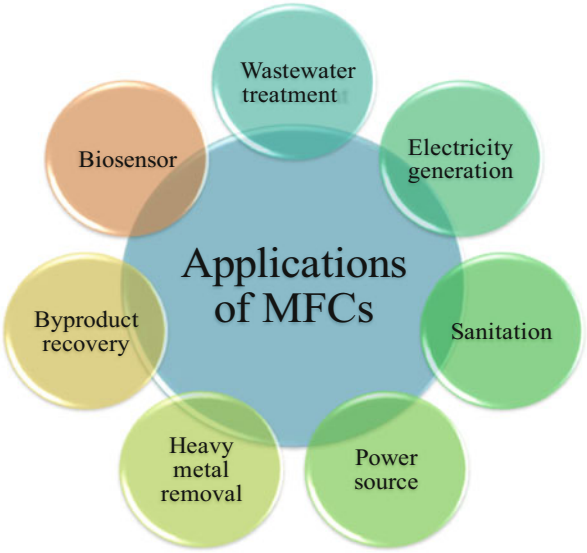

Fig. 2.2 Application niche of microbial fuel cell technology

of those remain untreated in the conventional biochemical treatment technologies. MFCs can deal with nearly all the COD present in wastewater and other contaminants (Luo et al. 2011; Zhang et al. 2009). Some attractive results are shown in Table 2.2. Although wastewater-fueled MFCs produce less energy than pure compounds, the cost of primary wastewater treatment would be reduced by the simultaneous energy recovery and wastewater treatment.

Dyes are the chemicals used for coloration in the textile, medical, cosmetic, rubber, photography, and paper industries (Zollinger 1991). Due to their complexity, most dyes are difficult to diminish. The appearance and the toxic nature of colors affect the effectiveness of some water treatment methods, and the quality of life through health problems (Carneiro et al. 2007). The development of treatment technologies for dyes is therefore an urgent necessity. Recently, many studies reported various dye removal/decolorization in MFCs (Zhang and Zhu 2011; Kalathil et al. 2012; Fang et al., 2013; Sun et al. 2015; Chen et al. 2016; Zou and Wang 2017; Rathour et al. 2019).

2.3.3 By-Product Recovery

Production of biofuels and recovery of valuable chemicals from renewable energy resources is one of the challenging tasks in present scenario. By virtue of this, third generation of microbial fuel cell has been focused on development of electrochemical reactions that simultaneously improve energy capture and recovery of useful compounds (Khunjar et al. 2012; Jadhav et al. 2017a). The production of bacterial assisted chemicals in MFCs, known as microbial electrosynthesis, provides a highly

Table 2.2 Application of MFCs for treatment of wastewater from various sources

Pollutant nature	Initial COD	HRT/ operating time	Wastewater removal	Reference
Human feces	5 g/L	190 h	71%	Fangzhou et al. (2011)
Cow waste slurry	1 g/L	72 h	84% BOD	Yokoyama et al. (2006)
Meat packing wastewater	1420 mg/L	250 h	86% BOD	Heilmann and Logan (2006)
Cassava wastewater	16,000 mg/L, 86 mg/L cyanide	120 h	88%	Kaewkannetra et al. (2011)
Cereal effluent	0.6 g/L	5 days	95%	Oh and Logan (2005)
Rice mill wastewater	1.1–1.1 g/L	288 h	96.5, 84, 81% COD; lignin; phenol resp.	Behera et al. (2010)
Dairy wastewater	4.44 kg COD/m^3	–	95.49, 78.07, 91.98, 99% COD, protein, carbohydrates turbidity, resp.	Venkata Mohan et al. (2010)
Dairy wastewater	1487 mg/L	10 days	81.29%	Mathuriya and Sharma (2009)
Whey	93.2 ± 0.4 gCOD/ L	3 m	92.8%	Kassongo and Togo (2011)
Hydrogen biofermenter effluent	6.3 g/L	23 h	97%	Sharma and Li (2010)
Starch processing effluent	4.9 g/L	140 days (4 cycle)	98 and 90.6% COD and ammonia-nitrogen removal	Lu et al. (2009)
Brewery wastewater	1.7 g/L	15 days	93.8%	Mathuriya and Sharma (2009)
Distillery wastewater	82.20 g/L 4.8 gTDS/L	–	72.84, 31.67, 23.96% COD; color; TDS	Mohanakrishna et al. (2010)
Biodiesel waste	1.4 g/L	–	90%	Feng et al. (2014)
Phenolic wastewater	Phenol (0.4 g/L) glucose mixture	60 h	95%	Liu et al. (2019)
Palm oil effluent	10 g/L	48 h	100, 96.5, 93.6% phenol; COD; NH_4^+-N	Cheng et al. (2010)
p-nitrophenol wastewater	NIF	12 h	Complete degradation	Zhu and Ni (2009)
4-chlorophenol	60 mg/L	45 h	Complete dechlorination	Gu et al. (2007)

(continued)

Table 2.2 (continued)

Pollutant nature	Initial COD	HRT/ operating time	Wastewater removal	Reference
Pentachlorophenol glucose and acetate as co substrate	PCP: 15 mg/L Glucose: 0.78 g/L	96 h	Removal rate 0.12 mg/Lh	Huang et al. (2018)
Ceftriaxone sodium (Cs)	0.05 g/L (Cs): 1 g/L glucose	24 h	96%	Wen et al. (2011a)
Synthetic penicillin (Pc) wastewater	0.05 g/L Pc:1 g/L glucose	24 h	98%	Wen et al. (2011b)
Selenite wastewater	0.05 and 0.2 g/L	48 and 72 h	99%	Catal et al. (2009)
Indole (Id) wastewater	0.25 g/L Id: 1 g/L glucose	10 h	89.5 & 100% COD; Id removal	Luo et al. (2010)
N-heterocyclic compounds	0.12 g/L of each	Indole: 122 h Quinoline: 102 h Pyridine: 63 h	COD-88% Id: 95% Quinoline: 93% Pyridine: 86%	Hu et al. (2011)
Pyridine	0.5 g/L	12 h	100% removal	Zhang et al. (2009)
Quinoline	0.5 g/L	6 h	100% removal	Zhang et al. 2019
Refractory contaminants (furfural)	0.3 g/L	60 h	96% COD; complete removal of furfural	Luo et al. (2011)
Food processing washdown water			84% of the soluble COD	Boghani et al. (2017)
Dairy industry wastewater			63 ± 5%	Faria et al. (2017)
Chocolaterie wastewater			70%	Subha et al. (2019)
Sea food processing wastewater			95% COD	Jayashree et al. (2016)
Brewery wastewater			94.6 ± 1.0%	Lu et al. (2017)
Active brilliant red X-3B	300 mg/L	48 h	Complete	Sun et al. (2015)
Acid orange 7	–	168 h	97% color removal	Zhang and Zhu (2011)
Congo red	0.3 g/L	–	90%	Hou et al. (2016)

attractive, modern way to produce useful resources from wastewater and generate electrical energy (Rabaey and Rozendal 2010).

Environmental concerns about MFC can be significantly increased when pollutants are used as electron acceptors in cathodic chamber. Based on this assumption, several studies have recently reported cathodic reduction as a method for the treatment of heavy metal contaminants (Liu et al. 2019; Choi and Hu 2013; Huang et al. 2018; Wu et al. 2019; Ezziat et al. 2019). Both the anodic and cathodic chambers of MFC serve as a platform for controlled electrochemical reactions for treatment and recovery of various heavy metals present in the industrial wastewater (Jadhav et al. 2017b). These heavy metals include chromium (Habibul et al. 2016; Wang et al. 2017; Vanadium Wang et al. 2017), arsenic (Leiva et al. 2018), copper (Wu et al. 2018), and many more (Gregory et al. 2004; Huang et al. 2018; Nancharaiah et al. 2016; Qian et al. 2018; Liu et al. 2019). Many of these studies reported to treat up to 100% of metallic treatment and recovery (Table 2.3).

Methane is an important greenhouse gas that can be generated by biochemical means during anaerobic digestion of wastewater and is considered as an environmentally friendly over other fossil fuels. CH_4 gas production is one attractive application of MFCs (Rizzo et al. 2013; Xiao et al. 2013; Yu et al. 2017a; Khudzari et al. 2019).

H_2O_2 is a natural environmentally friendly green reagent for the removal of contaminants and perhaps a source of oxygen-hydrogen. MFCs offer an efficient means for production of H_2O_2 (Fu et al. 2010; Dong et al. 2018; Asghar et al. 2017; An et al. 2019).

Biomass generation is another potential application of MFCs (Powell et al. 2011; Helder et al. 2010; Rashid et al. 2013). Previous studies reported that MFCs with algal biocathode utilize the CO_2 gas for algal biomass production and co-current electricity production. This offers sustainable technology for biomass production, carbon sequestration with simultaneous energy recovery due to the inexhaustible supply of CO_2. Recently many studies reported to produce the biomass in MFCs and in photosynthetic MFCs (Krishnaraj et al. 2015; Ma et al. 2017; Commault et al. 2017).

2.3.4 Biosensor Applications

Biosensors are the specific class of sensors that combine a physical transduction technique and microbial activities response detection or recognition mechanism to produce a signal, which is directly proportional to substrate characteristics and its concentration (Cornell et al. 1997). This response signal can be produced with variation in the proton load, the emission or absorption of gases, light emissions, absorption, etc., caused by the metabolism of species of interest through the biological recognition system. Transducer makes a measurable response from this biological signal, i.e. potential, electric current, and measured signals further enhanced, processed, and recorded to extract the exact data (Mulchandani and Rogers 1998).

Table 2.3 Heavy metal and other by-product removal and recovery in MFCs

Metal type	MFC architecture	Initial concentration	Inoculum source	Operating time/HRT	% removal	Bioelectricity produced	References
Electroplating wastewater (Chromium)	DC-MFC[a]	0.21 g/L	Mixed sludge	25 h	99.5% and 66.2% Cr(VI) and total Cr	1.6 W/m^2	Liu et al. (2019)
Chromium	DC-MFC[a]	0.1 g/L	Domestic wastewater	150 h	100% removal	0.15 W/m^2 at 0.2 g/L	Wang et al. (2011)
Chromium	DC-MFC[a] with rutile-coated cathode	0.026 g/L	Anaerobic mixed sludge	26 h	97%	800 mV	Li et al. (2009a)
Chromium	DC-MFC[a]	0.005 and 0.025 g/L	*β-Proteobacteria*, *Actinobacteria*, *Acinetobacter*	6 day	93% and 61%	0.5–0.6	Yeon et al. (2011)
$NaVO_3$	DC-MFC[a]	0.3 g/L	*Rhodoferax ferrireducens*	7 day	75.8%	0.6 mA	Li et al. (2009b)
Sulfide and Vanadium	DC-MFC[a]	Sulfide (anode): 50–200 mg/L; V(V) (cathode): 0.25–1 g/L	Anaerobic granular sludge	72 h	82.2% and 26.1% sulfide and vanadium	0.614 W/m^2	Zhang et al. (2009)
Chromium and vanadium	DC-MFC[a]	–	–	240 h	67.9% V; 75.4% Cr	0.97 W/m^2	Zhang et al. (2009)
Copper containing wastewater	DC-MFC[a]	1 g/L	$CuSO_4$ catholyte	288 h	97.8%	0.54 W/m^3 at 6.4g Cu/L	Liang et al. (2011)
Silver ion containing wastewaters		50–200 ppm		8 h	99.91–98.26%		Choi and Cui (2012)

Molasses wastewater					COD 81.22% Silver precipitation	65.82 mW/m^2	Zhang et al. (2013)
Tetrachloroaurate		200 ppm Au(III)			99.89% and 0.22 ppm		Choi and Hu (2013)

[a]*DC-MFC:* Dual chamber MFC

One of the most common biosensing application is the use of MFCs as biosensors (Kim et al. 2009).

Since the coulomb harvested is proportional to substrate concentration, MFCs can be deployed as a BOD sensor in wastewater streams (Kim et al. 2003). Such MFC-based BOD sensors offer excellent operating reliability, precision, good reproducibility, working capabilities in remote area, self-power potential, and a considerably longer service life over other BOD sensors (Kim et al. 2009; Kim et al. 2003; Di Lorenzo et al. 2009; Sun et al. 2015; Chouler et al. 2018). An accurate way of determining the BOD value is to calculate the Coulombic output of a liquid stream (Chang et al. 2004). Electric current also acts as an indicator for BOD monitoring (Du et al. 2007).

Chemical toxicants or suppressor inhibit the metabolism of bacterial population present on anode, which ultimately inhibit the of electron transfer rate to the electrode and therefore decrease the electrical output. MFCs can utilize this concept and can be utilized as a toxicity detection sensor for measuring the chemical toxicity present in the wastewater or substrate (Stein et al. 2012; Yu et al. 2017a, b). The MFC biosensor could be useful to assess river toxicity, to track sample contamination, or to conduct research on contaminated sites at the entry of effluent treatment plants as well as illegal dumping sites (Meyer et al. 2002; Chang et al. 2004). Such units are compactly designed to fit them either as a separate device or as a set of sensors for high-performance processing in measuring instruments. MFC biosensors have been applied to detect the Cr, Hg, Pb, or phenol, formaldehyde (Davila et al. 2011), anaerobic digestion (Liu et al. 2019), volatile fatty acid (Kaur et al. 2013), Cu (II) (Shen et al. 2013), p-nitrophenol (Chen et al. 2016), carbon monoxide (Zhou et al. 2018), and pesticides (Chouler and Di Lorenzo 2019).

2.4 Classification of MFC According to Applications

2.4.1 Microbial Carbon Capture Cells (MCCs)

Carbon capture, storage, and sequestration can be simultaneously obtained in MFC by providing algae as biocathode and algal biomass as a substrate for anodic oxidation. In microbial carbon capture cell (MCC), CO_2 generated during anodic oxidation utilized by algae to synthesize the algal biomass in cathodic chamber, upon harvesting which can act as feedstock for biodiesel production (Jadhav et al. 2019). MCC designed by coupling the MFC and algal species cultured in cathodic chamber, which can be useful for CO_2 utilization and O_2 production to enhance the cathodic reduction reaction rate. Use of algal species in cathodic chamber generates oxygen during photosynthesis, making it available for cathodic reduction, thus reducing the cost of external aeration as required in aqueous cathode MFC (Jadhav et al. 2017). Thus, MCC can serve as potential candidate for CO_2 sequestration, wastewater treatment, nitrogen removal, by-product recovery as well as for electricity generation.

2.4.2 Microbial Desalination Cell (MDC)

In conventional dual chamber MFC, additional middle chamber is provided for desalination in MDC by providing an anionic and cationic-exchange membrane (AEM & CEM) on either side. It integrates basic principles of MFC and electrodialysis process for organic matter removal and desalination. During anodic oxidation, decomposition organic matter by microbial consortia results into production of carbon dioxide, electrons, and protons released into anodic compartment. Current across the external load was produced due to flow of electrons to the cathode via external circuit arrangement. Cathodic terminal electron acceptor uses these electrons to produce water during reduction. Such phenomenon generates potential gradient as a driving force across the anodic and cathodic chambers. For maintaining the electro-neutrality in the electrolytes, the anions migrate from the middle chamber saltwater across the AEM into the anode, whereas the cations from saltwater transported across the CEM into the cathodic compartment. Such migration of ions from saltwater desalinates the salt from saline water and produces more energy than the external energy required to operate such system (Saeed et al. 2015).

2.4.3 MFC-Microbial Electrolysis Cell (MFC-MEC)

MFC system can be coupled with conventional wastewater treatment process or bioelectrochemical system to improve the wastewater treatment efficiency and/or resource recovery. It can be coupled with MEC to get higher amount of hydrogen yield during wastewater treatment by utilizing power output from MFC to drive the reactions. Such MEC-MFC-coupled system includes: anodic oxidation of substrate by bacteria; cathodic reduction through redox reaction; and proton reduction in the MEC cathode. Wang et al. (2011) proved about 41% improvement in overall H_2 production rate in coupled system as compared to fermentation process alone.

2.4.4 Sediment MFC (SMFC)/Benthic MFC/Marine MFC

SMFCs are bioelectrochemical cell responsible for conversion of chemical energy of sediment, wastewater rich in organic matter and sulfides, into electrical output through biocatalytic and metabolic activities of microorganisms and chain of electrochemical redox reactions (Reimers et al. 2001; Schamphelaire et al. 2008; Sajana et al. 2013, 2018). SMFC or benthic MFC constructed with an anode electrode fixed at 2–4 cm depth from water sediment interface and a cathode electrode at 2–4 cm depth from the water surface. Sediment–water interface serves as an alternative to proton exchange membrane and hence reduces the cost of membrane. Performance

of sediment MFC is governed by sediment properties, electrode spacing, water hydraulics, operating conditions, and design aspects (Jadhav and Ghangrekar 2013).

2.4.5 Bioelectric Toilet

Recently developed MFC centralized techniques, i.e., pee-power urinal, eLatrine, e-urinal, septic tank MFC, Green Latrine, microbial electrochemical septic tank, bioelectric toilet MFCs represent field scale applicability. In such system, human excreta, urine, and sludge settled in the septic tank can be used as a source of organic matter for bacteria during anodic oxidation (Jadhav and Chendake 2019). Such system offers effective human waste treatment, electricity generation, water reuse for flushing, energy saving, disinfection treatment, by-product recovery, and low area requirement than conventional septic tank, which makes it sustainable solution for onsite sanitation (Jadhav et al. 2020; Jadhav 2017). Such system focused on applicability of human waste as a substrate to improve the sanitation facilities with minimal utilization of resources. Implementation of such MFC into septic tank improved the treatment efficiency of existing sanitation practices and created a loop for adopting such system towards commercialization.

2.5 Practical Applications of MFC

Major limitation of MFC technology is it is still in laboratory scale due to lower power output and cost economics associated with its design. Several researchers have attempted to scale up the system to address challenges and issues associated with scaling up. Few researchers have implemented the MFC system in wastewater streams as well as implemented it for practical applications in sewage treatment (Feng et al. 2014; Ieroupoulos et al. 2013). MFC system of 250 L capacity was capable to produce electric power of 470 mW/m^3 in stacked arrangement of electrode assemblies (Feng et al. 2014). Stacked MFC system made up of ceramic separator of 26 L and 45 L developed at India was capable to produce power of 36 mW using sewage as a substrate (Ghadge et al. 2016). Ieropoulos et al. (2013) have developed stacked arrangement of MFC using pee as a substrate for oxidation. Similarly, field application of MFC as bioelectric toilet has been developed in IIT Kharagpur which treats human waste as a substrate and generated electricity to operate LED bulbs for illuminating the toilets at night time as well as for the mobile charging purpose (Jadhav 2017).

2.6 Outlook and Summary

Microbial fuel cell (MFC) technology provides flexible platform for both oxidation and reduction reactions and hence it has wide range of applications for contaminant removal from wastewater. In advance wastewater treatment system, MFC can be pre-treatment or post-treatment obtain for efficient WWT. It also provides solution for biological oxidation process and as a tertiary treatment for disinfection, denitrification, and aeration to make effluent suitable for discharge. Additionally, valuable resource recovery from wastewater makes this system competent with conventional WWT processes. Due to several applications, MFC can be suitable for implementing at the field scale for efficient effluent treatment and recovery of energy. As a bio-energy source, it can be suitable to operate electronic appliances with small power requirement.

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Chapter 3
Biofilms: Engineering Approaches to Enhance Process Efficiency

R. Reshmy, Deepa Thomas, Raveendran Sindhu, Parameswaran Binod, and Ashok Pandey

Abstract Electrochemically active biofilms (EABs) play a crucial role in the bioelectrochemical systems (BESs) in which they oxidize organic matter and manage the transfer of electrons via substrate oxidation to the anodic surface. The modification of organisms by genetic means leads to enhanced production of EAB and extra electron transfer (EET) mechanisms, which improve the process efficiency. Cell surface modification, operation parameter validation, and media optimization are some ways to accelerate the performance of BES. This chapter will provide an insight into the mechanism of biofilm formation and various methods to enhance the biofilm formation for improving electrocatalytic rates in bioelectrochemical systems.

Keywords Electrochemically active biofilms · Bioelectrochemical systems · Extracellular polymeric substances · Biofilm formation · Surface modifications

3.1 Introduction

The depletion of fossil fuels and environmental pollution had a serious impact on the sustainability and growth of life on the globe. The limited availability of conventional energy demands the development of alternative energy sources. One of the major efforts to resolve the above-mentioned crisis is the production of

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bioelectrochemical systems (BESs). Nowadays, BESs have drawn attention in three main aspects, namely the production of energy from organic substrates, generation of products and providing specific environmental services (Arends and Verstraete 2012; Chandrasekhar et al. 2015a). They are unique and can convert chemical energy into electrical energy and vice versa using microbes as catalysts (Bajracharya et al. 2016). The microorganisms can be present in these systems as planktonic cells or biofilms. The former can carry out extracellular electron transfer (EET) through mediators, whereas in latter by direct transfer from cell envelope to the electrode. Electrochemically active biofilms (EABs) produced by microorganisms play a vital role in BES. The well-studied types of BES include microbial fuel cells (MFCs) and microbial electrolysis cells (MECs). The MFCs are used in electricity production and wastewater treatment. The MECs are utilized in the field of generation of fuels, mainly hydrogen, ethanol or chemicals like hydrogen peroxide, caustics, etc. (Borole Abhijeet et al. 2011). The efficiency of BES depends on the capacity of EA microorganisms to couple their oxidative metabolism to the reduction of the anode electrode. The ability of microorganisms to attach to an electrode, either as a monolayer or multilayered biofilm, is significant for BES performance. One can induce active microbes to produce more EAB, and it is a better strategy to improve the power performance of MFCs (Angelaalincy et al. 2018).

3.2 Biofilms in Bioelectrochemical Systems (BESs)

Biofilms are surface-associated microbial colonies as well as microbes forming assemblies or aggregates (Halan et al. 2012). These microbes are integrated into self-produced extracellular polymeric substances (EPSs) in which they reside in a coordinated fashion and thus benefit from ecological niches created within the biofilm, develop all types of interfaces and are less affected by toxic substrates (Singh et al. 2006). The properties of these colonies are managed by their physiological performance. The complex biofilm attachment process is greatly influenced by various environmental and surface-related factors. Biofilm may usually be formed by a single bacterial species, even though it contains many bacteria, algae, protozoa and fungi (Enamala et al. 2018). In BES, biofilm controls both anode and cathode processes. In particular, mixed cultural bacteria act as a source of microbes in the anodic chamber so that biofilm is created from the natural sources (Sevda et al. 2018).

3.2.1 Constituents of Electrochemically Active Biofilms

In general, biofilms consist of a syntrophic consortium of microorganisms in which cells adhere together or to a surface. Biofilm's framework comprises two primary components, i.e., water channel for nutrient transport and tightly packed cell region

without prominent pores in it. The EPS components produced by biofilm cells comprise proteins, DNA, polysaccharides and RNA (Jamal et al. 2015). The cohesive, three-dimensional framework of the biofilm formed by EPS interconnects and immobilizes cells. It also provides mechanical stability and serves as a nutrient source. The composition of EPS greatly depends on the nature of microorganisms, the shear forces, nutrients availability and the temperature (Borole Abhijeet et al. 2011; Flemming and Wingender 2010; Babauta et al. 2012; Rabaey and Rozendal 2010).

The performance and activity of biofilms formed on the electrodes of BES depend on the electrochemical, biological, physical and chemical parameters. The open-circuit potential and measured current of electrodes greatly depend on biofilm electrode materials. Carbon materials such as graphite rod, carbon paper, carbon cloth, carbon felt, graphite fibre brush and reticulated vitreous carbon are commonly used electrodes in BES (Joo et al. 2002; Rae et al. 2007; Ishii et al. 2008; Ahn and Logan 2010; Liu and Cheng 2005; Pablo et al. 2010; Zhou et al. 2011; Wei et al. 2011). Busalmen et al. have also been successfully used gold electrodes for direct electron transfer studies (Pablo et al. 2010). In another study, Jain et al. used indium tin oxide as electrodes for EET (Anand et al. 2011).

3.2.2 Factors Affecting the Formation of Biofilms

The physical, environmental, surface and extracellular components of the organisms are the major factors which influence the biofilm formation. The effect of various electrochemical operating parameters like pH, temperature, ionic strength, flow rate and nature of substrate on EAB formation is discussed here.

3.2.2.1 pH

Acidity is one of the most significant environmental variables affecting the development and physiology of bacterial cells. The pH of the operational condition affects the current production and development of anodic microbial EAB. Biffinger et al. examined the effect of acidity on MFC containing *Shewanella oneidensis* and found that pH played an important role in bacterial growth (Biffinger et al. 2008). Patil et al. verified that only pH level ranging from pH 6.0 to 9.0 was suitable for biofilm growth. Any observable deviation from neutral pH conditions led to an observable decline in the performance of biofilm (Patil et al. 2011). High-stress levels also cause a reduction in the pH level (Franks et al. 2009).

3.2.2.2 Temperature

In microbial biotechnological processes, the temperature is an inevitable parameter. Patil et al. investigated the temperature dependence on biofilm formation and found that high temperature at initial biofilm growth favours the biofilm formation process and its bioelectrocatalytic performance (Patil et al. 2010).

3.2.2.3 Surface Topology and Flow Rate

The surface roughness of the electrode affects the cell attachment process. The rough surface allows more bacterial adhesion by offering enlarged surface, thus causes a reduction in sheer force on bacterial cells in flowing liquids at a high flow rate. Within the boundary layer, where turbulent flow is absent, the flow velocity is incapable to remove the biofilm. Whereas outside the boundary level, the turbulent flow is maximum and affects the cell attachment processes. The depth of the boundary layer mainly depends on the flow velocity. As velocity increases, a decrease in the size of the boundary layer and the cells experienced a high turbulence level. The formation, mass, structure, thickness, EPS production and metabolic activities of biofilms are regulated by hydrodynamic conditions (Prakash et al. 2003).

3.2.2.4 Divalent Cation

The initial adherence of microbial aggregates in the form of sludge flocks, granules and biofilms is controlled by divalent cations, such as calcium and magnesium. These ions facilitate the bridging of sites with a negative charge on extracellular polymers. The biofilm's thickness can be improved by providing more divalent cations which offer much mechanical stability (Min et al. 2005).

3.2.2.5 Quorum Sensing (QS)

During the initial cellular attachment process, there are up and down gene regulations. Cell-to-cell signalling or QS has also played a crucial role in cellular attachment and detachment process from the biofilm. It consumes a transcriptional activator protein that works in conjunction with tiny autoinducers signalling molecules to stimulate the expression of target genes resulting in chemical behaviour (Steyn et al. 2001; Dettweiler et al. 2019).

3.2.2.6 Nature of Substrate

The microbial growth rate also depends on substrate concentration (Pham et al. 2009). It was also observed that the microbial enrichment is greatly influenced by the type of the substrate used. Various studies demonstrated the influence of substrate on biofilm formation using different substrates such as acetate, potato wastewater, winery and domestic wastewater (Cusick et al. 2010; Kiely et al. 2011; Ganesh et al. 2018). It may be concluded that the type of substrate has a crucial role in biofilm formation.

3.2.3 Mechanism of Biofilm Formation

Biofilm is a community of microorganisms that depend entirely on the nature of the environment and adhering surface. The construction of biofilm is affected by numerous factors such as the concentration of nutrients, hydrodynamic conditions, bacterial motility, intercellular communication, EPS and proteins. Biofilm formation is a sequential process. The electron shuttle is considered as an essential component for the biofilm formation and its functioning as it permits microorganisms for electron transfer to the electrode. The mechanism involved in biofilm formation is depicted in Fig. 3.1.

1. Initial adherence of microbes on electrode surface: Microbial attachment to the surface by flagella or fimbriae is a reversible process. As discussed earlier, the adhering capacity of microbes greatly depends on surface topography. The nano- and micro-level surface roughness favours bacterial adhesion to substrates.
2. Irreversible attachment: Microbes are then split by binary fission on the surface. It secretes EPS, making the attachment stronger and irreversible. Since adherence is not always uniform, channels may grow on the surface to penetrate fresh nutrients

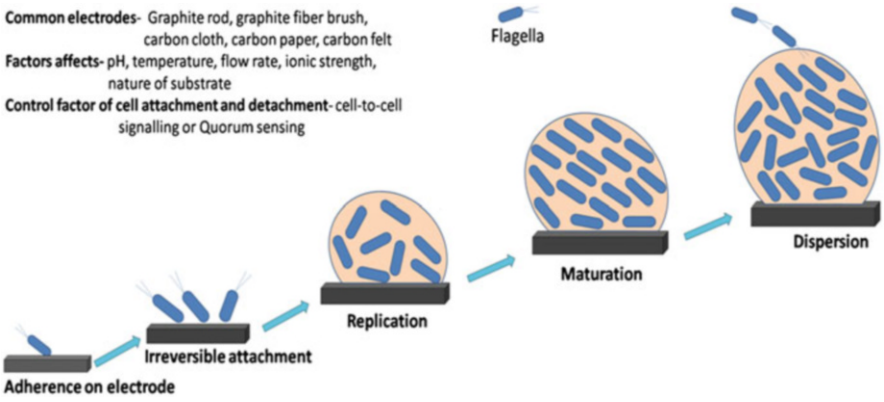

Fig. 3.1 Sequential process of biofilm formation in bioelectrochemical systems

and oxygen, which facilitates the metabolism of attached microbes. Thus, the rise in the quantity of biomass with improved EPS secretion resulting in an anaerobic gradient near the surface.
3. Replication: In this stage, the development of monolayer microcolony on the fixed surface occurs due to the replication after irreversible attachment.
4. Maturation to biofilm: Matured biofilm was formed by attaching debris from the adjacent environment in a three-dimensional structure and by employing new planktonic bacteria.
5. Dispersion: Dispersion or development through active and passive mechanisms in which sessile, matrix-enclosed biofilm cells transform through QS or a cell-to-cell signalling mechanism into freely swimming planktonic bacteria.

In MFCs, the microorganisms are utilized for energy harvesting by the electron transfer process. The biofilm formed on the electrodes of MFCs is called EAB. EABs are also known as electrochemically active microbes, electricigens, exoelectrogenic bacteria and anodophilic species (Marsili et al. 2010). In cases in which direct electron transfer or the formation of EAB is not possible, another strategy using bacteria that are physically immobilized can be considered (Flickinger et al. 2007).

3.2.4 Advantages and Applications of Biofilms in BES

The applications of biofilms in BES were explored in different fields, such as electrochemistry, microbiology, chemical engineering, biotechnology and sustainable energy development. These applications include wastewater treatment, bioremediation, power generation, biosensor design, biohydrogen production and production of value-added chemicals (Li et al. 2008; Sleutels et al. 2012; Du et al. 2007; Rozendal et al. 2008; Sivagurunathan et al. 2018; Chandrasekhar et al. 2015b).

In bioelectrochemical wastewater treatment, energy is harvested from wastewater using electrochemically active microorganisms. These microorganisms are capable of EET and oxidizing the organic materials in the wastewater. Thus, microorganisms function as a catalyst for the effective removal of organic matter. MFCs can improve the development of bioelectrochemically active microbes during wastewater treatment, so they have excellent operational stability. When low organic matter concentration and small volume are present, it was noted that the recovery of methane by anaerobic processes is not much economical. For such wastewater, MFC is beneficial because of its potential to recover energy directly in the form of electricity. Min et al. demonstrated the ability of MFC for energy production and wastewater treatment using swine water (Min et al. 2005). Various studies also proved the potential of MFC in wastewater treatment. BES is used for the generation of electric power or hydrogen from biomass with zero carbon emission into the ecosystem.

Due to their benefits over other bio-recognition components, such as electron transfer processes between microorganisms and electrodes in enzymes and cell

organelles, the chance of using microbes as biological identification components in biosensors has drawn significant interest over the years.

3.3 Methods for Enhancing Process Efficiency

The efficiency of BES mainly depends on the stability of bioanode electrochemical activity. This stability has been attributed to the factors such as facilitated electron transfer between electrode and bacteria, the improved surface area of electrodes and better electrode biocompatibility. The optimized strategies for improving their metabolic efficiency are the primary goal for BES development. The interactions between bacteria and anodic surface play an important role in biofilm growth. Many strategies can be adopted for enhancing this interaction leading to BES with many potential applications. The major engineering approaches for enhanced biofilm formation are given in Fig. 3.2.

3.3.1 Genetic Engineering Approaches

Although a number of factors affect fuel cell performance, a genetic engineering approach that would improve biofilm manufacturing in microorganisms is still regarded as a promising approach to improved fuel cell performance. To utilize genetic engineering approaches for enhancing BES performance, a detailed conception about microbe−electrode interactions at the molecular level is needed. In addition, the identification and monitoring of unique and flexible microbial catalysts can assist scientists to achieve superior microbial strains with elevated electron transfer levels that can improve the efficiency of BESs in a multitude of practical applications. A number of ways can be adopted to choose the right organism which can serve as the framework. This involves using a well-studied laboratory strain as a

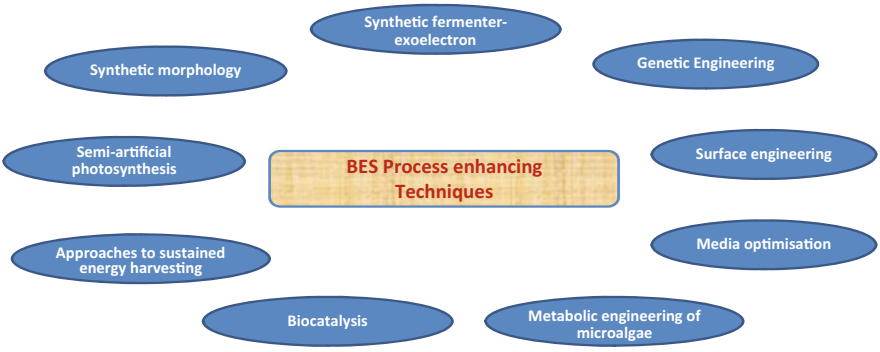

Fig. 3.2 Major engineering approaches to enhance process efficiency of bioelectrochemical system

framework and engineering it to express the electro-active proteins in right proportion, isolating a genetically traceable organism from the target setting and engineering it to express electro-active and protein assistance and isolating an organism from the target setting (Patil et al. 2014).

This strategy can make use of microbial-based biocatalysis for targeted bioelectrochemical applications such as biosensing and bioremediation. This approach also opens up possibilities for the development of versatile BESs (Rosenbaum and Franks 2014). Alferov et al. investigated the potential of cytochrome enriched *Escherichia coli* for enhancing electron transfer mechanism (Alferov et al. 2009). Another study by Coman et al. reported the successful use of engineered *Bacillus subtilis* containing cytochrome for increasing the overall BES performance (Coman et al. 2009).

3.3.2 Surface Engineering Approaches

The biofilm formation was largely affected by the surface physico-chemical properties such as roughness, composition, shear rate, lipophobic/lipophilic, hydrophobic/hydrophilic nature and charge density (Gallaway and Barton 2008) (Fig. 3.3). In addition, the electron transfer rate at the electrode−biofilm interface greatly depends on the surface functional group molecular structure. The unmodified electrodes based on carbon are well explored for BES applications. Nowadays, researchers focused on innovative methods for surface treatments for promoting the electrode−biofilm interaction (Marsili et al. 2010). It was reported that heat, plasma or acid treatment could improve the biofilm formation and its performance. Finkelstein et al. demonstrated the potential of pretreatments using sulphuric acid on graphite electrodes for improved performance (Finkelstein et al. 2006). Saito et al. reported the effect of nitrogen addition to the anode surface to improve the performance of MFCs (Saito et al. 2011). Guo et al. reported that the surface charge could impart a prominent effect on anodic biofilm formation. Electrochemically active microbes were more selective towards hydrophilic surfaces with positive charges. This was demonstrated using glassy carbon modified with different functional groups such as $-CH_3$, $-OH$, SO_3^- or $-N^+(CH_3)_3$ (Guo et al. 2013).

Increased surface roughness and high porous nature of electrode materials significantly improve the biomass concentrations and current generation in BES

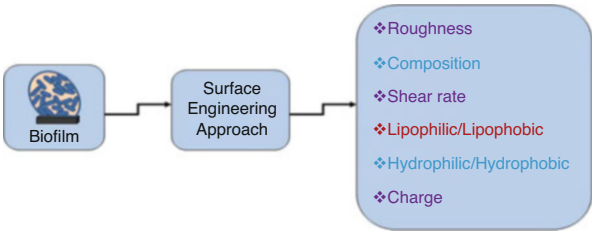

Fig. 3.3 Surface physico-chemical parameters affecting biofilm formation

(Dumas et al. 2008a). It was found that higher surface roughness of the electrode promotes faster initial attachment and leads to early biofilm formation. Dumas et al. investigated the correlation of surface roughness with biofilm formation (Dumas et al. 2008b). It was reported that shear rate also has a key role in improving the efficiency of BES. High shear rates cause strong aggregation and attachment of microbes which form more compact and denser biofilms (Pham et al. 2008).

3.3.3 Media Optimization Strategies

Mediator is employed for electron transfer from the surface of the electrode to the microorganism by electron shuttle. This approach could significantly alter the environmental factors such as pH, redox balance, etc. and leads to enhanced cell biofilm growth. Commonly used redox mediators are neutral red, thionin, methyl viologen, humic acid and riboflavin. Culture media are exclusively optimized for particular microbial strain, and its metabolism is greatly affected by precise concentrations and nature of nitrogen sources, minerals, buffers, vitamins, chelating factors and antifoaming agents. Angelaalincy et al. reported that media optimization is a promising tool for enhancing EPS production and this leads to the formation of a definite biofilm. From this study, it is evident that EPS production and biomass also depend on the nutrient supplementation (Angelaalincy et al. 2017). The role of media optimization in improving the production of EPS in bacteria and yeast systems has also been examined by several groups (Li et al. 2013; Joshi et al. 2013).

3.3.4 Metabolic Engineering Approaches

The metabolic engineering approach permits undeviating control over the microorganism's cellular machinery through mutagenesis or the introduction of transgenes. The growth of a number of transgenic algal strains with recombinant protein expression engineered photosynthesis and augmented metabolism foster designer microalgae possibilities (Singh et al. 2012; Saratale et al. 2017). The exploitation of metabolic processes can redirect cellular function towards the synthesis of preferred products and even expand microalgae processing capacities. One technique of coercing microalgae uses particular environmental variables, such as nutrient regimens, to cause required metabolism fluxes. It offers an alternative approach in which synthetic pathways are created into user-friendly hosts for biofilm production. It has been observed that the sheer power plays an important role in the growth of biofilm. Liu et al. reported that a certain detachment forces are necessary to produce a stable and compact biofilm structure (Atsumi and Liao 2008). To further increase the performance of biofilm, an engineered exoelectrogens with the novel biocompatible electrode can be a better choice.

3.3.5 Synthetic Fermenter−Exoelectrogen Strategies

The exoelectrogens, like *Shewanella* and *Geobacter* were extensively used in BES. A limited spectrum of carbon sources could only be used by these exoelectrogens. *Geobacter* primarily used acetate as a source of carbon, and *Shewanella* primarily used lactate, which considerably limited their practical applications. Fermenter−exoelectrogen strategy helps to extend the spectrum of carbon sources that could be used in BES. In this approach, a microbial consortium is constructed which contains a fermenter together with an exoelectrogen. Optimal coordination between fermenter carbon source metabolism and exoelectrogen extracellular electron transfer promotes the variety of carbon sources in BES (Kadier et al. 2016; Kumar et al. 2017).

Choi et al. made use of glucose as a carbon source for power generation by constructing a microbial consortium using *Shewanella oneidensis* strain along with two key genes from *Zymomonas mobilis*, glf (a glucose facilitator gene) and glk (a glucokinase gene) (Choi et al. 2014). *Escherichia coli, Shewanella oneidensis*-based microbial consortium utilizes xylose as a carbon source, is also reported. In this work, the genetically engineered *Escherichia coli* acts as a fermenter and the *Shewanella oneidensis* as the exoelectrogen. This strategy exploits xylose, cellulosic biomass and recalcitrant wastes as carbon sources in BES (Choi et al. 2014).

3.3.6 Synthetic Morphology Approaches

Synthetic biology is an evolving study field that focuses on engineering guidelines for the design of cellular features that can be regulated at the will of the user. Synthetic morphology approach allows sophisticated tools to specifically and wisely manipulate genetic programmes. This strategy makes use of engineering aspects and morphology of microorganisms for designing microbial cell factories for innovative applications.

In bacteria, the cell wall maintained the overall cell shape. The physical property of cells such as stiffness, strength and surface-to-volume ratio mainly depends on the size and shape of the cell. The structural diversity of bacterial cells can be exploited for practical applications. The engineering of the cell shape and morphology of microorganisms make them suitable for numerous biotechnological applications (Höltje 1998).

The cell membrane permeability can be enhanced by the synthetic porin. The protein with large pore sizes can improve the EET of bacteria and increases the efficiency of BES. The work expressed a porin protein OprF from *Pseudomonas aeruginosa* PAO1 into *Escherichia coli*. The results showed that synthetic porin protein can enhance the performance of *Escherichia coli* (Yong et al. 2013). Lee

et al. stated that the formation of poly (3-hydroxybutyrate) can be enhanced using recombinant bacteria, *Alcaligenes eutrophus*. The morphological modification was achieved by cloning *Alcaligenes eutrophus* polyhydroxyalkanoates synthesis genes into a wide host range plasmid pVK101, and the build vector system was transferred into *Alcaligenes eutrophus* (Lee et al. 1998).

3.3.7 Semi-artificial Photosynthetic Strategies

The semi-artificial photosynthetic strategy is a way to maximize the performance of BES by rerouting the natural photoelectrogenetic pathways. This strategy exploits the benefits of biocatalyst and synthetic materials for producing versatile chemicals which possess superior selectivity and efficiency (Nam et al. 2018). In this type of systems, inorganic photo sensitizer may provide bioavailable reducing equivalents and may enter into native or engineered metabolic pathways and convert simple inorganic salts to versatile compounds (Sakimoto et al. 2018). The schematic representation of the metabolic pathway is described in Fig. 3.4. Li et al. reported the successful fabrication of a hybrid photoanode system. This was achieved by integrating photo system II into nanotubular titania-modified indium tin oxide electrodes for photocurrent generation. The study indicated that this hybrid photo-BES enhances the direct electron transfer which leads to superior photocurrent generation (Li et al. 2016).

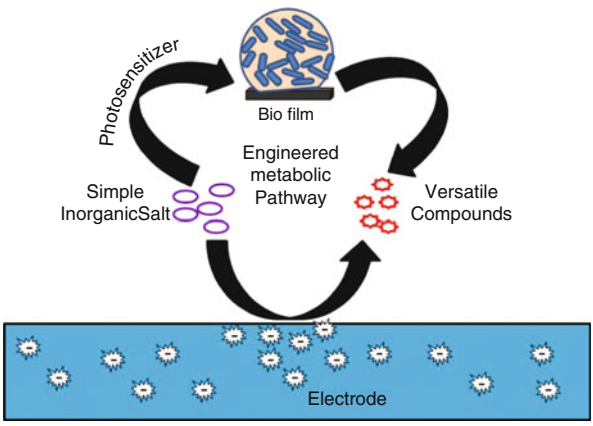

Fig. 3.4 Schematic representation of the metabolic pathway for the production of versatile compounds through biofilm

3.3.8 Approaches to Sustained Energy Harvesting

The main approaches for sustained energy harvesting in BES include Maximum Power Point Tracking (MPPT) and Power Management Systems (PMS). The energy harvesting technologies can be mainly categorized into charge pump-based, capacitor-based and boost converter-based systems. However, how energy harvesting affects microbial activity and community is studied to a lesser extent. Even though the energy harvesting by means of capacitors or charge pumps possibly will not affect the microbial activity and community, the booster-converter-based system largely affects these parameters and causes a change in electron transfer mechanism. While using the booster-converter-based system, it imparts a discriminating pressure on the microbes in order to control respiratory processes for an effective electron transfer and leads to an enhanced energy harvesting. Wu et al. successfully applied DC/DC booster circuit for enhancing the power efficiency of MFC using *Shewanella oneidensis* (Wu et al. 2012). Zang et al. investigated a bioelectrochemical desalination system for energy production and found that this system could effectively reduce electrodialysis energy consumption and desalination time to a large extent (Zhang and He 2012).

3.3.9 Biocatalytic Strategies

The term biocatalyst is generally used to depict the microorganisms in BESs. The biocatalytic approach could be used in BESs to more efficiently deposit reactive metallic catalysts on a microbial colonized electrode to improve the electrode's catalytic activity. De Windt et al. successfully applied this strategy to enhance the rate of catalytic dechlorination by incorporating biodeposited metals on the cell wall of microorganisms (Zhang and Hu 2017). The improvement of catalytic activity of microorganisms can be achieved by increasing the biofilm thickness or engineering the structure of the biofilms. Popov et al. examined the impact of butyrate and acetate enrichment on biofilm structure for enhancing the performance of BES and found that the butyrate enriched biofilm is more capable to improve the BES performance than the acetate (Popov et al. 2016).

3.4 Conclusions and Future Perspectives

BESs are versatile systems intended to convert chemical energy into electrical power using microbes as catalysts. The method of attachment of biofilm is too complicated and is significantly influenced by various surface-related, cellular and environmental factors. The electron shuttle is responsible for the development and performance of the biofilm as it allows microbes to transport electrons to the electrode within a small

distance of 40–50 mm. The development of optimized approaches for effective EAB formation is a prime factor in enhancing BES efficiency. This chapter mainly focuses on the mechanism of biofilm formation and various strategies that can be adopted for enhancing BES performance.

Several strategies can be adopted for improving the performance of BES, viz. genetic engineering approaches, surface engineering approaches, media optimization strategies, metabolic engineering of microalgae, synthetic fermenter−exoelectrogen strategies, synthetic morphology approaches, semi-artificial photosynthetic strategies, approaches to sustained energy harvesting and biocatalytic strategies. Most of the strategies modify the function of microorganism by engineering gene cytochromes or surfaces for enhanced system performance.

BES provides an attractive tool for sustainable energy and chemical production. In addition to this, it could also find applications in bioremediation, biosensing, biofuel production and wastewater treatment. Future research can be extended to produce promising results and reveal the possibility of BES in certain untapped areas like biomedicine, agriculture, corrosion prevention and mining.

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Chapter 4
Bioenergy Production in Bioelectrochemical System

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Abstract In current periods, increasing demands of fuel energy and more environmental issues are reported as big challenges for world people to sustain their life. These are due to exponential rate of population and more industrialization trends around the worlds. For life needs, we are depending on energy requirement and more amounts of non-renewable energy are utilized for that needs. In future periods, we are worried about fossil fuel availability stocks and its operation. Others, issue is more amount of waste organic compounds, present in water sources or other components of environment or nature. As mentioned earlier that more quantity of fossil fuel utilization can produce more toxic gases or by-products which could be components of greenhouse gases or other environmental pollution components. Now our efforts are putting to solve these challenges in world and efficient biological processes can be applied for biological processes mediated energy generation with simultaneous achievement of the wastewater treatment task. There are many efficient biological processes employed for bioenergy and waste organic matters utilization and bioelectrochemical system (BES) processes with methane-producing microorganisms can be applied for biogas mixtures generation and waste organic matters breakdown techniques. Microorganisms on electrodes in BES system are used as catalysts for the reactions carbon dioxide (CO_2) gas into generation of methane and electric power. In BES system, methane and hydrogen fuels are reported to produce and these are good examples of sustainable and renewable energy source with generation of least quantity of toxic gases or by-products. Author will emphasize more recent research development for generation of hydrogen and methane fuel with wastes utilization in BES system.

Keywords Biochemical system (BES) · Hydrogen · Methane · Organic waste matters · Biological processes · By-products · Fuels · Microorganism

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Abbreviations

Ag/AgCl	Silver/silver chloride
AM	Animal manure
BES	Bioelectrochemical system
BOD	Biological oxygen demand
CC/CB	Cloth coated with carbon black
CFTs	Carbon fiber textiles
CH_4	Methane
CO2	Carbon dioxide
COD	Chemical oxygen demand
GAC	Granular activated carbon
GFB	Graphite fiber brush
GG	Graphite granules
H_2	Hydrogen
HPA	Hydrogen-producing acetogenesis
HRT	Hydraulic retention time
MB	Methanogenic bacteria
MECs	Microbial electrolysis cells
MF	Methane fermenter
MFCs	Microbial fuel cells
MPPC	Maximum power point circuit
MRMC	Microbial reverse-electrodialysis methanogenesis cell
MSW	Municipal solid waste
MW	MegaWatts
NBES	Non-bioelectrochemical system
PRO	Pressure-retarded osmosis
RED	Reverse electrodialysis
SS/Pt	Stainless steel mesh coated with platinum
V	Volt
VFA	Volatile fatty acids

4.1 Introduction

Anaerobic modes of conversion processes with microbial cells or strains contribution had helped in utilization of organic waste compounds or other pollutants as waste resources into formation of some useful products (i.e., biofuels or other bioproducts or biochemical compounds) and these processes have reported as an established effective technology or approaches for environment protection via helping the waste matter or organic matters in wastewater treatment in our healthy environment. Some bioproducts examples are biogas mixture (different ratios of methane (CH_4) and CO_2 components) that is produced as beneficial, renewable

nature energy sources and these products are achieved in anaerobic digestion processes and is reported as technically manner simple process, with least quantity energy consumption via converting organic material (obtained as wastewater resources organic contents, solid or water soluble wastes, and biomasses) into CH_4 or other useful fuels (Zeeman and Sanders 2001; Parra-Orobioa et al. 2018).

System of anaerobic digestion (AD) processes is applied for degrading the wastes matter or components in wastewater via producing final or end products including different mixture of biogases in different components in varied volume percentages ($CH_4 \sim 55–75$ and $CO_2 \sim 25–45$) depending on bioprocesses nature. The processes of heating or upgradation for natural gas quality or co-generation of electric power energy or heat energy can be achieved by using of biogas fuel energy. Least quantity of energy consumption or less space requirement are needed for installations of processes of AD units plant plants processes and technologically it is simple processes. This digester has helped in anaerobic treatment tasks, reported as high-rate (i.e., biomass retention) or low-rate process system (without biomass retention) (Melis et al. 2000). High-rate systems are reported at short periods of retention time and long period of sludge retention time, applied for treating many types of wastewater where low-rate systems can help in digestion of slurries and solid wastes with longer hydraulic retention time and sludge retention time periods. These digesters are reported to produce different quantity of yield of biogases which is found due to variation in type or concentration of the feedstock and process conditions (Melis et al. 2000; De et al. 2003; Zhang et al. 2017).

In municipal solid waste (MSW) and animal manure (AM), organic matter proportions are found different and these waste matter are reported to produce different yields of biogas mixture (80–200 m^3/ton MSW) and 2–45 m^3/m^3 (AM). Co-digestion can improve reactor efficiency and economic feasibility as important or significant factor, reported in Netherlands co-digestion (Fruteau-de-Laclos et al. 1997; Garcia-Depraect et al. 2019). This process is limited to some range of substrates due to legislation rules and recommendation and it is commonly employed for digestion of substrate in agriculture. Renewability nature of energy biosynthesis can enhance the competitiveness of AD processes compared to aerobic composting processes. Operations or functions of AD systems in Europe are reported to produce total capacity of 1.5×10^3 MW, but in year 2010, its potential is estimated to enhance 5300–6300 or more MW electric energy (Fruteau-de-Laclos et al. 1997; De et al. 2003; Głąb et al. 2019).

It was reported for application of microbial fuel cells (MFCs) or microbial electrolysis cells (MECs) for treatment of wastewater and its technical or scientific advancement approaches can be achieved by rapid modes of biotechnology processes. Further, these devices operations are completed by utilization of bioelectrochemical reactions as integrated approaches under bioelectrochemical system (BES).) And these system operations are produced from integration of biological and electrochemical processes that generated huge quantity of electric power, biohydrogen (H_2), or other useful chemical products (Logan et al. 2008; Magnin and Seseure 2019). MFCs or MECs device as electrochemical cells has shown to consist of two electrodes (i.e., an anode or cathode electrodes) that are joint

by an external wire for completing an electron flow in electrical circuit system. MFCs unit had produced the electricity power, while MECs have capability for using electricity energy for driving chemical reactions at cathode site for biosynthesis of H_2 fuel or other nature of chemical products reactions at cathode for production of H_2 fuel or others chemicals by using wastewater via removing organic matters from sources of wastewater (Kim and Logan 2011; Zhao et al. 2017).

Abundance sources of entropic energy are present in huge quantity which is found from river water and seawater (salts), need to efficiently capturing and storing system. Hydrogen (H_2) production can be achieved by utilizing single process and it helped in capturing of salinity driven energy (Pant et al. 2010; Cao et al. 2019). Degradation processes of organic matters or compounds are achieved by using exo-electrogenic nature of bacterial cell system or fungal strains that reported in many pairs in seawater or river water systems. These microbial cells systems have been reported to sandwich between an anode and a cathode electrode for formation of microbial reverse electrodialysis or electrolysis cells. In waste matter degradation tasks and bioenergy synthesis processes, some exo-electrogen microbial strains are reported for adding of an electrode potential difference from oxidation of acetate substrate at reduced anode electrode overpotential. Stacks of reverse electrodialysis unit have helped in contribution of 0.5–0.6 V of salinity ratio (50 of seawater and river water mixture). Hydrogen productions are reported with increased rate (0.8–1.6 m^3 H_2/m^3.anolyte/day) (Mehanna et al. 2010; Wainaina et al. 2020).

Above-mentioned bioprocesses have maintained the flow rate (0.1–0.8 ml/min) of sea water or river water with the good hydrogen recovery. Different ratio of electron flow is used for biohydrogen production that released from substrate oxidation reactions (72–86%). Small scale of stack system (11 membranes) for reverse electrodialysis process are needed to only 1% of produced energy for pumping water and platinum at cathode side is used in these tests. H_2 biosynthesis rates (0.8 m^3/m^3/day) with good efficiencies of energy generation (51%) are reported from seawater or river water with biodegradable organic matter (Veerman et al. 2010; Kim and Logan 2011; Moustakas et al. 2020).

Bioelectrochemical systems are reported for methane generation and these systems are needed to require the additional voltage for overcoming large cathode overpotential values. Elimination of need for electrical grid energy can be achieved by constructing of microbial reverse electrodialysis (RED) methanogenesis cell (MRMC). This cell can be made by replacing a reverse electrodialysis stack system between an anode electrode with addition of exo-electrogenic microbial strain system and methanogenic microbes biocathode electrode (Bhanu et al. 2018).

MRMC can be converted to electrical energy as renewable salinity gradient bioenergy and it can provide additional potential, required for methane evolution at cathode. Succeeded in feasibility and efficiency of MRMC system are evaluated by using three different types of cathodes materials that are stainless steel mesh coated with platinum metal (SS/Pt), carbon cloth coated with carbon black (CC/CB), and plain graphite fibers brushes (GFB) with thermolytic solution (ammonium bicarbonate) with configuration of RED stacks (Luo et al. 2014; Chen et al. 2019).

These cells have reported to produce maximum methane yield (0.6 mol.methane/mol.acetate) by using of biocathode (SS/Pt) with Coulombic recovery (75%) and energy efficiency (7%). Biocathode of CC/CB in MRMC unit is reported less quantity of methane yield (0.55 mol.methane/mol.acetate substrate) with two-fold quantity of methane production at GFB of MRMC system. Further CC/CB material of biocathode is reported lower value of methane yield (0.55 mol CH_3/mol.acetate) that is double value of methane yield of methane yield of GFB biocathode system of MRMC unit. 89–91% of COD value removals and 74–81% of Coulombic efficiencies are reported to all types of cathode materials. Different analytical tests such as linear sweep voltammetry (LWV) and electrochemical impedance spectroscopy tests or analysis had reported for cathodic microbial cell functions and it can enhance the electrons movement flow from cathode electrode via compared to abiotic cathode nature as a control (Cao et al. 2019). MRMC system has shown significant potential for pure methane production via using poor grades waste heat energy and also organic matters resources at anode electrode (Huang and Hu 2018; Liu et al. 2016). In this chapter, biofuels biosynthesis (i.e., hydrogen and methane gases as a fuel energy formation) can utilize as cleaned or carbon free and sustainable biogases sources biogas resource for fulfilling of increased energy demand of world people.

4.2 Wastes or Wastewater Treatment System

In 2014, biological mode methane generation reported from wastewater treatment stations and from water treatment stations and for natural gas distribution network, the regulations are changed for authorization the injection network, well developed in France. SUEZ has shown his propose or technologies as an alternative by injection of bio-mode methane into natural gas distribution network locations (SUEZ report 2019; Moustakas et al. 2020).

BIOVALSAN has utilized 2/3 of carbon dioxide emissions, coming from the La Wantzenau wastewater treatment station as 4th biggest treatment in France country for treatment of organic or inorganic matters contaminated water for one million people. Combined innovative optimization approaches for sludge treatment and recovery of biogas can be good efforts for biofuel (bio-mode methane) synthesis from different stations and it can be smallest environmental foot prints in France (Parra-Orobioa et al. 2018). This energy generation can be shown as transition level towards a new local, sustainable and carbon absorber energy model in Strasbourg. SUEZ is operated for wastewater sources treatment station and the local natural gas distributors are involved in innovative BioVALSAN project (SUEZ report 2019; Bitz 2014).

BioVALSAN project consisted of injection of bio-mode methane (that produced from wastewater) into natural source gas network stations and these had shown to produce more quantity of purified methane (1.6 million Nm^3/year) and these biogases are reported to equivalent of their consumption of five thousands low economic housing units. BioGNVAL projects are demonstrated to produce a clean

fuel without emitting any fine particles with 50% less noise level and 90% less carbon dioxide gas emission to diesel fuel combustion (des Arcis and Euzen 2016). The BioGNVAL project has shown its capability for production of clean fuels that does not emit any fine particles with 50% and 90% lesser noise level and CO_2 emission, respectively, with compared to diesel engine.

The BioGNVAL has exhibited good efficiency for treating more amount of wastes matter for production of biogas (120 Nm^3/h). Energy content in 1 Nm^3 biogas is equal to around 1.1 l petrol, whereas energy content in 1 Nm^3 natural gas is equal to around 1.21.2 l petrol. It has shown 1 ton/day of BioLNG or the equivalent of two full tanks for a heavy vehicle. Wastewater generation is reported by disposal waste organic matters from 100,000 inhabitants that are utilized for synthesis of enough quantity of BioLNG to fuel energy for twenty buses and twenty trucks (des Arcis and Euzen 2016; Bitz 2014).

BioLNG or liquid form of biomethnae, that is generated from processing of organic wastes matters flow processes. Biogas can be generated by developing the anaerobic digestion and helped in breakdown the biological via emitting biogas. BioLNG is reported to practically CO_2 neutral fuels with other benefits. It has reduced carbon dioxide emission, lower engine sound. Lower nitrogen oxides or very less quantity PM (particulate matter) generation are reported (Hithersay 2018). BioLNG solution has increased the valuation of biogas via making Nordsol proposition unique property. Nordsol can build and operate its own BioLNG with facilitation of together with synthesis of biogases partners for solid business framework. It has shown in reported that Nordol company put their unique or specific efforts for achievement of mission of BioLNG mainstream and these have committed for developing sustainable nature to future via making bridge the gap between waste management facility of world and transport fuel generation. BioLNG in combination with retrieving $BioCO_2$ is found during the production process (Sapp 2018; Hithersay 2018).

BES process as innovative technology has been developed for efficient energy development and waste treatment task and it can influence the conventional AD processes for waste matter treatment. Benefits of BES technology to dark fermentation for hydrogen production are found with its effects analysis on two-stage fermentation processes with facility of H_2 and CH_4 biosynthesis (Sasaki et al. 2018). BES device is used as low-cost processes with lesser reactivity carbon sheets (for cathode or anode). In these processes, cathodic potential is controlled (at −1.0 V in ~Ag/AgCl) via a measurement via potentiostat device. During the operation of BES, glucose solution (concentration~10 g/L) is added as carbon source via generating electric current density with low value throughout (0.33–0.88 A/m^2/electrode) are added as main carbon source and it can generate high quantity of electric current density with low value throughout (0.3–0.9 A/m^3/electrode) and hydrogen production (0.5–1.5 mM/day) (Zhao et al. 2015; Zhang et al. 2017). Prevention of water electrolysis is reported and at hydraulic retention (i.e. 2 days), the substrate at pH 6.5 BES unit has reduced the quantity of gas mixture synthesis H_2 (52%) and CO_2 (47%) via comparing to non-bioelectrochemical system (NBES) and it is shown in Fig. 4.1. Methane

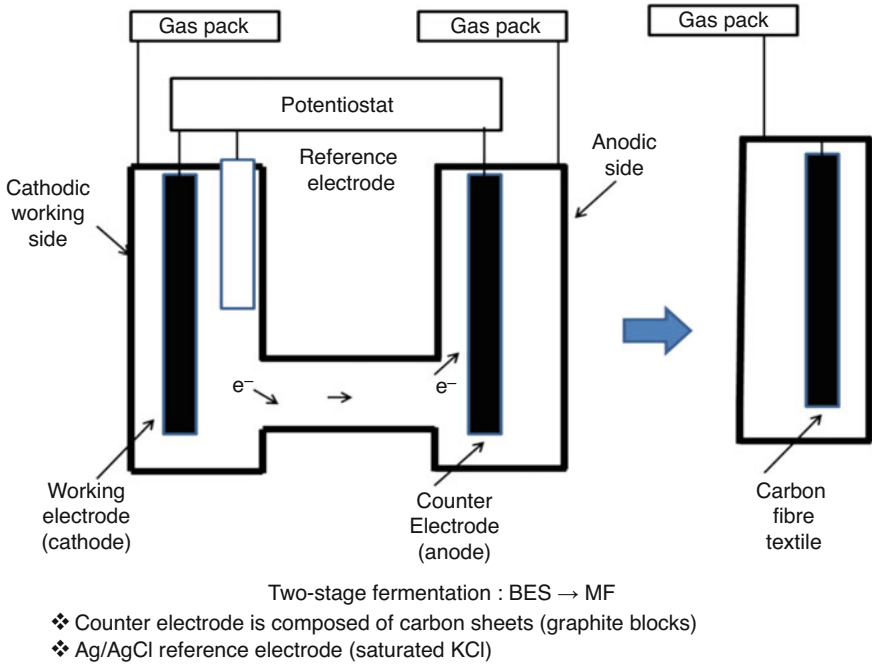

Fig. 4.1 Hydrogen generation from bioelectrochemical system (BES) by using two-stage fermentation processes. Effluents flow of cathode and anode sides of the BES reactor (500 ml) are mixed. And half portion of this mixture added to second stage (250 mL) for methane generation (Sasaki et al. 2018)

producer fermenter (MF) is applied after BES processes that enhanced the gas production (CH_4 ~ 85% and CO_2 ~ 15%) (Villano et al. 2017). MF has applied after the NBES. Performance of BES process is accelerated or enhanced by growth of *Ruminococcus* sp. or *Veillonellaceae* spp. But *Clostridium* or *Thermoanaerobacterium* species are reported to reduce the performance of BES.

Other biofuels such as ethanol, butyrate, and acetate generation are reported to produce in different quantity in the BES. Alteration in redox potential of BES unit is reported by application of modified structural microbial system via enhancing the methane generation with reduced level of CO_2 gas emission in the two-stage processes. BES functions can be improved by microbial consortium action that can result in improved gas biosynthesis from carbohydrates substrates degradation (Villano et al. 2017; Sasaki et al. 2018) and it is shown in Fig. 4.2.

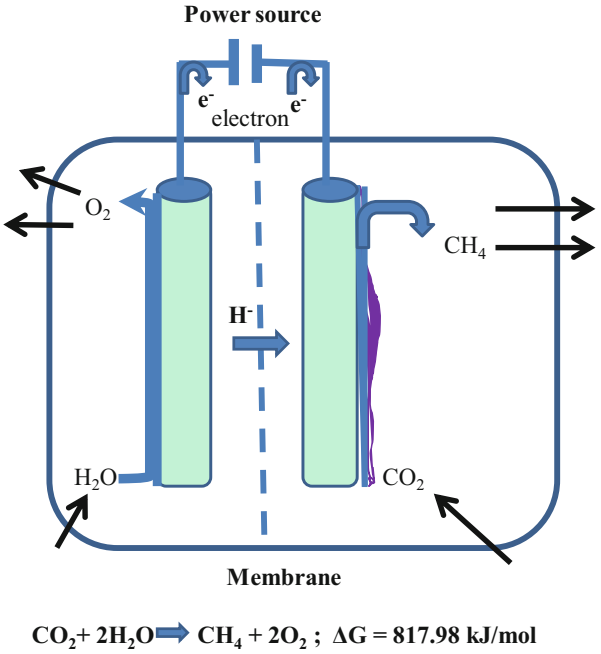

Fig. 4.2 Dual nature of BES system producing methane gas and water is shown as electron donor compound. Electrons released from water oxidation, and flow from anode to cathode driven by applied power (Liu et al. 2012; Liu 2018)

4.3 Hydrogen Follows to Methane Production and Its Operation

The microbial cells or strain systems in anaerobic condition wastewater treatment are highly complex bioprocesses via contributing fermentative nature bacterial cells (i.e., H_2 producing acetogen (HPA) or methanogenic bacteria (MB) with capacity for production valuable biogases. HPA microbial cells are added in an anaerobic mode of wastewater treatment processes as an alternative option to MB system. In MB system, SO_4^{2-} reducing bacterial cell or other H_2 degrading bacteria is involved (Zhang et al. 2018). Few strains of HPA are reported to isolate by doing the purification of this strain that are exhibiting the nature of obligate or facultative anaerobe. HPA microbial cell can convert the VFA and ethanol compounds into acetic acids, H_2, or carbon dioxide as products. The metabolic products or chemicals of HPA processes can enhance the production of CH_4 fuel by MB (Zhang et al. 2018; Li et al. 2009). HPA microbial system has shown transition type role in anaerobic mode wastewater treatment with induction of bio-augmentation processes. This process can enhance the removal rate COD level with high yield of CH_4 in anaerobic mode wastewater treatment processes (Amani et al. 2011; Garcia-Depraect et al. 2019).

HPA cultures have shown the high rate of degradation for propionic or butyric acids compounds with continuous subculturing in enrichment media. The processes of bio-augmentation processes with Z08 or Z12 microbial strains have shown

enhanced CH_4 production with glucose removal (1.58). In molasses wastewater, bio-augmentation with Z08 and Z12 has reported for enhanced COD removal rate (72–86%). Specific H_2 or CH_4 yields are found with COD removal (factors of 1.54 and 1.62, respectively). It has reported that bio-augmentation process with dominant microbial culture of HPA can help in enhancing methane biosynthesis with COS level removal. In anaerobic wastewater treatment, HPA process has exhibited as rate limiting step (Amani et al. 2011; Wang et al. 2018). Bioelectrochemical systems (BESs) are applied to various applications with broad range of application as well as economic and effective approach, An integrated system of BES processes is reported as microbial electrolysis and desalination cell (MEDC) system and can be applied for concurrently desalinating salt water via production of H_2 with achievement of potentially treating wastewater (Luo et al. 2011; Magnin and Seseure 2019).

This MEDC reactor is shown to make up of three chambers with insertion of a pair of ion exchange membrane that can apply with each chamber for any one of three functions. At voltage value of 0.8 V, lab scale batch mode MEDC system has shown to higher quantity of hydrogen production rate (1.5 m^3/m^3 day or 1.6 mL/h) at cathode chamber. This system has succeeded to removal 98.8% (10 g/L NaCl removal from the middle chamber) (Zuo et al. 2016). Anode recirculation can be alleviated at high pH or salinity that inhibits the bacterial function. But it can produce the highest quantity of current density (87–140 A/m^3) with improved desaline process rate (80%) and hydrogen (30%). Desalination processes change effect can change the H_2 production due to applied voltage as well as cathode buffer capacity. Reaction on cathode electrode can be influenced by applying external power in addition to side activity of microbes (Zuo et al. 2016; Luo et al. 2011). MFCs technology can harvest the electric power from biodegradable materials via their degradation. Energy production in MFCs system is reported to use the external resistors or charge pumps. And external resistors and charge pump can exhibit the properties of dissipation of energy via heating or receiving of electron in passive mode in MGCs system without any controlled action (Wang et al. 2012; Głąb et al. 2019).

Recent approach and innovative mode system can be applied for active extraction of the energy from MFCs reactor or device at any operating condition without using of any resistors parts. And this modified device operation can peak the power out level at maximum quantity of energy production. Harvesting power from a re-circulating flow of MFCs system can maintain at maximum power point circuit (MPPC) with its peak power point. A charge pump cannot change the operating condition due to current flow limitations. Energy is gained from the MPPC and it is found 76.8 J. These energy values reported the 76-fold higher value than the value of charge pump (1 J), that is used for evaluation of MFCs performance. At equal extent, organic removal rate, Coulombic efficiency has gained 21-fold high powers in MPPC than charge pump (Nikhil and Mohan 2019). Various number of capacitors have been applied in the MPPC system for various energy storage requirement and supply of power. Conversion of energy from MPPC has been characterized for identification of key factors for improving the system performance. MPPC is found as new perspective approach for energy harvesting devices via maximization

of energy production of MFC with controlled manners (Nikhil and Mohan 2019; Stoll et al. 2018; Wang et al. 2012).

Cathode potential value of granular activated carbon (GAC1) can change from −0.9 V to −0.5 V between 7 and 10 days. And other cathode potential value of GAC2 can change from 0.8 V to 0.5 V between the period of 30 and 37 days. The cathode potential of the graphite granules (GG) is attached with reactor and are found to stable around 0.9 V after long period inoculation. But slightly values of voltage for GG are found at 37 or 79 days of periods, due to enhanced current density (Huo et al. 2017; Guo et al. 2015; Wainaina et al. 2020). These potential differences between GAC GG biocathodes are reported on polarization curves at 30 or 90 days. The polarization value curve has exhibited the onset current value for GAC biocathodes that had positive potential values (−0.5 V to 0.4 V) during operation. Current densities value at GG biocathodes is very less in whole range of cathode potentials that test at −0.7 V to −0.3 V (LaBarge et al. 2017; Cao et al. 2019).

MECs system application is found for simultaneously production of H_2 from bioelectrochemical reaction or processes via treating wastewater with considerable energy consumption. This system can overcome the unfavorable thermodynamic value for sustainable mode and economic ways of feasible processes in practical application. Proof of concept system for H_2 biosynthesis in MECs can be made powerful by theoretically predicted energy value from pressure retarded osmosis (PRO) processes (Zhang et al. 2013). The MECs process consisted of a PRO system and it can extract high quality water via generating electric power from water osmosis processes. And a MECs device can remove the organic matter via hydrogen generation. The feasibility of MECs system is applied with stimulated PRO operation got energy and effluent load quality evaluation from practical MECs process (H_2 production or removal of organic matters) (Yuan et al. 2015). Low value yield of hydrogen is found due to low quantity of electron current flow that are induced by electrode potential in the BES system, operated at an HRT value of 2 days. Microorganisms with capacity of hydrogen synthesis are reported to belong to *Bacillus*, *Clostridium*, and *Thermoanaerobacterium* (Levin et al. 2004).

These microbial systems are found with dominating in BES or NBES system. These microbial systems are anaerobic fermentative bacteria with conversion monosaccharide sugars into hydrogen. *Clostridium* and *Thermoanaerobacterium* species are good H_2 producers and are reported during acetate or butyrate fermentation at high temperature or thermophilic conditions. It has reported to decrease in number of these genera and also decreased in butyrate biosynthesis in the BES. Microbial cells belong to the *Ruminococcus* species can produce ethanol with enhanced amount of H_2 and acetic acid in BES system (Ntaikou et al. 2008). In organic substrate model, BES is operated as first stage of two-stage process of fermentation that recovered hydrogen and methane metabolites by using glucose with low-cost carbon sheet and applied electric current. This BES system is very poor for preventing water electrolysis by electrode polarization processes (Call and Logan 2008).

The initial pHs of the first stage (6.5 or 7.3) are reported to reduce the cost via lowered pH value. In second stage, carbon fiber textiles (CFTs) in the reactors are reported to produce CH_4 efficient ways. Effects of electrode polarization in BES

system suppressed the methanogenesis processes at a long value of HRT. Electrode polarization has changed the structures or metabolic pattern of microbial consortium and at a short HRT value, BES system can increase the richness of microbial consortium species. These species are *Ruminococcus* genus and *Veillonellaceae* family that increased ethanol and propionate as metabolite production (Sasaki et al. 2013). *Clostridium* and *Thermoanaerobacterium* had decreased H_2 and butyrate biosynthesis in BES process. Higher quantity acetic acid production is found in BES process that can further trigger by modifying redox potential of the electrodes. BES process can reduce the gas production quantity in 1st stage or further increase the amount in second stage with increased methane generation and reduced CO_2 generation in second-stage processes (Villano et al. 2017; Sasaki et al. 2013).

4.3.1 Hydrogen Production

Hydrogen as a vehicle fuel can be used in chemical industry and it required the current largest or higher producer and consumer of H_2. Lots of other chemicals are reported in highly industrialized countries and Fe, steel, or foods are common need from 2016 report of U.S. Energy Information administration. Research associate scholar at the Andlinger Center has discussed for wastewater treatment via saving energy via use of H_2 generation process. Actual wastewater treatment is not lab-made solutions and these can be utilized for hydrogen produce using photocatalysis processes (Krupp and Widmann 2009).

Comparable and efficient systems can produce chemicals from wastewater. Processes of wastewater treatment can produce hydrogen with monitoring of the amount of electrons produced by bacterial strain. These electrons generations can directly co-relate to the amount of hydrogen production. It has reported that the process can be energy neutral. It can eliminate the need for fossil fuels via creating hydrogen fuel. Production of higher quantity of H_2 and other gases for future periods can be forwarded to moving this technology to industries. Hydrogen synthesis can be reported as critical metabolite in the manufacturing of the thousand of common products from plastic to fertilizers, and these can produce pure hydrogen which can be expensive and energy intensive (Foutes Lima et al. 2013).

Research team member at Princeton University is reported for harnessing sunlight energy via generation and isolation of hydrogen from industrial wastewater treatment. Special designed chamber of BES can apply "Swiss-cheese" black silicon interface for splitting water with isolation of hydrogen gas. These processes can be aided by bacteria via generating electrical current with consumption of organic matters in wastewater treatment. Wastewater treatment through the chamber can use a medium for simulating sunlight and it has found for organic matter breakdown via production of hydrogen through bubbling up of its gases (Lu et al. 2019).

Potential efficiency of domestic wastewater treatment is applied in anaerobic condition or states of H_2 biosynthesis. Test has done for analysis of real-time domestic wastewater and it has compared with two different strength (high and

ordinary level) of organic load in synthetic wastewater (with or without addition of food waste mixture). During treatment operation, waste matters are found with high strength of nutrient load at pH 7. These have been gradually decreased with pH of 5–5.5 at good experimental conditions. At pH (5–5.5), treatment processes are controlled during operation with ordinary organic load with maximum H_2 yield for high strength load (1.13 mol.H_2/mol. glucose) and for ordinary-strength load (1.0 mol.H_2/mol. glucose) (Paudel et al. 2015). COD removal is found during treatment at high value of organic matters (48 g COD/L day) from ordinary strength (3 g CS/L day) loadings. These processes have shown the good hydrogen contents (42–53%). Functioning environment of the H_2 biosynthesis process is very crucial due to effective metabolic pathways and intermediates production and also functional nature with the controlled environment (Barros et al. 2010; Masset et al. 2012). COD removals (30% and 26%) are reported for two different concentrations (high or ordinary) of organic matter load, respectively. Designing of onsite domestic wastewater treatment with recovery of energy system can help in calculation of organic mass balance (COD) and distribution of organic matter in the reactor system with its by-products (Masset et al. 2012; Paudel et al. 2015).

MECs system as promising way is reported for H_2 biosynthesis fuel. H_2 is an attractive source of renewable energy and also energy carrier. During hydrogen oxidation or combustion process, it produces heat and water and can be powered to cars. Hydrogen as a fuel source is reported as economical benefit in regard of eco-friendly processes for hydrogen fuel, produced sustainably from renewable sources. Numbers of biological or non-biological are reported for production of hydrogen. It has seen that major cases of large-scale processes are needed or consume the fossil fuels with expenditure of large amounts of energy (Wrana et al. 2010). Water is reported for splitting for production of hydrogen and oxygen and it needs large amounts of energy and expensive process. From bacterial fermentation of carbohydrates, hydrogen is also produced to limit quantity due to reduced ability of the bacteria for not completely degradation of sources of carbohydrates (Oh et al. 2010) and few biological approaches of H_2 generation in Table 4.1.

Application of MECs system is reported for high quantity of H_2 production (four-fold more) from any organic waste matter by use of electrically active bacterial cells. In this system, reduced amount of extra energy is needed and final balance of treatment process is shown the positive (+)value. Extra input energy is required for activating H_2 biosynthesis from clean and renewable sources. MECs are reported as variable source of renewable nature H_2 (Oh et al. 2010; Wrana et al. 2010).

4.3.2 Methane Biosynthesis

Anaerobic microbiological decomposition is utilized for getting microbial cell mediated deriving energy and is reported to grow with metabolizing the organic matters in an O_2 free environment. These processes are found more optimal and suitable biosynthesis of CH_4. AD process is reported in four phases by applying of

Table 4.1 Hydrogen production from different biological processes by different types of biological or microbial cells

S. No.	Microbial processes	Biochemical reaction	References
1.	Direct biophotolysis from microalgal or cyanobacteria species	$2\,H_2O$ + light energy → $2\,H_2$ + O_2	Ghiasian (2019); Nam et al. (2012)
2.	Photo-fermentations from purple bacteria (*Rhodobacter sphaeroides*) and microalgal species	CH_3COOH+ $2H_2O$ + Light → $4H_2$ + $2CO_2$	Gabrielyan et al. (2015)
3.	Indirect biophotolysis from microalgae and cyanobacteria, *Chlamydomonas reinhardtii*	A: $6H_2O$ + CO_2 + Light → $C_6H_{12}O_6$ + $6O_2$ B: $C_6H_{12}O_6$ + $2H_2O$ → $4H_2$ + $2CH_3COOH$ + $2CO_2$ C: $2CH_3COOH$ + $4H_2O$ + Light → $8H_2$ + $4CO_2$ Overall reaction: $12H_2O$ + Light → $12H_2$ + $6O_2$	Benemann (2000); Giannelli and Torzillo (2012)
4.	Water gas shift reaction from fermentative bacteria and photosynthetic bacteria	CO + H_2O → CO_2 + H_2	Najafpor and Younesi (2005); Markov (2012); Yu and Takahashi (2007)
5.	Two-phase H_2 + CH_4 fermentation from methanogenic bacteria and fermentative bacteria	A: $C_6H_{12}O_6$ + $2H_2O$ → 4 H_2 +$2CH_3COOH$ + CO_2 B: $2CH_3COOH$ → $2CH_4$ + $2CO_2$	Salem et al. (2018)
6.	High-yield dark fermentations from fermentative bacteria	$C_6H_{12}O_6$ + $6H_2O$ → $12H_2$ + CO_2	Mishra et al. (2019)

microbial systems of specific characteristics groups. First phase is started with hydrolysis process and it converts non-soluble biopolymers (complex nature) into soluble organic matters. Second phase is started with acidogenesis processes and this converts soluble organic matters into VFA and CO_2. Third phase is acetogenesis processes and it helps in conversion of VFA into acetate and H_2. And 4th phase is started with methanogenesis that converts acetic acids, CO_2, or H_2 molecules into methane field and can be shown in Fig. 4.3 (Kondusamy and Kalamdhad 2014).

Growth of bacteria with its conversion processes are found to slow rate at low temperature conditions and psychrophilic type of digestion is completed in longer periods of retention time in a large volume of reactors. And mesophilic temperature condition of digestion can complete the task in smaller size of reactor. Thermophilic type digestions are suitable wastewater treatment and it can discharge at a high temperature with pathogen removal. At thermophilic treatment, high loading is allowed. AD at minimum temperature (0 °C) rate of methane production is found to increase with increasing temperature (at 35–37 °C) (Lettinga and Haandel 1993; Kondusamy and Kalamdhad 2014).

Acidogenic bacteria are reported to excrete the various type of enzymes for hydrolysis the complex organic matter via conversion into soluble organic matters that later converted into VFA and alcoholic fuels. Further, these metabolites are

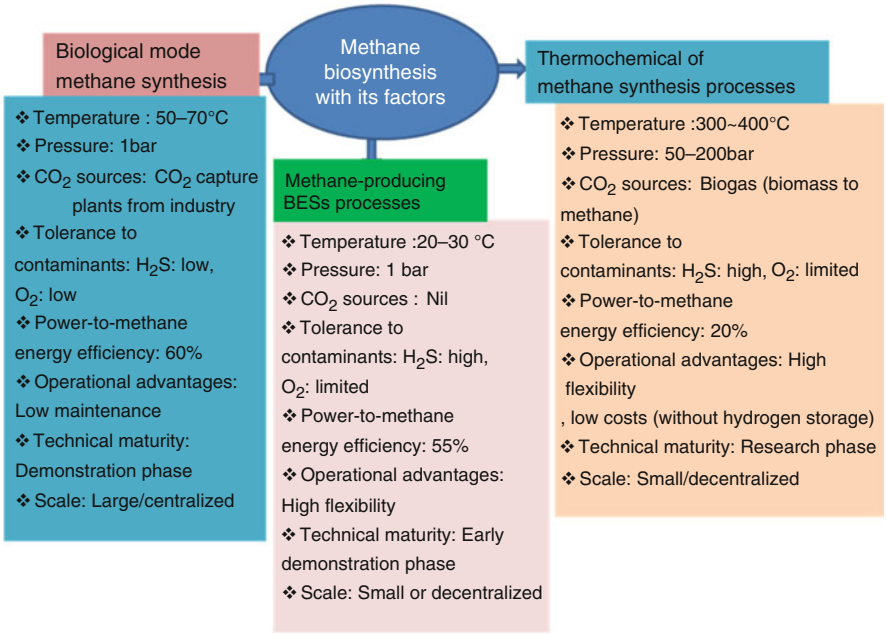

Fig. 4.3 Methane production processes and its critical factors for their yield and concentration (Liu 2018)

converted into acetic acid, hydrogen, or carbon dioxide molecules by acetogenic bacterial species. Methogenic bacteria can consume the acetic acid, H_2, and CO_2 for methane production. Various biological conversion processes are sufficiently coupled with membrane filter during processes that can prevent the accumulation of intermediates compounds. Accumulated VFA can decrease the pH that is not favorable for methane production. At high hydrogen pressure, reduced VFAs are formed via decreasing in pH (Cheng et al. 2009; Kondusamy and Kalamdhad 2014) and more examples of methane generation are shown in Table 4.2.

4.4 Conclusions

Sustainable fuel nature can help to maintenance of energy stocks in future and it will provide the alternative option for fossil fuel for increasing fuel energy. Nature of fuels can maintain the environment least destructive via utilization of renewable nature of energy (biogases or biofuels). Waste organic matter decomposition or reduction in our environments (water bodies or others) can be achieved by utilization of the waste treatment plants (anaerobic, aerobic, or BES) which can help in providing the bioenergy with reduction in organic matter in environments. In water bodies, these organic reductions can be tested by reduction in BOD and

Table 4.2 Methane production from biological processes via utilization of waste organic matters

S. No.	Biogases	Biological processes and wastes	References
1.	Volume (1800 mL) and composition (55% methane)	Anaerobic conditions of biodegradation capability of solid or liquid waste matter at controlled conditions. 5 g of substrates of both refuses (fresh or 1 year old waste matter), digested with 250 mL of microbial inoculums in 1 liter conical flask as bioreactors	Schirmer et al. (2014)
2.	Volume of biogas (5000 mL) from sludge of waste water and (500 mL) landfill waste with rich in methane (CH_4)	Large volume (tenfold) of biogas production occurred during AD of sludge wastewater via comparing with digestion of organic matter in landfill site for 30 days of times	Laskri et al. (2015)
3.	Yield of CH_4 with production of biogas and improved removal organic matter	Anaerobic co-digestion of sewage primary sludge organic matter (95%) with mixed olive or cheese whey wastewater (5%) in continuous mode reactors	Hallaji et al. 2019
4.	Biosynthesis of CH_4 (35–48%) with decreases hydraulic retention time (HRT) of 15 days	Co-digestion of food waste, grass clipping, garden waste with mixed municipal sludge organic matter is enhanced. Addition of higher quantity of carbon rich organic waste matters reduced the diversity of microbial community but enhanced the biogas synthesis	Nguyen et al. (2018)
5.	Cumulative methane yield (CMY) (20–36 mL/g VS)	Optimization of digestion performance with better understanding of mechanism of bentonite addition is reported and group M (WAS:KW = 1:2 based on VS) type waste organic matter is reported for higher cumulative methane yield (CMY). Waste activated sludge(WAS) and kitchen waste (KW) are common in China	Zhao et al. (2019)
6.	CH_4 yield (up to 7%) of calorific power of wood	AD (up to 61.3 g raw organic matters) in liter medium are 1.0×10^{-3} to 10^{-1} LCH_4/g VS of *Mangifera indica* (MI) and *Manihot utilissima* (MU) leaves had confirmed by BMP test at 10 °C	Ngoma et al. (2015)
7.	Methane yield (31mg CH_4.COD/g.VSS)	Integrated BES-AD system with a cathode potential (−0.9 V Ag/AgCl) has produced higher quantity (5.3–6.6-fold) CH_4 than AD reactor at 10 °C. CH_4	Liu et al. (2016)

(continued)

Table 4.2 (continued)

S. No.	Biogases	Biological processes and wastes	References
		production rate in integrated BES system at 10 °C than an AD reactor at 30 °C	
8.	Highest methane yield (408.3 ml CH_4/g COD glucose)	At applied voltage (1 V), highest quantity of CH4 yield (408.2 ml CH_4/g COD.glucose) and it is 30% increased quantity than the control tests (313.4 ml CH_4/g COD.glucose. MECs operated at various cell voltages (0.5, 0.7, 1.0 or 1.5 V) in anaerobic mode fermentation	Choi and KondaveetiS (2017)
9.	Methane production rate (5.6×10^{-2} m^3 methane/m^3 reactor*d) in the hybrid reactor	Enhanced CH_4 biosynthesis from digestion of WAS is reported and coupled with BES unit and potential use of bioelectrochemistry for biogas production improvement. Hybrid reactor is coupled bioelectrolysis and AD that can be developed for enhanced CH4 recovery from WAS organic matters	Cai et al. (2016)

COD values. CH_4 with other fuel gases (biogas) (biogases), and bio-mode H_2 (clean biofuel) synthesis is achieved by utilization of effective and efficient biological treatment processes (especially AD process and BES process). This sustainable fuel energy production can help in fulfilling of enhanced energy demand with solving of critical environmental issues (water pollution, reduced greenhouse gases, or other toxic products).

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Chapter 5
Hydrogen and Methane Generation from Biowaste: Enhancement and Upgrading via Bioelectrochemical Systems

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Abstract Bioelectrochemical systems (BESs) are emerging technologies that are based on catalyzing (bio-)anode and (bio-)cathode reactions from waste biomass by exoelectrogenic microorganisms. Microbial electrolysis cell (MEC), which is one of the BESs' technologies, is typically used to degrade organic wastes or wastewater for bioenergy recovery and biosynthesis. As one of the promising biotechnologies for resource recovery, value-added products have been obtained by MEC- or ME-integrated systems, such as hydrogen, methane, ethanol, etc. The fundamental reactions of (bio-)electron transport through anodic oxidation are well understood and allow us to increase reactor performance and efficiency. More attentions have

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been recently paid to cathode reactions on proton/electron transport and recovery, with or without microbial activities. Biogas upgrading systems have also been promoted in integrated systems, by combining bioelectrochemistry with various anaerobic processes. This chapter will focus on energy gas generation from waste organics involved in bioelectrochemical pathways and give an overview of bottlenecks and challenges related to this technology.

Keywords Bioelectrochemical systems · Microbial electrolysis cell · Hydrogen · Methane · Biowaste

5.1 Principle for Hydrogen and Methane Generation via Bioelectrochemical Systems

With the continuous stimulation of fossil fuel consumption and energy demand growth, the need to combine energy security with the development of a more sustainable energy sector is representing a severe global challenge. Some renewable resources are considered promising to harvest clean energy. Hydrogen is in principle an environmentally acceptable and clean energy vector that, however, is typically produced from non-renewable fossil fuels such as natural gas or water. In theory, bioelectrochemical systems (BESs) can be used to recover hydrogen from any biodegradable organics on the (bio-) cathode, by harnessing the biocatalytic electrolysis (bio-) anode, i.e., microbial electrolysis cell (MEC). Biocatalytic oxidation of organic matter to hydrogen can occur at both the anode and the cathode. In MEC, for instance, biodegradable organics can produce electrons through the biological oxidation at the anode. Electrons flow then through an external circuit (going from the anode to the cathode) and subsequently combine with protons to form hydrogen under a small voltage (0.2–1.2 V), necessary to overcome thermodynamic barriers (Cheng and Logan 2007). Additionally, H_2, being one of the most effective electron shuttles, can be exploited by microorganisms to produce small molecular compounds such as methane (Fig. 5.1). Compared with other H_2-/CH_4-producing technologies, MEC, as bioelectrochemical power-to-gas, which can convert renewable surplus electricity into hydrogen and methane, exhibits higher H_2-/CH_4-producing efficiency and wider diversity of substrate utilization, making it more advantageous, especially for the valorization of low concentration and/or complex organic matter (Logan et al. 2008).

5.1.1 Reactions Based on Extracellular Electron Transport

Based on whether there are microorganisms attached on the electrodes or not, MEC can be divided into four categories: (1) full-biological double-chambered (DC) bioanode/biocathode MEC; (2) full-biological single-chambered

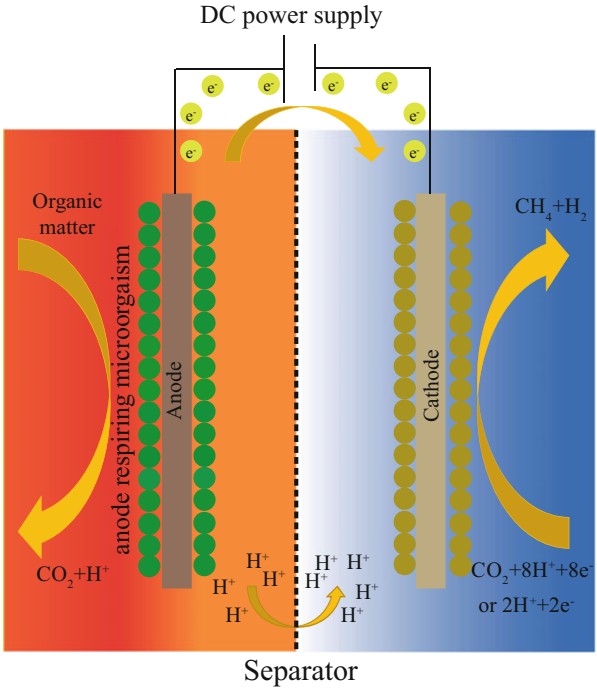

Fig. 5.1 Schematic of H_2 and CH_4 formation in the microbial electrolysis cell, MEC

(SC) bioanode/biocathode MEC; (3) half-biological double-chambered bioanode/cathode MEC; and (4) half-biological double-chambered anode/biocathode MEC.

In general, biodegradable organic matter is oxidized by anodic exoelectrogenic microorganisms and releases protons and carbon dioxide into the anolyte, while electrons transfer to the anode in a typical dual-chamber MEC (Hamelers et al. 2010).

[Anodic reaction]

$$CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-, E^0 = 0.187 \text{ and } E = -0.289 \text{ V vs.SHE} \quad (5.1)$$

Electrons flow typically through an external circuit, driven by an external voltage. Protons and carbon dioxide diffuse across the separator (such as ion exchange membranes, size-selective membranes, stacks of membranes, or the cloth) to the cathode, combining with electrons to be reduced to hydrogen or methane, depending on the cathodic potential, which drives the H_2/CH_4 production, affecting Gibbs' free energy (Jafary et al. 2015).

[Cathodic reaction]

Hydrogen formation:

$$8H^+ + 8e^- \rightarrow 4H_2, E^0 = 0 \text{ and } E = -0.412 \text{ V vs.SHE} \quad (5.2)$$

Methane formation:

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O, E^0 = 0.227 \text{ V and } E = -0.248 \text{ V vs.SHE} \quad (5.3)$$

Microbial electrolysis for hydrogen production can be considered as an advantageous combination of conventional hydrogen production pathways. First, compared to dark fermentation, "hydrogen-producing microorganisms" of MEC cannot directly produce hydrogen but are capable of extracellular electron transfer, which play a pivotal role. Besides, MEC can also handle more types of organic substrates without the problem of fermentation end products, which provides a possible way to thoroughly and fully degrade organic matter. In addition, the efficiency of hydrogen production via dark fermentation (such as treated carbohydrate-rich wastewater) is limited (Angenent et al. 2004), due to thermodynamical limitations involving endothermic reactions (Hawkes et al. 2002; Kim et al. 2004; Oh et al. 2003). Second, compared with photosynthetic biological hydrogen production (photosynthesis, photo-fermentation), it is not restricted by light. Notably, photo-fermentation requires enormous reactor surface areas to improve electron transfer and overcome the diffuse nature of solar radiation and thermodynamical barriers, which are clearly not economically viable (Hallenbeck and Benemann 2002). Third, the cathode reaction has the same reaction as the electrolysis of hydrogen in the electrochemical method, except that the electron source of the anode is different. As a consequence, the electrons provided by the relevant microorganisms can save input energy, as demonstrated by the minimum applied potential. The theoretical minimum potential of hydrogen production reaction in MEC is 0.10 V (shown in the following, Eqs. (5.4) and (5.5)), whereas the theoretical minimum potential of hydrogen production in industrial electrolysis is 1.2 V.

For the hydrogen production in MEC, the reaction between proton and electron occurs only in the cathode; the semi-cell reaction of its electrochemical system is briefly described as:

$$\text{Anodic reaction} : NAD^+ + H^+ + 2e^- \rightarrow NADH, E^0 = -0.320 \text{ V vs.SHE} \quad (5.4)$$

$$\text{Cathodic reaction} : 2H^+ + 2e^- \rightarrow H_2, E^0 = -0.420 \text{ V vs.SHE} \quad (5.5)$$

To enable a nonspontaneous reaction, electrons are required to flow from the anode potential (−0.320 V, Eq. (5.4)) to the cathode potential (−0.420 V, Eq. (5.5)), namely, from the high point to the low point. Thereby, MEC needs extra energy to execute the reverse flow of electrons. Theoretically, this process needs to provide a potential of at least 0.1 V to overcome the energy barrier, whereas in practice, an additional voltage supply of minimum 0.13 V is needed to perform the cathode hydrogen production. The reason for the extra voltage required is that electrochemically active microorganisms or electroactive microorganisms (EAMs) consume

some of available energy to sustain their own growth, resulting in microbes to release electrons with a higher potential than the equilibrium potential. On the other hand, the voltage applied to the reactor will also be lost as the consequences of ohmic resistance of electrochemical bias and occurrence of overpotential (Rozendal et al. 2006). Nevertheless, hydrogen generated through water electrolysis actually requires at least a voltage of 1.6 V or more (Crow 1994; Rasten et al. 2003).

5.1.2 Functional Communities Involved in Bioelectrochemical Systems

The term EAMs refers to those microorganisms that can directly or indirectly donate electrons to an electrode (called exoelectrogens), or that accept electrons from the electrode (known as electrotrophs) (Table 5.1). Thus far, the exoelectrogens isolated from natural environment belong primarily to the phylum Proteobacteria and Firmicutes, mostly facultative anaerobic microorganisms, which are capable of gaining energy to sustain growth via anaerobic respiration and fermentation metabolism. Most exoelectrogens are Fe(III)-reducing bacteria (FRB), viz., the oxidized iron is the final electron acceptor of the respiratory chain (Lovley 2006). There are different strategies for transferring electrons to the anode, such as the mediated interspecies electron transfer (MIET) (Cai et al. 2020) and the direct interspecies electron transfer (DIET) (Logan et al. 2019; Lovley 2017). The latter requires direct contact of the outer membrane cytochromes and electron transport proteins associated with outer cell surfaces on electrically conductive materials. MIET includes: (1) self-generated mediators that facilitate the shuttling of electrons from the cells to the anode; (2) electrically conductive pili, capable of long-range electron transfer; and (3) diffusive exchange of electrons between species via soluble electron shuttles such as H_2 (electron-accepting microbes are methanogens) (Table 5.1).

5.2 Hydrogen as the Main Product Using Microbial Electrolysis Cells

Previously, the process of hydrogen generation via electrolyzing dissolved organic matter, using EAMs acting as catalyst, was named “biocatalyzed electrolysis” (Rozendal et al. 2006); subsequently, it was referred to “electrochemically assisted microbial production of hydrogen” (Liu et al. 2005). In earlier research on hydrogen production in MEC, the reactor basically had the similar configuration as the MFC reactor, composed of typical bipolar chamber structure made of glass, where two electrode chambers were isolated by proton exchange membrane (PEM). These initial studies primarily focused on hydrogen generation with acetate as the model compound in MEC. The experimental results showed that the coulomb efficiency

Table 5.1 Electroactive microorganisms (EAMs) in bioelectrochemical systems, BESs

Species	Taxonomy	Information	Reference
Proteus vulgaris	Proteobacteria	Chemically immobilized onto the surface of graphite felt electrodes, supporting continuous current production	Allen and Bennetto (1993)
Shewanella putrefaciens IR-1	α-Proteobacteria	The first observation of a direct electrochemical reaction via Fe(III)-reducing bacteria in BES	Kim et al. (1999)
Clostridium butyricum EG3	Firmicutes	The first reported gram-positive bacterium (Fe(III)-reducing bacterium) in microbial fuel cell (MFC) can ferment glucose to acetate, butyrate, CO_2, and H_2	Park et al. (2001)
Desulfuromonas acetoxidans	δ-Proteobacteria Geobacteraceae (family)	Anaerobic marine microorganism oxidizing acetate with concomitant reduction of elemental sulfur or Fe (III)	Bond et al. (2002)
Geobacter metallireducens	δ-Proteobacteria Geobacteraceae (family)	Oxidize a variety of aromatic contaminants (benzoate, toluene) with the reduction of Fe(III)	Bond et al. (2002)
Geobacter sulfurreducens	δ-Proteobacteria Geobacteraceae (family)	The first report of microbial electricity production solely by cells attached to an electrode without electron transfer mediator (potassium ferricyanide; thionine; neutral red; anthraquinone-2,6-disulfonate, AQDS); oxidize acetate or H_2	Bond and Lovley (2003)
Rhodoferax ferrireducens	β-Proteobacteria	Isolated from anoxic subsurface sediments; dissimilatory Fe(III)-reducing bacterium; electricity generation by direct oxidation of glucose in electron-shuttling mediatorless MFC	Chaudhuri and Lovley (2003)
Aeromonas hydrophila	δ-Proteobacteria	A facultative anaerobic bacterium, Fe (III)-reducing bacterium, can reduce nitrate and sulfate	Pham et al. (2003)
Pseudomonas aeruginosa	γ-Proteobacteria	Excrete redox mediators (pyocyanin)	Rabaey et al. (2004)
Desulfobulbus propionicus	δ-Proteobacteria Desulfobulbaceae (family)	The first example of sulfate-reducing bacteria that can preserve energy to support their growth by electron transfer to insoluble electron acceptors, such as Fe(III) oxide and electrodes, without the addition of exogenous electron-shuttling compounds	Holmes et al. (2004a)
Geopsychrobacter electrodiphilus	δ-Proteobacteria Geobacteraceae (family)	The first organism retrieved from an anode, able to effectively oxidize organic compounds at an electrode,	Holmes et al. (2004b)

(continued)

Table 5.1 (continued)

Species	Taxonomy	Information	Reference
		gram-negative bacterium with abundant c-type cytochromes	
Geothrix fermentans	Acidobacteria	The first report of a Fe(III)-reducing bacterium from outside the *Proteobacteria* family capable of complete oxidation of organic compounds linked to electrode reduction and synthesizing a soluble compound to enhance electrode reduction	Bond and Lovley (2005)
Escherichia coli	γ-Proteobacteria	The first reported that *E. coli*-catalyzed MFC with a carbon-based anode exhibited a higher power density without electron mediators	Zhang et al. (2006)
Enterobacter dissolvens	Proteobacteria	Gram-negative bacillus capable of utilizing phenanthrene and degrading xenobiotic compounds	She et al. (2006)
Hansenula anomala	Ascomycota	Yeast cells with redox enzymes present in their outer membrane (ferricyanide reductase, lactate dehydrogenase) could communicate directly with electrode surface and contribute to current generation in mediator-less MFC	Prasad et al. (2007)
Shewanella oneidensis DSP10	γ-Proteobacteria	Live in anaerobic and aerobic environments; can reduce metals with/without oxygen	Ringeisen et al. (2007)
Shewanella oneidensis MR-1	γ-Proteobacteria	Gram-negative facultative anaerobic bacterium able to exploit a broad range of electron acceptors	Bretschger et al. (2007)
		The reduction of the highly toxic hexavalent chromium Cr(VI) via biocathodes	Xafenias et al. (2013)
Rhodopseudomonas palustris DX-1	α-Proteobacteria	The first reported power production of 2.72 ± 60 W m^2 by a newly isolated strain of a photo(hetero)trophic purple non-sulfur bacterium	Xing et al. (2008)
Ochrobactrum anthropic YZ-1	α-Proteobacteria	The first reported an *Ochrobactrum* species can produce electricity, isolated via using a special U-Tube MFC	Zuo et al. (2008)
Desulfovibrio desulfuricans	δ-Proteobacteria	A sulfate-reducing bacterium was used to simultaneously remove sulfate and generate electricity in MFC	Zhao et al. (2008)
Acidiphilium cryptum	α-Proteobacteria	The first reported used an acidophile as the anode biocatalyst in MFC	Borole et al. (2008)
Klebsiella pneumoniae L17	γ-Proteobacteria	Utilize directly starch and glucose to generate electricity (DIET)	Zhang et al. (2008)

(continued)

Table 5.1 (continued)

Species	Taxonomy	Information	Reference
Thermincola sp. strain JR	Firmicutes	The first gram-positive bacterium isolated from a thermophilic MFC	Wrighton et al. (2008)
Geobacter lovleyi	Proteobacteria	Reductive dechlorination of tetrachlorethene	Strycharz et al. (2008)
Comamonas denitrificans	β-Proteobacteria	An exoelectrogenic denitrifying bacterium isolated by dilution to extinction	Xing et al. (2010)
Acetobacterium (genus)	Firmicutes	Electroacetogenesis; autotrophic microbiome	Marshall et al. (2013)

(CE, total recovery of electrons from acetate) of MEC could reach up to 60%, which was much higher than that of MFC, reported in the same period. More than 90% of electrons and protons generated via bacterial acetate oxidation were converted to hydrogen. When further considering the maximum conversion rate of hydrogen, assuming 78% of CE and 92% of electron recovery efficiency, it can be easily calculated that 1 mol of acetate can produce approximately 3 mol of hydrogen. Moreover, comparing the hydrogen production capacity of different organic acids (acetate and butyrate), the results showed that acetate was more favorable to the metabolism of microbes in MEC, with hydrogen yield from acetate being higher than butyrate. Preliminary results showed that CE of acetate and butyrate could reach 50–65%, whereas that of glucose only reached 14–21%, which implied the feasibility of exploiting MEC to produce hydrogen and simultaneously degrade end products of dark fermentation (Liu et al. 2005).

$$C_6H_{12}O_6 + 6H_2O \rightarrow 12H_2 + 6CO_2 \text{ (thermodynamically unfavorable)} \quad (5.6)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CO_2 + 2C_2H_2O_2 \quad (5.7)$$

$$C_6H_{12}O_6 \rightarrow 2H_2 + 2CO_2 + C_4H_8O_2 \quad (5.8)$$

The stoichiometric yield of hydrogen production from glucose as the substrate would be 12 mol H_2/mol glucose, but this process (Eq. (5.6)) would require a large amount of energy and is unlikely to occur ($\Delta G^{0'}$ =+3.2 kJ mol). This would be translated into extremely low hydrogen yields when hydrogen is produced from glucose, with acetate and butyrate as the only fermentation by-products. Theoretically, 4 mol H_2/mol glucose can be obtained if only acetate is produced, while only 2 mol H_2/mol glucose when butyrate is the exclusive end product. Not surprisingly, usually only 2–3 mol H_2/mol glucose can be produced in actual fermentation process (albeit some thermophilic strains, e.g., *Thermothoga* can also reach yields around 3.5 mol H_2/mol glucose) (Logan 2004). In a combined process with glucose fermentation to (2 moles of) acetate and subsequent conversion of acetate in an

MEC, the overall hydrogen production yield reached 8–9 mol H_2/mol glucose, while the supplied energy requirement (by external voltage) was equivalent to 1.2 mol H_2/mol glucose (Liu et al. 2005).

In principle, MFC and MEC have similar functional microbes, including bacteria capable of extracellular electron transfer and other collaborative bacteria. Consequently, in the early stage of MEC research, the start-up mode was basically to first adapt the inoculum to obtain the corresponding functional flora by virtue of MFC electricity production, and then shifting into the MEC reactor operation (Cheng and Logan 2007; Call and Logan 2008; Hu et al. 2008; Call et al. 2009; Guo et al. 2010). Thus, in order to obtain an anodic syntrophic consortium between fermentative and anode respiring bacteria (ARB), Montpart et al., for example, first utilized the effluent from an already working MFC, composed of ARB (Montpart et al. 2015). The inoculum was fed with acetate and propionate, and subsequently with sludge from culture flasks, containing fermentative bacteria, in order to develop the syntrophic consortium. Once the syntrophic consortium had colonized well in MFC, the biologically enriched anode was transferred into a single-chamber MEC, treating synthetic wastewater (comprising different complex carbon substrates, i.e., glycerol, milk, and starch) to evaluate hydrogen production (Montpart et al. 2015). In the study by Liu et al., the authors unraveled the effects of different MEC start-up modes on hydrogen production and microbial communities (Liu et al. 2010). Interestingly, the results indicated that the start-up conditions with applied voltages (MEC mode) had a strong influence on the performances of MEC reactors, from the perspective of both CE and COD removal efficiency, and presented larger effect on gas composition, especially on the production of hydrogen. The hydrogen production of the reactor, directly started as MEC, was generally higher than that of the one initially operated in the MFC mode. Microbial community analysis results further demonstrated that microbial communities developed in MECs were well separated from those present under start-up conditions, implying that reactor operation affected microbial community composition (Liu et al. 2010). Subsequently, Lee et al. collected the effluent from an acetate fed-batch MEC operated for over 9 months as an inoculation to the upflow single-chamber MEC, reaching a production rate of 4.3 ± 0.06 m^3 m^{-3} d^{-1} of H_2 with 27–49 kg m^{-3} d^{-1} removal rate of COD (Lee and Rittmann 2010).

Various pure substrates have been well investigated in two-chamber MECs for hydrogen production (Cheng and Logan 2007), including glucose, cellulose, and various fermentative products (acetate, butyrate, etc.). Near-stoichiometric yields have been obtained by those MEC tests. However, mixed substrates or complex organic matters are still leading to low conversion yields. In summary, simple or pure substrates, like acetate as model substrate, are employed in MECs for mechanism analysis of electron transfer or electron flow calculation, while mixed or complex substrates are commonly studied for scaling up reactors or practical treatment.

5.2.1 Simple Carbon Sources for Hydrogen Production

Acetate, a by-product of dark fermentation of glucose, was typically used as a model substrate to ferment hydrogen in MEC research.

Rozendal et al. first reported biocatalyzed acetate for electrohydrolysis via EAMs, inoculated from the effluent of an electrochemical cell, which was previously acclimatized with sludge from a full-scale upflow anaerobic sludge blanket (UASB) reactor, treating sulfate-rich papermill wastewater for five months (Rozendal et al. 2006). A relatively large double-chamber MEC reactor with a volume of up to 3.3 L, separated by a cation-selective membrane, was operated under an applied voltage of 0.5 V, achieving a hydrogen production rate of 0.02 $m^3\ m^{-3}\ d^{-1}$. The CE and cathodic electron recovery efficiency reached 92% and 57%, respectively. It is worth mentioned that the previous research pointed out the existence of methanogens at the anode, resulting in hydrogen loss, and speculated about the possible impacts, viz., the loss of partial CE and the decreasing numbers of electrons delivered to the anode by competing for consumption of acetate. Unfortunately, this study did not focus on the phenomenon of the electromethanogenesis and did not further analyze microbial communities. In contrast, the authors assumed that the abovementioned consumption was insignificant compared to the H_2 recovery loss at the cathode, because the H_2 generated at the cathode would diffuse to the anodic chamber and be used as electron donor for biocatalysis.

Chae et al. employed a two-chambered MEC, fed with acetate, to elucidate the effects of applied voltages on the hydrogen production. They found that the hydrogen yields generally increased with applied voltages (from 0.1 to 1.0 V), obtaining a maximum H_2 yield of 2.1 mol/mol acetate. Moreover, the higher voltage implied a higher electron loss at the anode, compared to that of the cathode (Chae et al. 2008). Jeremiasse and colleagues obtained the maximum H_2 production rate using acetate and applying 1.0 V (Jeremiasse et al. 2010). In fact, the applied voltage is crucial for hydrogen formation and also significantly affects the H_2 conversion efficiency. Although hydrogen can theoretically be produced at the cathode by applying a circuit voltage greater than 0.14 V (Rozendal et al. 2006; Liu et al. 2005), in reality, higher voltages are required due to the overpotential. In practice, cathodic hydrogen generation can be considered negligible when applying below 0.30 V (Hu et al. 2008; Chae et al. 2008).

Table 5.2 presents a comprehensive overview of MEC performances obtained in different studies, using simple carbon sources.

5.2.2 Complex Carbon Sources for Hydrogen Production

Hydrogen can be generated via biocatalytic electrolysis (MEC) with the potential to efficiently convert a variety of dissolved organic matter and refractory wastes from wastes or wastewaters. Even substrates that were previously considered to be

Table 5.2 Simple substrates used for hydrogen production in the microbial electrolysis cell, MEC

Simple carbon sources[a]	Reactor configuration[b]	Reactor size (L)[c]	Operation mode	Electrode materials		Applied voltage (V)	Current density ($A\ m^{-3}$)[d]	Hydrogen production rate ($m^3\ m^{-3}\ day^{-1}$)	Cathodic H_2 recovery (%)	Coulomb efficiency (%)	Reference
				Anode	Cathode						
Acetate	DC CEM[e]	3.3	Batch	Disk-shaped graphite felt	Disk-shaped titanium mesh	0.5	$0.47 \pm 0.07\ A\ m^{-2}$	0.02	57 ± 0.1	92 ± 6.3	Rozendal et al. (2006)
Acetate	DC AEM[f]	0.014	Batch	High-temperature NH_3-treated graphite granules	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.6	99	1.10	NA[g]	NA[g]	Cheng and Logan (2007)
Acetate	DC PEM[h]	0.018	Batch	Carbon felt	Perforated titanium plate	0.1–1.0	$0.04–2.91\ A\ m^{-2}$	0.052 (0.8 V)	~80 (0.8 V)	68.4 (0.8 V)	(Chae et al. (2008)
Acetate	DC AEM[f]	0.2	Continuous	Graphite felt	Ni foam	0.50, 0.53, 0.62, 0.70, 0.80, 0.85, 0.90, 0.95, 1.00	5704 ± 32 (1.00 V)	50 (1.00 V)	NA[g]	NA[g]	Jeremiasse et al. (2010)
Acetate	DC CEM[e]	0.12	Batch	Carbon brush	Carbon cloth (CC) modified with ~5 mg cm^2 CNTs, Pt/C, Fe/Fe_3C@C	0.8	1.36 ± 0.10 (CC) 1.30 ± 0.09 3.50 ± 0.12 2.60 ± 0.07	0.087 ± 0.007 (CC) 0.075 ± 0.006 0.230 ± 0.031 0.181 ± 0.011	67.7 ± 10.6 (CC) 66.6 ± 6.6 81.6 ± 5.0 79.8 ± 8.4	26.9 ± 1.1 (CC), 24.1 ± 3.0 39.8 ± 2.6 43.6 ± 0.8	Xiao et al. (2012)
Acetate	SC CEM[e] AEM[f]	3.3	Batch	Graphite felt	Membrane electrode assembly Supported by Pt coated with 50 g m^{-2} titanium mesh	1.0	2.25 ± 0.05 $2.37 \pm 0.04\ A\ m^{-2}$	0.33 ± 0 0.31 ± 0.01	101.4 ± 0.7 101.3 ± 0.6	22.8 ± 0.2 23.2 ± 1.5	Rozendal et al. (2007)
Acetate	SC	0.028	Batch	NH_3-treated graphite brush	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.2–0.8 with an interval of 0.1 V	292 (0.8 V)	3.12 (0.8 V)	96 ± 1 (0.8 V)	98 ± 0 (0.8 V)	Call and Logan (2008)
Acetate	SC	0.5	Batch pH=7, pH=5.8	Carbon cloth without wetproofing	Carbon cloth with 30% wetproofing coated with 0.5 mg cm^{-2} Pt	0.3, 0.4, 0.6	9.3 14 $A{\cdot}m^{-2}$	0.53 0.69	82 87	75 73	Hu et al. (2008)
Acetate	SC	0.018	Batch	Carbon cloth	Carbon cloth without wetproofing	0.3, 0.35, 0.4, 0.6	270 200 300 (0.6 V)	2.0 1.5 2.3 (0.6 V)	86 75 85 (0.6 V)	75 73 56 (0.6 V)	Hu et al. (2009)

(continued)

Table 5.2 (continued)

Simple carbon sources[a]	Reactor configuration[b]	Reactor size (L)[c]	Operation mode	Electrode materials		Applied voltage (V)	Current density (A m^{-3})[d]	Hydrogen production rate (m^3 m^{-3} day^{-1})	Cathodic H_2 recovery (%)	Coulomb efficiency (%)	Reference
				Anode	Cathode						
				without wetproofing	coated with NiMo, NiW, or Pt						
Acetate	SC	0.028	Batch	NH_3-treated graphite fiber brush	Stainless steel brush	0.6	188 ± 10	1.7 ± 0.1	84	NA[g]	Call et al. (2009)
Acetate	SC	0.14	Batch	Graphite spheres	Carbon felt	1.06 ± 0.08	51.4 ± 1.6	0.057 ± 0.02	98 ± 2	60 ± 2	Lee et al. (2009)
Acetate	SC without PEM[h] with PEM[h]	0.05	Continuous	Carbon felt	Gas diffusion electrode coated with 0.5 mg cm^{-2} Pt	1.0	4.7 1.8	6.32 1.22	NA[g]	~90 ~44	Tartakovsky et al. (2009)
Acetate	SC	0.032	Batch	NH_3-treated graphite brushes	SS 304 SS 316 SS 420 SS A286 Ni 201 Ni 400 Ni 625 Ni HX Pt	0.6, 0.9	100 ± 4 116 ± 1 122 ± 10 222 ± 4 127 ± 8 116 ± 9 160 ± 22 124 ± 14 129 ± 7 (0.9 V)	0.59 ± 0.01 0.35 ± 0.008 0.58 ± 0.007 1.50 ± 0.04 0.38 ± 0.004 0.41 ± 0.10 0.79 ± 0.27 0.55 ± 0.11 0.6 ± 0.06 (0.9 V)	53 ± 1 2 ± 6 43 ± 2 61 ± 3 27 ± 4 31 ± 5 43 ± 9 40 ± 8 47 ± 2 (0.9 V)	92 93 88 102 96 100 95 95 98 (0.9 V)	Selembo et al. (2009a)
Acetate	SC	0.15	Batch	Graphite granules	Mipor titanium tube coated with platinum	0.2–1.0 with an interval of 0.1 V	170	1.58 (1.0 V)	88	95	Guo et al. (2010)
Acetate	SC	0.125	Continuous HRT 1.6 h HRT 3.1 h HRT 6.5 h	Graphite fiber	Graphite fiber	1.43 ± 0.04 1.47 ± 0.05 1.49 ± 0.03	1630 ± 50 1590 ± 70 1470 ± 60	4.32 ± 0.46 3.70 ± 0.03 2.64 ± 0.10	24 ± 2 21 16	190 ± 7 230 310 ± 3	Lee and Rittmann (2010)
Acetate	SC	0.064	Batch	Graphite felt	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.5–1.0 with an interval of 0.1 V	621.3 ± 20.6 (0.8 V)	5.56 (0.8 V)	~90 (0.8 V)	NA[g]	Liang et al. (2011)
Acetate	SC	0.5	Continuous	316 L SS fiber felt	Pt-coated titanium mesh tube	Anode potential at −0.2 V vs. Ag/AgCl	10.6 A m^{-2}	6.65	>80	64–88	Feng et al. (2018)

Acetate	SC	0.03	Batch	Heat-treated graphite fiber brush	Carbon cloth with 30 wt.% wetproofed coated with 0.5 mg cm^{-2} Pt	Anode potential of −0.2 V vs. Ag/AgCl	24.6 A m^{-2}	NA[g]	80 ± 1	NA[g]	Cho et al. (2019)
Glucose	SC	0.028	Batch	Graphite brush	Wetproofed carbon cloth coated with Pt	0.5 0.9	115 ± 4 182 ± 31	0.83 ± 0.18 1.87 ± 0.30	51 ± 4 88 ± 5	127 ± 23 105 ± 10	Selembo et al. (2009b)
Glucose	SC	0.026	Batch (4 °C)	Graphite brush	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.6 0.8	38 ± 5 50 ± 7	0.25 ± 0.03 0.37 ± 0.04	61 ± 4 68 ± 4	82 ± 13 74 ± 8	Lu et al. (2012a)
Glucose	SC	0.026	Batch (25 °C)	Graphite brush	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.6	113 ± 4	1.01 ± 0.05	82 ± 5	59 ± 6	Lu et al. (2012a)

[a]Sodium acetate is considered as acetate in this table
[b]SC refers to the single-chamber MEC; DC refers to the double-chamber MEC
[c]The reactor size of all double-chamber MEC reactor refers to the effective working volume of each corresponding anode chamber
[d]Current intensity is normalized by reactor chamber volume (A m^{-3}), or by anode electrode projected area (A m^{-2})
[e]CEM, cation exchange membrane
[f]AEM, anion exchange membrane
[g]*NA* not available
[h]PEM, Nafion 117 proton exchange membrane (PEM)

unfitting for producing hydrogen, according to the endothermic conversion reactions, can now be valorized by means of MECs.

There is a limited amount of carbohydrates from waste activated sludge (WAS) suitable for utilization by hydrogen-producing microorganisms; thereby, low H_2 yield is typically harvested from the WAS fermentation. Lu et al. obtained H_2 yields of 15.08 ± 1.41 mg-H_2/g-VSS from alkaline-pretreated WAS, which was 2.66-fold of that with raw WAS (5.67 ± 0.61 mg-H_2/g-VSS) in the two-chamber MEC (TMEC). However, more than 13 times higher H_2 production rate was achieved in the single-chamber MEC (SMEC) with alkaline-pretreated WAS, compared to TMEC (Lu et al. 2012b). Besides carbohydrates, there were other substrates (including proteins and their acidification products, such as volatile fatty acids), supporting hydrogen generation in MECs. In addition, it was further confirmed that electrohydrogenesis can react on both the exo-polymeric compounds and the intracellular ones.

Crop castoffs are considered to be a feasible feedstock for dark fermentation to generate hydrogen, thanks to the simple operation and low-energy requirements (Ghimire et al. 2015); however, this process is always associated to the formation of various by-products, mainly volatile fatty acids such as acetate and butyrate (Pan et al. 2010; Xing et al. 2011). Therefore, the integration of dark fermentation with MEC represents an effective way to convert biomass and main fermentation dead-end products into hydrogen (Marone et al. 2017). In order to further enhance hydrogen yield, Li et al. first investigated the effect of pre-adaptation and acclimatization strategies of the MFC anode biofilm grown on diverse substrates and subsequently transferred to the MEC. A maximum H_2 production rate of 4.52 ± 0.13 m^3 m^{-3} d^{-1} under the highest current density of 480 ± 11 A m^{-3} was achieved in a pre-acclimatized anode fed with butyrate (applying 0.8 V), while the one treated with acetate reached 3.56 ± 0.22 m^3 m^{-3} d^{-1} and 346 ± 11 A m^{-3} (Li et al. 2017). Notably, the H_2 yields and removal efficiency of butyrate were substantially higher than in the case of any other substrates (i.e., corn stalk fermentation, ethanol, propionate, or even acetate) (Li et al. 2017).

Table 5.3 presents a comprehensive overview of complex carbon sources that have been used in MEC studies.

5.2.3 *Hydrogen Loss Evaluation for Microbial Electrolysis Cells*

In practice, hydrogen production is boosted in MECs during the initial operation; however, the production of methane is an inevitable consequence for long-term operation of the mixed flora reactor, in most cases. Undesired H_2 sinks, especially by methanogens, have been a serious issue in MEC operations, although H_2 has a low solubility (i.e., 0.0016 g H_2 can be dissolved into 1 kg water at 293 K). In order to inhibit methanogens' growth, MEC reactors can be put in aerobic conditions for

10 min after each feed cycle, then replenished with fresh medium, and finally flushed with oxygen-free gas to reestablish anaerobic conditions (Selembo et al. 2009b). Except for bioelectrodes exposed to air intermittently (Call and Logan 2008; Call et al. 2009; Lu et al. 2010), there are other strategies adopted to avoid methanogenesis: (1) operation under lower pH (Hu et al. 2008) or lower temperature conditions (Lu et al. 2011), ultraviolet irradiation (Hou et al. 2014a); (2) washout of methanogens by lowering hydraulic retention time (HRT) (Wang et al. 2009); (3) reducing carbonate concentration (Rozendal et al. 2008); and (4) methanogen inhibitor addition (e.g., 2-bromoethanesulfonate) (Chae et al. 2010). Unfortunately, the abovementioned strategies only focus on repressing methanogenesis but overlook other routes of H_2 consumption, including H_2 oxidized by exoelectrogens, or homoacetogenic microorganisms utilizing H_2 and CO_2 to synthesize acetate ($2CO_2 + 4H_2 \rightarrow CH_3COOH + 2H_2O$) (Parameswaran et al. 2009). Both paths are commonly defined as hydrogen recycling between the anode and the cathode (Lee et al. 2009), which does not lead to dramatic H_2 loss, but improves the overpotential loss and prolongs duty cycle, eventually resulting in low H_2 recovery (Parameswaran et al. 2011). It seems to be essential to minimize the diffusion of H_2 toward the anode, to rapidly separate H_2 from the MEC reactor (Lee and Rittmann 2010). Instead of conducting top-down inhibition of methanogenesis, Lu et al. employed a novel approach to actively harvest H_2 by extracting it from the reactor, using a gas-permeable hydrophobic membrane and vacuum, leading to 3.32- to 4.29-folds higher H_2 yield than that of the conventional spontaneous release, without CH_4 detection (Lu et al. 2016). But the decreased biofilm growth, accumulation of foulants, and exorbitant cost related to the membrane will be a big challenge in the future.

5.3 Methane as the Main Product in Integrated Anaerobic Systems

Microbial electrolysis system can improve methane production by electrochemical enhancement process (Villano et al. 2011). Traditionally, the planktonic anaerobic bacteria (PAB) and electrochemically active bacteria (EAB) coexist in MEC and disperse in liquid and electrode surface, respectively (Cheng et al. 2009). Methane production partly depends on electron transfer function of PAB and EAB, which are responsible for the carbon dioxide reduction process. In addition, this process can also make use of electrons supplied by current. Hydrogenotrophic methanogens are generally regarded to exploit H_2 as the sole electron donor to reduce CO_2 for methanogenesis. In reality, recent research has clarified that the electron donor source of hydrogenotrophic methanogens is very extensive. There are mainly two ways through which methanogens directly acquire electrons: (1) supply of electrons through electrodes and (2) microorganisms with extracellular electron transport capability (Rotaru et al. 2014a; Fu et al. 2015).

Table 5.3 Complex substrates used for hydrogen production in the microbial electrolysis cell, MEC

Complex carbon sources	Reactor configuration[a]	Reactor size (L)[b]	Operation mode	Electrode material		Applied voltage (V)	Current density (A m^{-3})[c]	Hydrogen production rate (m^3 m^{-3} day^{-1})	Cathodic H_2 recovery (%)	Coulomb efficiency (%)	Reference
				Anode	Cathode						
Domestic wastewater	DC CEM[d]	0.192	Batch	Non-wetproofed carbon paper filled with graphite granules	Carbon paper coated with 0.5 mg cm^{-2} Pt	0.23 0.32 0.41 0.50 0.59	~0.18, ~0.2, ~0.26, ~0.38, ~0.40	0.0125 g-H_2/g-COD (0.50 V)	4.2 3.5 19.0 42.7 37.5	10.4 19.4 25.8 23.2 26.4	Ditzig et al. (2007)
Cellulose	SC	0.028	Batch FEI-Syn, SSI-Syn, SSI-Pr[e]	NH_3-treated graphite fiber brush	Flat carbon cloth coated with Pt (10% Pt/C)	0.5	NA[f]	0.59 ± 0.21 1.11 ± 0.13 1.02	49 ± 16 86 ± 7 89	110 ± 20 73 ± 3 90	Lalaurette et al. (2009)
P-glycerol	SC	0.028	Batch	Graphite brush	Wetproofed carbon cloth coated with Pt	0.5 0.9	116 ± 5, 221 ± 12	0.80 ± 0.008, 2.01 ± 0.41	64 ± 15 79 ± 18	99 ± 10, 104 ± 7	Selembo et al. (2009b)
B-glycerol	SC	0.028	Batch	Graphite brush	Wetproofed carbon cloth coated with Pt	0.3 0.5 0.6 0.8 0.9	15 ± 3 35 ± 8 59 ± 10 87 ± 11 63 ± 14	0 0.14 ± 0.06 0.30 ± 0.01 0.55 ± 0.28 0.41 ± 0.13	1 ± 0 45 ± 15 72 ± 19 52 ± 15 65 ± 14	37 ± 5 84 ± 11 65 ± 8 103 ± 11 91 ± 10	Selembo et al. (2009b)
Effluent of an ethanol-fed dark-fermentation CSTR[g]	SC	0.026	Batch	Carbon fiber brush	Wetproofed carbon cloth (30%) coated with Pt 0.5 mg cm^{-2} Pt (Pt/C: 20 wt.%)	0.2–0.8 with an interval of 0.1 V	135 (0.6 V)	1.41 ± 0.08 (0.6 V)	94 ± 4 (0.6 V)	87 ± 2 (0.6 V)	Lu et al. (2009)
Swine wastewater	SC	0.028	Batch D-S ND-S D-L ND-L[h]	Graphite-fiber brush	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.5	93 ± 22, 106 ± 6, 92 ± 13, 112 ± 25	0.8 ± 0.2 0.9 ± 0.2 1 ± 0.1 1 ± 0.1	53 ± 6 61 ± 12 58 ± 1 29 ± 2	43 ± 2 29 ± 17 48 ± 9 70 ± 2	Wagner et al. (2009)

Protein	SC	0.014	Batch	Graphite fiber	Carbon cloth coated with 0.5 mg cm^{-2} Pt	0.6, 0.8	132 ± 2 (0.6 V)	0.42 ± 7 (0.6 V)	29 ± 5 (0.6 V)	114 ± 9 (0.6 V)	Lu et al. (2010)
Potato-processing wastewater	SC	0.028	Batch	NH_3-treated graphite fiber brush	Carbon cloth with 30 wt.% wetproof coated with 0.5 mg cm^{-2} Pt	0.9	6.4 A m^{-2}	0.74	NA[f]	80	Cusick et al. (2011)
Winery wastewater	SC	1000	Continuous	Heat-treated graphite fiber brush	Stainless steel 304	0.9	7.4	0.19 ± 0.04	NA[f]	NA[f]	Cusick et al. (2011)
Methanol-rich industrial wastewater	SC	0.028	Batch	Heat-treated graphite fiber brush	Stainless steel 3004, carbon cloth coated with MoS_2, Pt	0.7	1.5 1.2 2.1 A m^{-2}	0.12 ± 0.02 0.08 ± 0.01 0.17 ± 0.03	NA[f]	12 ± 2 7 ± 0.5 10 ± 0.5	Tenca et al. (2013)
Food-processing wastewater	SC	0.028	Batch	Heat-treated graphite fiber brush	Stainless steel 3004, carbon cloth coated with MoS_2, Pt	0.7	1.0 1.0 2.4 A m^{-2}	0.12 ± 0.02 0.5 ± 0.06 0.41 ± 0.02	NA[f]	29 ± 2 26 ± 4 35 ± 2	Tenca et al. (2013)
Glycerol	SC	0.04	Batch	Graphite fiber brush	Graphite fiber cloth coated with 5 mg cm^{-2} Pt	0.8	NA[f]	NA[f]	NA[f]	NA[f]	Montpart et al. (2015)
Starch	SC	0.04	Batch	Graphite fiber brush	Graphite fiber cloth coated with 5 mg cm^{-2} Pt	0.8	NA[f]	NA[f]	NA[f]	NA[f]	Montpart et al. (2015)
Milk	SC	0.04	Batch	Graphite fiber brush	Graphite fiber cloth coated with 5 mg cm^{-2} Pt	0.8	NA[f]	NA[f]	NA[f]	NA[f]	Montpart et al. (2015)

(continued)

Table 5.3 (continued)

Complex carbon sources	Reactor configuration[a]	Reactor size (L)[b]	Operation mode	Electrode material		Applied voltage (V)	Current density (A m^{-3})[c]	Hydrogen production rate (m^3 m^{-3} day^{-1})	Cathodic H_2 recovery (%)	Coulomb efficiency (%)	Reference
				Anode	Cathode						
Corn stalk fermentation effluent	SC	0.064	Batch	Graphite felt	Carbon cloth coated with 0.5 mg cm^{-2} Pt (Pt/C: 20 wt%)	0.5 0.8	480 ± 11 (0.8 V)	2.41 ± 0.12 4.52 ± 0.13	NA[f]	72 ± 3 76 ± 2	Li et al. (2017)

[a]SC refers to the single-chamber MEC; DC refers to the double-chamber MEC

[b]The reactor size of all double-chamber MEC reactor refers to the effective working volume of each corresponding single anode/cathode chamber, not the total effective working volume, which is twice of that

[c]Current intensity is normalized by reactor chamber volume (A m^{-3}), or by anode electrode projected area (A m^{-2})

[d]*CEM* cation exchange membrane

[e]*FEI*, fermentation effluent inoculum, that is the MECs with brushes acclimatized to individual substrates or a synthetic effluent; *SSI*, the mixtures of solutions (in equal volumes) obtained from the MFCs acclimatized to individual substrates (that is the MECs with brushes acclimatized to a synthetic effluent); and a single-substrate inoculum, *SSI-Pr*, the predicted results for the synthetic effluent, which were calculated on the basis of the solution composition and the performance in MECs with individual substrates (Lalaurette et al. 2009)

[f]*NA* not available

[g]*CSTR* continuous stirred-tank reactor

[h]*D-S*, diluted wastewater with short cycle; *ND-S*, non-diluted wastewater with short cycle; *D-L*, diluted wastewater with long cycle; *ND-L*, non-diluted wastewater with long cycle. Short cycle, the batch ended immediately after max gas production; long cycle, batch ended when gas production finished

According to the type of substrate utilization, the potential mechanisms of methane formation in a bioelectrochemical system can be divided into two categories: one is through electron transfer, i.e., by means of DIET from the (bio-)cathode, and the other one is through interspecies hydrogen transfer among hydrogen-producing and hydrogen-consuming methanogens. Furthermore, in some cases, acetate and formic acid are formed by means of DIET from electrodes or via acetate- and formate-producing microorganisms (along with H_2 production); hence, acetate and formic acid can be further decarboxylated by acetotrophic methanogens to produce CH_4. Possible paths of methane formation on the (bio-)cathode are shown in Fig. 5.2.

5.3.1 *Microbial Extracellular Electron Transfer Driving Methane Production*

Carbon dioxide, methyl compounds, or acetate can be converted into methane in the microbial methanogenesis process. The fundamental pathways are shown in Fig. 5.3. There are two principal ways of obtaining electron donors for acetoclastic or hydrogenotrophic methanogens to generate methane: one is to directly harvest electrons through electrodes and the other is to utilize microorganisms capable of extracellular electron transport to capture electron donors.

Methanosaeta is a typical acetoclastic methanogen that exclusively uses acetate for methanogenesis. Morita et al. found that *Geobacter* was the dominant bacteria in microbial aggregates in cultured anaerobic digestion (AD) reactors, whereas *Methanosaeta* is the most abundant methanogen, indicating for the first time a possible DIET process with methanogenic wastewater aggregates. Microbial aggregates possess metallic-like conductance similar to the conductive pili of *Geobacter sulfurreducens* (Morita et al. 2011). Among microbial aggregates formed by the combination of *Geobacter metallireducens* and *Methanosaeta harundinacea*, *the former* can provide electrons to *the latter*. DIET between *Geobacter* and *Methanosaeta* can be used for methane formation (Rotaru et al. 2014b), which changed the viewpoint that the archaea *Methanosaeta* exclusively uses acetate to produce methane. In addition, metatranscriptomic analysis further revealed that *Methanosaeta* also has the capacity to reduce carbon dioxide for methane production in AD reactors. The relationship between *Geobacter* and *Methanosaeta* is similar to that between fermentation bacteria and syntrophic methanogens, which is based on electron transfer. Kaur et al. found that *Geobacter* exhibited a clear overwhelming competition for acetate utilization, compared to *Methanosaeta* in the open circuit (Kaur et al. 2014). Further, Jung et al. demonstrated positive correlation between external resistance and methanogenesis, also showing that the substrate competition among exoelectrogens and methanogens might be influenced by the same external resistance, thus suggesting that the anode potential can regulate the competition

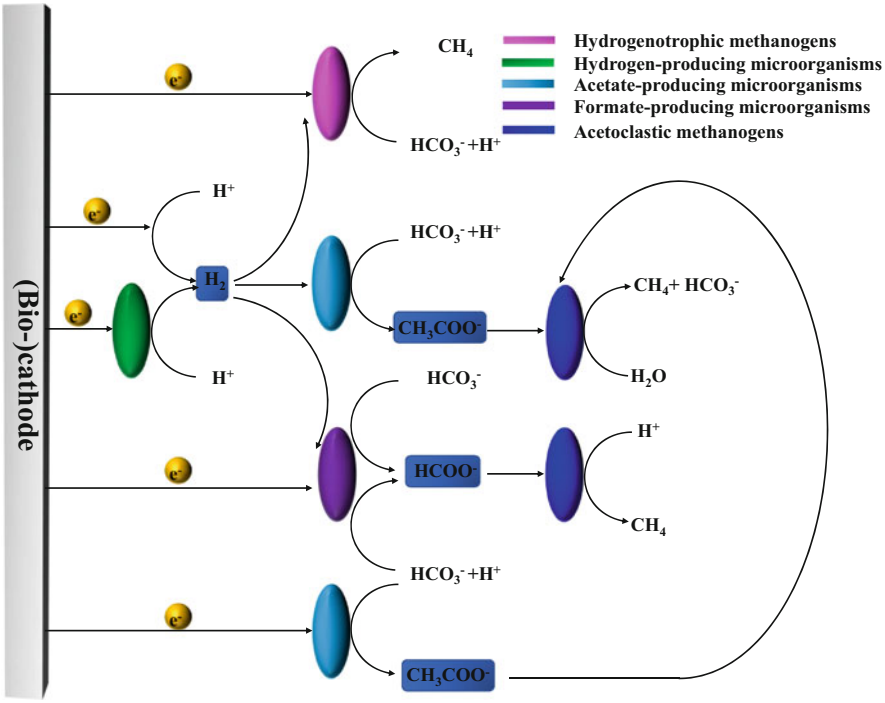

Fig. 5.2 Possible pathways for methane generation in the microbial electrolysis cell, MEC

between extracellular electron transfer bacteria and methanogens (Jung and Regan 2011).

Methanosarcina belongs to the facultative acetoclastic methanogen, which can utilize a wide variety of substrates, including acetate, methanol, methylamine, and hydrogen. Rotaru et al. found the evidence that *Methanosarcina barkeri* participated in DIET with *Geobacter metallireducens*. The study showed that the exploitation of activated carbon particles can replace pili for long-range electron transport, which implied that conductive materials can act as a substitute carrier for pili to perform electron transfer between species (Rotaru et al. 2014a). The close contact is necessary for the implementation of DIET, which may be attributed to the conductivity of pili, whose conductivity is ~5 mS cm^{-1} (Malvankar et al. 2011). The applied voltage, driving electrons through the external circuit and stimulating methane formation, is comparable to the transmission method through the pili. Nevertheless, on the contrary, the external circuit is not limited by the protein structure and the transmission scale, which can attain long-range electron transfer and display excellent conductivity.

Hydrogen and formic acid at the cathode can also become electron transfer mediators. This is different from DIET achieved by the conductive mediator, and the (bio-)cathode methanation process is in a way more controllable and expandable. On the other hand, the microbial electrosynthesis process based on carbon dioxide

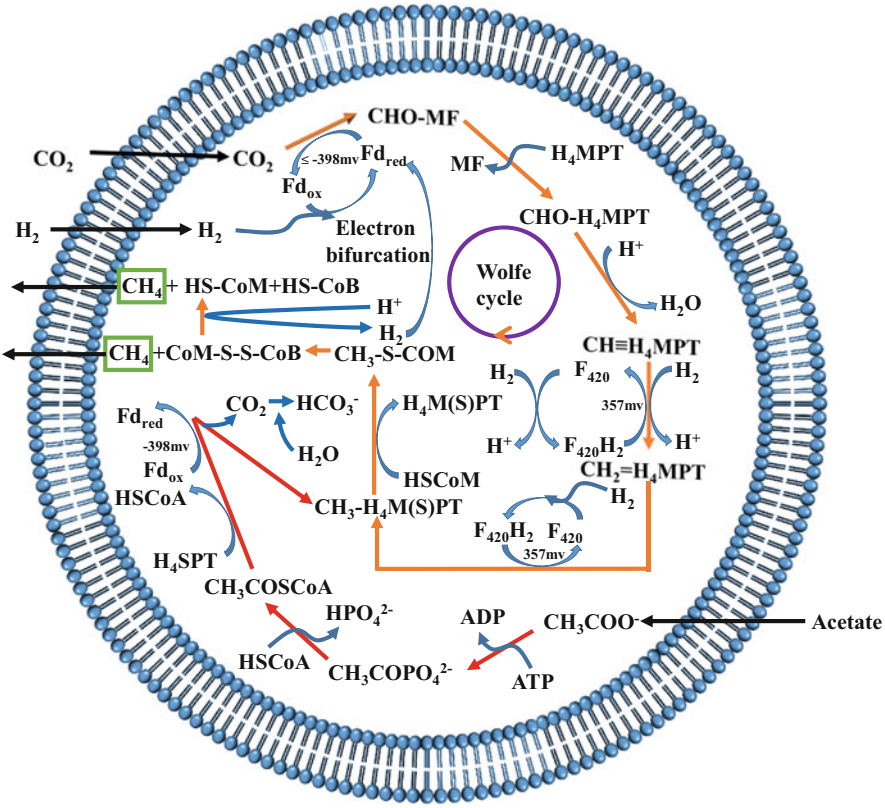

Fig. 5.3 Metabolic pathways of methanogens

reduction at the cathode can also promote the synthesis of chemical substances such as acetate and formic acid (Lee et al. 2017). Altogether, the (bio-)cathode methanogenesis process through DIET prevailingly comprises: (1) hydrogen produced by electrochemical processes that can diffuse into microorganisms to maintain microbial metabolism; (2) hydrogen produced by redox proteins (such as hydrogenase) then used as electron transport mediators; (3) since electrons can pass through the redox transmembrane protein, such as cytochrome c, they can be transferred from the electrodes into the microbes (Kumar et al. 2017). From a macro perspective, we can conclude that: (1) hydrogen evolution reaction (HER) occurs directly at the cathode (or from microorganisms on the electrode surface, $E^0 = -0.41$ V) and then hydrogen is absorbed by hydrogenotrophic methanogens and combined with carbonate to form methane (Wang et al. 2009); (2) certain methanogens receive electrons directly from the cathode and combine it with carbonate to generate methane ($E^0 = -0.24$ V) (Cheng et al. 2009; Van Eerten-Jansen et al. 2012); (3) homo-acetogens attached to the cathode surface receive electrons from the

electrode and synthesize acetate utilizing carbonate (Nevin et al. 2011), and acetotrophic methanogens utilize acetate to generate methane ($E^0 = -0.28$ V).

5.3.2 *Electricity-Stimulated Anaerobic Methanogenesis Process Using Different Substrates*

Conventional methanogenesis primarily depends on the hydrogen or formic acid for interspecies electron transfer in methanogenic environments (Stams and Plugge 2009). It is energetically difficult to complete proton reduction due to negative redox potential of NADH/$FADH_2$ (NAD^+/NADH, $E^0 = -0.32$ V, FADH/$FADH_2$, $E^0 = -0.22$ V), when hydrogen is used as the electron transport carrier. Therefore, anaerobic microorganisms usually use ferredoxin Fd (Fd_{ox}/Fd_{red}, $E^0 = -0.398$ V or lower), as a common redox mediator for catalyzing hydrogen evolution reaction (Stams and Plugge 2009). However, some methanogens do not possess cytochromes; thus, Fd and coenzyme F_{420} ($F_{420}/F_{420}H_2$, $E^0 = 0.357$ V) can help these methanogens, as the most vital hydrogen scavengers, without cytochrome to oxidize hydrogen at extremely low concentrations. Hydrogen is the common product at the cathode of BESs and is also the electron donor for hydrogenotrophic methanogens, as a bridge for converting biohydrogen to biomethane in MEC. Furthermore, hydrogenotrophic methanogens can be highly enriched at the cathode (Lovley 2017; Siegert et al. 2015), and there is also DIET that does not require hydrogen for catalysis (Cheng et al. 2009). Obviously, *Methanococcus maripaludis* with knocked out hydrogenases was able to directly obtain electrons from the cathode to reduce carbon dioxide into methane (Lohner et al. 2014).

In addition to the diffusion of hydrogen, formic acid can also be used as an electron intermediary to achieve interspecies electron transfer. The discovery of formic acid transfer pathway was due to the fact that the sole hydrogen transfer rate could not match the methane production rate from butyrate degradation in the bioreactor (Thiele and Zeikus 1988). However, only part of the methanogens can utilize formic acid, even though the transfer diffusion rate of formic acid is 100-fold that of hydrogen. Hence, formic acid also becomes an electron loss during the methanogenesis process. Moreover, it is extremely difficult to evaluate the contribution of hydrogen and formic acid to methanogenesis; on the other hand, the electron transfer process in traditional AD relies on two pathways, resulting in restricted possibilities for methane yield enhancement.

MECs, as emerging technologies for anaerobic wastewaters/wastes treatment and energy recovery, can be regarded as a practical integrative step to address some obstacles of AD, such as poor operational stability, low biogas yields, and qualities (Wang et al. 2020a). Importantly, the integrated electricity-stimulated anaerobic system can treat multiple wastes, regulate the establishment of microbial community structures and electron transfer paths, and dramatically improve energy efficiency and overall systems stability. Bo et al., for instance, employed waste activated sludge

as substrate in a MEC reactor (at an applied voltage of 1.0 V) for methane generation and obtained 2.3 times higher rates than the conventional AD (Bo et al. 2014). Furthermore, various kinds of biowastes can be employed in such microbial electrolysis integrated anaerobic systems for methane production, including black wastewater (Zamalloa et al. 2013), digested pig slurry (Cerrillo et al. 2016), distillery wastewater (Feng et al. 2017), food waste leachate (Lee et al. 2017), beer wastewater (Sangeetha et al. 2017), food waste (Zhi et al. 2019), etc. Table 5.4 presents a comprehensive overview of different substrates that have been tested so far.

5.4 The Energy Efficiency Calculation Formulas Involved in Microbial Electrolysis Cells

The performances of MEC reactors are typically evaluated using various parameters. In this section, we will present the main calculations, which are primarily based on the products associated to hydrogen and methane metabolism.

5.4.1 Essential Parameters Calculation

H_2 or CH_4 production rate (Y_{H_2}, Y_{CH_4}):

The daily volumetric H_2 or CH_4 production rates are obtained by dividing the produced volumetric H_2 or CH_4 yield by each cycle and normalize it with the effective working liquid volume of the reactor per day.

$$Y_{H_2} = \frac{A_{V_{H2}}}{t \cdot V_{\text{liquid}}} \tag{5.9}$$

$$Y_{CH_4} = \frac{A_{V_{CH4}}}{t \cdot V_{\text{liquid}}} \tag{5.10}$$

where Y_{H_2} and Y_{CH_4} are the H_2 or CH_4 production rate ($m^3\ m^{-3}$ reactor d^{-1}); $A_{V_{H_2}}$ and $A_{V_{CH_4}}$ are the average H_2 or CH_4 production for each batch (mL/batch); t represents the residence time of each batch (d, day); and V_{liquid} is the effective working volume of the reactor.

Coulomb Efficiency (CE)

Coulomb efficiency is used to measure the electron recovery efficiency of the microbial anode (Wang et al. 2020b). CE can be used as the metrics for evaluating the performance of the electrodes in BESs, combined with overpotential, which shows the energy loss at the electrodes (Hamelers et al. 2010). Coulombic efficiency can be an indicator to differentiate the involvement of anodic oxidation and acetoclastic methanogenesis in the removal of acetate. Calculations for the

Coulombic efficiency (CE) and COD removal efficiency (CRE) can be found in previous studies (Yang et al. 2019).

$$\mathrm{CE} = \frac{Q}{Q_T} \times 100\% \tag{5.11}$$

$$Q = It = \int i\mathrm{d}t \tag{5.12}$$

$$Q_T = \frac{b \cdot F \cdot m}{M} = \frac{b \cdot F \cdot (\mathrm{COD_{in}} - \mathrm{COD_{out}}) \cdot V_{\mathrm{liquid}}}{M_{\mathrm{O_2}}} \tag{5.13}$$

where Q is the actual amount of organic matter via anode microbial degradation; Q_T is the theoretical calculated amount of substrate oxidation; I is the current (A or C s^{-1}), which is calculated by Ohm's law ($I = V_{\mathrm{voltage}}/R_{\mathrm{ex}}$) from the voltage ($V_{\mathrm{voltage}}$) drop measured across the resistor (R_{ex}); t is the time (s); b is the total number of electrons transferred by oxidation (O_2) of 1 mol substrate, $b = 4$ (acetate); $F = 96{,}485$ C mol^{-1} is the Faraday constant; $\mathrm{COD_{inf}}$ is the initial chemical oxygen demand of substrate, before the reaction (mg L^{-1}); $\mathrm{COD_{eff}}$ is the final chemical oxygen demand of substrate after the reaction (mg L^{-1}); M is the relative molecular mass of the substrate (g mol^{-1}); $M_{\mathrm{O_2}} = 32\ \mathrm{g \cdot mol^{-1}}$ is the relative molecular mass of O_2.

Current Density (CD)

There are two approaches to express current density: the first is based on the projected electrode (anode or cathode) area (I_A, A m^{-2}) and the other is based on the total reactor effective working volume (I_V, A m^{-3}) (Wang et al. 2020c).

Electrode overpotential (EO), taking hydrogen generation as an example:
Anode and cathode overpotential are calculated as ($E_{\mathrm{eq,\ anode}}$, $E_{\mathrm{eq,\ cathode}}$):

$$\mathrm{EO} = E_{\mathrm{meas}} - E_{\mathrm{eq}} \tag{5.14}$$

where E_{meas} represents the measured anode/cathode potential (V vs. NHE), while E_{eq} is the equilibrium/theoretical anode/cathode potential (V vs. NHE), using the Nernst equation (based on the acetate).

$$\mathrm{CH_3COO^-} + 4\mathrm{H_2O} \rightarrow 2\mathrm{HCO_3^-} + 9\mathrm{H^+} + 8\mathrm{e^-}$$

$$E_{\mathrm{eq,anode}} = E^{0'}_{\mathrm{anode}} - \frac{\mathrm{RT}}{n_{\mathrm{substrate}}F} \ln\left(\frac{[\mathrm{CH_3COO^-}]}{[\mathrm{HCO_3^-}]^2[\mathrm{H^+}]^9}\right) \tag{5.15}$$

$$2\mathrm{H^+} + 2\mathrm{e^-} \rightarrow \mathrm{H_2}$$

Table 5.4 Different substrates used for methane production in the microbial electrolysis integrated anaerobic systems

Substrates	Reactor configuration[a]	Reactor size (L)	Operation mode	Retention time (day)	Electrode material		Applied voltage (V)	CH_4 production rate increase[b]	COD removal efficiency increase[b]	Reference
					Anode	Cathode				
Simulated black wastewater	DC without membrane	AD: 20 MEC: 24.2	Batch	AD: 20, 40 d; MEC: 3.5 d	Stainless steel mesh		2.0 ± 0.1	5	0	Zamalloa et al. (2013)
Anode: NaCl cathode: CO_2	DC CEM[c]	0.4	Batch	0.25	Carbon stick		Cathode potential from −0.6 to −1 V vs. Ag/AgCl	2.30 ± 0.34 mL (−0.9 V)	NA[e]	Zhen et al. (2015)
AD: pig slurry; anode: filtered digested pig slurry from AD; cathode: NaCl	DC CEM[c]	AD: 4, MEC: 0.5	Continuous	10 8 6.7 5 (AD)	Carbon felt	Stainless steel mesh 304	Anode potential at 0 V vs. SHE	1.35	1.65	Cerrillo et al. (2016)
Distillery wastewater	DC (UASB with SEA[d])	5.5	Continuous influent pH: 3.6 5.6 7.0 7.5	NA[e]	Fabricated with graphite fiber fabric, nickel chloride, multiwall carbon nanotube, and exfoliated graphite		0.5	1.719 1.604 1.783 1.7	1.062 1.123 1.115 1.099	Feng et al. (2017)
AD: pig slurry; anode:	DC CEM[c]	AD: 4, MEC: 0.5	Continuous	AD: 10 day, anode:	Carbon felt	Granular graphite	Cathode potential at −0.8 V vs. SHE	1.21	1.95	Cerrillo et al. (2018)

(continued)

Table 5.4 (continued)

Substrates	Reactor configuration[a]	Reactor size (L)	Operation mode	Retention time (day)	Electrode material		Applied voltage (V)	CH_4 production rate increase[b]	COD removal efficiency increase[b]	Reference
					Anode	Cathode				
filtered digested pig slurry from AD; cathode: $NaHCO_3$				32.4 h, cathode: 14.1 h						
Anaerobic activated sludge	SC	0.15	Batch	NA[e]	Ti/Ru alloy mesh plates		1.4 1.8	11.41 13.58	NA[e]	Guo et al. (2013)
Synthetic wastewater (sucrose)	SC (UASB)	2	Continuous	1	Carbon felt		0.8	3.9	1.31	Zhang et al. (2013)
Sodium acetate	SC (UASB)	10	Batch	0.25	Graphite rod		−0.4 −0.35 −0.3 −0.25 V (anode potential vs. Ag/AgCl)	1.39 1.57 1.67 1.93	1.35 1.57 1.72 1.97	Zhao et al. (2014)
Waste activated sludge	SC	0.18	Batch	0.5	Carbon felt	Stainless steel	0.4 1.0	2.3 (1.0 V)	3 (1.0 V)	Bo et al. (2014)
Waste sludge	SC	2	Batch	22 (digestion)	Fe tube	Graphite pillar	0.3 0.6	1.22 (0.3 V)	1.11 (0.3 V)	Feng et al. (2015)
Waste activated sludge	SC	0.5	Batch	2	Graphite brush	Carbon cloth coated with	0.8	3	NA[e]	Liu et al. (2016a)

						0.5 mg/cm^2 Pt				
Sodium acetate	SC	0.02	Batch	14	Granular activated carbon		0.55	5.3-6.6	5.6	Liu et al. (2016b)
Glucose	SC	0.27	Batch	6	Carbon fiber brush		0.5 0.7 1.0 1.5	1.15 1.24 1.27 1.16	1.19 1.21 1.25 1.40	Choi et al. (2017)
Food waste leachate	SC	15	Batch	20	Graphite mesh coated with Ni		0.3	1.7	NA[e]	Lee et al. (2017)
Artificial beer wastewater	SC	NA[e]	Continuous	0.5 0.75 1 1.5	Carbon brush	Nickel mesh	0.80 ± 0.01	1.54 2.10 3.04 based on the lowest MEC-AD	1.05, 1.16, 1.21 based on the lowest MEC-AD	Sangeetha et al. (2017)
Glucose and waste sludge	SC	0.8	Batch	24 h	Reticulated vitreous carbon		0.3 0.6	Glucose: 1.09, 1.10 Sludge: 1.131, 1.134	Glucose: 1.10 (0.3 and 0.6 V)	Gajaraj et al. (2017)
Food waste	SC	20	Batch (SBR[f])	20 d	Graphite carbon mesh coated with Ni		0.3	1.03	1.02	Park et al. (2018)
Raw sludge from the aeration tank of WWTP	SC	0.15	Batch	NA[e]	Ti/Ru alloy mesh		0.6, 0.8, 1.3, 1.8, 2.3	1.20–1.79 (0.6–1.8 V)	1.03–1.28 (0.6–1.8 V)	Xiao et al. (2018)
Food waste and sewage sludge	SC	0.18	Continuous	15 (SRT[g])	Carbon brush	Ti/RuO_2 graphite felt	0.2, 0.4, 0.8, 1.2	2.8 (0.4 V)	NA[e]	Zhi et al. (2019)

(continued)

Table 5.4 (continued)

Substrates	Reactor configuration[a]	Reactor size (L)	Operation mode	Retention time (day)	Electrode material		Applied voltage (V)	CH_4 production rate increase[b]	COD removal efficiency increase[b]	Reference
					Anode	Cathode				

[a]SC refers to single-chamber MEC; DC refers to double-chamber MEC
[b]The increased folds of CH_4 yield or production rate and COD removal efficiency (%), compared to the control
[c]*CEM*, cation exchange membrane
[d]*SEA*, the separator and electrode assembly that was stacked with the anode
[e]*NA*, not available
[f]*SBR*, sequencing batch reactors
[g]*SRT*, solids retention time

$$E_{\text{eq, cathode}} = E^{0'}_{\text{cathode}} - \frac{\text{RT}}{n_{\text{H}_2}F} \ln\left(\frac{P_{\text{H2}}}{[\text{H}^+]^2}\right) \tag{5.16}$$

where $E^{0'}_{\text{anode}} = 0.187$ V is the equilibrium anode potential at standard conditions; $T = 298$ K is the absolute temperature (10^5 Pa) (Logan et al. 2006); $n_{\text{substrate}} = 8$ is the number of electrons needed to generate H_2 by oxidizing one mol acetate (Table 5.5); $R = 8.314$ J K^{-1} mol^{-1} is the ideal gas law constant, under the standard biological condition, $E_{\text{eq, anode}} = -0.279$ V (Logan et al. 2008). $E^{0'}_{\text{cathode}}$ is the equilibrium cathode potential at standard conditions, assuming $[\text{H}^+] = 1$ mol L^{-1}, then $E^{0'}_{\text{cathode}} = 0$ V; $n_{\text{H}_2} = 2$ is the amount of electrons needed to generate 1 mol H_2 for hydrogen evolution reaction, under the standard biological condition, $E_{\text{eq, cathode}} = -0.414$ V (Logan et al. 2008). When at unit partial H_2 pressure and 30 °C (303 K), the cathode overpotential reduces to (Jeremiasse et al. 2010):

$$E_{\text{eq, cathode}} = -0.060\ \text{pH} \tag{5.17}$$

5.4.2 The Contribution for Production of Hydrogen and Methane in MECs

The source of hydrogen and methane is evaluated by comparing the volume generated by the current or detected by gas chromatograph, that is, the different contributions of H_2 or CH_4 production coming from the current or the substrate (acetate).

The theoretical production of hydrogen generated based on the measured current and substrate consumption ($T_{V_{\text{H}_2-\text{current}}}$, $T_{V_{\text{H}_2-\text{acetate}}}$) is given by:

$$T_{V_{\text{H}_2-\text{current}}} = \frac{\int It}{n_{\text{H}_2}\text{F}} V_m \tag{5.18}$$

$$T_{V_{\text{H}_2-\text{acetate}}} = \frac{b_{\text{O}_2}\Delta\text{COD}V_{\text{liquid}}}{2\text{M}_{\text{O}_2}} V_m \text{ (based on the mearsured COD conceration)} \tag{5.19}$$

$$T_{V_{\text{H}_2-\text{acetate}}} = \frac{b_{\text{H}_2}V_{\text{liquid}}\Delta C_{\text{acetate}}}{M_{\text{acetate}}} V_m \text{ (based on the mearsured acetate conceration)} \tag{5.20}$$

The theoretical production of methane generated based on the current and substrate consumption ($T_{V_{\text{CH}_4-\text{current}}}$, $T_{V_{\text{CH}_4-\text{acetate}}}$) is given by:

Table 5.5 The number of moles of electrons per mole of common substrate (n), based on the half-cell reactions

Substrate	$M_{substrate}$[a]	Half-cell reaction	$n_{substrate}$[b]
Acetate	60.05	$C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$	8
Formate	46.03	$CH_2O_2 \rightarrow CO_2 + 2H^+ + 2e^-$	2
Propionate	74	$C_3H_6O_2 + 4H_2O \rightarrow 3CO_2 + 14H^+ + 14e^-$	14
Lactate	90.08	$C_3H_6O_3 + 3H_2O \rightarrow 3CO_2 + 12H^+ + 12e^-$	12
1,3-Propanediol	76.09	$C_3H_8O_2 + 4H_2O \rightarrow 3CO_2 + 16H^+ + 16e^-$	16
Glycerol	92.09	$C_3H_8O_3 + 3H_2O \rightarrow 3CO_2 + 14H^+ + 14e^-$	14
Glucose	180.16	$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 24H^+ + 24e^-$	24

[a]$M_{substrate}$, the relative molecular mass of the substrate
[b]$n_{substrate}$, the number of moles of electrons per mole of common substrate consumed

$$T_{V_{CH_4-current}} = \frac{\int I\mathrm{d}t}{n_{CH_4}\mathrm{F}} V_m \tag{5.21}$$

$$T_{V_{CH_4-acetate}} = \frac{\Delta C_{acetate} V_{liquid}}{M_{acetate}} V_m \text{ (based on the mearsured acetate concentration)} \tag{5.22}$$

where $T_{V_{H_2-current}}$ and $T_{V_{CH_4-current}}$ represent the theoretical production rate of H_2 or CH_4 generated by the current for every batch, after being normalized to the effective working liquid volume of the reactor, per day ($m^3\ m^{-3}$ reactor d^{-1}); I is the current (A or C s^{-1}), calculated by Ohm's law ($I = V_{voltage}/R_{ex}$) from the voltage ($V_{voltage}$) drop, measured across the resistor (R_{ex}); t is the time (s); $\int I\mathrm{d}t$ is the coulombs produced by the current, (C); $n_{H_2} = 2$ for H_2 and $n_{CH_4} = 8$ for CH_4 are the number of electrons needed to generate 1 mole H_2 ($2H^+ + 2e^- \rightarrow H_2$) or CH_4 ($CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$); F = 96,485 C mol^{-1} is the Faraday constant; b_{O_2} is a conversion factor based on the stoichiometric relation between electrons in COD and H_2 gas, equaling to 1 mol H_2 per 16 g O_2; $b_{H_2} = 4$ is a conversion factor based on the stoichiometric conversion of the amount of one mol acetate consumed to generate the amount of mol equaling to 4 mol H_2 per 1 mol acetate ($CH_3COOH + 4H_2O \rightarrow 2CO_2 + 4H_2$); $V_m = 22.4$ L mol^{-1} is the gas constant. $T_{V_{H_2-acetate}}$ and $T_{V_{CH_4-acetate}}$ represent the theoretical volume of H_2 or CH_4 generated by the substrate (acetate) for every fed-batch, based on the acetate converted to methane ($CH_3COOH \rightarrow CH_4 + CO_2$) ($m^3\ m^{-3}$ reactor d^{-1}); ΔCOD and $\Delta C_{acetate}$ are the changes in substrate concentration with every fed-batch (mg L^{-1}); V_{liquid} is the effective working volume of the reactor, mL; $M_{acetate} = 60.05$ g mol^{-1} is the relative molecular mass of the substrate (acetate).

Thus,

$$\mathrm{CE} = \frac{T_{V_{\mathrm{H_2-current}}}}{T_{V_{\mathrm{H_2-acetate}}}} \text{ (based on the mearsured acetate conceration)} \tag{5.23}$$

5.4.3 The Energy Calculation Involved in MECs

The overall hydrogen recovery (R_{H2}) is given by:

$$R_{\mathrm{H2}} = \frac{A_{V_{\mathrm{H2}}}}{T_{V_{\mathrm{H_2-acetate}}}} = \frac{n_{\mathrm{H_2}}}{\frac{\int It}{nF}}$$
$$= \frac{2Fn_{\mathrm{H_2}}}{\int It} \text{ (based on the mearsured acetate conceration)} \tag{5.24}$$

Cathodic hydrogen recovery ($R_{\mathrm{H_2,cat}}$) is given by:

$$R_{\mathrm{H_2,cat}} = \frac{A_{V_{\mathrm{H_2}}}}{T_{V_{\mathrm{H_2-current}}}} \tag{5.25}$$

The overall hydrogen recovery is used to evaluate the ratio of recovered H_2 compared to the maximum potential H_2 recovery, based on the substrate utilization (Wagner et al. 2009). Cathodic hydrogen recovery is used to evaluate the fraction of electrons that form H_2 from the overall amount of electrons reaching the cathode, namely generating current.

Electron reduction efficiency (E_e) is given by:

$$E_e = \frac{Q_{\mathrm{CH_4}}}{Q} \times 100\% \tag{5.26}$$

$$Q_{\mathrm{CH_4}} = 8 \cdot n_{\mathrm{CH_4}} \cdot F \tag{5.27}$$

Ideal gas law:

$$n_{\mathrm{CH_4}} = \frac{PV}{RT} \tag{5.28}$$

where this equation is used under experimental condition (25 °C, 1 atm, i.e., 1011.325 kPa).

Electron reduction efficiency (E_e) is used to measure the capacity of electron reduction by catalysis at the cathode surface.

The total input energy (W_{input}) is given by:

$$W_{\text{input}} = W_{\text{electricity}} + W_{\text{substrate}} \tag{5.29}$$

where W_{input} is the total amount of energy added to the entire system, kJ; $W_{\text{electricity}}$ (W_E) is the amount of energy added to the circuit by the power source, kJ, adjusted for losses across the resistor; $W_{\text{substrate}}$ (W_S) is the amount of energy added by the substrate (kJ).

The input electricity energy (W_E) is given by:

$$W_E = \frac{\sum_1^n \left(IE_{ap}\Delta t - I^2 R_{\text{ex}}\Delta t\right)}{1000} \tag{5.30}$$

where E_{ap} is the voltage applied, using the power source (V); Δt is the time increment for n data points measured during a batch cycle (s); and R_{ex} is the external resistor (Ω).

The input substrate energy (W_S) is given by:

$$W_S = n_S \Delta H_S \tag{5.31}$$

where n_S represents the substrate consumed (in terms of number of moles) per batch cycle; ΔH_S is the heat of combustion of the substrate, $\Delta H_{\text{acetate}} = 870.28$ kJ mol^{-1}, $\Delta H_{\text{glycerol}} = 1655.4$ kJ mol^{-1}, $\Delta H_{\text{glucose}} = 2802.7$ kJ mol^{-1} (Selembo et al. 2009b).

The total gained energy (W_{gained}) is given by:

$$W_{\text{gained}} = W_{\text{H}_2} + W_{\text{CH}_4} = n_{\text{H}_2}\Delta \text{H}_{\text{H}_2} + n_{\text{CH}_4}\Delta \text{H}_{\text{CH}_4} \tag{5.32}$$

where W_{H_2} and W_{CH_4} are the energy content generated from H_2 or CH_4 (kJ); n_{H_2} and n_{CH_4} are the number of moles of H_2 or CH_4 produced during a batch cycle; $\Delta \text{H}_{\text{H}_2} = 285.83$ kJ mol^{-1} and $\Delta \text{H}_{\text{CH}_4} = 890$ kJ mol^{-1} are the calorific values of H_2 and CH_4, based on the heat of combustion (upper heating value).

The methane revenue (R_{CH_4}) is given by:

$$R_{\text{CH}_4} = P_e \frac{Y_{\text{CH}_4}\Delta \text{H}_{\text{CH}_4}}{V_m}\eta \tag{5.33}$$

where $P_e = 0.10$ £ kW^{-1} h^{-1} is the standard price of electricity (referenced from business rates in the UK) (Aiken et al. 2019); R_{CH_4} is the revenue from methane (£·m^{-3} reactor day^{-1}); $\eta = 35\%$ is the electrical efficiency with a combustion engine as converter.

5.4.4 The Energy Recovery Efficiency

The total energy recovery efficiency (η_{total}) is given by:

$$\eta_{\text{total}} = \eta_E + \eta_S \tag{5.34}$$

where η_{total} is the ratio of energy output evaluated by produced H_2 or CH_4 to the total energy input composed of electricity input and substrate (acetate) consumption in the entire system; η_E is the ratio of the energy content of H_2 or CH_4 produced to the input electrical energy required; and η_S is the ratio of output energy evaluated by produced H_2 or CH_4 to the input energy from the consumed acetate.

The electrical energy recovery efficiency (η_E) is given by:

$$\eta_E = \frac{W_{H_2}}{W_E} \text{ (based on } H_2 \text{ as the main biogas)} \tag{5.35}$$

$$\eta_E = \frac{W_{CH_4}}{W_E} \text{ (based on } CH_4 \text{ as the main biogas)} \tag{5.36}$$

The substrate energy recovery efficiency (η_S) is given by:

$$\eta_S = \frac{W_{H_2}}{W_S} \text{ (based on } H_2 \text{ as the main biogas)} \tag{5.37}$$

$$\eta_S = \frac{W_{CH_4}}{W_S} \text{ (based on } CH_4 \text{ as the main biogas)} \tag{5.38}$$

The conversion efficiency of substrate ($\eta_{\text{substrate}}$) is given by:

$$\eta_{\text{substrate}} = \frac{n_{H_2}\frac{V2}{V_m}}{n_{\text{substrate}}\frac{C_{\text{substrate}}V_{\text{liquid}}}{M}} \times 100\% \text{(based on } H_2 \text{ as the main biogas)} \tag{5.39}$$

$$\eta_{\text{substrate}} = \frac{n_{CH_4}\frac{V4}{V_m}}{n_{\text{substrate}}\frac{C_{\text{substrate}}V_{\text{liquid}}}{M}} \times 100\% \text{(based on } CH_4 \text{ as the main biogas)} \tag{5.40}$$

where $\eta_{\text{substrate}}$ is the substrate conversion efficiency; $n_{\text{substrate}}$ is the electron per single mole of substrate; n_{H_2} and n_{CH_4} are the electrons yielded by H_2 or CH_4; and $C_{\text{substrate}}$ is the substrate concentration (mg L^{-1}).

5.5 Efficiency Improvement for Electron Transport

The electron transfer process is critical for the methanogenesis on the microbe-electrode interface. The electron transfer process of the cathode is similar to that of the anode, except for the direct electron transfer by direct contact and an indirect electron transfer process using hydrogen as a mediator (Miriam et al. 2011). Previous studies have illustrated that hydrogen is an important electron intermediate in the formation of cathodic methane, as well as an important electron donor for basophilic hydrogenotrophic methanogens. Compared with other pathways (Fig. 5.3),

hydrogen as an electron donor has the advantage of facilitating the enrichment of a wider range of hydrogenotrophic methanogens by diffusion, which is beneficial to improve the methane production rate. Therefore, it is more advantageous to enhance the hydrogen-to-methane pathway by strengthening the hydrogen evolution reaction for the enrichment of hydrogenotrophic methanogens, viz., promoting the hydrogen-mediated electron transfer process.

The formation of hydrogen at the cathode primarily depends on the electrochemical reaction process. Different electrode materials can affect the electron transfer rate and thus restrict the rate of HER. Furthermore, the characteristics of the biofilm also trigger differences in electron recovery efficiency, which further affect hydrogen yield. On the reaction interface between electrodes and microorganisms, the final electron acceptor is influenced by microorganism types and material properties. Under the conditions of non-pure cultures and non-specific materials, the electrons transmitted to energy metabolism and anabolic processes are different (since diverse microorganisms have different electronic respiratory chains), consequentially resulting in a variety of products. As a consequence, many undesirable by-products are ultimately produced, which affects the electron recovery efficiency.

The microbial community structure plays a decisive role in the distribution of reactive products, and the properties of the electrode materials can cooperate with the microorganisms to capture certain specific electron acceptors, subsequently resulting in high electron transfer recovery. In order to further strengthen bioenergy recovery, the electron transfer process is primarily facilitated in terms of electrode material modification and microbial community regulation. On the one hand, it can promote the rate of electron transfer on the interface of the bioelectrodes, improving the ability of catalyzing HER on the cathode. Furthermore, it can increase the recovery efficiency of electron transfer to the target end products.

5.5.1 Cathode Materials Upgrading

To date, one of the main drawbacks of BESs large-scale application, particularly with MEC, is the demand for costly materials, e.g., platinum in cathode. These materials are often favored due to the dramatic electrocatalytic activity for H_2 evolution, although the performance is negatively influenced by a number of different components that can be found in waste streams. Therefore, more sustainable and low-cost cathodes for bioenergy production via BESs are becoming urgent (Villano et al. 2010). Recently, microbial biocathodes have exhibited more widespread applications, e.g., bioremediation systems for biological reduction of oxidized contaminants (Aulenta et al. 2008, b), biological reduction of nitrates to nitrogen (Clauwaert et al. 2007), or electrochemical reduction of CO_2 to CH_4 (Villano et al. 2010).

In general, upgrading electrode materials primarily focus on reducing the mass transfer resistance of materials and the catalytic resistance. Based on the low hydrogen evolution potential of nickel foam (NF), the high catalysis efficiency of

earth-abundant transition metal phosphides, and low cost, Cai et al. studied a one-step phosphorization of NF; the authors used phosphorous vapor to fabricate a 3D biphasic Ni_5P_4-NiP_2 nanosheet matrix, acting as an electron transfer cathodic tunnel for H_2, coupled with a bioanode (Cai et al. 2018). A productivity of 9.78 ± 0.38 mL H_2 d^{-1} cm^{-2} was obtained, which was 1.5-fold higher than NF alone, and even higher than that described for commercially available Pt/C of 5.28 mL d^{-1} cm^{-2} (Cai et al. 2016a) and 4.94 mL d^{-1} cm^{-2} (Hou et al. 2014b). In addition, in order to replace the precious metal Pt, many transition metals, e.g., molybdenum, stainless steel, nickel foam, and other materials, are used as the matrixes, which can be further modified to improve the electrocatalytic activity of the electrode. Selembo et al. compared the effects of different stainless steel alloys (SS 304, 316, 420, A286) and nickel alloys (Ni 201, 400, 625, HX) using sheet metal cathodes in MEC, on hydrogen production, and found SS A286 displayed the best performance of 1.5 m^3 H_2 m^{-3} d^{-1} at 0.9 V (Selembo et al. 2009a). Call et al. confirmed that the stainless steel brush cathode with specific surface area can achieve the maximum productivity of 1.7 ± 0.1 m^3 H_2 m^{-3} d^{-1} at 0.6 V, compared to graphite brush cathode and flat stainless steel cathode (Call et al. 2009). Similarly, Su et al. also found that 3D macroporous stainless steel fiber felt cathode with high electrochemical active surface area has superior catalytic properties for H_2 evolution, achieving 3.66 ± 0.43 m^3 H_2 m^{-3} d^{-1} (current density of 17.29 ± 1.68A m^{-2}) at 0.9 V (Su et al. 2016). Hrapovic et al. successfully electrodeposited Ni on porous carbon paper, as cathode, obtaining the maximum H_2 production rate of 5.4 m^3 m^{-3} d^{-1}, when Ni loaded between 0.2 and 0.4 mg cm^{-2}, on the contrary, no any increase of hydrogen production under the coelectrodeposition of Pt and Ni (Hrapovic et al. 2010).

Figure 5.4 presents a comprehensive overview of cathode materials that have been used in MEC studies to enhance hydrogen and methane production.

5.5.2 *Functional Microbial Community Regulation*

In the traditional methanogenesis process, various volatile acids (such as propionate, butyrate, valerate, etc.) need to be converted into acetate and hydrogen by acetogenesis, before being used by acetoclastic and hydrogenotrophic methanogens, respectively. It is generally believed that 70% of the source of methane is derived from acetate and 30% from the contribution of hydrogen (Angenent et al. 2004). However, the growth rate of methanogens is slower than that of fermentative microorganisms, which limits the increase in the rate of methanogenesis. In view of this limitation, a methanogenic process based on extracellular electron transfer is developed inside a conventional anaerobic bioreactor (Liu et al. 2016a). This pathway allows direct electron transfer at the anode, using coenzyme cytochrome c with both oxidized and reduced states, extending the substrate types for methanogenesis. During extracellular electron transport of methanogenesis, the EAB can utilize various types of substrates, such as acetate and propionate, while

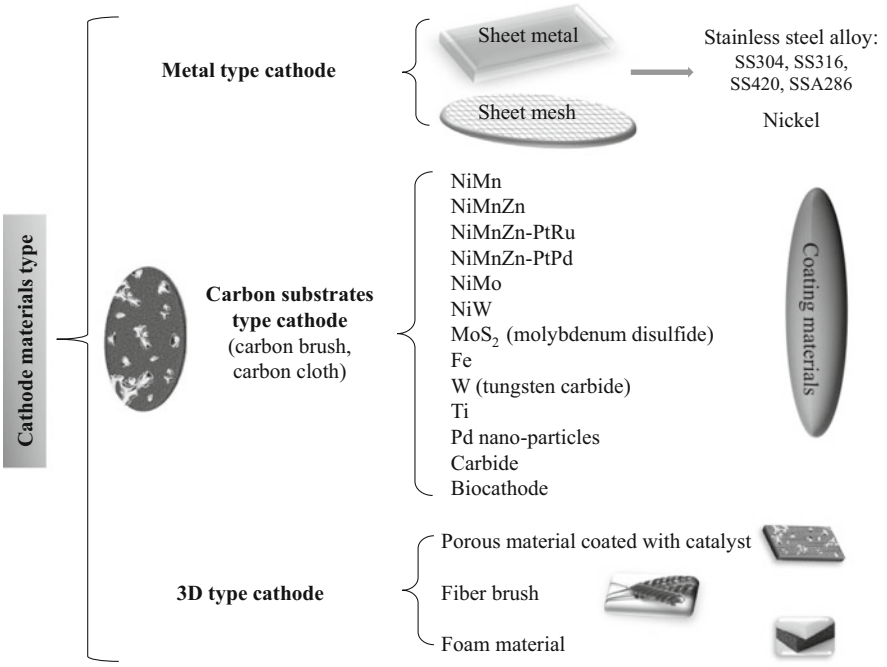

Fig. 5.4 Different cathode types of materials used in the microbial electrolysis cell, MEC

the electrons generated by oxidizing substrates can pass through an external circuit to drive the reduction of carbon dioxide at the cathode to form methane. Meanwhile, hydrogen also generates from electrons and protons, which can promote the growth of hydrogenotrophic methanogens, counteracting the limitations of traditional acetoclastic methanogens.

Cai et al. coupled the AD with the MEC to treat waste activated sludge and enhance methane generation (Cai et al. 2016b). Based on the results of Illumina MiSeq sequencing, methanogens were enriched in the cathode biofilm (particularly hydrogenotrophic *Methanobacterium* and acetoclastic *Methanosaeta)*, with two primary methanogenic pathways taking place at the cathode. This implied the possibility of increasing methane production, while reducing WAS digestion time, by controlling the bioelectrochemisty of the process.

Quorum sensing is an essential strategy for microbial community regulation and cell-to-cell communication in biofilms. The principle is that microbes secrete signaling molecules to affect the physiological activities of surrounding microorganisms, such as mobility, sporulation, biofilm formation, virulence, but also symbiosis, competition, toxicity, antagonism, antibiotics production, etc. (Miller and Bassler 2001). Acylated homoserine lactones (AHLs) are a representative signaling molecule that can also be used to regulate the interspecies communication process. AHLs

can be synthesized by bacteria like *Pseudomonas* sp. and degraded via chemical or biological pathways. In recent studies, it has been shown to enhance the respiratory activity associated with electron transfer, facilitating the capacity of electron transfer between cells and electrodes (Toyofuku et al. 2007). This increase in respiratory activity is mainly attributed to two strategies: one is by changing the physiological characteristics of microorganisms, including the cell membrane transmittance (Yong et al. 2013) and gene expression (Hu et al. 2015) (acting directly on the microorganism itself), and the other is by regulating processes related to electron shuttles for the biosynthesis, such as phenazines (Rabaey et al. 2005) (acting on the extracellular synthesis). Both strategies have been demonstrated to effectively increase the electrochemical activity of microorganisms. Cai et al. employed short-chain AHLs (3OC6), as intraspecific signaling molecules to modulate the biofilm community of bioelectrodes in single-chamber MECs. Surprisingly, the overall performance parameters of MECs with AHLs addition were significantly enhanced, including hydrogen yields, CE, electron recovery efficiency, and energy efficiency (Cai et al. 2016c). The lower internal resistance of reactors was verified via electrochemical impedance spectra (EIS). Noticeably, more EAB and fewer hydrogen scavengers, especially homo-acetogens *Acetoanaerobium* and *Acetobacterium*, and methanogens, especially hydrogenotrophic methanogen *Methanobrevibacter*, were detected in cathodic microbial aggregation (Fig. 5.5), which further confirmed the potential of regulating microbial communities by AHLs for strengthening electron transfer and hydrogen production in MEC, and impeding methanogenesis without any chemical inhibitors added (Cai et al. 2016c).

5.6 Bottlenecks and Challenges

MECs provide a promising potential to boost renewable hydrogen and methane generation from biowastes, possibly providing a new horizon to address imminent challenges in the energy sector, related to the rapidly growing population and fast developing industries. Importantly, MEC as an environmental-friendly technology not only displays a sustainable role in bioenergy recovery, but also simultaneously disposes and valorizes wastes. Undoubtedly, there are big challenges, such as fluctuating performances of MECs and costs of large-scale units, significantly constraining the transfer of bioelectrochemical technology from the laboratory to full scale. For example, system architecture, operating parameters, biological parameters determination, and techno-economic evaluation (such as products revenue, reproducibility, durability, scalability, etc.) need to be implemented and optimized, in order to bring this technology closer to the market.

Noticeably, there are four significant issues that need to be taken into consideration: (1) seeking alternative renewable energy, like solar, wind, waste heat, geothermal and marine energy, to improve sustainability or input energy saving; (2) increasing purities of biogas (H_2 or CH_4); (3) optimizing the electrode space layout to maximize efficiency in the limited reactor space; and (4) catalyzing the

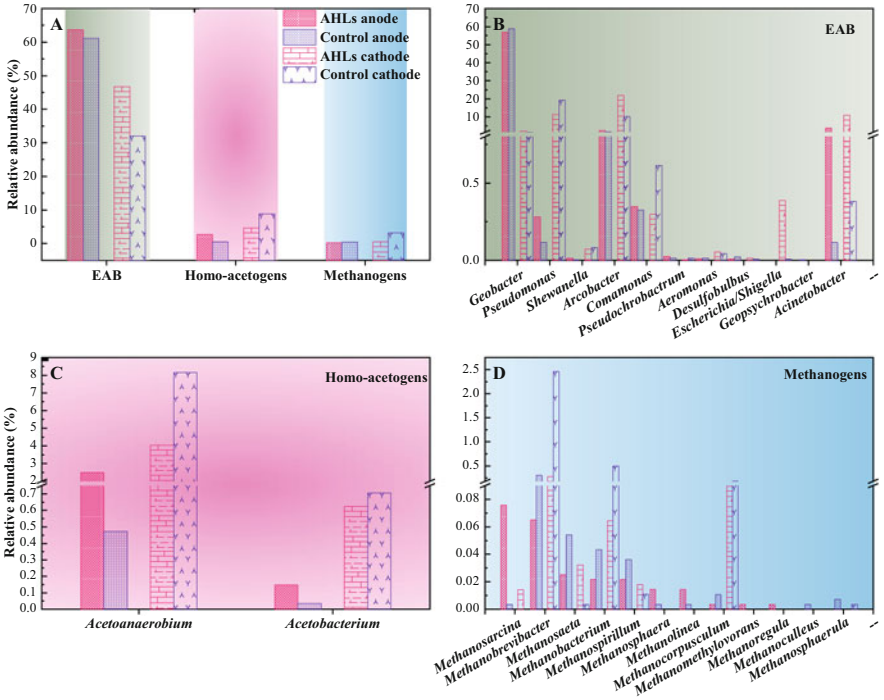

Fig. 5.5 The comparison of specific functional microbial community compositions on genus level between the anode and the cathode in the presence or absence of acylated homoserine lactones, AHLs. (**a**) is based on three functional microbial categories; (**b–d**) are subdivided into the specific categories of functional genera classification, including electrochemically active bacteria, EAB, homo-acetogens, and methanogens (reproduced from Cai et al. 2016c)

surface characteristics of the electrode and evaluating the electrochemical parameters of the composites. In the future, it will be strategic to integrate MECs with other waste treatment technologies to expand their scopes and applications. According to reactor configurations, alloy metal materials (like stainless steel mesh) will allow to form different configurations with large surface area, low overpotential, and low internal resistance of the system, at the same time promoting functional microorganisms adhesion to the electrodes. For the development of the electrode module system, it is necessary to pay more attention not only to the microstructure and the material properties of the electrodes, but also to three-dimensional electrode structure configuration with engineering application potential. The engineering application of MEC technology is promoted by modifying the conductive polymer on a single substrate, applying nanomaterials to improve electrode conductivity and specific surface area, and enhancing electron transfer performance and catalytic activity of bioelectrodes.

In conclusion, new assembly strategies will be explored, based on micro-nano interface of micro-electrode. Also, high-throughput sequencing, stable isotope

labeling, and other scientific methods are comprehensively employed to further reveal the function and structure of electrode microbial flora in depth, and shed light on microbial interactions, as well as extracellular electron transfer mechanism, based on electron mediator, nanowire, and cytochrome. Overall, these approaches are expected to provide technical and theoretical understanding for the development of viable and sustainable applications of MEC technology.

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Chapter 6
Bioelectrochemical Technology for Sustainable Energy Production and Waste Treatment

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Abstract Over the years, the demand for energy worldwide is increasing sporadically and considering that the major sources of energy are being gradually depleted, the need to explore other renewable sources is the desire of society in recent times. In addition, environmental pollution has been a global topic for quite some time, bearing in mind the extent of its adverse effects worldwide, the necessity to treat it has become more critical. These challenges have compelled researchers to employ bioelectrochemical technologies (BETs), which involve innovative techniques using bioelectrochemical systems (BESs) to aid in simultaneous energy production and waste treatment. This chapter firstly introduces the challenges of waste treatment and the demand for energy worldwide, presenting BETs and various classes of BETs. This precedes the use of BETs for efficient waste treatment, which is thoroughly addressed and this culminates in other uses of BETs in environmental remediation. Finally, the production of energy from BET, as well as biohydrogen and biofuels is discussed with numerous references presented.

Keywords Bioelectrochemical · Technology · Microorganisms · Bacteria · Energy · Systems

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6.1 Introduction

Sustainable and consistent supply of fuel, power, and water is crucial for the robust development and well-being of humankind. Environmental pollution due to the widespread of chemical contaminants continues to be a subject of great concern worldwide, as it threatens human health and ecology (Wang et al. 2015a). With increasing environmental contamination, human exposure to harmful chemical pollutants has been elevated substantially following the massive increase in their use in agricultural, domestic, as well as technological industries. There have been considerable investments by many countries in an attempt to remediate different contaminants in water, soil sediments, and the atmosphere at large (Yin et al. 2011; Di Lorenzo et al. 2009). Notwithstanding these huge investments and active remediation practices adopted by governments around the globe, the challenge of the contemporary world is the desire to clean up these environmental contaminants by eco-friendly, sustainable, and economically adaptable technologies. Nevertheless, the challenges of generation of secondary pollutants, high cost, significantly vast quantities of energy and chemicals associated with the use of traditional remediation technologies make this clean-up process ineffective (Wang et al. 2015a; Huang et al. 2011).

Alongside waste treatment, there is an ever-growing demand for new, clean, reliable, and sustainable sources of energy due to insufficient accessibility and environmental concerns brought by the overutilization of fossil fuels as a dominant energy source. Projections of global energy expenditure established in various studies predict that the annual energy demand is likely to keep growing, reaching an estimated 23 terawatts by the year 2050 in comparison to about 13 terawatts in 2010 (Chae et al. 2009; Villano et al. 2012). At the same time, overutilization of the existing resources in industrial, municipal, as well as agricultural activities is expected to continually degrade the environment causing threats of global warming and associated effects (Roy and Pandit 2019). Considering all these, it becomes crucial to design and develop alternative renewable sources of energy to ensure the sustainability of our planet by diversifying the sources for concurrent waste treatment and energy production (Resch et al. 2008). At the forefront of waste treatment and this energy puzzle, bioelectrochemical technologies (BETs) have recently been proposed as an emerging and sustainable platform for concurrent waste treatment and energy production (Mohan et al. 2010). Primarily, the beneficial use of BETs emanates from their flexibility in converting different forms of waste (Fig. 6.1), providing opportunities for clean and efficient production of energy, broad spectrum of products including biofuels, useful chemicals, and electricity in a reliable way using microorganisms as catalysts (Mohan et al. 2016; Butti et al. 2016; Bajracharya et al. 2016). It is established that organic wastes present in diverse wastewater resources have higher internal energy content than the quantity of energy needed for the wastewater treatment process (Heidrich et al. 2010). Thus, wastewater could potentially be utilized as a renewable resource, economizing a considerable amount of money and energy, since it contains pollutants which are organic and can be

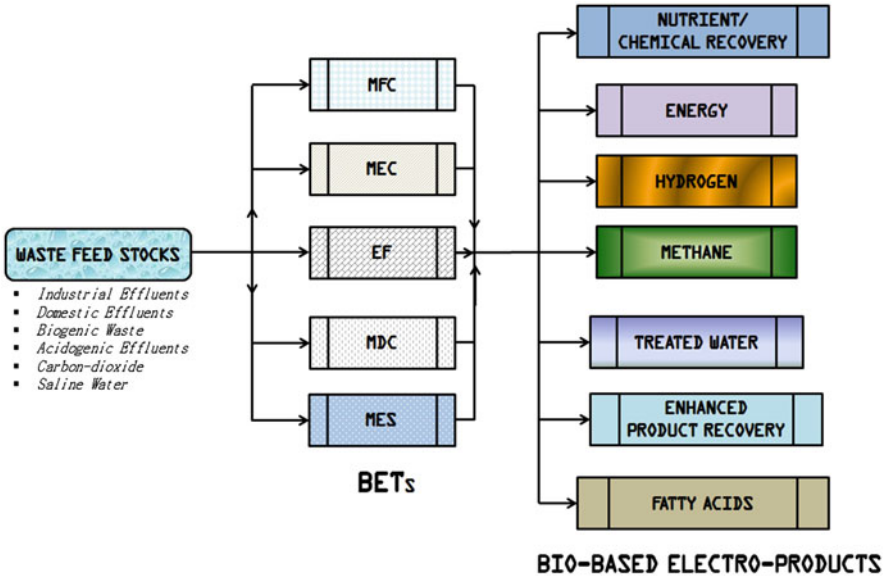

Fig. 6.1 Diverse application of BETs for wastewater treatment and bio-based product recovery from diverse waste feedstocks (adapted from (Mohan et al. 2019))

employed to generate hydrogen, electricity, and other useful chemicals. To achieve this, the organic matter present in wastewater is concurrently decomposed by electrogens within an electrochemical cell while helping to clean up wastewater at the same time.

Basically, these bioelectrochemical platforms are hybrids and interdisciplinary in nature encompassing different disciplines such as material sciences, electrochemistry, environmental engineering, microbiology, and biochemistry (Aulenta et al. 2018; Villano et al. 2017; Choi and Sang 2016). In these systems, the change in the redox potentials between the cathode and anode generates a difference in potential that causes the spontaneous movement of electrons from a region of lower to higher potential (Patil et al. 2015). The perceived movement of electrons is measured via an external circuit in terms of electric current. In an event where enzymes or microbes are used to catalyze the electrode reactions in electrochemical technologies, the system is called microbial electrochemical system (MXC) or in general terms BET (Harnisch and Schröder 2010). Based on their applications, BETs can be categorized, viz. microbial electrolysis cell (MEC), for the formation of useful chemicals including hydrogen, hydrogen peroxide, methane, caustic soda, and acetate; microbial fuel cell (MFC), for waste treatment and generation of bioelectricity; microbial electrosynthesis system (MES), for generation of platform chemical products; electrofermentation (EF), for enhancement of products synthesized by using biological approaches and microbial desalination cell (MDC), for the separation of ionic substances (Bajracharya et al. 2016; Mohan et al. 2014; Kim and Logan 2013; Nikhil et al. 2015). MFCs are a special class of BETs which produce

electricity from the decomposition of organic materials at the anode compartment. In summary, it occurs as a result of a combination of low electrode potential involved when organic materials at anode and a relatively high redox potential for reduction of oxygen at the cathode lead to production of bioelectricity (Rabaey and Verstraete 2005). MECs require an external potential to be supplied so as to activate the cathode electrode potentials in driving the production of important chemical products (Singh et al. 2015). The BET in which carbon dioxide or organic substances undergo cathodic reduction to produce valuable organic molecules is termed as microbial electrosynthesis (MES) (Rabaey and Rozendal 2010). Additionally, MDC is another kind of BETs with potential applications in removing salts from water and sediment, while in plants, MFCs and microbes combine with plant roots to produce electricity (Cao et al. 2009; Chiranjeevi et al. 2012; ElMekawy et al. 2014). Over the years, BETs have been confirmed as powerful bioreactors for the remediation of recalcitrant contaminants and noxious wastewaters under microbial electroremediation processes or bioelectrochemical treatment (Mohanakrishna et al. 2010).

In this chapter, we give an extensive overview of bioelectrochemical technology as an emerging and sustainable platform with potential applications in waste treatment and the production of energy.

6.2 Bioelectrochemical Technology for Efficient Waste Treatment

Addressing issues related to environmental pollution, global climate change, and environmental remediation in practical terms is imperative for the sustainable development of our planet earth (Omer 2008). Over the years, countries all over the world have been spending billions in treating trillions of liters of wastewater containing various unmanageable wastes including explosives, dyes, heavy metals, organic pollutants, and pesticides which in turn consume considerable amounts of energy (Wang et al. 2015a). However, this wastewater can potentially be used renewably, salvaging not only substantial amounts of energy but also a great deal of money considering that the organic pollutants embedded in it can be utilized to generate hydrogen, useful chemical products, and electricity (Sleutels et al. 2012) (Fig. 6.2a, b). Due to an exponential rise in energy demand and as a consequence of depletion of fossil fuels dominating the energy sector, the search for new reliable, renewable, and clean energy solutions for waste treatment has increased enormously.

Wastewater remediation is among the principal applications of bioelectrochemical technologies. Currently, bioremediation of low concentrated wastewaters employs conventional protocols which are energy-intensive and not friendly to the environment. Hence, it is crucial to treat wastewater sources by using cost-effective, energy-saving, as well as self-sustainable remediation protocols (Gavrilescu and Chisti 2005; Mohan et al. 2007; Li et al. 2014). These protocols will aid as efficient methods to offset considerable amount of energy incurred in

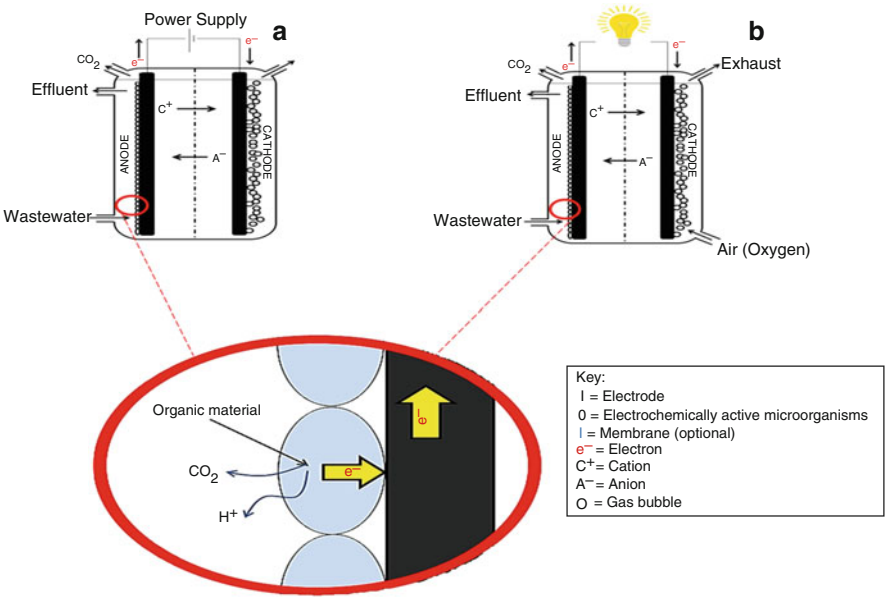

Fig. 6.2 BETs schematic representation of the two most common wastewater treatment systems: (**a**) MFCs and (**b**) MECs for hydrogen production (adapted from (Rozendal et al. 2008))

traditional wastewater remediation approaches. In solution to flaws observed in traditional waste treatment technologies, BETs are appealing as fundamentally important platforms wherein biomass undergo decomposition by the action of electrically active microorganisms, achieving an effective and efficient wastewater clean-up. In wastewater treatment, BETs are beneficial because of their potential in converting conventional energy-intensive remediation techniques into concurrent energy production processes and wastewater treatment. It is a worthy mention to indicate that wastewater contains an appreciably high amount of energy, approximately 2–4 times that utilized for its treatment, creating the possibility for a self-sufficient treatment process by integrating complete BETs in wastewater treatment plants (McCarty et al. 2011). BETs consolidate the benefits of wastewater remediation from various origin via the generation of bioelectricity and other useful products (Pandey et al. 2016). Contained in wastewater is organic matter, which is regarded as a valuable material serving as an energy source that is renewable. Consequently, the energy present in wastewaters containing organic substrates can possibly be tapped in form of electricity or any other invaluable product by using bioelectrochemical systems (Duteanu et al. 2010).

6.2.1 Removal and Decolorization of Dyes

The extensive use of dyes in different manufacturing and processing industries contributes greatly to water contamination (Cardenas-Robles et al. 2013), Azo dyes containing an azo functionality (–N=N–) are among synthetic dyes which are extensively employed in textiles, leather, plastics, food, as well as cosmetics industries. The expulsion of effluents from the plants that use these dyes contains various chemicals and persistent coloring substances which are harmful to human and aquatic life. Some researchers have indicated that the effluent containing dyes are considered detrimental due to their mutagenicity (Selvam et al. 2003; de Aragao et al. 2005), carcinogenicity, toxicity, and pollution effect on the environment, hence necessitating their proper treatment before being discharged in water bodies (Pant et al. 2007). Owing to their chemical stability, uneasy biodegradability upon microbial action, the remediation of wastewaters contaminated with dyes is a challenging endeavor. Different physico-chemical techniques including oxidation, adsorption, coagulation, flocculation, precipitation, ozonation, alkalinization, filtration, and electrochemical processes have been reportedly used for removing dyes from effluents (Mo et al. 2008; Malik et al. 2007). However, most traditional strategies suffer several shortcomings including the need for a lot of chemicals, high energy input, as well as sludge accumulation which may cause disposal problems in the environment (Matto and Husain 2007; Pak and Chang 1999). Recently, the successful treatment of wastewaters polluted with dyes using bioelectrochemical systems has been recorded by scientists (Cui et al. 2011; Mu et al. 2009a; Sun et al. 2009). Investigations on concurrent degradation of azo dye and production of bioelectricity by employing MFCs have been established (Sun et al. 2009). Mu and co-workers conducted an investigation on the use of a bioelectrochemical system to decolorize Acid Orange 7 (AO7) at cathode, under anodic microbial oxidation of acetate (Mu et al. 2009a). It was observed that decolorization of AO7 was effectively attained at a rate of about 2.64 ± 0.03 mol m^{-3} NCC day^{-1} (net cathodic compartment, NCC), with simultaneous energy production. The results further demonstrated that the required dosage of organic substrates in bioelectrochemical systems was reduced compared to other conventional anaerobic biological methods.

In another study Cui et al. (Cui et al. 2014) designed a method of treating wastewater containing azo dye, wherein "*Alizarin Yellow R*" was employed as a prototype dye, achieving proficiency of ($97.5 \pm 1.0\%$). This decolorization efficiency was reported to be higher when compared to that obtained when other biocathodes were used. In an evaluation of the feasibility of bioelectrochemical platforms using different co-substrates for removing Congo red, Cao et al. (2010) reported a simultaneous increase in the efficiency of bioelectricity production and dye removal when glucose, sodium acetate, and ethanol were employed as substrates in MFC. The results showed that in 36 h using dye concentration of 300 mg/L, decolorization of Congo red (98%) can be achieved. Of all substrates used, glucose achieved the fastest decolorization rate followed by ethanol and sodium acetate. In addition, they indicated that the production of electricity in the system was not

significantly influenced by the degradation of the dye. The optimum power density of 63.2 mW/m^2, 103 mW/m^2, and 85.9 mW/m^2 was reportedly produced from ethanol, glucose, and sodium acetate, respectively. These results proved the practicability of employing different co-substrates for bioelectricity production and concurrent Congo red removal in the MFC.

6.2.2 Sequestration of Heavy Metals

Heavy metals constitute those toxic transition metals with high atomic masses. Also, these metals cannot be processed by living organisms and they are considered to possess definite gravity which is five times that of water (Srivastava and Majumder 2008). They include vanadium, nickel, copper, lead, cobalt, chromium, mercury, cadmium, and arsenic. Heavy metals are everywhere, but the metals prevalent in soil, water sediments, and air are pollutants of huge concern due to their grave danger to the environs, thus disadvantageous to human well-being (Mathuriya and Yakhmi 2014). These metals are generally characterized by their non-biodegradability, toxicity, and bioaccumulation (Xue et al. 2009). The discharge of heavy metal ions in water streams at a considerably greater concentration than the accepted limit compromises the quality of domestic water, thus leading to serious health hazards (Edition 2011; Zwain et al. 2014). In this regard, it is crucial to get rid of these noxious metals from wastewaters and domestic water before they are discharged into the surroundings to safeguard and improve public health. Different techniques ranging from physical, biological, and chemical have been reportedly developed to aid in the remediation of poisonous heavy metals from contaminated water. The methods include adsorption (Zaini et al. 2010), chemical precipitation (Hui et al. 2005), ion exchange (Ijagbemi et al. 2009), solid-phase extraction (Dalida et al. 2011), and membrane separation (Qdais and Moussa 2004) among other methods. Despite their widespread applications, most of these methods are associated with high cost, complicated set-ups and they are ineffectual in situations of low concentration of the metal ion, making them unsuitable to some pollutants (Kurniawan et al. 2006).

The use of bioelectrocatalysts driven bioelectrochemical technologies appears promising for the wastewater treatment, since the metal ions are reduced and deposited by microorganisms including fungi, algae, bacteria, and yeasts (Mathuriya and Yakhmi 2014). In addition, treatment of wastewater polluted by poisonous heavy metals can be performed in bioelectrochemical systems comprising of cathode and anode compartments. More specifically, MFCs have been demonstrated as outstanding biological platforms in comparison to conventional methods concerning wastewater treatment, owing to their exceptional ability to transform chemical energy present in substrates into electricity with improved efficiency, remediate low-strength wastewater (Rittmann 2008; Watanabe 2008), operate safely and quietly at ambient temperature (Rabaey and Verstraete 2005). Initially, the performance of MFCs was observed to be poor hence giving a relatively low efficiency,

but with considerable efforts being invested in tailoring the architecture of MFCs, most of these platforms now demonstrate higher efficiency of about 100% in removing contaminants from different wastewater sources (Liu et al. 2011; Zhang et al. 2010a). Consequently, MFCs can be employed as effective systems for treatment of wastewater due to their superb performance surpassing other wastewater treatment techniques like conventional anaerobic digestion (Pham et al. 2006) and up-flow anaerobic sludge blanket reactor methods (Duteanu et al. 2010).

In an attempt to address the effectiveness of bioelectrochemical systems in the simultaneous elimination of several heavy metals present in an integrated system, Huang and co-workers designed a BES consisting of MFC and MEC platforms for removal of noxious metals by accommodating chromium or copper to the biocathodes in MFCs, because they possess high redox potentials and MECs for reducing cadmium, because of its more negative redox potential (Huang et al. 2015). The experimental results showed a decrease in the number of various microbes. Also, different communities of bacteria were observed on the reactors biocathodes with a mixed solution of heavy metals compared to reactors with a single metal. This observed reduction in variety is supported by a considerable reduction in the Shannon indices ranging from 4.62, 4.60, 4.08 to 4.22, 4.15, and 3.86 for chromium, copper, and cadmium, respectively. The decrease in diversity was attributed to greater toxicity contributed by mixing different metals of different toxicity values compared to the toxicity of single metals. The study therefore revealed that BESs with biocathodes can potentially be employed to treat wastewater polluted by chromium, copper, and cadmium, either individually or collectively in mixtures, by utilizing cathodes of MECs and MFCs (Kamika and Momba 2013; Horvat et al. 2007).

In another study, Jiang et al. (2013a) recorded the successful removal of several heavy metals such as selenium (98%), barium (97%), strontium (95%), zinc (81%), molybdenum (77%), copper (67%), chromium (45%), and lead (33%) in MFC during the treatment of wastewater from oil sand industry. The results in comparison to those by indigenous microalgae showed the diverse elimination proficiencies of some heavy metals. The microalgae *P. Kessleri* revealed greater removal proficiencies for zinc (100%) and copper (76%), but registered lesser efficiency for chromium (27.4%), molybdenum (27.2%), and strontium (53%) as recounted by Mahdavi et al. (2012).

The elimination of copper ions present in wastewater was demonstrated by Cheng et al. (Cheng et al. 2013). Their results showed an efficiency of 87% copper obtained with a cathodic efficiency of 80% and all-out power density of 2.0 W m^{-2}. This, in addition to other reported studies, proves that recovering metals during the production of electricity eradicates the demand for energy in the treatment process. Additionally, it shows that MFCs possess the capacity to recover and remove metals of concentrations which are very low and this is the desire of environmentalists in recent times.

6.2.3 *Removal of Sulfide*

The discharge of wastewaters and organic wastes from many processes prevalently contains sulfur compounds. It is important to note that the existence of sulfides in toxic gaseous form (H_2S) presents an immediate danger to human health and well-being usually at concentrations above 100 mg L^{-1}. In wastewaters, the method of biologically converting sulfur compounds through sulfate-reducing bacteria (SRB) causes the release of corrosive, pernicious, and odorous sulfides. These SRBs which reduce sulfate such as *Desulfovibrio desulfuricans* are normally present in the wastewaters with organic content which is high. Remediation of wastewater containing sulfates by using biological sulfate reduction processes is regarded as a proficient practice. However, the process is hampered by obstruction of bacteria metabolism caused by sulfur-based gases and other sulfides, leading to the letdown of the process due to inherent toxicity and harsh nature of sulfides (Zhao et al. 2008). The removal of sulfides in MFCs is achieved by electrochemical oxidation of sulfides at the anode with simultaneous power generation (Rabaey et al. 2006; Habermann and Pommer 1991). The presence of sulfides in different wastewaters and their electrochemical removal have been presented by Pikaar et al. (2012, 2015, 2011) and Dutta et al. (2010).

The biological process of reducing sulfate to sulfide was followed by catalytic oxidation to sulfate as revealed in Eqs. (6.1) and (6.2), as well as Fig. 6.3. Other researchers have investigated the use of various anodes including charcoal (Cooney

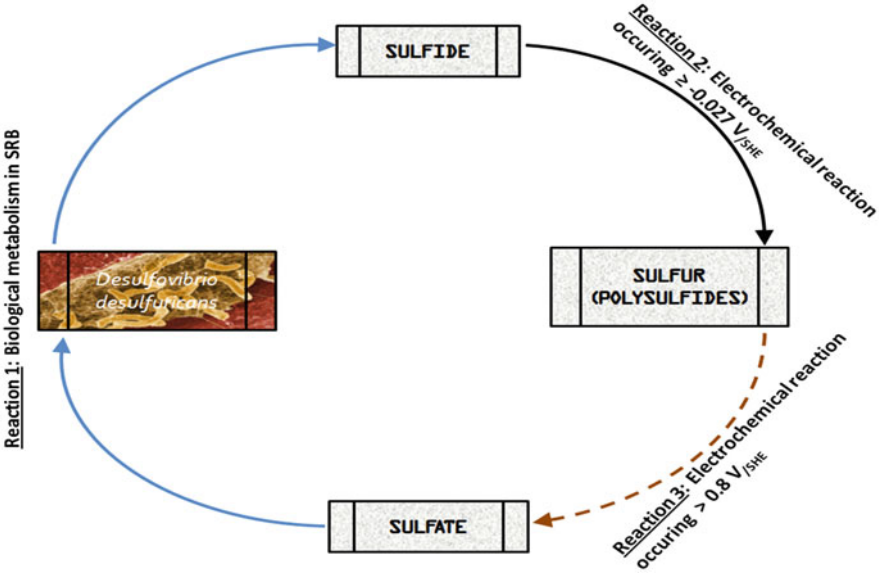

Fig. 6.3 Schematic representation of reaction pathways for removing sulfide and sulfate in MFCs (adapted from (Bajracharya et al. 2016))

et al. 2008), metal hydroxide-modified graphite (Habermann and Pommer 1991), activated carbon cloth and graphite foil (Zhao et al. 2008). In order to maintain the movement of electrons via an external circuit, oxygen was successfully reduced at the cathode.

$$\text{Sulfate} \xrightarrow[\text{Biocatalytic reaction}]{\textit{D.desulfuricans}} \text{Sulfide} \xrightarrow[\text{Electrochemical reaction}]{\text{Anode}} \rightarrow \text{Sulfate} \tag{6.1}$$

$$\text{Sulfate} \xrightarrow{\textit{Paracoccus}\ \text{spp}} \text{Sulfide} \xrightarrow{\textit{Paracoccus}\ \text{spp} + \textit{anode}} \text{Sulfur} \tag{6.2}$$

The efficacy of BETs in removing sulfides from different wastewater streams together with production of electricity was presented by Rabaey et al. (2006). In particular, the researchers employed MFC containing hexacyanoferrate cathodic electrolyte to convert dissolved sulfide to elemental sulfur. The designed bioelectrochemical platform consisted of square and tubular types of MFCs. Their results revealed the change of sulfide to sulfur in MFCs was dependent on the cell potential. The tubular system was able to achieve sulfide removal of about 514 mg sulfide L^{-1} net anodic compartment (NAC) day^{-1} (241 mg L^{-1} day^{-1} total anodic compartment, (TAC)), with concurrent electricity production with power outputs of about 101 mW L^{-1} NAC (47 W m^{-3} TAC). In summary, the two MFCs achieved total sulfide removals of approximately 98%.

While studying the variables that influence the performance of MFCs in the treatment of synthetic wastewater containing sulfur, Zhao and co-workers used *Desulfovibrio desulfuricans* in reducing sulfite and thiosulfate (Zhao et al. 2009). In their study, it was concluded that the metabolism of sulfur-reducing bacteria was not confined to the reduction of sulfate since a number of species can promote the production of sulfide through reduction of sulfite and thiosulfate. MFC constituting of two air-breathing cathodes, carbon-based anode, and the sulfate-reducing species (*Desulfovibrio desulfuricans*) was fabricated in order to obtain high power outputs and efficiently removing thiosulfate and sulfite. The results obtained showed the removal of 0.97 g dm^{-3} (86%) and 1.16 g dm^{-3} (91%) of both thio and sulfate, respectively, from contaminated water at an ambient temperature. The results of this study strongly confirm nearly complete removal of sulfur species from the simulated wastewater using MFC technique.

The application of bioelectrochemical techniques in treating different gaseous contaminants including H_2S has been reportedly patented by various researchers including Borole (Borole 2010) and Borole and Tsouris (Borole and Tsouris 2013). Also, bioelectrochemical systems for remediation of saltwater containing high amount of sulfate were previously investigated by Zheng et al. (2014). Another study by Bajracharya et al. (2016) was conducted purposely to remove both sulfides and sulfates from polluted water using BES, wherein the use of *Desulfovibrio desulfuricans* and *Paracoccus* organisms was confirmed to sequestrate sulfate and sulfide, respectively. Therefore, this bioelectrochemical technology serves as a

prospective breakthrough for concurrent electricity generation and treatment of sulfur-based wastes in an efficient, economic, and reliable manner.

6.2.4 *Bioelectrochemical Removal of Nitrates*

The presence of nitrates in both portable water and wastewaters has been a subject of serious concern to both human and animal health. Nitrate is among the hazardous water contaminants known to cause gastric cancer when reduced to nitrosamines in the stomach (Gálvez et al. 2003; Glass and Silverstein 1999). Additionally, the presence of nitrate poses detrimental effects to pregnant women and infants because of the conversion of nitrate to nitrite within the foetus's stomach of a pregnant mother after possible ingestion of nitrate containing meals. In the blood, the reaction between nitrite and hemoglobin encourages the conversion of hemoglobin to methemoglobin, which restricts the flow of oxygen to the cell tissues. When this happens in an expectant mother, the infant develops a bluish color on the skin, a disorder termed as a blue baby syndrome or methemoglobinemia (Shrimali and Singh 2001).

Both surface and ground water resources are easily contaminated by nitrates in different ways. Studies have shown that pollution of groundwater by nitrate is basically due to many leaching agrochemicals resulting from excessive use of fertilizers (Feleke and Sakakibara 2002). Through agro-activities, nitrate salts get into groundwater as they penetrate through the soil. Apart from agro-activities, nitrates both in surface and ground water could also emanate from uncontrolled land effluents of wastewater from landfills, industrial, domestic wastes (Islam and Suidan 1998), and animal wastes (Terada et al. 2003). Owing to their detrimental effects, the removal of nitrates from wastewater is a mandatory step in environmental remediation practices.

Numerous techniques have been reportedly employed in treating wastewater streams contaminated with nitrate but most of them are unable to completely remove nitrate, besides biological denitrification which has been shown to assist in reducing inorganic nitrate compounds to nontoxic nitrogen gas. Researchers have reported the potential of bioelectrochemical technology in biological denitrification of wastewater containing nitrates. The method has the advantage of being flexible toward the treatment of nitrate at varying concentrations. Biological denitrification in BESs can be implemented through in situ production of hydrogen at the surface of the cathode compartment. Electrolysis of water as a means of achieving in situ hydrogen production is preferable because of the added advantage of generating electricity in the process. More importantly, electricity produced is clean, meaning that there is no interference with residual by-products and the process can be easily controlled ensuring easy handling of hydrogen formation. Hydrogen and the lower redox potential environment produced via cathodic reaction can potentially be used by hydrogenotrophs in reducing nitrate into nitrogen gas (Zhang et al. 2005). Biological denitrification in bioelectrochemical system can be improved by rational design in a way that ensures befitting contact between hydrogen and microorganisms.

Bioelectrochemical reactors (BERs) in which denitrifiers are directly immobilized on the cathode promote effective access of hydrogen to the microorganisms during hydrogen production (Park et al. 2005). BERs furnish immobilized microorganisms with a constant supply of electron donors, encouraging a quick denitrification process. In BERs, organic or inorganic carbon sources can be used as heterotrophic or autotrophic hydrogenotrophs, respectively. Generally, the use of autotrophs in BERs offers all benefits ascribed to electrolytic formation of hydrogen, autotrophic denitrification, and hydrogenotrophic donation of electrons, within one platform (Ghafari et al. 2008). Thus, the focus of researchers has been on bioelectrochemical technology by employing autotrophic microorganisms in BERs as a promising approach to remediate diverse water resources contaminated with nitrate compounds.

A novel BES consisting of multi-electrodes was developed by Sakakibara and Nakayama (2001) aiming at treating wastewater using autotrophic denitrifying biofilm placed on the cathodes. The experiment was continuously performed for a long time of almost 500 days to improve the performance of the designed platform to treat synthetic wastewater having nitrate concentration of 20 mg N/L. In this research work, researchers successfully reduced nitrate's concentration in the simulated effluent to a smaller amount, lower than 0.5 mg N/L.

Another interesting study was conducted by Park et al. (2005) employing autotrophic microorganisms that obtained electrons from the cathode to denitrify artificial wastewater. These researchers reportedly treated wastewater containing high content of nitrate utilizing hydrogen as the source of electrons. The acclimatization of denitrifying biofilm was achieved by subjecting it to 200 mA for a period of 60 days, where 98% nitrate reduction was achieved. The outcomes of this study showed that quite a lot of nitrate were successfully reduced to nontoxic nitrogen gas, and this was attributed to the activities of the denitrifiers that were not dependent on the volume of hydrogen generated as an electron source.

6.2.4.1 Denitrification Pathway in Bioelectrochemical Systems

For the complete process to reduce nitrates (NO_3-) to nitrogen gas (N_2), the denitrification pathway presumably occurs in four consecutive steps as shown in reaction Eqs. (6.3)–(6.10) (Feleke and Sakakibara 2002; Killingstad et al. 2002).

$$NO_3^- \rightarrow NO_2^- \rightarrow NO \rightarrow N_2O \rightarrow N_2 \tag{6.3}$$

In BERs, nitrates decompose in a series of seven reaction equations including electrolysis of water.

Electrolysis of water:

At anode:

$$5H_2O \rightarrow 2.5O_2 + 10H^+ + 10e^- \tag{6.4}$$

At cathode:

$$10H_2O + 10e^- \rightarrow 5H_2 + 10OH^- \tag{6.5}$$

Reduction of NO_3- to N_2 by denitrifiers is shown below. Hydrogen gas is used as a source of electrons as indicated in this decomposition:

$$2NO^{3-} + 2H_2 \rightarrow 2NO_2^- + 2H_2O \tag{6.6}$$

$$2NO_2^- + 2H_2 \rightarrow N_2O + H_2O + 2OH^- \tag{6.7}$$

$$N_2O + H_2 \rightarrow N_2 + H_2O \tag{6.8}$$

Net reaction equation of denitrification on cathode obtained from Eqs. (5), (6), (7), and (8):

$$2NO_3^- + 6H_2O + 10e^- \rightarrow N_2 + 12OH^- \tag{6.9}$$

The overall reaction in BER is a combination of Eqs. (5) and (9):

$$2NO_3^- + H_2O \rightarrow N_2 + 2.5O_2 + 2OH^- \tag{6.10}$$

6.2.5 Removal of Hydrocarbons and Their Derivatives

Hydrocarbons are a group of organic compounds consisting of carbon and hydrogen only in their chemical structure. In a reaction where there is substitution of hydrogen by other functional groups occur, the resulting compound is referred to as hydrocarbon derivatives. Contamination of groundwater and soil by petroleum hydrocarbons is an environmental problem which is widely spread. The main hydrocarbons that have reportedly been considered for causing serious health and environmental problems are PAHs (polycyclic aromatic hydrocarbons), BTEX (benzene, toluene, ethylbenzene, and xylene), and total petroleum hydrocarbon (TPH). Over time, these main hydrocarbons have been used to characterize hydrocarbon concentrations. Compared to chemical and physical remediation systems, bioremediation methods including biostimulation, bioaugmentation, and biosparging have been proved efficient and environmentally friendly. However, the slow kinetics/demand for external supply of electron acceptor, competition between the native dominant microbial strains/injected ones, and the poor contact or scattering of amendments with the target contaminants limit their application (Das and Chandran 2011; Tyagi et al. 2011).

Numerous researchers have recounted the effectiveness of BESs particularly MFCs to tackle challenges of scarce electron donors or acceptors for provision of conducive environment to considerably reinforce degradation of hydrocarbons complemented by energy generation. This is attributed to the use of the electrodes as sustainable electron acceptors for oxidizing the hydrocarbons, encouraging elimination of aeration while sustaining cathodic aerobic-based metabolic process (Wang et al. 2012; Lu et al. 2014a, b). Different hydrocarbons and their derivatives have been investigated for their treatment in different wastewater resources using bioelectrochemical systems. They include petroleum (Morris and Jin 2007; Jin et al. 2013), phenol (Luo et al. 2009), benzoate (Zhang et al. 2010b), benzene (Rakoczy et al. 2013; Wu et al. 2013), furan derivatives and phenolic compounds (Catal et al. 2008), furfural (Luo et al. 2010), and nitrobenzene (Mu et al. 2009b).

Inspired by the power of BETs in environmental remediation, Daghio et al. (Daghio et al. 2018) conducted an investigation on the removal of BTEX mixture present in contaminated water by varying reaction voltage. For the purpose of examining the degradation of the mixtures of BTEX at potential range of 0.8–1.2 V between the electrodes, the researchers employed single-chamber BESs. They observed that at all the tested voltages; the degradation of hydrocarbons was related to the reduction of sulfate and energy production. Considering the applied potential and the performance of BES in terms of hydrocarbons removal, the researchers presented maximum current density of 480 mA/m^2 using an external potential of 0.8 V. In addition, the external potential was shown to increase the degradation of *p*-xylene, *m*-xylene, and toluene with the optimum removal rate constants of 0.16 ± 0.02 $days^{-1}$, 0.34 ± 0.09 $days^{-1}$, and 0.4 ± 0.1 $days^{-1}$, respectively.

MFCs are considered to possess high efficiency in the treatment of different wastewater including complex industrial wastewater, with concurrent production of power (Pant et al. 2010) making them an effective replacement for the conventional biological wastewater treatment methods (Raghavulu et al. 2009). However, very limited findings have been reported for the remediation of refinery wastewater, oil-contaminated soil, petroleum sludge, which employ BES and successfully simultaneously generate power (Guo et al. 2016, 2014; Majumder et al. 2014; Ren et al. 2013; Zhang et al. 2014).

Considering the contribution of the oil industry in contamination of water sources, researchers from Indian Oil Corporation Limited (Srikanth et al. 2016) conducted a study aiming at bio-electrocatalytic treatment of wastewater from petroleum refinery using MFC. Refinery wastewater treatment employing MFC was performed under batch mode (BM) followed by 8 h of continuous mode (CM) and 16 h hydraulic retention time (HRT). Evaluation of the MFC platform's performance was executed with regard to power density, energy conversion efficiency with reference to operational mode, specific contaminants (sulfide, phenol, grease, and oil), and organics removal. The study achieved maximum power density of 225 ± 1.4 mW/m^2 during CM operation with 16 h HRT coupled with substrate degradation efficiency of $84.4 \pm 0.8\%$ including the 95 ± 0.6 of oil content. The BM operation similarly registered excellent substrate degradation of $81 \pm 1.8\%$ despite

the longer HRT. Furthermore, it was confirmed that 99% of hydrocarbons in the MFC were lower than limits detectable, indicating the superiority of MFCs in waste treatment.

6.2.6 Removal of Chlorinated Organic Compounds

Chlorinated organic compounds are yet another group of pollutants which needs to be addressed. Chlorinated organic compounds including trichloroethene (TCE) and perchloroethene (PCE) have been extensively utilized as degreasing agents and solvents in different processes. These organic contaminants pose a threat to human well-being since they are both carcinogenic and are spread widely in soil and groundwater. It has been established that in BESs, the cathode chamber ensures steady donation of electrons for reductive dechlorination while the anode compartment facilitates further degradation by oxidation of lower chlorinated organic compounds (Aulenta et al. 2011). The in situ treatment of PCE and TCE from contaminated wastewater requires donation of electron from an external source for stimulation of microbial dechlorination, encouraging sequential reduction of PCE to TCE, vinyl chloride, *cis*-dichloroethene, and ethane or ethane (Lohner et al. 2011).

While investigating the probability of employing graphite electrodes as a direct source of electrons for microbially catalyzed reductive dechlorination using *Geobacter lovleyi*, Strycharz et al. (Strycharz et al. 2008) discovered that the potential to couple dechlorinating microorganisms with electrodes is advantageous for bioelectrochemical treatment of subsurface chlorinated pollutants, particularly where sources of electron donation are challenging. The results of their study showed that *G. lovleyi* can utilize an electrode as the main source of electrons for reduction based dechlorination of PCE. Additionally, they asserted that the ability of *G. lovleyi* to obtain electrons directly from graphite electrodes is a good indication toward enriching other microorganisms with electron-donating electrodes for broad range treatment of chlorinated environmental contaminants.

Despite the promise shown by the dechlorination process in the treatment of chlorinated environmental contaminants, the challenges of incomplete reduction and the development of more toxic derivatives of vinyl chloride through the detoxification procedure cannot be ignored. However, it is advised to operate at more reducing electrode potentials to achieve a more complete reduction to ethene, although there still exists a challenge of obtaining low Coulombic efficiencies from very negative potentials (Aulenta et al. 2011).

6.2.7 Removal of Micropollutants

Micropollutants are bioactive and persistent anthropogenic chemicals, also known as trace organic compounds that occur in the marine environs at concentrations which

are quite low particularly in μg/L and ng/L (Virkutyte et al. 2010). Although conventional treatment techniques are not intended to remove micropollutants, their discharge to water sources has considerable negative consequences on the ecosystem and human health particularly. The existence of micropollutants in aquatic systems comes from diverse anthropogenic sources including personal care products, pharmaceuticals, pesticides, and disinfection by-products (Drewes et al. 2005; Ratola et al. 2012; Hamid and Eskicioglu 2012). The removal of micropollutants from wastewater has been largely dependent on expensive conventional methods including the use of activated sludge, activated carbon membrane filtration, and advanced oxidation processes. In the course of traditional processes for treatment, a portion of the micropollutants can possibly be removed via volatilization, chemical precipitation, primary settling, and biodegradation or activated sludge sorption (Virkutyte et al. 2010; Stevens-Garmon et al. 2011; Z-h et al. 2009). The removal of micropollutants by using high-pressure membranes is reliant on the kind of pollutant and the properties of the membrane (Xu et al. 2006; Drewes et al. 2005). The efficacy of advanced oxidation processes in removing micropollutants varies depending on the properties of the target pollutant, water chemistry, oxidants, doses, as well as reaction time (Pérez-González et al. 2012; Hey et al. 2012; Dickenson et al. 2009). However, it has been established that the conventional methods are ineffective in completely removing the vast majority of micropollutants such as pharmaceuticals (Gros et al. 2010).

It is noteworthy to mention that few studies have been employed to investigate the usefulness of bioelectrochemical technology in removing these pollutants. Bioelectrochemical systems such as MFCs are capable of efficient elimination of micropollutants from water due to the fact that they offer a special environment for redox reactions using microorganisms. To prove the effectiveness of MFC in micropollutants removal, Wang et al. (2015b) conducted an investigation on the removal mechanisms of an array of 26 trace organic compounds selected on the basis of their properties including hydrophobicity, feasibility for biodegradability, and charge. The study revealed that both biodegradation and sorption mechanisms encouraged micropollutants removal. The efficacy of removing neutral micropollutants was determined by the hydrophobicity and biodegradability capacity of the compounds, while the removal efficiency of positively charged micropollutants was found to be affected by electrostatic interactions in the MFCs. In addition, sequestration of positively charged trace organic compounds was comparably higher than that of negatively charged micropollutants.

In another study, two bioelectrochemical systems, namely MFC and MEC were assessed for their ability to mitigate micropollutants in municipal wastewater, with or without current flow, using acetate as the carbon source (Werner et al. 2015). The study employed a biocide to examine reduction with respect to biotransformation and sorption processes. The findings revealed that 8 out of 10 micropollutants demonstrated an equivalent reduction in both MEC and MFC, except for trimethoprim and caffeine which recorded higher reductions in the MEC. It was highlighted that the flow of electric current was unable to influence the reduction of micropollutants except for caffeine, which registered higher reduction in the

presence of current flow in both MEC and MFC. Significant sorption of micropollutants took place at the biocathode, although no clear trend could be detected concerning the tested micropollutants physico-chemical properties, in addition to that of the magnitude of sorption. The study showed that it is practically feasible to use BESs for energy generation during wastewater treatment without negatively impacting the reduction of trace organic compounds.

6.2.8 Removal of Perchlorate

Over the years, perchlorates (ClO_4^-) have been considered as an impending environmental threat, being discharged into soils, surface water, and consequently leaching into groundwater (Butler et al. 2010). These pollutants have high mobility and can obstruct the production of hormones by the thyroid gland and cause destruction of the nervous system. The degree to which perchlorates are constantly released into the surrounding environs was unknown until recent advancements in analytical techniques which can assist in detecting low concentration of ClO_4^- as low as 4 μg/L. Thus, as a consequence, people have been identified to be prone to perchlorate exposure through drinking water as a result of contamination from different water sources (Bender et al. 2005). The treatment of perchlorate contamination water using conventional technologies is somewhat difficult because of its inherent high solubility and chemical stability (Logan 2001). The use of microbes to reduce perchlorates has been employed by modifications of electron donors including molasses, acetate, as well as other substrates rich in organic content. However, in such processes of reduction of perchlorates, the existence of nitrates as the favorite electron acceptor consumes a huge amount of chemicals, hence limiting the overall process (Tang et al. 2012). Researchers have investigated using electrodes as an inexhaustible source of electron for the stimulation of microbial perchlorate reduction. Thrash and co-workers recorded in a study geared at investigating the bioelectrochemical reduction of perchlorate in the cathode of a BER that perchlorate-reducing bacteria including *Dechloromonas* and *Azospira* species are able to receive electrons either straight from the cathode or via mediators to get perchlorate reduced at the cathode compartment (Thrash et al. 2007). They reportedly found out that 90 mg L^{-1} of perchlorate was successfully reduced by *Dechloromonas* and *Azospira* species washed cells using 2,6-anthraquinone disulfonate as a mediator in the BER. Additionally, identical results were obtained by inoculating natural microbial community into the BER for 70 days, encouraging isolation of novel DPRB and strain VDY to accomplish/aid the reduction of perchlorate in the absence of mediator.

A group of researchers performed an investigation on the performance of MFC consisting of a denitrifying biocathode for the reduction of perchlorate (Butler et al. 2010). This decrease in the MFC was influenced by varying the amount of perchlorate and nitrate at the biocathode. After varying the experimental parameters such as increasing perchlorate loading and decreasing nitrate loading, it was observed that

perchlorate can be reduced without the need for mediators or fixed voltages. The removal of perchlorate was achieved at a conversion efficiency of 84% and an optimum concentration of 24 mg/L-d at the cathode. These results indicate clearly that perchlorate-reducing bacteria can make use of a cathode as a source of electrons for simultaneous generation of usable electrical power and treatment of perchlorate.

6.3 Other Uses of BETs in Environmental Remediation

6.3.1 Reduction of Carbon Dioxide (CO_2)

CO_2 is a major greenhouse gas released from anthropogenic sources, and the increase in its concentration is estimated to have grown further by 40%, i.e. from 280 ppm to roughly 400 ppm since the industrial revolution. The existing view concerning CO_2 accumulation as the primary cause of climate change has prompted research work into its capture and conversion to valuable products. To this end, the BET system can be judiciously designed to capture CO_2 through algal removal or bioelectrochemical reduction at the cathode, and hence encouraging simultaneous production of energy and other useful chemicals or by-products. Carbon dioxide reduction to methane or other different organic substances using electrons produced from the cathode is termed as microbial electrosynthesis (MES) (Rabaey and Rozendal 2010; Rabaey et al. 2011; Villano et al. 2010). In a study by Cheng et al. (Cheng et al. 2009), methane was successfully obtained from the MES platform using abiotic anode and biocathode conditioned with *Methanobacterium palustre*. The results indicated an overall efficiency of 80% after generation of electric current on bioanode of the MEC. These results give an indication that electromethanogenesis can be utilized in converting electrical current generated from energy sources which are renewable into biofuels while implementing the capture of CO_2.

Apart from methane, CO_2 can be reduced into other useful chemicals. For example, researchers at the University of Massachusetts (Nevin et al. 2011) achieved the reduction of CO_2 to 2-oxobutyrate, acetate, or formate by taking advantage of electrons derived from the cathode when acclimated with *Sporomusa ovata* in MFC. The study focused on determining the feasibility of different microorganisms for the reduction of CO_2 to other useful products. They reported that acetonic bacteria including *Sporomusa* species, *Clostridium aceticum*, *Clostridium ljungdahlii*, and *Moorella thermoacetica*, were observed to consume electric current and achieve organic acid production. Specifically, acetate, formate, and 2-oxobutyrate were successfully formed with 2-oxobutyrate identified as the dominant product of electrosynthesis by all investigated *Sporomusa* species with efficiency of greater than 80%. Hence, from their findings, a known variety of microorganisms can be further researched into as potentials for the electrosynthesis process, giving several alternatives for further optimization of this process. Another fascinating study indicated that by utilizing CO_2 as the lone carbon source in an MEC led to the production of methane acetate and hydrogen when employing brewery waste's

autotrophic microbial community (Marshall et al. 2012, 2013). However, there are still some challenges regarding the kinetics and the underlying mechanisms of electrosynthesis and its by-products; economic and logistical feasibilities for industrial-scale applications (Rabaey and Rozendal 2010; Lovley and Nevin 2011; Zhou et al. 2013). In summary, Table 6.1 depicts research findings on BESs for waste remediation and energy production.

6.4 Bioelectrochemical Technology for Energy Production

Over time, the demand for sustainable, renewable, and clean sources of energy has increased drastically due to the upsurge in population and per capita energy consumption (Yuan et al. 2014; Imam et al. 2013; Mohan et al. 2013). Currently, the dominant and major sources of energy are natural gas, petroleum, and coal, collectively known as fossil fuels (Imam et al. 2013). However, their long-lasting environmental pollution, non-renewability, and rapid consumption pose a serious impact on the future public health, ecology, and world economics; hence, there is a critical need for clean and alternate sources of energy (Reddy et al. 2012). Out of the many technologies available for energy and water production, bioelectrochemical technologies are proposed as an efficient and sustainable alternative means for simultaneous waste treatment and energy production by employing microorganisms as bioelectrocatalysts (Fig. 6.3) at both cathode and anode electrodes (Ogugbue et al. 2015; Pant et al. 2012).

Bioelectrochemical platforms are accompanied with oxidation–reduction energy transfer reactions which are collectively termed as redox reactions. In biological electrochemical systems associated with energy generation, the second law of thermodynamics is crucial as it gives a comprehensive view of bioenergetics (Mohan et al. 2018). Also, the law is useful in determining the connection existing between the change in standard electrode potential and free energy of bioelectrochemical reactions. In a general perspective, according to this law, the spontaneity of a specific redox reaction is established based on the corresponding redox potential and the change in free energy. The movement of electrons between two compounds depends heavily on reduction–oxidation potentials existing between the source of electron and electron accepting-species. Moreover, whether a certain redox reaction will be feasible or not is dependent on the relative electron affinity of the electron accepting-species within redox couple, promoted either via organic or inorganic compounds (Karube et al. 1977). The transfer of electrons between two compounds happens in a way that the flow of electrons will start from the compartment with lower to higher standard electrode potential. It is worth mentioning that relative concentration of electrons and electrode potential between the two half cells determine the direction in which electrons will flow. Essentially, species having positive standard electrode potential possess high electron-accepting behavior due to their strong oxidizing power than species with less positive electrode potential. Since the establishment of biological interventions in electrochemical technology, some

Table 6.1 BESs for waste remediation and energy production

Pollutants		BESs used	Electrode	Treatment efficiency	Power/energy/ current density	References
Dyes	Congo red	Dual-chamber MFC consisting of anode and cathode chambers separated by proton exchange membrane	Graphite-granular cathode and anode	92.7%	552.2 mW m^{-2}	Li et al. (2010)
	Acid Orange 7	Cylindrical single-chamber MFC constructed from polyvinyl chloride	Both cathode and anode constructed from carbon fabric	>90%	125 mA m^{-2}	Fernando et al. (2014)
	Reactive blue 160 (RbU160)	Single-chamber air cathode, mediator-less, membrane-less MFC	Carbon cloth as anode and hydrophobic carbon cloth as cathode	97.2%	–	Liu and Logan (2004)
Heavy metals	Pb^{2+}	Two-chamber MFC with cathode and anode separated by cation exchange membrane	Anode made up of carbon felt, and titanium wire as cathode	47.5%	–	Modin et al. (2012)
	Cu^{2+}	Metallurgical MFC	Rough graphite plate as anode and graphite foil as cathode	>99.88%	0.80 W/m^2	Heijne et al. (2010)
	Cd^{2+}	Single-chamber MEC constructed using polypropylene block	Bioanode prepared from graphite fiber brush and piece of stainless-steel mesh as a cathode	50–67%	–	Colantonio and Kim (2016)
	Cr (IV)	Plant–microbial fuel cell (PMFC) consisting of a single Plexiglas cylinder anode chamber	Granular graphite anode and carbon felt cathode	>90%	55 mA/m^2	Habibul et al. (2016)
Chlorinated organic compounds	Trichloroethene	Continuous-flow bioelectrochemical reactor consisting of two identical chambers separated by proton exchange membrane	Polarized graphite cathode and granular graphite anode	>90%	–	Aulenta et al. (2011)
	Perchloroethene	Two-chamber bioelectrochemical reactor	Bare graphite felt electrode and Granular graphite cathode	88.4%	–	Xie et al. (2014)
Nitrates		Biofilm-electrode reactor	Dimensionally stable anode and graphite felt biocathode	98%	–	Park et al. (2005)

Sulfides		Square and tubular MFCs with a hexacyanoferrate cathodic electrolyte	Granular graphite anode and woven graphite felt cathode	98%	47 W m^{-3}	Rabaey et al. (2006)
Hydrocarbons and derivatives	Phenol	Single-chamber mediator-less MFC	Carbon cloth anode with carbon coating and carbon cloth cathode with platinum coating	99%	371–544 mA/m^2	González-Pabón et al. (2018)
	Benzene	Dual-chamber cylindrical MFC	Anode and cathode filled with graphite fibers and separated by cation exchange membrane	18–49%	300–550 μA	Schnitzler et al. (2003)
	Petroleum	Both single-cell and dual-cell MFCs	Cathode and anode	–	120 mW/m^2	Morris and Jin (2007)
	Toluene	Single-chamber BES reactors	Graphite plates anode and cathode connected to stainless-steel wire	81–84%	200–480 mA/m^2	Daghio et al. (2018)
	Xylene	Single-chamber BES reactor	Graphite plates anode and cathode connected to stainless-steel wire	75–84.3%	200–480 mA/m^2	Daghio et al. (2018)
	Nitrobenzene	Batch BES reactor with microbially catalyzed cathode	Graphite fiber anode and carbon cloth cathode	98.93%	–	Wang et al. (2011)
Perchlorate		BES reactor	Graphite cathode and silver reference electrode	90%	–	Thrash et al. (2007)
		Two well-mixed and continuously fed MFCs constructed from rectangular Plexiglas frames	Granular biocathode and anode separated by Ultrex cation exchange membrane	84%	17–18 mA/m^2	Butler et al. (2010)
Micropollutants	26 trace organic compounds	Both single-chamber MFC and double-chamber MFC	Plain carbon cloth as anode and water-proof carbon cloth as air cathode	38–99%	147 mW m^{-2}	Wang et al. (2015b)
	10 trace organic compounds	Cube-shaped single-chamber MFC and MEC reactors	Graphite fiber brushes as anodes and carbon cloth as cathodes	14–100%	3.59–6.73 A/m^2	Werner et al. (2015)

(continued)

Table 6.1 (continued)

Pollutants		BESs used	Electrode	Treatment efficiency	Power/energy/ current density	References
Carbon dioxide		Dual-chamber MECs consisting of an abiotic anode and biocathode	Graphite fiber brush anode and carbon cloth cathodes	96%	–	Cheng et al. (2009)
		Microbial electrosynthesis system	Anode and cathode containing varying amounts of freshwater medium	–	2.5 A m^{-2}	Marshall et al. (2013)

experimental works have been performed by using enzyme or whole organism catalyzed redox pairs under normal experimental conditions for energy production (Mohan et al. 2018; Butti et al. 2016).

6.4.1 *Electroactive Microorganisms for Electron Transfer and Energy Conservation in BESs*

Before adopting BESs for commercial purposes there is a need to improve the existing challenge relating to microorganisms and performance of electrodes to boost electron transfer. Enhancement of electron transfer within BESs will promote bioelectrochemical reactions leading to higher generation of electricity, hydrogen, or other important by-products (Lovley 2008). Among other factors, the performance of BESs depends essentially on particular electroactive species of microorganisms such as yeast, bacteria, microalgae, plant, and rhizosphere (Fig. 6.4), which are capable of discharging electrons from within their cells to the respective electrode surface(s). The fashion with which these electroactive microorganisms (electrogens) transfer electrons while conserving energy is the practice designated as extracellular electron transfer (EET) (Rozendal et al. 2008; Harnisch et al. 2011). Both direct and indirect transfer of electrons from bacteria which are physically and not physically linked to an electrode, respectively, have so far been pinpointed as the two principal methods of electron transfer in BESs (Fig. 6.5). The direct electrons transfer among microorganism (bacteria) and the corresponding electrode may take place in two main ways. In the electron transfer's first mode, physical contact exists among the surface of the electrode and the outer structures of the microbial cell's membrane. Such microorganisms' outer membrane structures are also joined to inner structures, thereby encouraging the transfer of electrons from inside the microbial cell via the walls of the membrane and then to the electrode directly. The second mode of electron transfer is achieved between the electrode and microorganism via tiny structural projections (such as nanowires or pili) extending from the microorganism's outer membrane and attaching themselves to the electrode surface. In this approach, while the nanowires possess the ability to reach over tens of microns, the direct contact between the electrode and microorganisms which are distant from the electrode can still be maintained (Pham et al. 2009).

Another mode of electron transfer is the indirect transfer which occurs via electron shuttle compounds which are long-ranged and may either occur naturally (for example, in wastewater) or formed by microorganisms (bacteria) (Velasquez-Orta et al. 2010). In this indirect mechanism, electrons are firstly conveyed to the bacterial cell surface where they are gathered by shuttle compounds and transported to the electrode.

It is noteworthy to mention that by employing either one or more of these electron transport modes for electron transfer toward the electrode, microorganisms can grow around the surface of electrode and manage to form biofilms. This formation of

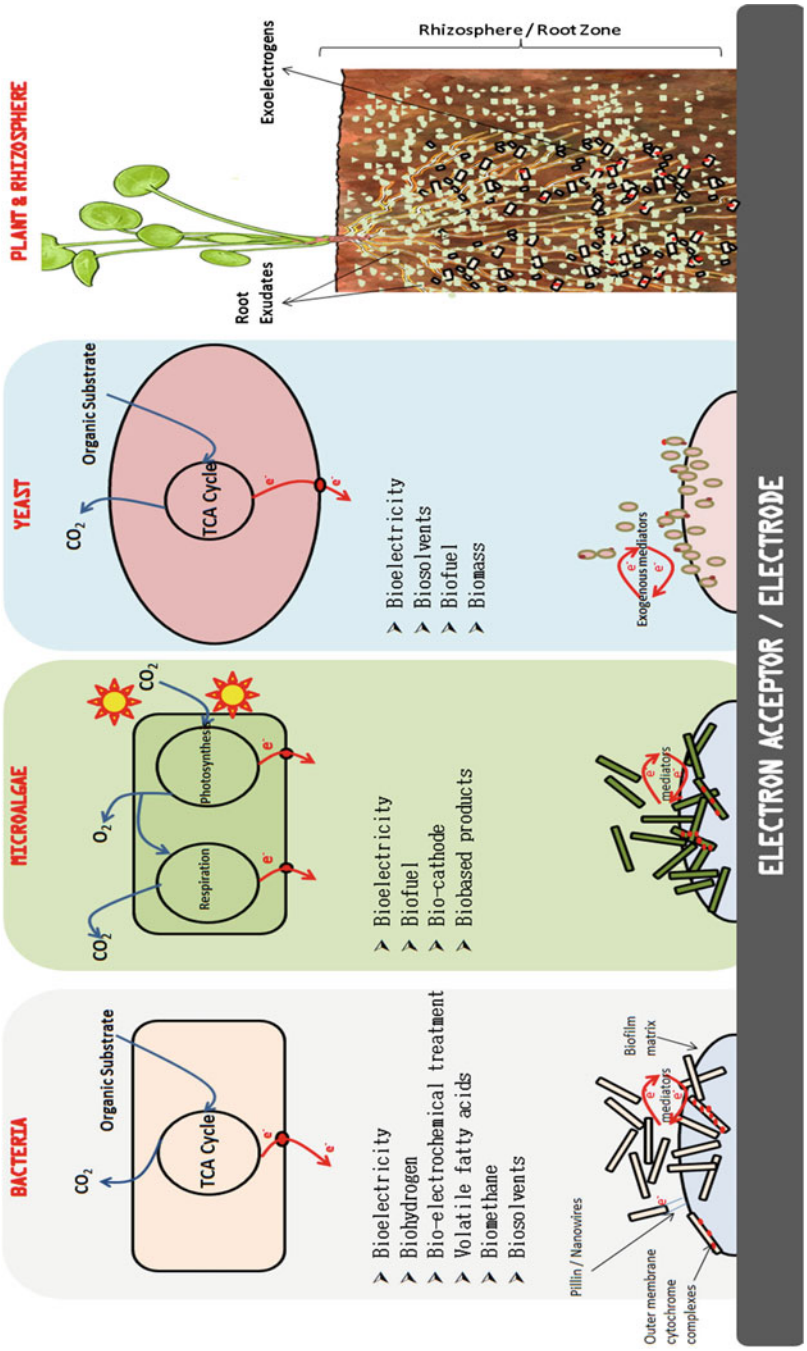

Fig. 6.4 Different biocatalysts employed in BETs and their approaches to extracellular electron transport (adapted from (Mohan et al. 2019))

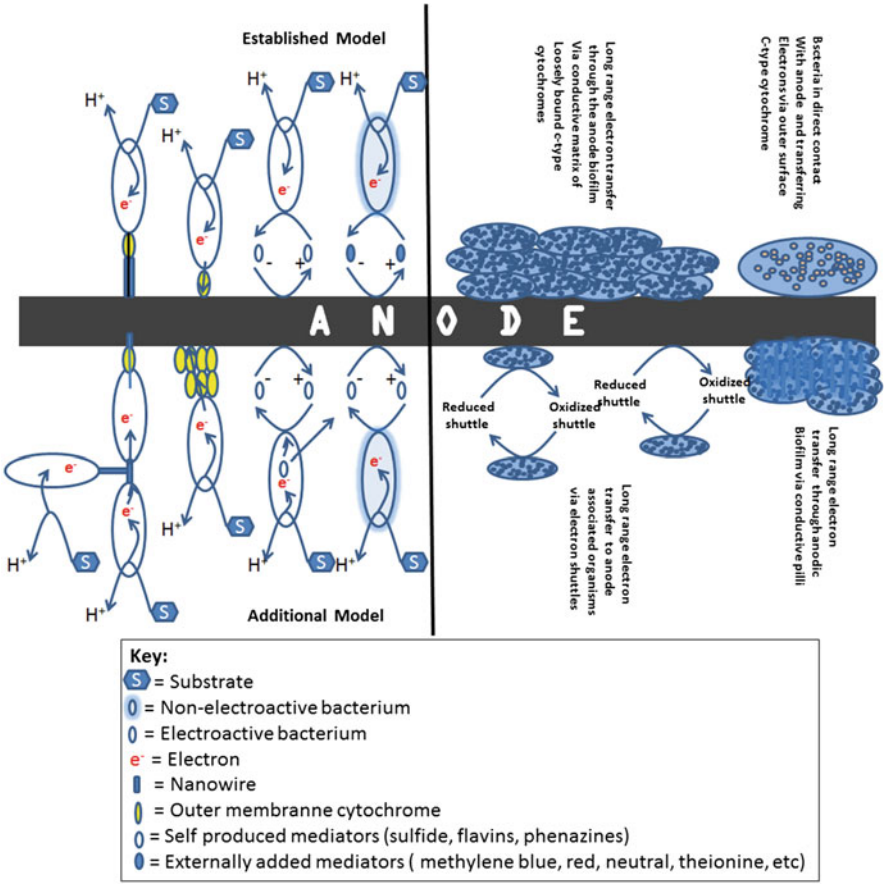

Fig. 6.5 Proposed mode of electron transfer in BESs and interaction between microorganisms (bacteria) and electrode (adapted from (Pant et al. 2012))

biofilms is dependent on the electrodes surface area. Simply, an electrode with an appreciably larger surface area facilitates greater possibility for the accumulation of bacteria and hence formation of biofilms. The likelihood of biofilm formation indicates higher possibility for production of electrons, signifying a large yield of electricity, hydrogen, or other useful by-products from the bioelectrochemical cell (Tender et al. 2002).

6.4.2 *Generation of Electricity from Organic Waste and Wastewater Substrates in BESs*

Production of electrochemical energy is under considerable attention by researchers as an alternative source of energy or power, owing to its sustainability and environmental benignity (Zabihallahpoor et al. 2015). Generation of electricity from waste streams can simultaneously assist in meeting the global energy demand, minimize pollution and costs resulting from water and wastewater remediation (Zabihallahpoor et al. 2015; Deval et al. 2017). BET is a special technology which transforms the existing chemical energy in biodegradable substrates straight into electrical energy by employing microorganisms. In comparison to conventional technologies, BETs provide a platform which is flexible for both reaction processes occurring at cathode and anode linked together by an external wire for a complete electrical circuit (Ren 2013). This is attributed to the fact that any biodegradable material, particularly waste materials, undergoes anodic oxidation, generating current that can be directly collected as electricity or utilized for production of valuable chemicals, water desalination, and removal of contaminants. The incorporation of biological organisms for catalyzing electrochemical reactions presents BESs with a certain level of complexity equivalent to other elaborate electrochemical systems like fuel cells, batteries, and supercapacitors.

Among different types of BES, MFCs and MECs are considered as important biotechnology processes which are rapidly developing. These bioelectrochemical technologies utilize an elegant combination of electrochemical and biological processes to produce bioelectricity, biohydrogen, or other invaluable products (Rozendal et al. 2008). While MFCs are intended to produce electricity, MECs promote chemical processes at the cathode by electricity to produce hydrogen and/or other valuable chemicals. Utilizing wastewater as the substrate, organic matter decomposition in MFC and MEC employs a particular kind of microorganisms in an anaerobic environment with bacteria normally at the anode compartment. While the organic material is decomposed at the cathode and the bacteria releasing protons, carbon dioxide as well as electrons into solution, the electrons are gathered at the anode, reaching the cathode through an external circuit (Harnisch et al. 2011). When the electrons moving to cathode chamber from the anode compartment are accompanied by the flow of electric current, the movement of protons to the cathode is made possible via the electrolytic solution in the cell. The carbon dioxide present in the solution can also possibly be captured and reused. Production of electricity by MFC is attained from the external circuit which conveys electrons. Meanwhile under aerobic environment, electrons combine with oxygen and protons when reaching the cathode chamber, usually forming water from the air. Production of hydrogen can also be made possible through combination of protons and electrons obtained from bacterial-decomposition of organic material with exclusion of oxygen from the cathode (Cusick et al. 2010; Wrana et al. 2010).

It has been recognized that electricity production from BESs comes from a myriad of waste sources such as food processing, municipal, brewery, paper

recycling, refinery, and agricultural wastewater (Pant et al. 2010). Also, the power output depends on various parameters such as conversion efficiency, loading rate, and biodegradability of the material. In principle, simple substrates offer greater power output, while complex wastes provide much lower electricity. For instance, when a bioelectrochemical system with sludge inoculum and a 4 cm^3 cathode reactor was employed for concurrent generation of electricity and wastewater treatment, it was able to achieve the maximum power density of 766, 205, and 225 W/m^2 from acetate, brewery wastewater, and swine wastewater, respectively (Min et al. 2005; Feng et al. 2008; Cheng et al. 2006).

It is noteworthy to mention that MFC is among the bioelectrochemical technologies that can utilize a different kind of system frameworks, materials, and substrates with bacteria to produce bioenergy even though power output in these systems has been observed to be relatively low (Logan 2008). This technology is specifically desirable for reliable long-lasting energy applications, with potential safety and health issues (Du et al. 2007). Stimulated by the desire to generate renewable and environmentally friendly energy via oxidation of biodegradable materials in MFCs, Rahimnejad et al. (2012) used an active biocatalyst, *Saccharomyces cerevisiae* for generation of power. The effectiveness of MFCs stack was investigated with respect to the quantity of electricity produced. The results showed power generation of about 2003 mW m^{-2} and an optimum current of about 6447 mA m^{-2} in MFC. The observed high electrical efficiency of MFCs can be explained by the uniform dissemination of microorganism on the surface of graphite and was supported by the images obtained from atomic force microscopy.

6.4.3 Bioelectrochemical Production of Biofuels

Due to the rapid depletion of global fossil-fuel reserves, global demand for energy, greenhouse gas emission, growing call for energy security and concerns related to exponential increase in the price of oil and diversity, significant attention is being directed toward developing carbon-free and sustainable energy sources (Imam et al. 2013; Zhu et al. 2014; Enamala et al. 2018). Biofuels refer to a variety of fuels that in a certain way are obtained from inexhaustible bioresources. Recently, biofuels have gained enormous research interest as a promising alternative fuel to existing fuels derived from petroleum since they can be employed as transportation fuels with minor alteration to the existing technologies (Zhu et al. 2014). Important biofuels including gaseous (methane or hydrogen) and liquid (ethanol, biodiesel) are produced from biomass particularly organic materials (Lebaka 2013).

6.4.3.1 Biohydrogen Production

Various bioelectrochemical systems available for energy production such as MECs offer an alternative and hopeful method of generating hydrogen fuel as future energy

resource (Rabaey and Rozendal 2010; Kadier et al. 2018; Deval et al. 2017). Biohydrogen is considered as an attractive renewable resource and energy carrier containing high energy density and can readily be transformed by using fuel cells into electricity (Rabaey and Rozendal 2010; Turner 2004). It is well-known that the utilization of biohydrogen as a dominant fuel source toward future hydrogen economy is highly encouraged due to its production from sustainable, renewable, and environmentally friendly sources (Schrope 2001). Hydrogen is widely utilized as chemical and fuel in diverse procedures; hence, it is unique compared to other chemicals like methane. Based on chemical oxygen demand, production of hydrogen from wastewater is estimated to be 7 times more valuable compared to methane obtained from an equivalent quantity of wastewater (Rozendal et al. 2007a). Most methods used to produce hydrogen utilize fossil fuels and significant amount of energy is consumed, hence limiting their potential for upscaling. It is widely known that water splitting can be employed to produce oxygen and hydrogen, but the process is expensive and large amount of energy is needed to achieve it. Yet, another way of generating hydrogen is through bacterial fermentation of carbohydrates, but the inability of bacteria to break down the carbohydrate sources completely limits the quantity of hydrogen to be produced. Thus, biological treatment technologies can aid in the production of energy from wastewater. Acetogenic fermentation has also been utilized in producing hydrogen from wastewaters (Mohan et al. 2012), but the method suffers from low yield of hydrogen and thermodynamic limitations in microbial metabolism which hampers its further applications.

For the successful generation of biohydrogen, there is a need for developing innovative proficient methods, making use of improved and up-to-date advances in microbial technologies. Bioelectrochemical systems such as MECs (Fig. 6.2b) provide an alternative platform which can improve the yield of hydrogen at relatively smaller quantities of external energy in comparison to traditional electrolysis of water and other fermentation processes (Liu et al. 2005). More specifically, MECs can possibly generate an immense amount of hydrogen from any organic waste material at an appreciable rate (Wrana et al. 2010; Liu et al. 2005). This method of producing hydrogen using bioelectrochemical technology is also known as bioelectrohydrogenesis (Logan 2004; Pasupuleti et al. 2015; Rozendal et al. 2010).

The mode of action for bioelectrochemical systems including MEC requires a little supply of extra energy to activate the process and drive hydrogen production (Logan et al. 2008). Interestingly, the amount of energy produced is large enough to compensate the energy supplied to activate the process, achieving overall positive energy at the end of the process. More importantly, the small amount of energy supplied for the process of activation for hydrogen production is obtained from sources which are renewable and clean, making MECs a feasible source of renewable hydrogen.

Diverse sources of wastewaters such as industrial, agricultural, and municipal sources are applicable feedstock for bioelectrochemical systems because they have abundant amounts of dissolved organic matter which serve as possible sources for chemicals and fuel production (Rozendal et al. 2007a). The production of hydrogen from MEC could be achieved from different organic sources such as wastes and

non-fermentable materials (Logan et al. 2008). Essentially, organic compounds such as acetates undergo oxidation through microorganisms such as bacteria to produce carbon dioxide, protons, and electrons at the anode of MEC. The electrons get conveyed to the solid anode by the aid of electrochemical interaction of bacteria. Hydrogen is created when the flow of electrons toward the anode combines with protons under the influence of external voltage, as illustrated in Eqs. (6.11) and (6.12) (Logan et al. 2006). In order to balance the charges, the protons move to the cathode compartment from anode compartment within the solution. In fact, only an applied potential greater than 0.2 V is needed for generation of hydrogen in MEC, which is less compared to the normal potential of greater than 1.6 V needed for electrolysis of water (Logan et al. 2006, 2008). In some instances, mixing of the product and substrate may occur, therefore in order to prevent them from mixing, a membrane is exploited to separate the cathode chamber from the anode compartment. The chemical equations below represent possible reactions occuring at the cathode and anode in MEC using acetate as substrate.

$$\text{At cathode} : 8H^+ + 8e^- \rightarrow \ 4H_2 \text{ or } 8H_2O + 8e^- \rightarrow 4H_2 + 8OH^- \tag{6.11}$$

$$\text{At anode} : CH_3COO^- + 4H_2O \rightarrow 2HCO_3{}^- + 8e^- + 9H^+ \tag{6.12}$$

Technically, the production of hydrogen at the cathode electrode happens after surmounting the probable endothermic barrier of −0.414 V against the standard electrode potential by supplying a small difference in external potential of 0.14 V to the MEC, while the oxidation process furnishes the remaining overpotential performed by the anodic bacteria which is estimated to be −0.279 V (Logan et al. 2008; Rozendal et al. 2006). Simultaneous treatment of wastewater and hydrogen formation in MEC is a proficient strategy to produce energy which is clean. This ability to use various organic substrates by electrochemically active bacteria in MECs makes it reliable and economically beneficial. Nonetheless, the process is challenged by sluggish hydrogen evolution reaction (HER) on MEC's carbon cathodes and the need to surmount the large overpotential. To increase the process kinetics at low overpotentials, the use of platinum as electrocatalyst has been investigated, where electrodes loaded with varying amounts of platinum are obtained either from commercial shops or prepared in laboratories. The coating of titanium–platinum is employed as a cathode electrode to evade the problem of potential barrier. However, the high cost of noble metal-based electrodes including platinum limits their large-scale application. In the quest for other suitable electrodes for hydrogen evolution reaction, inexpensive non-noble metal-based cathodes such as nickel/nickel alloys and steel have been studied for hydrogen generation in MEC (Hu et al. 2009; Manuel et al. 2010; Selembo et al. 2009, 2010). Studies have shown that nickel and nickel molybdenum electrocatalysts exhibited promising results with somewhat larger overpotential than platinum (Hu et al. 2010; Rozendal et al. 2010). In a study performed to explore the production of valuable, clean hydrogen in a MFC, Jeremiasse et al. (Jeremiasse et al. 2010a) noted that the specific surface area

increase of the nickel cathodes resulted in lowering of the cathode overpotential, encouraging production of high hydrogen of about 50 m^3 m^{-3} d^{-1}.

Researchers have also been concerned with the potential of microorganisms such as bacteria in catalyzing the HER at biocathodes (Jeremiasse et al. 2010b; Rozendal et al. 2007b). Stimulated by the desire to ascertain the potential of microorganisms in biohydrogen production within bioelectrochemical systems, Rozendal et al. developed a microbial biocathode that was centered on a natural culture of microorganisms that are active electrochemically and tested it for production of hydrogen (Rozendal et al. 2007b). They recounted that the microbial biocathode successfully registered a higher current density of about -1.2 A/m^2 obtained at voltage value of -0.7 V, which was comparatively 3.6 times higher than that of a control electrode (-0.3 A/m^2). Moreover, in the course of hydrogen yield tests, the microbial biocathode yielded 0.63 m^3 H_2/m^3 cathode liquid volume per day at hydrogen efficiency of 49%, compared to 0.08 m^3 H_2/m^3 cathode liquid volume per day produced by the control electrode at a cathodic hydrogen efficiency of 25%. From these findings, the researchers concluded that the use of microbial biocathode in BESs permits the utilization of cheap electrode materials and these present huge potential for affordable generation of hydrogen gas from wastewaters.

Another study was conducted to demonstrate the possibility to operate a full biological MEC without using expensive electrocatalysts at both cathode and anode electrodes (Jeremiasse et al. 2010b). In this study, all reactions at the two electrodes were catalyzed by microorganisms. Evaluation of the results showed that, with cathode voltage of -0.7 V, the biological cathode in MEC achieved a higher current density (MEC 1: 1.9 A/m^2, MEC 2: 3.3 A/m^2) than that of control graphite cathode (0.3 A/m^2) in an electrochemical half-cell. These results provide an indication that the production of hydrogen is catalyzed at the biocathode, by the action of electrochemically active microorganisms.

Upscaling and improving the production of hydrogen in bioelectrochemical systems such as MECs has been the focal point of some researchers, by searching alternative cathode materials with higher performance. A pilot study of 4 L MEC reactor employing graphite felt as anode and as cathode stainless steel generated 0.9 m^3 H_2 m^{-3}-MECd^{-1} when used for desalination of wastewater (Carmona-Martínez et al. 2015). Even though the applied potential controlled the electric current, the obtained current densities reported in MECs were found to increase with the increase in specific surface area of electrodes. Anodes with the larger specific surface area were reported to possess higher current densities. In the same way, at the cathode of MEC, the production was reported to have been enhanced by the increase in the cathode surface area. In summary Table 6.2 shows overview of hydrogen production in MECs by a few researchers.

6.4.3.2 Production of Bioethanol

Bioethanol refers to ethanol produced from renewable substrates. Being environmentally friendly and renewable, bioethanol is deemed a promising option, leading

Table 6.2 An overview of hydrogen production in MEC

Type of MEC	Hydrogen production rate (m^3 H_2/ m^3 day^{-1})	Current/ power density	Efficiency and recovery	References
Dual-chamber reactor with biocathode system containing graphite felt, operating under continuous mode	0.63	1.2 A/m^2	49% H_2 recovery	Rozendal et al. (2007b)
Dual-chamber continuous-flow MEC consisting of graphite felt anode and nickel foam cathode	>50	22.8 A/ m^2	90% cathodic H_2 recovery	Jeremiasse et al. (2010a)
Two chambers active MEC with anode made of graphite felt and carbon felt, while the cathode was coated with platinum	5.6	16.4 A/ m^2	43% overall, 41 cathodic H_2 recovery	Sleutels et al. (2009)
Two chambers BER with graphite plate cathode and autotrophic biocathode	9.2	1.88 A/ m^2	39.4% H_2 recovery	Jourdin et al. (2015)
Single-chamber MEC consisting of graphite fiber brush anode	0.9–1	93–112 mW/m^2	77% overall H_2 efficiency	Wagner et al. (2009)
Double-chamber MEC consisting of graphite granules as anode and platinized carbon cloth as cathode	0.03–1.5	90–450 mW/ m^2	54–91% overall efficiency	Cheng and Logan (2007)
One-chamber MEC with graphite fiber brush as anode and carbon cloth air cathode	0.59–1.11	1.15 A/ m^3	52–63% H_2 recovery	Lalaurette et al. (2009)
Biocatalyzed electrolysis cell with carbon felt electrodes separated by cation selective membrane	0.63	1.2 A/m^2	49% H_2 efficiency	Rozendal et al. (2007b)
Single-chamber biocatalyzed electrolysis cell	0.3	2.39 A/ m^2	23% overall H_2 efficiency	Rozendal et al. (2007a)

to a striking surge in research into increasing its production capacity. The use of bioelectrochemical platforms in reducing acetate with hydrogen is considered a promising and sustainable way of producing ethanol from wet biomass waste (Pant et al. 2012). Various studies have reported the bioelectrochemical reductions of organics to produce ethanol (Peguin et al. 1994; Shin et al. 2002; Steinbusch et al. 2009). Owing to the slow kinetics and low concentration of ethanol observed in previous studies, new studies on ethanol production have focused on investigating the feasibility of biological acetate reduction by employing an electrode in place of hydrogen, as an electron source in the presence of an electron mediator. In principle, whenever mediators are included in the bioelectrochemical system, they accelerate electron transport from the cathode (electrode or hydrogen) to microorganisms. In addition, mediators can influence the metabolism of organisms and obstruct parasitic processes such as methanogenesis (Steinbusch et al. 2009). In recent years, BET

with methyl viologen mediator at the cathode electrode was employed in the reduction of acetate to ethanol (Steinbusch et al. 2009). It was presented that methyl viologen successfully accelerated the production of ethanol by 6-fold and increased its concentration by twofold to 13.5 ± 0.7 mM compared to the control. In addition, methyl viologen mediator inhibited *n*-butyrate and methane production, encouraging high ethanol production efficiency (74.6 ± 6%). It was also shown that the addition of methyl viologen to an inoculated cathode in the bioelectrochemical system led to concurrent production of ethanol (1.82 mM), methane, butyrate, and hydrogen at the cathode (0.0035 Nm^3 hydrogen m^{-2} day^{-1}), though it was not clear through which mechanisms ethanol was produced. In production of ethanol, it is important to consider the role played by mass transport in the overpotential because the reaction process depends on transport of ethanol, acetate, and protons (Hamelers et al. 2010).

According to the study by Steinbusch et al. (Steinbusch et al. 2009), the cathode which is responsible for the production of ethanol has two possible flaws: (a) the ethanol formed could be lost to the anode via the membrane and (b) a decrease in cathode performance can occur due to loss of the mediator. They recommended that the problem can possibly be rectified through immobilization of the mediator.

6.4.3.3 Production of Biomethane

Bioelectrochemical systems consisting of microbial biocathodes provide opportunities for fuels such as methane and valuable chemicals from organic substrates (Jiang et al. 2013b). Bioelectrochemical production of methane production is preferable considering its advantages of being easy to store, transport, and compress. Additionally, the compression and transportation of methane in pipes, as well as its storage, is achieved by employing modern technologies and can easily be integrated into an existing infrastructure (Cheng et al. 2009).

The cathodic production of methane in MEC is being regarded both as an attractive alternative energy effluent refining step for digester effluents involving low production of sludges and absence of aeration expenses (Hamelers et al. 2010; Clauwaert and Verstraete 2009). The peculiarity of electromethanogenesis via MEC is in the simultaneous production of methane and wastewater treatment. This method has added advantages compared to conventional methanogenesis regarding higher methane yield as well as the use of effluents released from anaerobic digestion processes (Clauwaert and Verstraete 2009; Wagner et al. 2009). In a study geared toward employing membrane-less design using a working voltage of −0.6 or −0.8 V against standard hydrogen electrode, an efficient formation of methane (57.4–74.1% in 3 days and 95.9–96.3% in 7 days) was observed and prevailing methanogenesis on the cathode compartment was highly efficient than those in control reactors (15.4% in 3 days and 64.2% in 7 days) (Sasaki et al. 2011). Generally, bioelectrochemical production of methane from organic wastes has gained tremendous attention as described by other researchers (Cheng et al. 2009; Clauwaert and Verstraete 2009; Rader and Logan 2010; Wang et al. 2009).

6.5 Conclusion

Research into the development of BETs as emerging and versatile platforms with potential impact in the areas of bioenergy production, resource recovery, and waste remediation is increasingly widespread in recent years. The recovery of electricity from diverse sources of wastewater using BESs remains a promising alternative because it offers the feasibility of lowering the total cost of treatment while decreasing the biomass production and ensuring the sustainability of the surrounding environment. In view of production of power and generation of electricity, it becomes apparent that researchers need to judiciously explore novel, cost-effective, and efficient bio-based materials that can improve the performance and broaden the MFCs applicability. Additionally, the advantage of providing reliable and easy to get biofuels containing high energy density, BETs possess the capacity to be fabricated for other applications such as biosensors and robots. To be specific, MFCs are presently being considered as reliable sources of power for robots (gastro-bots), where biomass is used to produce electricity in artificial stomachs with the focus to extend the development of autonomous robots capable of producing their own energy from environmental substrates. Furthermore, researches have demonstrated the potential applicability of MFCs to power biosensors for real-time and on-site observation of water quality through detection and quantification of toxic contaminants existent in the water systems. In contrast to past research findings which focused on BESs consisting of bioanodes, recent developments in the research field of BETs have seen considerable improvement in the use of biocathodes for bioremediation in BESs, wherein the treatment of diverse environmental pollutants from soil, sewage, industrial, or agricultural wastewater can be achieved, thus meeting society's expectations for clean and reliable supply of water. For the purpose of wastewater treatment, integrating MFCs within existing remediation technologies is considered more sensible, cost-efficient, and feasible. This is due to large internal energy in the organic wastes present in wastewater compared with the small amount of energy required for wastewater treatment. Thus, wastewater can be employed as a feedstock in BESs to produce energy by direct conversion of biodegradable organic matter into hydrogen, electricity, or other useful chemical products.

Despite BETs abundant technological promise, it still faces challenges of high overpotentials, lower power production, high ohmic resistance, and limited performance of some biocatalysts. However, researchers are working hard to improve on these challenges to ensure availability for commercial application.

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Chapter 7
Hydrogen Production and Contaminants Removal Using Microbial Electrochemical Cells

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Keywords Microbial electrochemical cells · Hydrogen production · Wastewater treatment · Perchlorate removal · Chromate reduction · Dechlorination

7.1 Introduction

Microbial electrochemical cells (MEC) constitute an emerging technology between electrochemistry and microbiology. MEC have been designed for various product recovery and reduction of oxidized contaminants. They are based on the bacterial interaction with insoluble electron acceptors, relying on the exchange of metabolic electron removed e-donor or supplied to the electrode. In this chapter, we will discuss the cathodic H_2 production and the removal of oxidized contaminates in MEC.

7.2 H_2 Production on Biocathode

An alternative to precious metal catalysts for H_2 production in MEC is the use of biocathodes. It came with the development of MEC from microbial fuel cell (MFC) and bioelectrochemical system (Kim et al. 2004). However, biocathodes are still in the early stages and require a deep understanding of the bioelectrochemical mechanisms involved.

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A bioelectrochemical degradation is represented in Eq. (7.1), the simple substrate like acetate metabolize by living microorganisms. This releases H^+ and CO_2 into the anolyte and e^- to the anode as an oxidation half reaction of two-chambered MFC (Hamelers et al. 2010). Electrons then flow to the cathode through the external circuit. H^+ diffuses across the proton or cation exchange membrane and reacts with e^- and terminal electron acceptor like O_2 to form H_2O and complete reduction half reaction as in Eq. (7.2) (Oh et al. 2004). This reaction is thermodynamically favorable and spontaneous, so it produces energy.

$$C_2H_3O_2^- + 2H_2O \rightarrow 2CO_2 + 8e^- + 7H^+ \left[E^\circ_{acetate/CO2} = -0.289 \text{ V vs.NHE at pH} = 7\right] \quad (7.1)$$

$$O_2 + 4H^+ + 4e^- \rightarrow 2\,H_2O \left[E^\circ_{H+/H2O} = +0.818 \text{ V vs.NHE at pH} = 7\right] \quad (7.2)$$

This simple system was further modified by eliminating O_2 and supporting H^+ with an extra applied voltage to reduce it to H_2 in a MEC reactor with oxidation half reaction as in Eq. (7.3) (Liu et al. 2005; Rabaey and Rozendal 2010). Such voltage turns this thermodynamically unfavorable, non-spontaneous reaction into a thermodynamically favorable and spontaneous reaction.

$$H^+ + 2e^- \rightarrow H_2 \left[E^\circ_{H+/H2} = -0.412 \text{ V vs.NHE}\right] \quad (7.3)$$

MEC provides the energy (–0.412 V) required for H^+ to H_2 reduction via microbial electron supply of 0.289 V, along with external applied voltage. Theoretically, the external applied voltage is around 0.14 V and more than 0.2 V in practice, considering electrode overpotential and Ohmic losses (Call et al. 2009). However, this applied potential is still less than the one required in water electrolysis for hydrogen production. MEC have an advantage over fermentation for producing pure H_2 due to the CO_2 and H_2 gases produced separately in anodic and cathodic chambers separated by a membrane (Rabaey and Rozendal 2010). Additionally, there are no propitious conditions for methanogens to consume H_2 gas.

Overpotential is a major issue in MEC operation. The use of platinum electrode showed promising results own to its low activation overpotential. However, it is expensive, non-renewable, imperiled to be disillusioned by carbon monoxide and certain pollutants like sulfur, and has negative effects on the environment (Chae et al. 2009). Since this electrode contributes to 47% of the total cost of MEC reactor, it definitely does not favor economic operation (Logan 2010; Rozendal et al. 2008a). A promising alternative to this catalyst is to improve the functionality of biocathode in terms of overpotential, H_2 productivity, and start-up time, while cathodic H_2 production has led to ecofriendly electrode discovery.

7.2.1 Biocathode MEC Categories

According to Jafary et al. (2015), Biocathode MEC can be developed and categorized as follows: (1). Half biological two-chambered biocathode MEC; (2). Full biological two-chambered biocathode MEC; (3). Full biological single-chambered biocathode MEC.

Half biological two-chambered biocathode MEC (MEC-I) was first developed from bioanode using acetate-fed bioanode with electrochemically active mixed culture, accompanied by flushing the headspace with H_2. Later, it was followed by acetate replacement with sodium bicarbonate, persistent hydrogen flushing, and reversing the polarity of the electrodes. Concerning the biological element/treatment, initially microorganisms were allowed to grow in batch mode for 50 h and then switch to continuous flow using a nutrient medium. After 250 h of inoculation and polarity reversal, the biocathode achieve a current density of –1.1 A/m^2 and a potential of –0.7 V. This current density was four times higher than the one obtained by using a titanium electrode coated with platinum (Rozendal et al. 2006). For the same value of current density (–0.47 A/m^2), the comparison of the applied potential for platinum-coated (with –0.7 V) and biocathode (with –0.65 V) MECs is encouraging. Measurements demonstrated a hydrogen yield of 0.63 m^3 H_2/m^3/day; however, there was a 67–94% of hydrogen loss, mostly due to its diffusion through the membrane. This approved the application of microorganisms as the cathode catalyst and still functions under half biological conditions (bioanode and abiotic cathode during the first two steps, and biocathode and abiotic anode after polarity reversal in the third step).

Jeremiasse et al. (2010) studied the first full biological electrolysis cell (MEC-II) in which both oxidation and reduction reactions were biocatalyzed with electrochemically active microorganisms (biocathode and anode). In a study by Tartakovsky et al. (2009), a current density of 3.3 A/m^2 was achieved at a cathode potential of –0.7 V and an applied voltage of 0.8 V. This result were comparable with a continuous membrane-less MEC at 3.2 A/m^2 catalyzed with 5 g/m^2 of platinum-load electrode (Tartakovsky et al. 2009). Low hydrogen rate of 0.04 N m^3/m^3/day and cathodic hydrogen recovery of 21% were reported, which were lower than those obtained in the same experimental setup for the first MEC-I (0.63 m^3 H_2/Volume/day and 49%, respectively) (Rozendal et al. 2008b).

In the third category, full biological single-chambered biocathode MEC (MEC-III) the highest hydrogen production rate of about 24 mmol/h was reported at a cathode potential of –1.0 V with 56% cathodic hydrogen recovery in the biocathode MEC, and with ferricyanide in the anode. The results for membraneless MEC at a similar cathode potential of –1.0 V (0.7 V applied voltage) were 10.8 mmol/h of hydrogen production rate and 36% of cathodic hydrogen recovery. However, it was unclear whether any biocatalytic (biocathode) activity by microorganisms in one compartment of MEC was present in this research (Liu et al. 2005).

7.2.2 Substrate

MEC recorded lower product yield when compared to chemical methods but it still remains as a promising technology due to the use of renewable resources, wastewater as a feedstock, and less susceptibility to poisonous components present in real wastewater. To develop a biocathode MEC, a carbon source is necessary to supply cathodic biocatalyst growth; furthermore, they have shown an effective impact on main/side product formation (Jafary et al. 2015).

In an initial MEC experiment, while reversing the polarity, sodium acetate in anode was replaced by sodium bicarbonate and converted to the cathode. It resulted in methane as product and no H_2 production was observed. In this case, bicarbonate served as a carbon source for hydrogenotrophic methanogens to consume hydrogen and produce CH_4 (Eq. (7.4)). Methane has been also reported in MEC-II fed with sodium bicarbonate in cathode and sodium acetate in the anode. It has been hypothesized that CH_4 may have been produced in bioanode and then diffuse across the membrane to the cathode or, that there exist methanogens in the biocathode and they utilize the CO_2 from the anode and H_2 from the cathode chamber to produce methane (Eq. (7.5)):

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \tag{7.4}$$

$$CH_3\,COOH \rightarrow CH_4 + CO_2 \tag{7.5}$$

Besides, the replacement of bicarbonate (autotrophic) with acetate (heterotrophic) as carbon source helps improving the start-up time and H_2 production rate up to seven times, about 2.2 m^3 H_2/m^3 reactor/day (Jeremiasse et al. 2012; Rozendal et al. 2008a). In another biocathode study performed by Marshal et al., carbon dioxide presented a capability as the sole carbon to produce hydrogen at a rate of 11.8 mM/day at cathode potential of –1590 mV; aside from acetate and methane as two other coproducts in an electrosynthetic system (Marshall et al. 2012). The results improved to around 100 mM/day at the same cathode potential of –590 mV in a similar experimental setup operated in a semi-batch mode over 150 days in a later study (Marshall et al. 2013).

7.2.3 Challenges

Hydrogen loss across the membrane is a considerable fact that maintains both membrane-less and two-chambered configurations as an appealing research focus. Using a membrane in an abiotic cathode MEC was preferred due to the prevention of methanogens to consume H_2 in the product chamber. Concerning membrane-less setups, they resulted in low cathodic H_2 recovery which was probably due to the utilization of H_2 products by exoelectrogens to produce electricity on the anode.

Other challenges include a long adaption and start-up time of the biocathode, along with a low production rate in comparison to metal catalysts.

7.3 Perchlorate Removal

Perchlorate is a kind of persistent chemical included in the US EPA candidate list as an emerging surface and groundwater contaminant (Yang et al. 2019). The main concern is its mobility in the environment and its inhibitory effect on thyroid function (Butler et al. 2010). Among the various process developed for its treatment, the biological option is highly effective and economical (Hatzinger 2005). Most of the biological processes rely on the ability of perchlorate-reducing bacteria (PCRB) which are ubiquitous in the environment and are mainly facultative anaerobes and denitrifies (Shea et al. 2008). They are used as terminal electron acceptor and an organic carbon as electron donor; however, the use of this donor creates the issue of secondary pollutants (Logan 1998). Among the biological processes, perchlorate reduction using biocathodic microbial fuel cell (MFC) setups have the distinct advantage of decoupling oxidative (anodic) reactions from reductive (cathodic) reactions across a proton exchange membrane (PEM), thereby minimizing any secondary water quality effects.

A study done by Shea et al. (2008) investigates a functioning MFC with a denitrifying biocathode for perchlorate reduction, as a means to confirm the existence of biocathode-utilizing PCRB and the possibility of perchlorate remediation. The maximum perchlorate removal was 12 mg/L-d, contributing 64% to the 0.28 mA produced by the cell. This result suggests that PCRB are utilizing the cathode as an electron donor without exogenous electron shuttles. Butler et al. did a similar study by increasing the concentration of perchlorate and decreasing the nitrate concentration at a fixed potential. They achieved a maximum perchlorate removal of 24 mg/L-d and a cathodic conversion efficiency of 84% (Butler et al. 2010). When the concentration of sole perchlorate and sole nitrate was 0.40 mmol/L and 0.32 mmol/L respectively, both MFCs showed excellent performance on removal efficiency and current stability. As the influent molar ratio of NO_3^-/ClO_4 was 1:1, the holistic substrate reduction (40.97% for perchlorate, 86.03% for nitrate) and electricity generation performance (3.10 A/m^3) reached the optimum (Jiang et al. 2017).

7.3.1 Kind of Electrode

Yang et al. also studied the effect of electrode material on the perchlorate removal without external energy supply of perchlorate-reducing microbial pre-enrichment in MESs. They analyzed dual-chambers MESs with four kinds of cathode materials including Fe/C particles (Fe/C), zero valent iron particles (ZVI), blank carbon felt

(CF), and active carbon (AC). The highest perchlorate ClO_4^- removal rates in these reactors were 18.96 (Fe/C), 15.84 (ZVI), 14.37 (CF), and 19.78 mg/L/day (AC) at 100 Ω. A previous research about the electrochemical characteristics of perchlorate and iron group metals reported that ClO_4^- was adsorbed on the surface of iron material and then reduced to nontoxic Cl^- (Lang et al. 2005). However, since in the MES electrons of anode could be transferred to the cathode and ClO_4^- reduced directly by it, the removal of ClO_4^- was not only an adsorption process, but also a redox reaction process (Yang et al. 2019).

In the Fe/C and ZVI MES reactors, iron materials were used as the cathode materials, where ClO_4^- could be adsorbed and then transformed into other products under the reduction of iron materials (Yang et al. 2019). In these systems, ClO_4^- is reduced to Cl^- by Fe^0 under anoxic conditions as in Eq. (7.6) (Im et al. 2011). Fe^0 was oxidized to Fe^{2+} and then further oxidized into Fe^{3+}, therefore, cathodes were consumable.

$$ClO_4^- + 4Fe^0 + 8H^+ \rightarrow 4Fe^{2+} + Cl^- + 4H_2O \tag{7.6}$$

7.3.2 *Effect of pH*

Perchlorate reduction in the biocathode of MFC depends on both: cathode potential and pH. The maximum perchlorate reduction was observed at a cathode potential of −375 mV vs. Ag/AgCl and pH of 8.5. With an increase in pH from 6.2 to 8.1, the perchlorate reduction nearly tripled, increasing from 19% to 57%. When the pH of the influent was slightly raised to 8.5, complete perchlorate removal was achieved (Butler et al. 2010). While using the Fe/C ZVI, electrodes are consumable. The generation of hydroxides (e.g., ferrihydrite with high surface sites) when basic pH excursion occurs in the cathode chamber likely induces the adsorption and/or co-precipitation of ClO_4^- in the Fe/C and ZVI reactors.

7.3.3 *Microbial Community*

The perchlorate-reducing biocathode bacterial community, which contained putative denitrifying Betaproteobacteria, shared little overlap with a purely denitrifying biocathode community, and was composed primarily of putative iron-oxidizing genera.

The bacterial community presents in the perchlorate-reducing biocathode contains putative denitrifying Betaproteobacteria. However, it shared little overlap with the purely denitrifying biocathode community and was composed primarily of putative iron-oxidizing genera. On comparing the microbial community in the

chambers of perchlorate-reducing MFC and denitrifying MFC, the anode communities are similar to each other but highly distinct in their biocathode communities (Butler et al. 2010). At a broad phylogenetic scale, the biocathode communities in denitrifying MFC were dominated by Betaproteobacteria, including established denitrifying lineages and predominantly composed of sequences affiliated with iron-oxidizing bacteria (FeOB) from the Betaproteobacteria genera *Ferritrophicum* and *Sideroxydans.* In perchlorate-reducing MFC, the microbial community within the perchlorate-fed biocathode was significantly more diverse than its denitrifying counterpart and was primarily composed of bacterial sequences more similar to a clone from a dioxin-dechlorinating microcosm (Yoshida et al. 2005) and to sequences from bacteria of the genera *Chryseobacterium* and *Kaistella* (phylum Bacteroidetes). *Proteobacteria* and *Chloroflexi* were dominant in biocathode of perchlorate-reducing MFC. Among Proteobacteria, phylum, b-Proteobacteria, and a-Proteobacteria were identified as the most significant classes in biocathodes (Jiang et al. 2017).

7.4 Chromate Reduction

Chromium is a metallic species widely used in industrial applications including metal plating, leather tanning, and dye manufacturing. Its two most common species found in the environment are hexavalent, Cr^{VI}, and trivalent, Cr^{III} (Fonseca et al. 2012). The former is highly mobile and soluble which results in contamination of soils, surface waters, and groundwater; while the latter is generally considered harmless to the environment due to its lack of mobility as an insoluble species (Dong et al. 2013; Hsu et al. 2012). Thus, the reduction of Cr^{VI} to Cr^{III} has been proposed as an effective mechanism for limiting exposure and movement in the natural environment.

A study was done by Hsu et al. using *Shewanella* strains as biocatalysts for chromate reducing in a microbial fuel cell shows a maximum power generation of between 10.2 and 59.4 nW cm^{-2}. *Shewanella* acting as the sole biocatalysts at the cathode are capable of achieving the reduction of chromium concentrations to less than 5 ppb, well within acceptable guidelines established by regulatory agencies (Hsu et al. 2012). The chromate reduction takes place as per the following reaction:

$$HCrO_4^- + 7H^+ \, 3e^- \rightarrow Cr^{3+} + 4H_2O \; [E^\circ = +0.382 \text{ V vs.SHE at pH} = 7]$$

A facultative electroactive bacterium (*Bacillus*-accession number MH782060) was aerobically isolated from the biocathode of a Cr^{VI} reducing MFC. This strain showed efficient reducing ability in both heterotrophic (aerobic LB broth) and autotrophic (anaerobic MFC cathode) environments. Cr^{VI} removal reached 50.6% after 20 h in LB broth supplemented with Cr^{VI} (40 mg/L).

7.5 Dechlorination

MEC are progressively considered for bioremediation applications like the reductive transformation of chlorinated hydrocarbons, where a polarized solid-state cathode serves as an electron donor for reductive dechlorination catalyzed by microbes (Di Battista et al. 2012; Strycharz et al. 2008). The dichlorination of these chlorinated hydrocarbons can be achieved via two different electron transfer mechanisms. The first one is in situ electrolytic generation of molecular hydrogen, which in turn serves as an electron donor for the reductive dichlorination. The second one is the direct exchange of electrons between the electrode and the dechlorinating bacterial growth attached to the cathode surface in form of an electroactive biofilm (Aulenta et al. 2010). However, some non-organohalide respiration microorganisms, such as methanogens and denitrifying bacteria will also participate in electron competition, reducing the efficiency of electron utilization for organohalide respiration (Aulenta et al. 2011). The relative amount of electron transfer towards the dichlorination mainly depends on the set cathode potential in the MEC reactor (Rosenbaum et al. 2011).

7.5.1 Effect of Applied Potential

A study was done by Aulenta et al., proven that the set cathode potential effect the rate of dichlorination of TCE, competition for available e^- and spectrum of lower chlorinated by-products. According to them, when cathode potential was maintaining at −250 mV no abiotic H_2 production was detected, and TCE dichlorination progress slowly. Methanogenesis was almost completely suppressed and dechlorination accounted for nearly all the electric current flowing in the system. However, at the lower electrode potential of −450 mV and below higher rate of TCE dechlorination was achieved that result in the formation of vinyl chloride and ethane. At this potential, very active methanogenesis occurred that accounting for over 60% of the electric current (Aulenta et al. 2011).

A continuous study was done by the same group of authors also concluded that cathode potential maintained at −250 a negligible amount of CH_4 produce and as the potential was maintained further low the electric current of −750 mV gradually increased and higher rates of TCE dechlorination were achieved, along with the presence of very active methanogenesis due to abiotically produced H_2. The dominance of dechlorinators also at the cathode in MEC is also affected by the applied potential. All known dechlorinating bacteria are heterotrophic in nature and require organic compounds. Study done by Di Battista et al. (2012) shows that acetate concentration in the cathode effluent was always below the analytical detection limit, it cannot be excluded that some acetate was still being produced by homoacetogens by using H_2 and CO_2 and then rapidly absorbed up by dechlorinators (Di Battista

et al. 2012). This reaction occurs at the lower applied potential from −550 mV to 750 mV where H_2 production is possible (Eq. (7.7)).

$$4H_2 + CO_2 + \text{Homoacetogens} \rightarrow CH_3\,COOH \rightarrow \text{Dechlorinators} \tag{7.7}$$

In case of higher applied potential of −250, no H_2 was available and growth of dechlorinators are slow and their slow growth maintained by organic carbon released from the decay of the biofilm, grown during previous runs at more reducing cathode potentials. Under open circuit conditions (no current flowing in the circuit), during which a slow dechlorination was observed, clearly indicating that biofilm decay supplied the reducing power needed to drive the reductive dechlorination process (Aulenta et al. 2011).

So, dichlorination is good at lower potential but it also results in the production of CH_4 and bacterial biomass which consume the redox equivalent. Study was done by Chen et al., reveal the electron fluxes in biocathode BES performing dichlorination of chlorinated aliphatic hydrocarbon (CAHs), 1,1,2,2-tetrachloroethene (PCE), 1,1,2-trichloroethene (TCE), and 1,2-dichloroethane (1,2-DCA) with a constant cathode potential of −0.26 V, open circuit and abiotic cathode. The biocathode (99%) had significantly higher dichlorination efficiency than the opened circuit (17.2%) and abiotic cathode (5.5–10.8%), respectively, indicating the improved CAHs dichlorination capacities. The dichlorination pathway in biocathode systems was consistent with the pure microbial system, that with cis-1,2-dichloroethene and ethene as the primary products for PCE/TCE and 1,2-DCA, respectively. Besides, methane was the main by-product of heterotrophic biocathode, and methane production was enhanced to some (Chen et al. 2018).

Apart from chlorinated aliphatic hydrocarbon, complex chlorinated nitroaromatic antibiotic chloramphenicol (CAP) is also a priority pollutant in wastewaters (Liang et al. 2013). A fed-batch bioelectrochemical system (BES) with biocathode with an applied voltage of 0.5 V (served as extracellular electron donor) and glucose as an intracellular electron donor was applied to reduce CAP to amine product ($AMCl_2$). The biocathode BES converted 87.1 ± 4.2% of 32 mg/L CAP in 4 h, and the removal efficiency reached 96.0 ± 0.9% within 24 h. Conversely, the removal efficiency of CAP in BES with an abiotic cathode was only 73.0 ± 3.2% after 24 h. One intermediate, CAP-acetyl, was found in biocathode BES, indicating that it was produced from bioactivity since CAP acetyltransferase catalyzes acetylation of 3-hydroxyl of CAP while 3-hydroxyl acetylated CAP can also be transformed reversibly to 1-hydroxyl acetylated CAP (Shaw and Leslie 1991).

When the biocathode was disconnected (no electrochemical reaction but in the presence of microbial activities), the CAP removal rate was dropped to 62.0% of that with biocathode BES. Acetylation of one hydroxyl of CAP was noted exclusively in the biocatalyzed process, while toxic intermediates, hydroxylamino (HOAM), and nitroso (NO), from CAP reduction, were observed only in the abiotic cathode BES. Electrochemical hydrodechlorination and dehalogenase were responsible for the dechlorination of $AMCl_2$ to AMCl in abiotic and microbial cathode BES, respectively (Liang et al. 2013).

7.5.2 *Microbial Population Responsible for Dechlorination*

CARD-FISH analysis done by Battista et al. shows the presence of *Chloroflexi* in the whole range of explored cathode potentials. Notably, they accounted for a major share (from 65% to 100%) of total Bacteria and most probably played a key role in the bioelectrochemical dechlorination process (Di Battista et al. 2012). *Chloroflexi* phylum is capable of growth-linked reductive dechlorination of aliphatic and aromatic compounds (Krzmarzick et al. 2012).

Among them, *Dehalococcoides* sp. is the only one capable of dechlorinating toxic chloroethenes all the way to harmless ethene. For such a unique metabolic feature, the presence of Dehalococcoides is often regarded as a prerequisite of successful bioremediation systems based on reductive dechlorination processes. Dehalococcoides a well-known TCE to ethene dechlorinating microorganism was the predominant dechlorinating bacterium when TCE dechlorination was supported by abiotically produced H_2 gas. Interestingly, *Chloroflexi* phylum seemed to play a key role in the reductive dechlorination of TCE, at cathode potentials in the range from −250 mV to −450 mV, when the reaction was most probably driven by direct extracellular electron transfer from the cathode to the microorganisms (Di Battista et al. 2012).

7.6 Conclusion

MEC constitute a relatively recent innovation approach for wastewater treatment. Laboratory scale MEC operation shows remarkable results in terms of removal of organic matter and oxidized contaminates of interest. However, there are still several challenges that must be overcome for scaling up the technology. This effort should be focus on detail molecular understanding, development of new electrode material, inoculum, and MEC configuration.

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Chapter 8
Bioelectrochemical System for Wastewater Treatment for Energy via Suitable Microbial Systems

Rajesh K. Srivastava

Abstract Wastewater treatment with capacity of trillion of liters is reported to spend a billion of Euro currency with some amount of energy. Now, it can be utilized as renewable organic matter resources for production of electric power, hydrogen and caustic soda chemicals with saving of more amounts of money and energy.

Bioelectrochemical system is utilized for synthesis of electricity power or hydrogen biomolecules or chemical products via application of microbial fuel cells (MFCs) or microbial electrolysis cells (MECs) that are used for the chemical energy of waste organic matter from low strength stream of wastewaters or lignocelluloses biomass. And microbes are worked as catalyst with utilization of different types of organic compounds without need of expensive metal as catalyst. This system helps in breaking down of complex organic in wastewater system via electrically active microbial cell application with cleaning of wastewater system. New useful products formation is reported from microbial electrolysis process in bioelectrochemical system with recovery of nutrients and metals or removal of toxic and recalcitrant compounds. Microbial fuel cells are used for synthesis of hydrogen fuel. European Union has started with many innovative projects for wastewater treatment with acylamine function amine function of carbon anode for improved or enhanced microbial electro-catalysis. Improvement in environmental and energy performance has reported via wastewater treatment with biochemical or biofuel production. The author will discuss the new concepts and invention of alternative materials development for electrode, separator, or catalyst and also innovative design for bioelectrochemical system with emphasis on recent development for electric power or other products formation with its limitations.

Keywords Bioelectrochemical systems · Biofuels · Microbial systems · Wastewater · Treatment · Anode · Cathodes

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Abbreviations

AD	Anaerobic digestion
AM	AM radio waves of 540–1600 kHz
BES	Bioelectrochemical systems
BMP	Biochemical methane potential
BOD	Biological oxygen demand
CH_4	Methane
COD	Chemical oxygen demand
CSIR	Continuous stirred tank reactor
EPA	Environmental Protection Agency
HWW	Hospital wastewater
IC	Internal circulation
IPB	Integrated photo-bioelectrochemical
LDP	Loss of dairy product
MEC	Microbial electrolysis cells
METs	Microbial electrochemical technologies
MFCs	Microbial fuel cells
NAC	Net anodic compartment
OTUs	Operational taxonomic units
PCR	Polymerase chain reaction
PHCs	Petroleum hydrocarbons
PPW	Petroleum produced water
PW	Pure whey
SBR	Sequencing batch reactor
SCIE	Science citation index-expanded database
TAC	Total anodic compartment
TDS	Total dissolved solids
TNT	TiO2 nanotube
TS	Total solid
TS	Total solid
TW	Tera Watt
VFA	Volatile fatty acid
VS	Volatile solid
WHO	World Health Organization
WTE	Waste to energy
WWTPs	Wastewater treatment plants

8.1 Introduction and Basic Concepts

Suspended solids, various types of nutrient substrates, biodegradable plastic or other organic matter, microbial pathogen, toxic heavy metals, refractory organic compounds or other dissolved inorganic matter or solids are reported as contaminant in wastewater stream (Souza et al. 2018). Wastewater stream is good source of refractory organic compounds which are agricultural pesticides, surfactants, and phenols and they create big challenge as exhibiting the resistance toward conventional nature of wastewater treatment methods. Heavy toxic metals as major contaminant come from waste generated by commercial or industrial activities. Waste products from these places are reported to deposit in water bodies and become polluted domestic water or wastewater resources containing inorganic solid, Na^+, Ca^+, or SO_4^{2-}. This wastewater contained various types of biodegradable organic compounds including carbohydrates, protein, or fats. Biodegradable organic matter can destabilize the natural oxygen in the river, pond, or other ecosystem. Discharged wastewater into lakes and stagnant waters are needed to go for proper treatment (Taha and Al-Saed 2017).

Bioelectrochemical system can be a new technology for wastewater with improved efficiency for energy synthesis. For this application some limitations for BES anode electrode are found for not directly discharging electron via using wastewater effluents. But this problem has solved BES cathodes with enhanced treatment for additional selected contaminants (Gul and Ahmed 2019). Investigation for a number of effective approaches is reported that grouped the cathode-supported waste or cathode-stimulated treatment for waste matter. Cathode-stimulated treatment is proceeded with involvement of electron transfer facility directly with reduction of various contaminants (nitrate or dye removal). But cathode-supported treatment approach is completed with contamination removal that utilized aerobic oxidation, algal biomass growth, or strong oxidant generation in advanced oxidation approach or membrane supported treatment (Kaur et al. 2018).

This BES system is operated at mild conditions with microbe's involvement (responsible for electron transfer from an electron donor of lower potential value to electron acceptor of high potential). In this process, at anode site the oxidation process takes place whereas at cathode site, electrochemical reduction processes take place and result in other products formation at this site (Jain and He 2018).

Seven units of full scale biological wastewater plants are discussed with their location identification at the Polar Arctic circle region in Finland. These places are reported with archaea, bacteria, or fungi as potential community's structure that can be utilized in bioreactor operation. Different analysis approaches such as quantitative PCR, massive parallel sequencing, or multivariate reduction are applied for identification of respective genes or pathways of respective microbial system and these approaches can help in effective wastewater treatment in bioreactors. These biological approaches can help in activated sludge system. Activated sludge system is a strong source of effective and dominated bacteria compared to archaea or fungi

species that are confirmed by diversity analysis approaches (Cydzik-Kwiatkowska and Zielin 2016).

A core operational taxonomic unit (OTU) in influent feed and bioreactor is reported. Several microbial strains such as *Methanobrevibacter*, *Methanosarcina*, *Thamumarchaeota Trichococcus Leptotrichiaceae* (archaea), *Methylorosula* (bacteria), or *Trichosporonaceae* (fungi) are reported as dominant organisms. Oligotype structure of core OTUs is reported with ubiquitous fungi type oligotype in sewage influent and bioreactors both. For these (i.e. above mentioned microbial species) microbial system confirmations, multivariate redundancy analyses performed for core OTUs, related organic or nutrient matter removal. Competition among archaea or fungi species is reported in OTUs. And bacteria species in OTUs are positively correlated at extremely cold temperate operated bioreactors (Gonzalez-Martinez et al. 2018).

On a daily basis, wastewater generation is reported from domestic or industrial sources all over the world posing the water crisis and environmental deterioration as big challenge for society. Developing of sustainable energy from efficient ways of wastewater treatment plants can provide best solution for energy shortage issues in everyday life and success is possible with the help of recent advance in microbial electrochemical (MEC) technology development (Krieg et al. 2014). MEC technology can help in wastewater treatment as well as synthesis or recovery of clean energy with usable water purification. Various types of designs and configurations of MFC units can help in treatment of wastewater organic matter. In addition, they help in treatment of waste matter, come or desposited, from domestic or industrial activities. This process needs indigenous or enriched electrogenic microbial system including some fungal or bacterial species. Design performance improvement has reported by using conventional or simple nature single chamber or dual chamber MFCs unit to integrated hybrid or engineered MFCs units via application at lab scale to pilot scale level. These MFCs design has applied for wastewater treatment with more amounts of electric power and clean water generation via removal of toxic or waste organic compounds (Krieg et al. 2014; Rathour et al. 2019).

A tubular shaped, single chambered MFCs unit in continuous mode has generated high power outputs. Granular graphite matrix anode electrode with ferricyanide solution containing cathode is reported to be effective wastewater treatment with energy generation (maximum power outputs). MFCs system with 66 or 90 W m^{-3} as maximum power outputs is reported for net anode compartment (NAC) but 38 or 48 W m^{-3} power outputs is found at total anodic compartment. For digester effluent and domestic wastewater, feed streams are reported to contain acetate or glucose as organic matter and is reported to generate power output of 59 and 48 W m^{-3}, respectively, at NAC. Total Coulomb conversions efficients are 75 and 59% for acetate and glucose, respectively, with loading rate (1.1 kg COD m^{-3} NAC volume/day). Improved MFCs performance enhanced the conversion of non-rapid biodegradable organic matter with better facility of direct electron flow from anode to cathode electrode. Sustainable and open air cathode has shown critical issues for its practical implementation in MFCs (Rabaey et al. 2005; Blanco-Aguilera et al. 2019).

Conversion of waste nature matter to energy fuel (WTE) technology is reported and incineration process, AD (anaerobic digestion), pyrolysis, and gasification are good examples of WTE but they suffered from low efficiency with high energy needs. MFC technology is applied for production of renewable or sustainable energy sources with more opportunities for current period global energy crisis problem. In India, the total wastewater generation is reported almost 250% or more with least or minimal total treatment capacity (Pan et al. 2015).

Government is putting efforts on developing sustainable mode of solution for degradation or treating of waste matter and world human population is reported to consume seven billion cubic meter of water quantity every year with further rise these value to 950 and 1422 billion m^3 in coming periods (year 2025 or 2050). Wastewater treatment is a big or critical challenge with serious concern or problem and it can provide more amount of energy recovery potential. In this regard, MFC technology would be generated nearby 23.3 or 40 terawatt (TW) power in year of 2025 or 2050, respectively, via achieving proper wastewater treatment with simultaneously generation of electric power throughout the urban areas of India (Khan et al. 2017). Bioelectrochemical systems (BES) have shown their potential with capability of performing the simultaneous mode of wastewater treatment and electricity or fuel energy or valuable biochemical synthesis. Performance evaluation of global level scientific outputs of BES related research is reported on more research publication or papers in science citation index database (SCIE) from years 1991 to 2014. Further, published in journal as an output in subject categories is found in countries and institute analysis on BES design or configuration in world research works also reported. More number of annual publications on BES design or operation are now increased steadily after the year 2004. MFCs system is studied as bioreactor or devices for electric power synthesis with wastewater treatment tasks as dominant or broad applications. Carbon nanotubes and grapheme have been reported as nano-structured materials in the BES field (Wang et al. 2015). The author will emphasize in this chapter on recent development on wastewater treatment via various designs of BES with more efficiency for clean energy generation.

8.2 Wastewater Sources and Its Components

Wastewater is generated due to various human activities at domestic or industrial level and it contained liquid waste matter from various sources and people from domestic or homes, agriculture, commercial. Pharmaceutical sectors with hospitals sites are principal sources for wastewater generation. Hospital wastewater (HWW) has reported to contain pharmaceutical wastes or residues, hazardous chemicals matter or pathogens as well as radioisotopes as dangerous substances. They create different nature of risks (physical, chemical, or biological) for public or environment components health issues and currently, without any legal procedure for effluent treatment in hospital sites prior to discharge into municipal side collector or directly disposed onto surface water sources (Carraro et al. 2017).

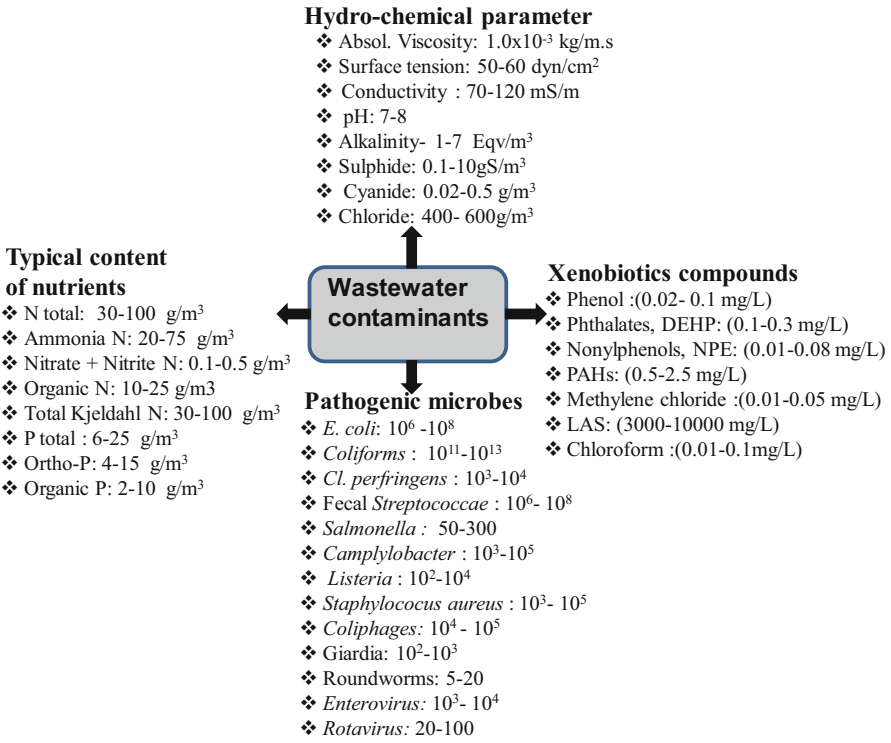

Fig. 8.1 Contaminants in wastewater from different activity of society (Henze 2008)

Hospital wastewater (HWW) is full of hazardous substances and pharmaceutical wastes or residue, chemical nature of hazardous substance or compounds, pathogens and radioisotopes are main contaminants. To minimize the adverse effects, these compounds need to be neutralized by wastewater treatment plants (WWTPs) (Wang et al. 2020). Wide ranges of concentration for hazardous contaminants is reported due to depending on the size of a hospital or bed density, numbers of impatient or outpatients crowd rate. Further, number or types of wards or services and seasonal effects of country also affected the contamination risks. Some hazardous substance come from dental amalgam or medication and these are also produced in hospital facilities and follow to regulatory status, proper treatment for different types of wastes is done before dispose to environment (Carraro et al. 2016) and shown Fig. 8.1.

Role of hospital wastewater is reported for environmental contamination and different legislation around the world are needed for main principles on the hospital wastewater treatment. Guidelines from WHO and EPA guidelines for radionuclide hazard have discussed with guidelines of legislation side for proper disposal of hospitals wastewater to the environment. Biological wastewater treatment processes can be preceded via exploitation of the concerted microbial activities with

microorganism's communities' structure that linked to changing at environmental conditions. Development of optimal biological system is reported in engineered microbial system and this engineered microbes development can be done by application of molecular techniques. This technique can help in inadequacy of culture dependent methodologies for identification of microbial diversity in sludge samples (Kassem et al. 2020). Culture independent technology and application of omics in wastewater system can help in understanding of microbial strains diversity and their functions in wastewater treatment processes (Ferrera and Sanchez 2016).

Industrial wastewater is reported to vary great extent in flow rate and pollution strength and it is very difficult to determine the fixed values for industrial wastewater constituents. It is reported to contain various nature of suspended, colloidal, or dissolved solids (inorganic or mineral and organic compounds) that exhibited either excessively acidic or alkaline pH conditions (Choudhary and Parmar 2013). High and low concentrations of colored waste matter are reported with their nature of inert, organic or toxic nature materials with containing of bacterial, Industrial wastes are discharged into sewer system with exhibiting of negative effects on treatment efficiency or undesirable effect on the sewer system (Panagopoulos et al. 2019). It is necessary to proceed proper pretreatment for the waste matter nature prior to release into municipal system. It needs fully treated waste before disposing directly into the surface or groundwater systems (Das et al. 2012).

Wastewater is now reported for good resources of organic nutrients than as a waste mattes, that utilized for plant nutrient and also for energy generation. Generation of energy is obtained from wastewater organic matter degradation. And nitrogen organic matter and P containing nutrient is essential and is utilized for biofertilization. Due to high cost of energy consumption in synthetic fertilizers synthesis, MFCs can provide direct biological conversion of organic matter from wastewater resources into electric power. And significant improvement for this conversion process is reported with competitive to anaerobic digester (AD). The anaerobic mode of biological conversion processes is also utilized for biofuel production which in turn is used for renewable fuel nature electric power generation. Membrane coupled with complete anaerobic treatment system is applied for net generator of energy for larger quantity of consumer energy needs today (McCarty et al. 2011; Gosset et al. 2020).

Municipal and industrial origin wastewater has exhibited high levels of toxicity condition for aquatic life or biotic components and proper treatment is necessary for waste matter before discharged into natural ecosystem in developing countries. Physical, biological, and chemical methods have been employed in water treatment plants in most developed nations and helped in cleaning the wastewater resources. During the effective treatment, each step is analyzed using bioassays and compared to toxicity extent of the input wastewater matter. Industrial origin wastewater is reported to be more toxic in nature and its proper treatment needs to be ensured before sending to municipal wastewater treatment plants. Due to high sensitivity, fast response time and case use and bioassays are employed to monitor progress of each step in wastewater treatment processes in order to provide early warning

periods (Donde et al. 2018). Several types of bioassay are conducted in most cases with good comparison capability and high sensitivity (Hader 2018).

Phototactic green algae (*Chlamydomonas reinhardtii*) are reported to generate photoinduced channel rhodopsin-mediated current flow across the cell membrane that is measured by a simple population assay. Process modification of instantaneous measurements is reported due to phototactic degree and orientation of gravitactic attraction. And addition of heavy toxic cations or organic nature polluting agents can rapidly (in one or several minute time) change the photocurrent movement. Opened route of the flagella mediated voltage gate for calcium ion channels can be sensitive routes to the tested heavy metals cations (Chan et al. 2019).

Photoreceptor currents are generated by channel rhodopsins and sensitive capability of photocurrents to heavy metals cations side is several fold more than detected by swimming velocity technique or other physiological parameters of flagella containing algal species. Measurements of photoelectric flow in algal species suspension are ideally suitable options for low cost process with detection of contamination in water due to heavy toxic metal (Govorunova and Sineshchekov 2018).

Wastewater is reported as a combined form of water carried waste that is come from residences or institutions. It also contained waste matter that is created by commercial or industrial activity, which can contaminate the water from underground and surface water (storm water). Municipal, agricultural, or industrial origin or generated waste matter is reported as categories of wastewater sources (Donde et al. 2018). Municipal wastewater can be generated from residential, commercial, or institutional activities and also contained wastes with water from street drainage or runoff sources (Almeida et al. 1999). Commercial or institutional activities can generate more volume of wastewater that comes from hospitals, clinics, departmental stores, offices, or public recreations. Contaminants of wastewater resources are reported with various nature of suspended solids, nutrients, biodegradable nature or pathogens, heavy metals of toxic nature, and refractory organics or dissolved inorganic solid compounds. Ca^{2+}, Na^{+}, or SO_4^{2-} ions in most domestic supplied water are reported and are also shown in Fig. 8.1. Proteins, carbohydrates, and fats as contaminants of wastewater can destabilize natural oxygen in the ecosystem (Hu et al. 2007; Gosset et al. 2020).

Agricultural wastewater includes fertilizers and biomass wastes (animal or cattle dung, tree branches, vegetation fumes, or other agricultural residues). Industrial wastewater contained complex nature or type of waste matter. Waste treatment or selection of the best treatment type or process can be reported in combination of effectiveness and cost (WERF Report 2003; Kassem et al. 2020). Agricultural or industrial waste matter treatments are more complex and are to be treated separately or individually in the concerned premises. Municipal water wastes are generated from wastes with water from cities or urban centers. Solid wastes and sewage are found from municipal wastes and wastes in rural villages are made up of fecal or urine (excreta) feces and urine (excreta) and refuse is done for the garbage or rubbish waste matter (Henze 2008; Wang et al. 2020).

Municipal solid waste matter includes rubbish or garbage fraction from residences and food wastes. Municipal solid wastes include rubbish or garbage from

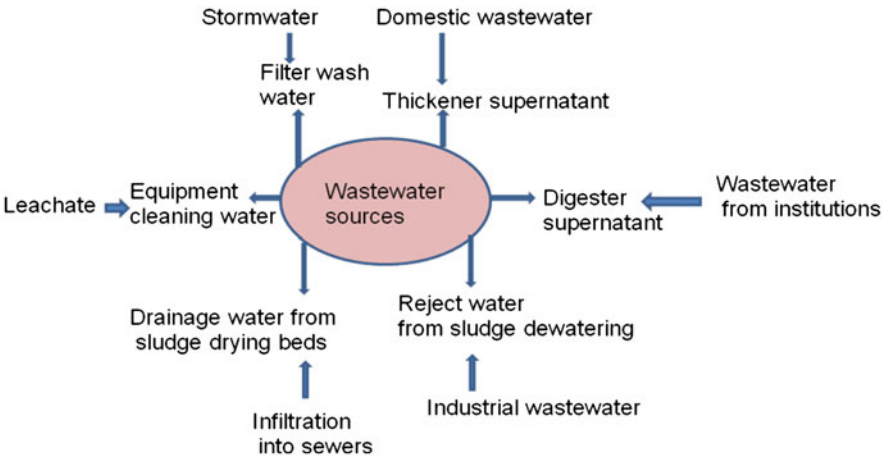

Fig. 8.2 Wastewater generated internally in various treatment plants from society (Henze 2008)

residences and food waste, papers, plastic bags, or glasses from commercial and other institutional centers. Centers are shown in Fig. 8.2. Harmful chemicals from hospitals and commercial centers are reported and need to separately sort with disposition of this waste with special care. Solid waste fraction can be incinerated and also put or keep into landfill sites (Blanco-Aguilera et al. 2019). Sewage organic matter is human excreta and wastewaters are flushed along sewer pipes. Waste matter from kitchen sink, bath, toilet flushes, laundries, and runoff water are needed for sewage treatment. Domestic sewage is contained 99.9% and 0.1% impurities and suspended, colloidal or dissolved solid proportions with gases, pathogenic microorganism and other materials are reported (Roeleveld and van Loosdrecht 2002). There are reports on some wastewater treatments for wastewater cleaning, generation of energy or valuable products as well as solving environmental issues.

8.3 Anaerobic Treatment for Wastewater

Anaerobic wastewater treatment has been applied for two effluents (containing pure whey ~PW) components or loss of dairy product ~LDP) generated from dairy cooperative and methane production was reported. These effluents are found to rich in organic matter (97% for LDP and 87% for PW) and microbiological analysis has shown to obtain the germs and lactic acid bacteria in effluents (Gul and Ahmed 2019). Total coliforms are presented only in LDP and the sulfate-reducing bacteria are absent in both substrates. Low loads (25% and 50%) have reported best CH_4 yields in PW (25.546 ml STP/gVS) and LDP (79.1 ml STP/gVS), confirmed via biochemical methane potential (BMP) assay at 38 °C. Reduction of the volatile solid

(VS) and total solid (TS) is reported more than 80% from two effluents with decrease of organic pollution also (Lhanafi et al. 2017).

Mechanical treatment is used in dairy waste treatment. It proceeded with screen, grit chamber, skimming tank or primary sedimenting process tanks or clarifiers. Chambers are applied for removal of heavier inorganic matter (i.e. sand, grit, or others). Skim processing tanks helped in removal of oils, grease, wood pieces, fruit skin, etc. (Kaur et al. 2018). Settling tanks or clarifier equipment can permit the matter fraction at low velocity rate or at rest parts of sedimentation tanks to settle down at the bottom region of sediment processing tanks. Collection of materials at bottom is sludge organic matter and sludge or effluent fraction is needed for additional treatment for making it harmless nature compounds (Sengil and Ozacar 2006; Kolhe et al. 2009; Kaur et al. 2018).

Comprehensive manner of dairy volarization model is applied as decision support tool for midterm allocation of raw material waste from final products or during production periods. This developed model can help in identification of optimal concentration of product portfolio composition via allocation of raw milk products to most profit gained dairy products (Wazed et al. 2010). This model can help in important constraints identification such as recipes details, composition variations, dairy products formation, interdependencies level, season changes, demand or supply capacity, or flow rate of transport facility This developed model has been analyzed at the international level dairy processor centers (such as Friesland, Campina, or the Netherlands) (Souza et al. 2018). The model structure and its output elements structure are considered to optimal level volarizing the raw products. Comprehensive study and functional nature of this model can be tested the effect of seasonal change on milk volarization process for profit and a shift in the allocation of milk (Banaszewska et al. 2013; Wazed et al. 2010).

Practical or experimental application of biotechnological waste processing or treating of milk production plants is achieved by development of a process for biogas production with application of anaerobic bioreactors. Laboratory scales installations of these reactors are conducted at laboratory studies (Wang et al. 2020). Principal technological scheme of biofuel production is carried out at appropriate material, technical, or economic calculations. Using the information on produced biogases as fuel energy is reported at boiler system with reduced natural gas consumption as well as cost of recycling processes at dairy industry plants (Panfilova et al. 2016).

Anaerobic condition methane from fermentation has found to complete in four steps. And its first step involves the enzymatic hydrolysis of undissolved complex organic compound into simpler or monomer dissolved substance and its second is proceeded with acid formation and also release of short chain volatile fatty acids (VFA), amino acid, alcohols and H_2, CO_2 molecules (known as acidgenic step) (Kassem et al. 2020). And its third step is started with acetogenic steps for conversion of VFA, alcohol and amino acids into acetic acids, dissociation into hydrogen cation and acetate ions where its fourth step is started with methanogenic stage for generation of methane from acetic acids and also result in the reduction reaction of CO_2 by H_2 molecule (Goblos et al. 2008).

The precipitation process is chemical treatment for wastewater treatment and it is completed by adding flocculants (flocculating material) to wastewater and mixed vigorously ad agitators. Precipitation is occurred for insoluble phosphate ions in form of fine particles aggregation into large size flocks formation. Sedimentation basins are continuously scraping the sediment into sump or oblique gutter sites and it carried off-water from clarifier surface layers (Kushwaha et al. 2011). Biological treatment provides benefits via performing the microbial strain mediated transformation of complex organic compounds as well as possible adsorption of heavy metals with the help of suitable microbial cells system. Biological modes of treatment of milk waste treatment are carried out by combining different types of scheme or strategy of biological mode for selective nature of constituent removal (Sengil and Ozacar 2006).

8.4 Bioelectrochemical System (BES) and Its Operation

Petroleum industry application is reported as one of the biggest and fast growing fuels with waste producing industries that is fulfilling the continuous increase of fuel sources (for energy) demand as a form of non-renewable energy nature. Petroleum refinery is reported to produce huge quantities of different types of waste organic matter (oily sledges, huge wastewater, volatile nature organic matter, non-usable catalysts, heavy toxic metals, or others) due to its huge quantity and continuous operation modes in many other refinery operation (including dairy processing units) and it has shown big challenges or issues for managing of huge quantities of generated or produced waste matter from its different petroleum industries processes (Kassem et al. 2020). This industry has generated the complex nature of wastes with the report of changing stringent environmental regulations. This waste quantity can be decreased with reduced energy loss via treatment with conserving the energy loss with utilization of accumulated energy in chemical bonds of these waste organic matter. BES is considered as an efficient tool for reduction of waste disposal with economic benefits via transformation of waste organic matter into energy pool sources. Feasibility of using BES operation has shown more potential alternative for harnessing or generating the huge energy quantity from different waste matter degradation from various petroleum refineries (Srikanth et al. 2018).

It has reported that oily sludge is found as significant solid fractions of waste generated during different types of processing in petroleum industries. This waste is found as complex emulsion of various nature of types of petroleum hydrocarbons (PHCs), water, heavy toxic metals, or solid particles and are reported as hazardous compounds with huge quantities generated at world level. It needs an effective treatment with widespread attention. This waste has shown many negative environmental impacts and many effective treatment methods have been employed for neutralizing this waste with PHCs before its oil recovery or sludge disposal. In this waste, various heavy metals need to treat properly before oil recovery and sludge matter disposal to open environment. And no single or specific processes are

reported that are found an effective treatment approaches that is associated with their different advantages or limitation. Improvement in recent process technologies and their combined oil recovery approaches with sludge waste disposal can be implemented with resources reuses recommendation and environment protecting regulation. The comprehensive mode of examination of oily sludge waste treatment via BESs can apply for both objectives (such as advanced developments with future research directions) (Hu et al. 2013).

Treatment strategy of petroleum industries produced water (PPW) is done by application of BES unit with application of uplifted cathode electrode potential. It has shown good treatment capability with reduced levels of waste matter (in terms of COD or hydrocarbon removal). And removal of 91% COD and 77% hydrocarbon is found with reduction in TDS levels that is reported during BES operation at appropriate cathode potentials (400 mV). Reduced sulfate concentration is not reported at significant levels due to oxidative reactions that are reported at anode electrode. Enhanced oxidation processes of PPW waste fraction at anode electrode have reported with generation of good power output (negative value of 20.47 mA) with increased fuel cells behavior. Electrochemical mode of analysis (cyclic or linear sweep nature voltammeter) has done with good correlation and good capability of treatment and dynamic microbial cells of the BES unit with loading of real field wastewater has shown dominant nature with effectiveness for petroleum crude oil degradation (Jain et al. 2016).

BESs tools or approaches are applied for wastewater treatment purposes but its organic waste degradation capacity (on BES anode electrode) BES anodes) is found to be poor in nature without directly discharging of electrons or reusing of system. For enhancing the degradation of waste matter performance, BES cathode electrode is applied as additional components treatment for selected or specific contaminant via investigating the number of approaches and these have been grouped into cathode-stimulated treatment (involved electron transfer directly with reduction of contaminants like NO_3^- ion or dye compounds) and cathode-supported treatment (accomplished with toxic contaminant removal occurred by aerobic condition oxidation, algal strain biomass cultivation as well as strong oxidant compounds production for advanced mode of oxidation or membrane mediated treatment). Cathode promoted wastewater treatment process via BES reactions can judge the challenge or problems, with offering good suggestion or recommendations on the future period development of BES designs or operations for best wastewater treatment performances (Jain and He 2018) and shown in Fig. 8.3.

A lot of quantities of wastewaters generation are coming from domestic or industrial resources, posed several challenges (water crisis issue or environmental deterioration) to our healthy environments. Sustainable nature of energy generating, effective wastewater treatment system can provide plausible solution via application of microbial electrochemical technologies with completion of simultaneous treatment of wastewaters. These systems efforts can be big achievement in recovery of clean energy with reclamation of usable water. Various types of configurations, electrode assemblies, and effective designs of MFCs system have been reported for degradation of industrial or domestic origin wastewater via application of indigenous

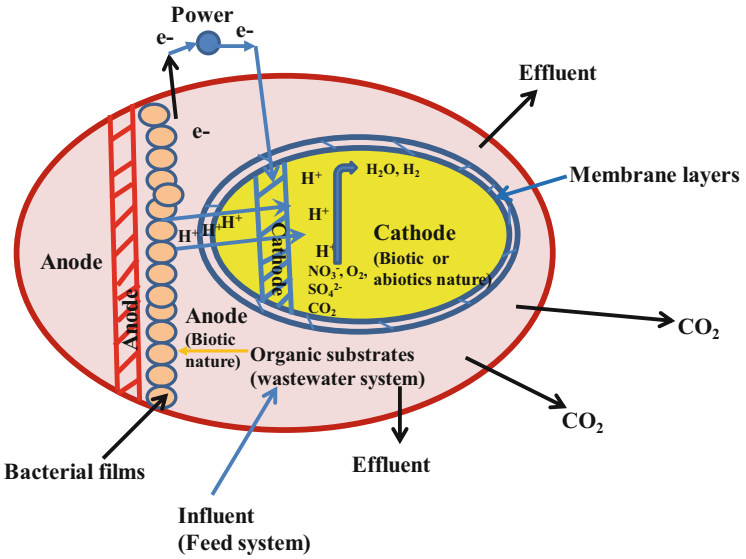

Fig. 8.3 Biochemical system (BES) design utilized for clean energy and energy and electricity power generation via proceeding wastewater treatment (Pant et al. 2012)

or enriched electrogenic microbial systems. Conventional mode of single or dual chambered MFCs system has shown to integrate hybrid MFCs and is applied at scaling up from laboratory level to pilot level with making of various modes of advancements in technical or scientific operation that can be applied for wastewater treatment as well as power generation tasks from few recent years (Rathour et al. 2019) and some waste concentration with its values after bioprocesses treatment is shown in Table 8.1.

8.4.1 Bioelectrochemical Systems (BESs) and Energy Generation

Anaerobic digestion (AD) is normally performed at low temperature and it is shown as attractive technology. At moderate climates (low temperature), this AD is reported for low microbial activity with facilitation of low rates of methane formation. And bioelectrochemical systems (BESs) are reported for enhanced methane production at low temperature via utilization of organic matter in anaerobic digestion (AD) processes. At 10 °C, this methane (CH_4) production is occurred at bioelectrochemical reactor and reported to operate with granular activated carbon as electrodes. Bioelectrochemical systems have been resulted for enhanced CH_4 yield with accelerated rate of CH_4 production as well as increased acetate removal efficiency at 10 °C (Liu et al. 2016).

Table 8.1 Reduced wastes or toxic compounds concentration in bioelectrochemical systems (BESs)

S. No.	Wastes in effluent	Reduced value of wastes in processes	References
1.	Nitrate or dye compounds	Cathode-stimulated treatment reported with reduced contaminants (nitrate or dye compounds) at is operated at mild conditions with microbes involvement	Jain and He (2018)
2.	Toxic or waste organic compounds	A tubular shaped single chambered, continuous MFCs can generate high power outputs having granular graphite matrix (the anode) and a ferricyanide solution (cathode) with reduction of toxic or waste organic compounds	Krieg et al. (2014), Rathour et al. (2019)
3.	Nitrogen and P nutrients in wastewater	Membrane coupled with complete anaerobic treatment of wastewater for renewable fuel (electricity) generation via direct biological conversion of organic matter containing N or P components	McCarty et al. (2011)
4.	Volatile solid (VS) and total solid (TS) reported from two effluents	Mechanical treatment is used in dairy waste treatment with reduction (more than 80%) of the volatile solid (VS) and total solid (TS) reported from two effluents	Lhanafi et al. (2017)
5.	Petroleum produced water (PPW) and petroleum crude oil	Application of BES system under uplifted cathode potential has shown good treatment efficiency with reduced waste concentrations in terms of COD (91%) and hydrocarbon removal (71%)	Jain et al. (2016)
6.	Hydrolyzed product and soluble organic residue with higher COD (3600 mg COD/L)	The biogenic conversion of coal to methane improved from polarization of the electrode. In the bioelectrochemical (BEC) reactor with diluted hydrolysis of product reported with improved methane yield	Song et al. (2016), Piao et al. (2018)
7.	More COD with ammonium nitrogen and phosphate ions	The IPB system helps in removal of more value of (more than 92%) more than 92% COD with ammonium nitrogen (98%) and phosphate (82%). This system has produced a maximum power density (2.2 W/m^3) and algal biomass growth (128 mg/L)	Xiao et al. (2012), Sun et al. (2019)
8.	Higher chemical oxygen demand (COD) with ammonium compounds	Combined process of bioreactor succeeded with removal of chemical oxygen demand (COD ~85%) with	Hu et al. (2017), Lee et al. (2007)

(continued)

Table 8.1 (continued)

S. No.	Wastes in effluent	Reduced value of wastes in processes	References
		ammonium (80%). It has shown resultant effluent concentrations of COD (1591 mg/L) and ammonium (61 mg/L) as more reduced values	
9.	BOD5/COD ratio	Ozonation process enhanced the biodegradability of the anaerobic effluent and is also reported with reduced BOD5/COD ratio (0.15–0.33) from CSTR and IC bioreactors. And SBR has shown more reduced BOD5/COD ratio (0.07) by using biological processes. Sulfate removal efficiency (65%) was also reported from this bioreactor	Hu et al. (2017), Lee et al. (2007)

Highest CH_4 yield (31 mg CH_4. COD/g.VSS) is reported from combined BES-AD process with cathode electrode potentials (−0.9 V-Ag/AgCl) and it can be helped to achieve the 5.3 to 6.6-fold higher or more methane production than AD reactors at temperature of 10 °C. CH_4 (methane) generation rate in integrated BES-AD process at 10 °C has slightly lower value than the AD reactor at 30 °C. External circuit system between the acetic acid oxidizing reaction at bioanode and methane production at cathode electrode is found by utilization of alternative pathways. Electrons from acetate ion to methane are reported during hydrogen production. Methanogenesis from acetate as alternative pathway has helped in higher quantity of methane production rate at low temperature. Combination of BES with AD system is shown as attractive alternative strategy for enhanced performance of AD process in cold areas via performing wastewater treatment (Feng et al. 2017; Liu et al. 2016). In other reports, it has shown enhanced biogenic processes for coal conversion to methane gas and it is reported for bioelectrochemical reactions in anaerobic reactor at polarized electrodes. This electrode (1.0 V polarization) is reported for higher methane yield from coal (52.5 mL/g) lignite. Application of electrode (2.0 V polarization) has minimized the adaptation periods for generation of methane from coal substrates and has slightly less methane yield than 1.0 V value of electrode (Piao et al. 2018).

CH_4 generation from coal resources in the BEC reactor is reported with hydrolysis processes of methane products with soluble organic residues for higher COD value (3600 mg COD/L value). The hydrolyzed product is reported with substrate inhibition effects and can be further inhibited from coal conversion to CH_4. It has reported that diluted hydrolysis product can mitigate the substrate inhibition to methane production. 5.7-fold diluted hydrolyzed product can be inhibited by the methane conversion rate (50%). CH_4 yield (55.3 mL/g lignite) is reported from hydrolyzed product (diluted tenfold) via confirmed anaerobic condition toxicity test.

The biogenic mode of conversion of coal to methane is enhanced from polarization of the electrode in BEC anaerobic reactor with diluted hydrolysis product improved with methane yield (Song et al. 2016; Piao et al. 2018). BES is reported to utilize the MFCs and MECs units and these systems have capability for conversion of biodegradable organic matter into electric energy (Kim et al. 2009).

These systems are found for production of hydrogen bioenergy via application of a microbial cells biofilm on the electrode (biocatalyst). Waste to energy (WTE) technology via MFCs system can be found for treatment of organic contaminant waste coming from domestic or industrial sources wastewater via simultaneous mode production of electric power. From these systems, the maximum power densities (up to 1 kW/m^3) are reported based on reactor volume or size (Rozendal et al. 2008).

8.4.2 Energy from Bioelectrochemical Systems (BESs) with Microbial Fuel Cell (MFC)

Integrated photo-bioelectrochemical (IPB) system has been applied via installing MFCs with algal species containing treatment processes and it has achieved simultaneous removal of organic matter in synthetic solution of MFCs system and also provided nutrients for algal bioreactor. It has shown bioenergy production in form of electric energy with algal biomasses via utilization of BEC and microbiological processes (Kakarla and Min 2019). During the 1 year operation time, EMP system has helped in removal of more value of (more than 92%) COD with ammonium nitrogen (98%) and phosphates (82%). These systems have produced a maximum power density (2.2 W/m^3) and also algal biomass (128 mg/L) (Xiao et al. 2012; Sun et al. 2019). Algal biomass cultivation has produced more dissolved oxygen (DO) value to the cathode reaction side in the MFCs system whereas BES, oxygen reduction is found at MFC cathodes using buffered pH for best medium for algal biomass (known as catholyte). The performance of BES system is affected by illumination periods and DO level. Initial level energy analysis has shown for IPB system that can generate huge quantity of energy in theoretic value via covering its consumption and improved electric power generation. The analysis of the attached or suspended microbial strain at cathode electrode is revealed that diverse nature of bacterial cell texa group of typical aquatic or soil microbial communities. These can achieve the functional roles in contaminant degradation or removal with nutrient cycling (Sun et al. 2019; Kakarla and Min 2019).

Bioelectrochemical systems have shown their application via reduction of energy consumption during wastewater treatment and helping in replacement of energy intensive aeration in waste treatment systems. These systems are also generating electrical energy. Biomass productions in MFCs are reported in range of 10–50%, depending on the microbial cell nature in conventional wastewater treatment with reduction in environmental impact or disposal costs. In this regard, various

electrochemically active bacteria have shown their capability for metabolizing biodegradable organic compounds via discharging the electron to an extracellular mode of electron acceptor during bacterial respiration processes. From these bacteria, it has transferred the electron to electrode site via direct mode of electron transfer, electron mediator or shuttles, and electric mode conductive nanowires (NWs) as electron mediator systems (Reguera et al. 2005).

Bacterial electron transport mechanisms have shown better understanding of the biomaterial functions or roles that involved or utilized metabolic pathways for improvement in power generation from MFCs. Biofuel cell systems information can be improved via performing necessary interdisciplinary research works via involving the electrochemistry, microbiology, materials sciences, or surface chemistry with engineering approaches to reactor designs or operation and modeling. From these fields, integration of research systems can generate via increasing the performance or efficient and feasible capacity of BES process for sustainable mode energy generation (Kim 2009; Reguera et al. 2005).

The production of electric power from microbes has been demonstrated with scientific enquiries and electrical power generation is associated with the decomposition of organic compounds. Use of electrochemical devices can be done for harvesting electric energy, coming from the microbial decomposition of organic substrates due to concept of a bioelectrochemical processes. Alternative options for fossil fuels have been intensified with advanced research and significant interest in the scientific community. Driven the prospective application is reported with production of sustainable energy and other synergetic benefits via doing the wastewater treatment and resource recovery. Perspective of production of fuels, electricity, and chemicals is reported by using bioelectrochemical systems (Schirmer et al. 2010).

Production of biofuels and valuable chemicals is found on the basis of working principles of bioelectrochemical systems. Integration of biorefineries with bioelectrochemical systems for the enhanced synthesis of biofuel or other variable chemical production can be shown state-of-the-art thermodynamic feasibility models and methods for evaluating the economic viability of the integrated systems of wastewater treatments (Shemfe et al. 2017; Schirmer et al. 2010).

8.4.3 Energy from Microbial Electrolysis Cells (MEC) and Bioelectrochemical Systems (BESs)

Bio-H_2 synthesis is found from MEC system via performing effective treatment of wastewater and has reported more potential for its application with wastewater generated from industries. These have shown in reduction energy consumption and more economical costs of operation and contained both electrodes and are performed at anaerobic nature. Engineering approach of these systems can be shown effective strategy for microbial fuel cell counterparts, retrofitting into the present or current day status of infrastructure of wastewater treatment. Critical

parameters for assessment of MEC system performance are analyzed with assessment of research on MEC function that rhetorically matches the reality. It has shown to generate valuable product (hydrogen) with further testing at plausible level scale under real process conditions (Cotterill et al. 2019).

Application of a MEC system is used for enhancing biogas production at AD reactor with its effect analysis on rate of methane production is reported with utilization of food waste organic matter. AD reactor performance has compared with combined forms of an AD bioreactor integrated with A MEC system (AD +MEC) and these combinations of systems have shown the accelerated CH_4 generation with stabilized and fast organic matter oxidation processes and fast speed of methanogenesis bioprocesses. CH_4 biosynthesis rate with its stabilization periods for AD and MEC bioreactor has reported faster (1.7- and 4-fold) than single AD reactor. At final steady state, the CH_4 yields are found to have same value of the theoretical maximum methane yield. MEC alone cannot increase or enhance the CH_4 yield over theoretic calculation value but it has enhanced the CH_4 biosynthesis and stabilization of BES reactors (Park et al. 2018).

MECs systems are reported to consume the chemical energy from organic matter and it helped in bio-H_2 synthesis. New and hybrid MECs design has shown its improved performance for this fuel generation via achieving the wastewater treatment. This MEC design is reported with externally aligned TiO2 nanotube (TNT) array of photoanodes that is fabricated by anodization of Ti foil and it is supplied for photogeneration of electron current to the MECs mediated external circuit with improved overall performance. The photo-process mediated electron generations have helped in reduction of electron depletion at bioanode. These approaches have improved the proton reduction reactions at the cathode electrode. This 28 mL hybrid MEC operations are performed under stimulated (AM 1.5) illumination (100 mW/cm^2) and shown to exhibit a H_2 evolution rate (1.4×10^3 mmol m^{-3} h^{-1}) a maximum current density (0.371 mA cm^{-2}) and power density (1.4×10^3 mW m^{-2}) and these show 30.8%, 34% or 26% more value than a MEC reactor under dark fermentation condition (Kim et al. 2018).

8.4.4 Bioenergy from Wastewater Treatment via Other Approaches

The bioethanol industry has reported with more demand of water supply and present periods water consumption rate (11–15 dm^3 m^{-3}) in corn grains dry grinded in ethanol production plant is reported for ethanol production in cellulosic ethanol plants capacity (23–38 dm^3 m^{-3}). Feasibility of use of treated wastewater effluent is reported for cellulosic mode or advanced ethanol synthesis with help in making potable freshwater. Two different sources of filtered treated effluent are reported for case study of Bloomington- normal (IL) as sources from resident locations and Decatur IL as mix type waste fractions from industrial and residential nature. It

has evaluated and compared the fermentation rate and end product (ethanol) yield from pure form of cellulosic substrates. Analysis for characterization of components has been done for both types of effluent water samples after completion of fermentation processes with ethanol production and reduced quantity of toxic elements. Final ethanol yield (0.36–0.37 g g^{-1} and 0.36 g g^{-1}) reported from Bloomington normal and Decature effluent, respectively, in controlled conditions treatment via applying deionized water. Proper ways of characterization studies under suitable conditions can help in effective treatment of effluent water from production pg cellulosic ethanol in feasible quantity (Ramchandran et al. 2013).

The treatment of wastewater has been achieved with cellulosic ethanol production in biorefinery industries, shown as special challenges. In this regard, a pilot-scale process ethanol production has utilized by using some bioreactors such as CSIR system, an internal circulation (IC) reactor, sequencing batch mode reactor with enhanced ozone oxidation. These have been innovated for effective treatment of biorefinery wastewater which is a challenging task. And very interesting reports have come from CSTR and IC bioreactor application with reduction of COD value in anaerobic treatment. SBR process has shown with nitrogen removal via application of alternating aerobic nitrification and anaerobic denitrification (Lee et al. 2007).

Combined process of bioreactor has been succeeded with removal of 85% of COD value with 80% of NH_4^+ ion. Resultant effluent with COD (of 1.6×10^3 mg/L) and NH_4^+ (61 mg/L) value has reduced values. Further, ozonation process enhancing the biodegradability of the anaerobic effluent is reported with reduced values of BOD5/COD ratio (0.15–0.33) from CSIR and IC bioreactors, respectively. And SBR has shown more reduced BOD5/COD ratio (0.07) by using effective biological process by using a biological process. SO_4^{2-} ion removing efficiency (65%) has reported in using of alternating anaerobic and aerobic processes with final effluent with SO_4^{2-} ions concentration (217 mg/L) (Hu et al. 2017; Lee et al. 2007).

Ethanol concentrations using conventional dry grind procedure have determined for some interval periods (every 2 weeks) for 1 year and these have reported with variations in ethanol concentration with variability pattern for commodity and corn supply. Highest concentrations of ethanol have reported in the month of January due to storage time variation and it has considered for significant factor, affecting ethanol concentrations. It has also shown the effect of various enzymes treatment on mean value of ethanol concentration over a period of year. Two enzymes of liquefaction enzymes have applied at optimal range (pH of 5.8 or 5.1 and two enzymes of saccharification process are used at optimal pH (5). And protease has used in five enzymes treatment for 1-V type treatment. It has reported that the final ethanol concentration (17.5 %v/v) with enzyme treatment V (0.6% more) compared to enzyme treatment 1 has applied for additional amount of ethanol synthesis (600,000 to 100 million gallons/year) in an ethanol plant. More effective enzymes have helped for increased overall dried corn based ethanol plant with more profit value (Ramchandran et al. 2015) and some examples are shown in Table 8.2.

Table 8.2 Energy from wastewater via utilization of bioelectrochemical systems (BESs)

S. No.	Energy	Waste or wastewater treatment	References
1.	CH_4 yields from PW (25.546 ml STP/gVS) and LDP (79.1 ml STP/gVS)	Effluents containing pure whey (PW) and loss of dairy product (LDP) in anaerobic wastewater treatment	Lhanafi et al. (2017)
2.	CH_4 yield (31 mg CH_4.COD/gVSS) 31 mg CH_4-COD/g VSS)	From combined BES-AD system at 10 °C with bioelectrochemical reactor	Liu et al. (2016), Feng et al. (2017)
3.	Methane yield (55.3 mL/g lignite)	From hydrolyzed product (diluted tenfold) with polarization of the electrode in the BEC anaerobic reactor via confirmed toxicity test	Song et al. (2016), Piao et al. (2018)
4.	Methane production rate	1.7 times higher for AD + MEC reactor	Park et al. (2018)
5.	H_2 evolution rate (1.4×10^3 mmol m^{-3} h^{-1}) with a current density (0.37 mA cm^{-2}) and a power density (1.4×10^3 mW m^{-2})	28 mL hybrid MEC operations are performed under simulated AM 1.5 illumination (100 mW cm^{-2})	Kim et al. (2018)
6.	Ethanol concentrations (0.36–0.37 g g^{-1}) and 0.36 g g^{-1})	From Bloomington normal and Decature effluent, respectively, in control conditions treatment using deionized water of ethanol production in cellulosic ethanol plants capacity (23–38 dm^3 m^{-3}) via fermentation processes	Ramchandran et al. (2013)
7.	Ethanol concentration (17.5%v/v)	Two liquefaction enzymes have applied at optimal range (pH 5.8 and 5.1) with saccharification by two enzymes at optimum pH (5). And enzyme protease used in five enzymes treatment (1-V)	Ramchandran et al. (2015).
8.	Production rate (81 ml/L/day) of hydrogen	In a two chambered MRCs used for mixture of VFA in the efficient of a dark fermentation	Rivera et al. (2015)
9.	Maximum hydrogen production (0.53 L/L day), after 3 days	2.5 L MECs with eight separate electrode pairs (graphite fiber brush anodes pre-acclimated) for current generation using acetate compound with 304 stainless steel mesh cathodes (64 m^2/m^3)	Rader and Logan (2010)
10	Increase of 12% in biogas production over the control (1353 mL CH_4 day^{-1} at an injection flow rate of 1938 mL H_2 day^{-1})	Hydrogen pulse addition on digestion performance of sewage sludge reported with increase in efficiency of methane production in theoretical process of coupling bioelectrochemical systems (BES) along with H_2 biosynthesis and subsequent AD process	Martinez et al. (2019)

8.5 Conclusions

Wastewater generation in huge quantity is reported from various sources such as municipal, domestic, or institutional places with food processing, agricultural, or industrial industries. Lot of money is reported to consume for its efficient treatments. Many toxic or useful waste sources in form of organic compounds or elements are present in wastewater effluents and it has posed many environmental issues with big challenges. It needed to apply effective treatment for biodegradable compounds before disposal to open into open environment. Currently many effective treatments (i.e., bioelectrochemical system ~BES) are employed for treatment of different types of wastes in water or other industrial effluent via generation of electric power or other biofuel generation (hydrogen, methane, and ethanol). BES is reported to utilize the MFCs or MECs for generation of different types of bioenergy with reduction in toxic compounds or useful nutrients. BOD and COD value in polluted water bodies is reported to reduce with generation of sustainable energy. Sometimes some saccharfication enzymes are used for hydrolysis of complex waste residues (cellulose) during ethanol production.

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Chapter 9
Harvesting Energy Using Compost as a Source of Carbon and Electrogenic Bacteria

Fabio Flagiello, Edvige Gambino, Rosa Anna Nastro, and Chandrasekhar Kuppam

Abstract Compost is widely used to improve soil fertility for its chemical–physical properties, with particular regard to the abundance of humic substances. Compared to the untreated organic solid waste, the use of compost in microbial fuel cells (MFCs) could offer different advantages like the strong reduction of fermentative processes. The use of compost in MFCs in combination with soil or mixed with other substrates had been reported by some researchers to improve the performance of MFCs fed with agro-industrial residues and plant MFCs. In this chapter, we report the results of an experiment carried out using a compost of vegetable residues as feedstock in a single chamber, air cathode MFCs. We investigated the behavior of two MFCs serially connected, the possibility to use compost as a long-term source of energy in MFCs, the influence of cathode surface/cell volume ratio on MFCs performance in terms of power and current density. Our results showed for MFCs serially connected a maximum PD and CD of 234 mW/m^2 and 1.6 A/m^2, respectively, with a maximum OCV of 557 mV. Unexpectedly, the compost-based MFCs kept significant electric outputs (854 mV, 467 mW/m^2 kg, and 114 mA/m^2 kg) after being reactivated 2 years later its set-up, thus demonstrating its potential as long-term operation energy system.

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Keywords Compost · Microbial fuel cells · Voltage reversal · Waste management · Waste-to-energy systems

9.1 Introduction

Since the Kyoto protocol agreement, the production of green energy to sustain the cities growth was the focus of worldwide public opinion and, because of this, an interesting topic of political debates. Despite the big evolution of green energy technology and the amount of money invested in research and development, we are still so far from reaching a worldwide stable economy based on green energy plants. We still need the "old good oil" to perform most of our daily tasks and support the big amount of overall energy request. Great efforts are being performed to replace fossil fuel, with some recent encouraging results: Costa Rica, using a very interesting mix of green technologies was recently able to fulfill the energy demand covering 300 days/year. This achievement did not include the local transport system, still based on fossil fuel. The secret of oil success can be summarized in two simple sentences: flexibility of use (Ferreira Coelho and Szklo 2015), enough amount to sustain the modern civilization growth for years and affordable costs, even for developing countries. Among renewables, biomass-based systems are very good candidate technologies to obtain both energy and energy vectors (H_2 and CH_4) for the availability of substrates produced in the agroindustry, agriculture practices, and everyday life (Florio et al. 2019).

9.1.1 Organic Waste: A Modern Gold Mine

Billions of tons of food are produced in every corner of the Earth (Food and Agriculture Organization of United Nations 2018), with the consequent increasing amount of waste. For this reason, waste management is becoming of outstanding importance to reduce environmental damages due to leachate leaking, greenhouse gas emission, microplastics diffusion in the environment, etc. (UNEP 2015). In recent years, organic waste has been considered more and more like a resource for both energy and commodity chemical production. The new approach towards waste management have to be essentially focused on the 3R concept (reduce, reuse, and recycle), cleaner productions, circular economy establishment, waste prevention, and, finally, the transformation of waste into a source of energy and commodity chemicals (Nastro et al. 2016; Florio et al. 2019; Venkata Mohan et al. 2016). With the advances in the green chemistry and consequent advances in biorefinery, the range of molecules obtainable from biomass is increasing more and more, thus changing the "waste" into "raw material for new biosynthesis processes." Different biosynthetic routes are already available like acidogenic bacteria-based processes: biohydrogen and biohythane production and carbohydrates fermentation are two

examples. Like oil, organic waste is mainly composed of carbon. Both of them can be used to produce fuel for the automotive sector (Basso et al. 2016) and, both of them, can be used to produced plastic compounds with the advantage, in the case of the organic waste, of the biodegradability (Sharma et al. 2018) and a very low carbon footprint. Furthermore, the organic waste can be converted in compost, a very well-known fertilizer useful for damaged soils and to recover bacteria communities able to act as biochemical refinery under different environmental condition (Aresta et al. 2012). These examples of use open to a wide range of growing economic opportunities as highlighted by Levidow (2018).

9.1.2 Compost: From Where All Start

Whenever we want to recover a portion of our garden's soil or we want to improve the performance of our cultivar, we buy a fertilizer. Not all the fertilizers are the same, and they gave rise to a lot of scientific debates on their long-term effects on the environment (Xin et al. 2016). In the last decade, even thanks to the circular economy philosophy, the focus of the scientific community has been mainly on the organic fertilizers that can be recovered from organic waste. Compost is one of the best examples of this recovering and is widely used as a soil amendment (Adugna 2016). The composting is a three-phase process: intensive decomposition, stabilization, and maturation, with the stabilization as the shortest phase (Dimambro et al. 2016). In Fig. 9.1 we report a diagram of the German Rottegrad classification of the different phases succeeding each other during the composting process.

In the first step, organic matter is degraded by thermophile bacteria at a temperature near 60 °C. The most common bacteria strains at this stage are *Bacillus* spp., *Thermoactinomyces* sp., *Stearothermophilus* sp. (Daas et al. 2016). Soon after the oxidation phase (intensive decomposition), the temperature cools down (stabilization stage): in this phase, compost becomes more rich in aromatic structures while aliphatic and alcoholic structures are degraded. During the maturation phase the temperature goes down at 30 °C, the organic elements are already completely converted and lots of mesophilic biochemical reactions complete the chemical enrichment of the matter. The big complexity of these reactions is still under investigation by scientists, who see the opportunity to use biowaste as a "biorefinery" (Fava et al. 2015), able to produce biodegradable chemical compounds in a change of the ones coming from oil. Anyway, there is another aspect not well known about the compost: the ability to participate, directly or indirectly to electrical energy production.

9.1.3 The Blood of Our Society: Electrical Energy

According to the definition of the famous "Encyclopaedia Britannica (2019)," energy is: "*the capacity of doing work*. . ." and all we know there are lots of different

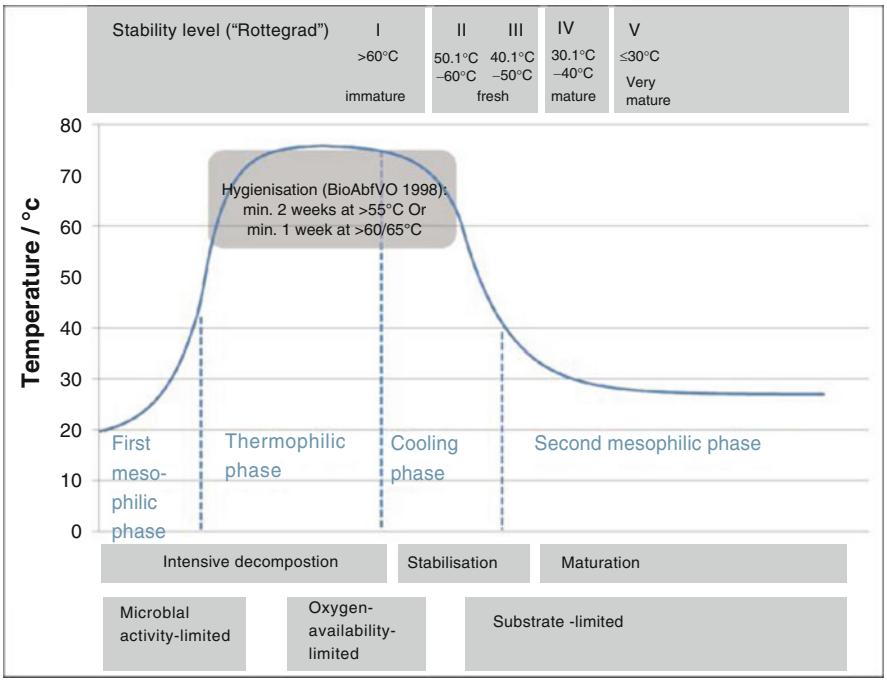

Fig. 9.1 A diagram of the German Rottegrad classification for compost maturity. The graph shows the temperature plotted over time (from Dimambro et al. 2016).

types of energy, as potential, chemical, thermal, and so on but, probably, the best-known energy is the electrical energy. Electricity is the "blood" of modern society; its flow powers our lamps, notebooks, smartphones, medical devices, cars, our houses. Without electricity we could never had the big development of the last centuries, so taking into account this, it is worth noting that the production of electricity was, and actually is, always based on the use of oil and its derivate products that are, unfortunately, very pollutant. Limiting pollution is a modern mission of governments and scientists have discovered different alternative sources to produce electricity with a nearly zero environmental impact like solar panels, wind farms, biomasses. One of the problems with these technologies is related to their low energy production efficiency and their discontinuous working operation. If we add also the big costs and the maintenance of the production plants, it becomes understandable why so difficult a definitive worldwide shift is. So, scientific research needs to face the above problems by finding new ways to produce electrical energy, limiting greenhouse gas emissions and costs while granting the satisfaction of world energy needs. In this framework, a combination of biology and engineering could give an important contribution to shift from a fossil-fuel-based to a green energy society.

9.1.4 *Microbial Fuel Cells: A New Paradigm in Green Energy Production*

The production of electrical current from bacteria was known since 1911, when Potter experienced for the first time *Escherichia Coli* as "bio-extractor" of electrons from organic matter. The results obtained were discouraging compared with the energetic alternative performances of the historic period. We had to wait until 1993 to obtain a serious interest in the argument, when Allen and Bennetto (1993) realized the first prototype of an electrochemical bioreactor able to reach interesting results in terms of current density using *Proteus vulgaris* as microorganism and, as substrate, glucose. That reactor was one of the first modern double chambers "microbial fuel cell" (MFC). A basic MFC is a bio-electrochemical device composed of two physical elements, an anode and a cathode, which can be placed in two chambers where different environmental conditions are realized (Logan et al. 2006). Each chamber contains an electrode, generally made by carbon-based material. An ion exchange membrane divides the two compartments, thus preventing the flow of unwanted substances among the two chambers while allowing anions or cations (according to the chosen membrane) to pass from the anode to the cathode compartment. In this last configuration, very useful for research tests, the catholyte and the anolyte are independent, and parameters as pH, bioecological dynamics, kind of substrate, the effect of different electrolytes can be easily taken under control. Also, the internal resistance is very low due to the lack of physical or chemical obstacles as unexpected biofilm formation on the electrodes or chemical electron competitors. The side effects are related to the high costs of production and maintenance in the long run, the efficiency of the membrane, and its durability (Stoll et al. 2016). In most part of cases, proton exchanging membrane, like Nafion, is used for lab-scale experiments (Khan et al. 2017; Koók et al. 2017). Cations exchanging membrane made up by ceramic, eggshell, and other cheap materials are becoming more and more popular and recommended for an in-field application of MFCs to liquid/solid waste treatment (Ortiz-Martínez et al. 2016; Chouler et al. 2017; Khan et al. 2017; Nastro 2014). Unlike two-chamber MFCs, in a single-chamber MFC both anode and cathode share the same compartment (Fig. 9.2). In a single-chamber MFC, both electrodes are soaked in the same feedstock. A wide range of substrates could be used to power MFCs: pure solutions containing an organic molecule acting as a source of chemical energy for bacteria or more complex substrates like municipal/industrial wastewater, biomass, and even compost (Nastro et al. 2016; Khan et al. 2017; Santoro et al. 2017; Florio et al. 2019; Zhao et al. 2017; Gambino et al. 2017; Moqsud et al. 2015) are examples of energy sources for bacteria in MFCs.

Regardless of the carbon source available, all MFCs are based on the metabolism of electroactive or exoelectrogenic bacteria and on their ability to exchange electrons with the anode placed into an anoxic/anaerobic environment (Logan 2009). One of the factors affecting MFCs performance is the nature of the molecule acting as electrons acceptor at the cathode, i.e. the step of potential established between the anode and the cathode. Such a step is the force driving the electrons to flow towards

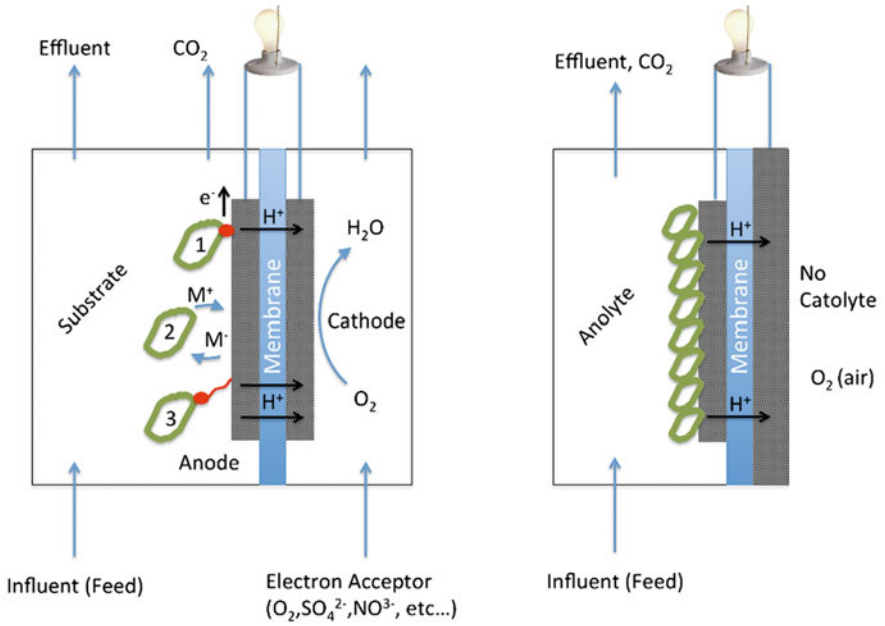

Fig. 9.2 Schematic of the double-chamber and single-chamber MFC (from Nastro et al. 2016)

the cathode: the higher is the step of electric potential between the electrodes, the higher is the current density potentially produced (Ucar et al. 2017). Other factors that can limit MFCs performance are: activation, concentration, and ohmic losses (which can be revealed during polarization experiments (Chandrasekhar et al. 2017)), pH of the substrate used as source of chemical energy, environmental temperature (Xu et al. 2018) as well as biofilm age (Paitier et al. 2017). Both biological and electrochemical processes in MFCs result in electrons able to power electrical devices like biosensors (Chouler et al. 2018), robots, small medical instruments and so on (Santoro et al. 2017; Dong et al. 2013).

9.1.5 Compost as a Source of Electrogenic Bacteria

Compost can be considered an important source of exoelectrogenic bacteria, thanks to its unique biochemical genesis. During the thermophilic and mesophilic phases, it is possible to isolate bacteria belonging to *Geobacillus* and *Bacillus* genera, using simple microbiological culture techniques and amplification/sequencing of rDNA 16S. It is worth noting that all these genera are also involved in other green chemical processes, like the production of biofuels or water depuration (Novik et al. 2018). *Geobacillus* spp. are widely used among different industrial fields, producing various metabolites of commercial use like enzymes, ethanol, and antibiotic substances

like Geobacillin I and II (Garg et al. 2012). *Bacillus licheniformis* and *Geobacillus thermoglucosidasius* can easily survive and carry out their metabolism at high temperatures and, for this reason, can be used in reactors operating at $T > 50^{\circ}$ (Choi et al. 2004). Many microorganisms among the *Bacillus* genus and other gram-positive bacteria have proved their electrogenicity in MFCs. For example, *Bacillus subtilis and Bacillus licheniformis* were used in amperometric biosensor systems for water BOD values (Su et al. 2011), increasing the speed of the analysis. *Bacillus firmus* was, instead, tested in a membraneless single-chambered MFC, working in batch mode using glucose, hydrolyzed potato peel, and hydrolyzed cyanobacterial biomass substrates. A maximum power density (PD) of 16.46 mW/m^2 at 62.48 mA/m^2 was achieved using cyanobacterial biomass as the substrate (Singh et al. 2016). Pure culture of *Brevibacillus borstelensis* STRI1 was tested with sugarcane molasses (1.15 g/L) removing up to 82% of COD and reaching 188 mW/m^2 of PD (Hassan et al. 2019). *Bacillus subtilis* was also tested in a double-chamber CEM membrane provided, as electrogenic bacteria to harvest energy from wastewater using graphite electrodes. The results in terms of COD removal and PD were, respectively, of 90% and 270 mW/m^2 (Ismail and Jaeel 2013a).

9.1.6 *Compost as a Source of Energy in MFCs: A Short Overview*

Recently, some papers confirmed the efficiency of bacteria from compost as inoculum in MFCs fed with different substrates, dairy and food waste included (Cercado et al. 2013; Reiche and Kirkwood 2012; Cercado-Quezada et al. 2010a, b). In some other papers, they report the utilization of compost as substrate alone or in combination (Wang et al. 2013, 2015; Khudzari et al. 2016; Moqsud et al. 2015; Nastro et al. 2016). In all cases, the authors report a significant increase in power production when compost is used as inoculum or substrate in MFCs. Moqsud et al. (2015), for example, report an increase of, respectively, two and three times in voltage and in power when compost is mixed in soil in plant MFCs (Carmalin and Sreeja 2017). Wang et al. (2015) explored the possibility to join composting of vegetable residues with power generation in MFCs, giving evidence that electrogenesis can occur during composting conditions. Even though the authors explored the influence of C/N ratio and moisture content on power generation, there is no data about the influence of temperature. Moreover, the whole process occurred in the anode compartment of MFCs, i.e. in the absence of oxygen, so even though they address the whole process as "anaerobic composting" that is not comparable to the well-known compost process. Nevertheless, Cercado-Quezada et al. (2010a) reported for MFCs fed with yogurt waste optimal working temperatures of 40 °C and 60 °C (maximum current density of 1450 mA/m^2 at 40 °C). In this chapter, we explored the utilization of homemade compost as a substrate for energy recovery in a single chamber, air cathode MFCs. We carried out two distinct yet connected experiments:

the first one was to investigate power production in two homemade compost MFCs serially connected in a stack and the possibility to use compost as a long-term source of power. The second experiment investigated the performance of MFCs fed with compost from a solid waste treatment plant, with particular regards to cell volume/cathode surface.

9.2 Materials and Methods

9.2.1 Homemade Compost-MFCs Stack

As a first step, we set up 500 mL MFCs by using a common PET bottle for sampling activity as cell and graphite rods as electrodes, as reported by Gambino et al. (2017). The feedstock was prepared by mixing 100 g of soil, 300 g of homemade compost, 200 mL of sodium acetate solution (10% w/v), 100 mL of PBS. The anode was buried in the mix of soil and compost at about 2 cm below the surface of the solid layer. The cathode was placed at about 5 cm from the anode, soaked in the liquid phase of the feedstock (Fig. 9.3). pH was set at 7.2 ± 0.2. Voltage was measured by a Keithley Multimeter and the current produced was calculated according to Ohm's law, normalized to the cathode surface (mA/m^2). Polarization experiments were carried out on a two-weeks base, using resistors in a range of 256 kΩ–100 Ω. These first MFCs (named $MFCs_0$) were prepared in double replica, connected to a 200 Ω external resistor for 24 h and, then, serially connected. The stack was kept at maximum power and incubated at 30° C for 4 weeks. When a voltage reversal occurred, 10 mL of the liquid substrate containing a source of energy/carbon (10% acetate solution, Trypticase Soy Broth purchased from Oxoid®, 10% glucose solution) was added to the $MFCs_0$ and the stack was left in OCV for 24 h. Once the data collection ended, each MFC was left in OCV for 72 h, then connected to a 1000 Ω resistor and stored at 20 °C. During the following months, the leachate level was

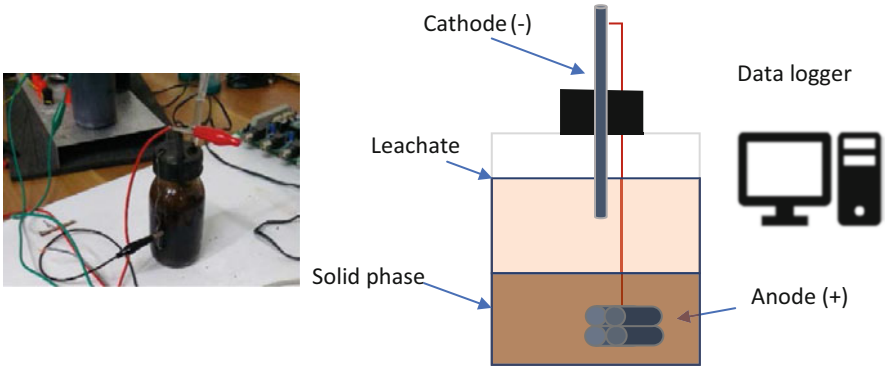

Fig. 9.3 A compost-MFC (on the right). On the left, a schematic of the MFC-data logger system

kept constant by adding sodium acetate solution (10% w/v) and PBS in a mix of 2:1 ratio. From time to time, the voltage was measured to verify whether both $MFCs_0$ were able to keep long-term performance.

9.2.2 Industrial Compost-MFCs

In order to collect more data about single compost-MFCs behavior and performance, after 2 years from the first experiment, we set up MFCs fed with fresh compost sampled at a waste treatment plant in Naples District (Italy). More in detail, we provided glass and plastic bottles of 100 mL (MFC_1 and MFC_2) and 250 mL (MFC_3 and MFC_4) in volume with an anode made up of four graphite sticks (5 cm in length, 0.5 cm in diameter). Like in previous MFCs, the anode was buried in compost and placed at an approximate distance of 5 cm from the cathode. This last one was made of a 5 cm graphite stick (0.5 cm in diameter, 8.0 cm^2 total surface) and put at the interface between the feedstock and the air (Fig. 9.3). An insulated copper wire was used to connect the electrodes. MFCs feedstock was a suspension of compost/saline solution (1:2 w/v ratio). The solution was prepared using NaCl in distilled water (0.9% w/v) and phosphate buffer solution (PBS, Oxoid) in a 1:2 ratio. The final pH was set at 7.5 ± 0.2. For this second experiment, we added no acetate but the organic compounds in compost were the only source of energy since the very beginning. Nevertheless, in order to reduce the MFCs start-up period, an inoculum of 25 mL and 10 mL of leachate from $MFCs_0$ was added to MFC_1/MFC_3 and MFC_2/MFC_4, respectively. To investigate the electrochemical performances, a data acquisition system made up of an ARDUINO based MEGA 2560 was set up to record the values of voltage. The current was calculated according to Ohm's law. MFCs were monitored for 4 weeks, with no nutrients refill. Polarization experiments were performed every week using a range of 256 kΩ to 100 Ω resistors as well. After every polarization experiment, MFCs were set at the maximum power for 5 days before being set in OCV for 6 h. Then, the same cycle was repeated and data was collected.

9.2.3 Anode Biofilm Screening

Anode biofilms were sampled with a cotton swab and treated according to Florio et al. (2019). A basic screening on viable microorganisms was carried out with media for bacteria culture (Oxoid©). A PureLink™ Microbiome DNA Purification Kit (INVITROGEN©) was used to extract genomic DNA from microbial isolates and 16S rDNA sequences were amplified by Real Time-PCR (UNO96 HPL Thermocycler, VWR). A sequencing similarity search was performed using the BLAST algorithm referring to the GenBank database.

9.3 Results and Discussion

9.3.1 Homemade Compost-MFCs Stack

The stack of two $MFCs_0$ serially connected, during the first month, achieved a maximum PD and CD of 234 mW/m^2 and 1.6 A/m^2, respectively, with a maximum OCV of 557 mV. pH ranged between 6.0 and 7.8. We occasionally observed a voltage reversal in one of the two MFC_0. We, then, added 10 mL of 10% acetate solution, TSB, and 10% glucose solution to the feedstock, at different times, to verify if such reversal could have been ascribed to nutrients depletion. Our results seem to support our hypothesis (Fig. 9.4). We observed MFCs highest performance after glucose addition, thus signifying the prevalent utilization of glucose as a source of energy and electrons by the electroactive bacteria. As to pH, we measured a decrease in values soon after adding glucose. Voltage, power, and pH trends suggested the presence of electroactive bacteria able to carry out mixed acid and/or lactic acid fermentation pathways. These last ones allow many microorganisms like bifidobacteria, enterobacteria, clostridia, bacilli, and lactobacilli to recover energy from glucose and other carbohydrates in anaerobic conditions (Ciani et al. 2013). Microbiological analyses of anode biofilm confirmed even in this case our hypothesis, showing the prevalence of Enterobacteria, with *Escherichia coli* and *Acinetobacter* spp. as prevalent strains. As to gram-positive bacteria, *Bacillus subtilis* and *Bacillus* spp. were present, even though in a less concentration. The growth of strictly aerobic bacteria like bacilli at the anode is not unexpected. Some strains like *Bacillus subtilis* can grow anaerobically, either by using nitrate or nitrite as a terminal electron acceptor or by fermentation (Nakano and Zuber 1998) and the

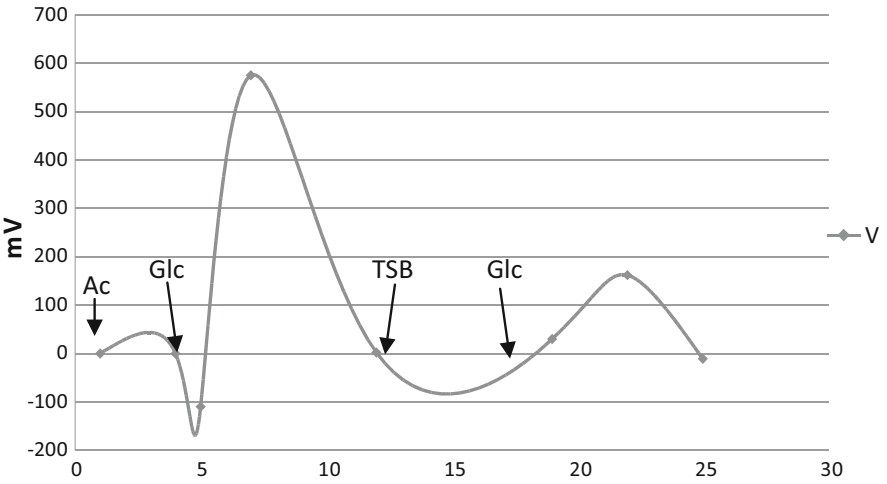

Fig. 9.4 MFC° stack trend over time according to added carbon sources. *Ac* acetate, *Glc* glucose, *TSB* tryptic soy broth

electroactivity of bacilli has been demonstrated by different researchers (Florio et al. 2019; Ismail and Jaeel 2013b). The quite unusual composition of anode microflora can find an explanation in the not perfect process occurring in the home compost-bin. Nevertheless, the performance achieved by the use of not mature compost is far higher than the outputs obtained by the same authors in MFCs fed with the Organic Fraction of Municipal Solid Waste (Nastro et al. 2017; Jannelli et al. 2017; Florio et al. 2019).

A certain voltage instability, with also negative values, was observed in one of the two MFCs° even after its disconnection from the stack, at the end of the experiment. It required several months to obtain stable positive voltages at 1000 Ω external load. After 2 years, we reactivated one of the two MFCs° by replacing 100 g of the spent substrate with fresh compost sampled at the solid waste treatment plant in Naples District. This MFC° after few hours in OCV achieved 413 mV, increasing to 854 mV 3 months later. Polarization curves were performed monthly for 4 months. The MFC° was kept at maximum power for 5 days before being put in OCV for 6 h and, then, perform a new polarization experiment. In Fig. 9.5 we report the polarization and power curves obtained from the 1st till the 4th month of operation, after the reactivation. PD increased with time, achieving 35 mW/m^2 the 4th month and a maximum CD of 140 mA/m^2 the 3rd month (Table 9.1).

9.3.2 *Industrial compost-MFCs*

Polarization experiments of MFC_1, MFC_2, MFC_3, and MFC_4 were performed on a weekly base. Power and polarization curves are reported in Figs. 9.6 and 9.7. MFC_3 and MFC_4 power curves revealed a certain irregularity, with more than one peak and the presence of overshoots. A constant increase in power production and stability was, instead, observed in 100 mL MFCs (MFC_1 and MFC_2), even though the highest PD was obtained in MFC_4 (4.2 mW/m^2) the third week of operation and the maximum CD was achieved in MFC_3 the 1st week (Table 9.1). In all MFCs, pH values ranged between 7.2 and 7.8. It is interesting to notice that, besides MFC_0, the highest performance was achieved in 100 mL MFCs. This result confirmed what was reported by Santoro et al. (2018): "*smaller the microbial fuel cell reactor is, the greater is the power output both density (express in function of the electrode geometric area) and volumetric (express in function of the reactor empty volume).*" In the case of compost-MFCs, the lower is the electrodes volume (or surface)/cell volume, the higher are the energy losses due to fermentative activities taking place in the feedstock to the detriment of electrogenesis.

Anodes biofilm qualitative analyses revealed the presence of *Bacillus licheniformis, Bacillus firmus, Bacillus subtilis, Geobacillus thermoglucosidasius, Brevibacillus borstelensis.* All these strains are among the most characteristic microorganisms in compost and, some of them are proved electroactive bacteria as reported in Sect. 1.5.

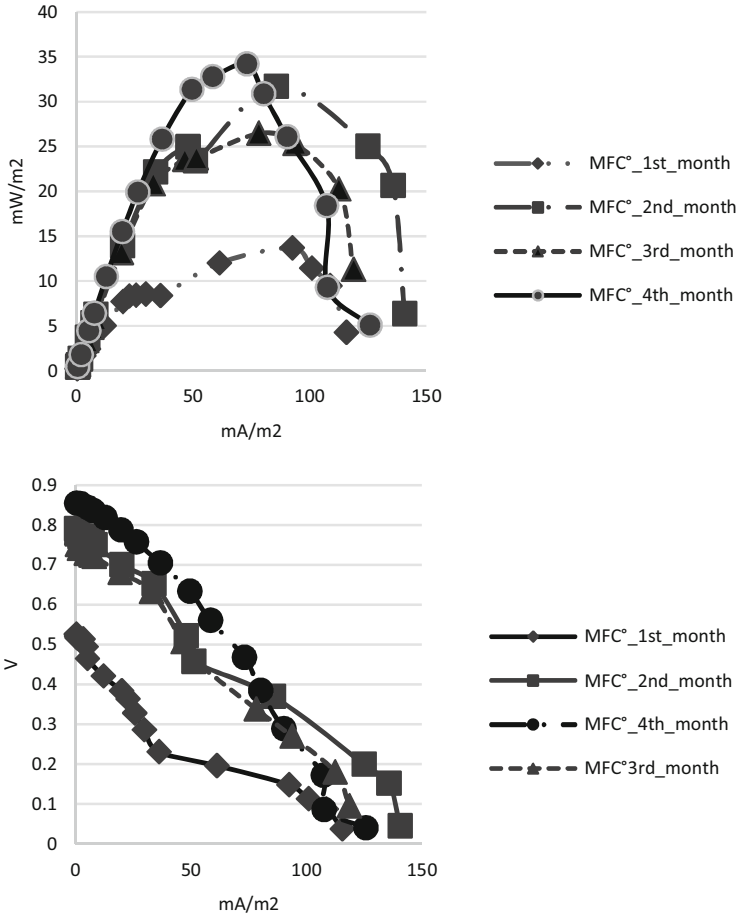

Fig. 9.5 Power and polarization curves after MFC reactivation

Table 9.1 MFCs electric outputs

	Cell volume (mL)	Compost (g)	CD_{max} (mA/m^2)	CD_{max} (mA/m^2kg)	PD_{max} (mW/m^2)	PD_{max} (mW/m^2kg)	V_{max} (mV)
MFC1	100	30	20.8	693	2.3	76.7	230
MFC2	100	30	11.5	383	1.9	63.3	532
MFC3	250	80	38.7	484	1.6	20	207
MFC4	250	80	37.4	467	4.2	52.5	520
MFC°	500	300	140	467	34.2	114	854

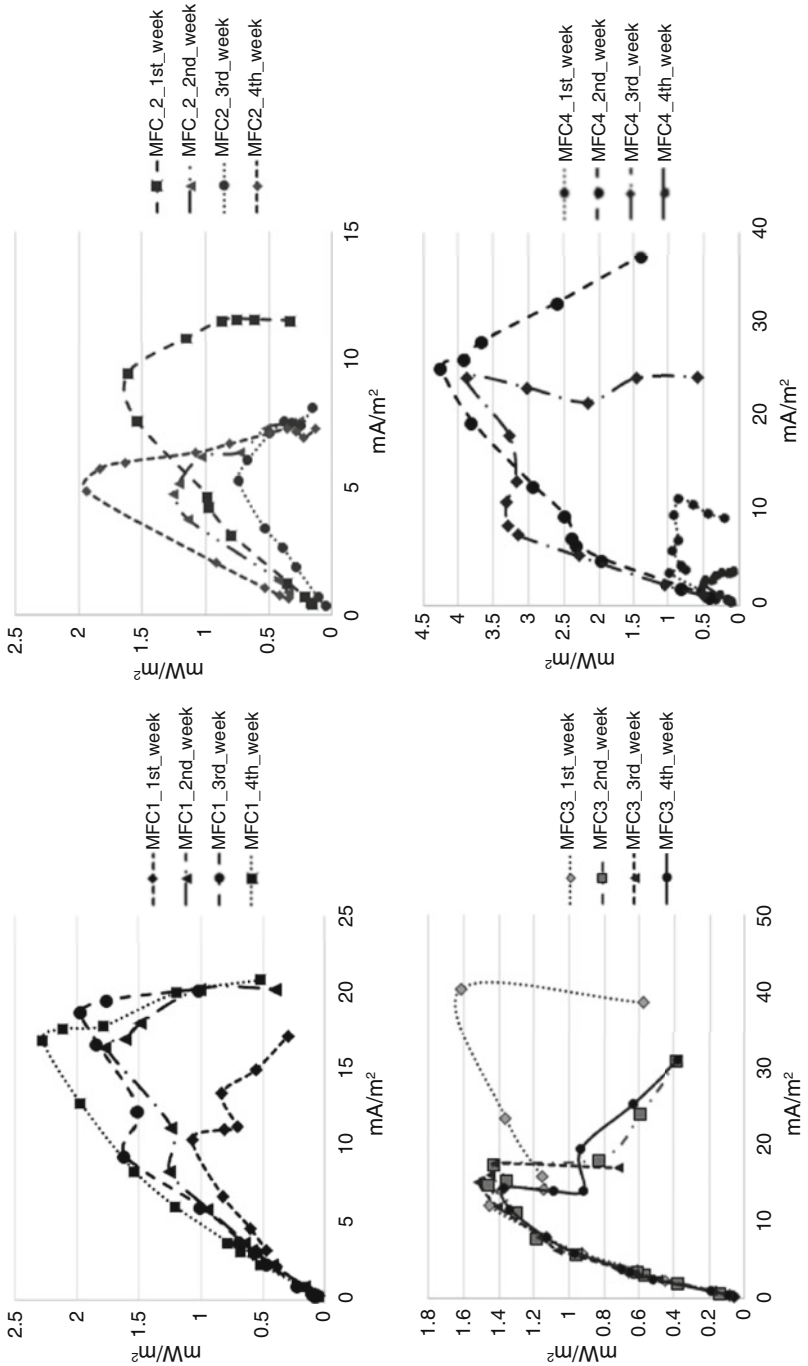

Fig. 9.6 Power curves of MFC1 and MFC2 (100 mL), MFC3 and MFC4 (250 mL)

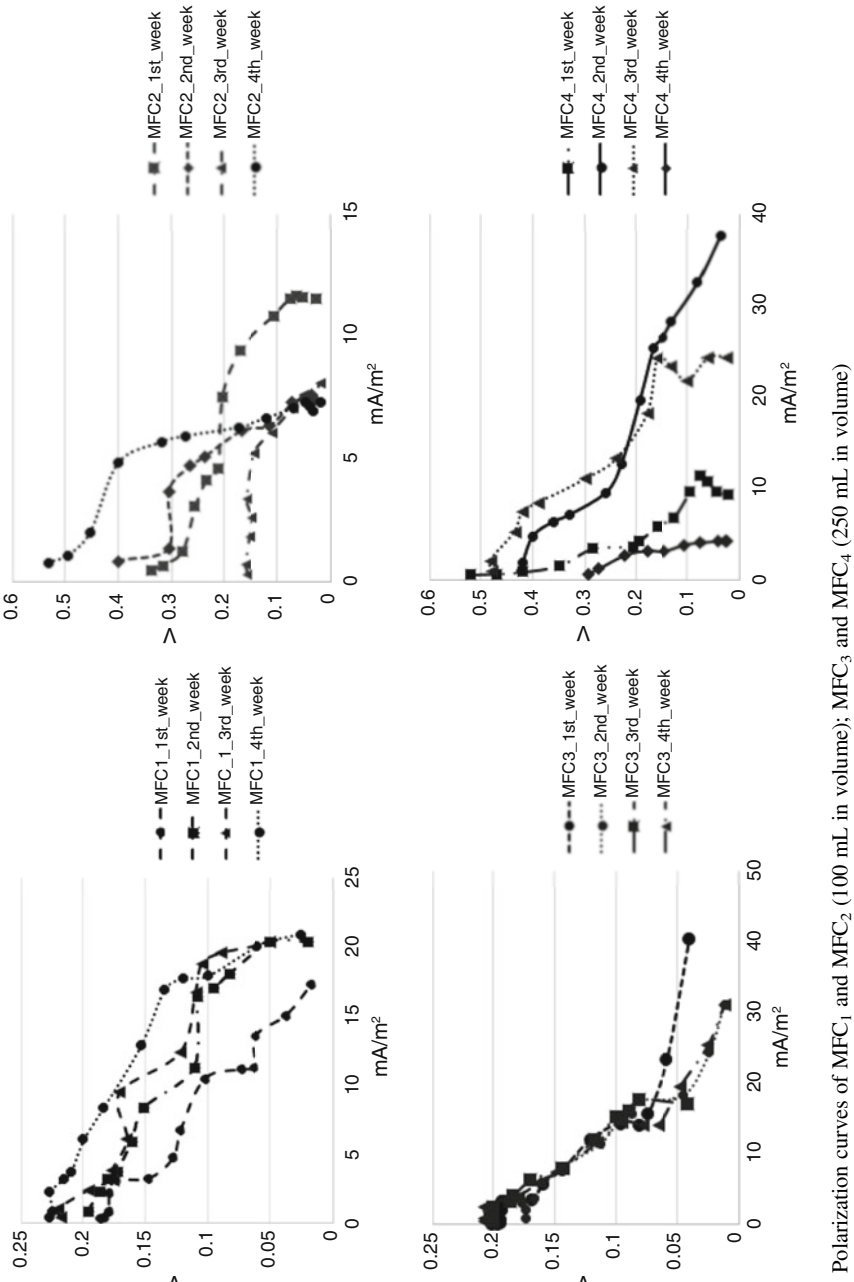

Fig. 9.7 Polarization curves of MFC_1 and MFC_2 (100 mL in volume); MFC_3 and MFC_4 (250 mL in volume)

9.4 Conclusions

Our research confirmed the potentialities of compost as feedstock in MFCs to be operated on both a short and a long-term basis. Interesting results in terms of PD and CD have been measured in MFCs with a lower electrode surface/cell volume ratio, confirming what is observed in MFCs fed with wastewater. As to MFC°, high power outputs were achieved after its reactivation, two years later its set-up. The high power and current outputs could be explained by the development of a robust electroactive biofilm ant both anode and cathode. The feedstock composition should have changed after such a long time. This last issue and how it could have contributed to increase in MFC_0 performance are still to be investigated as well as the dynamics of anode microflora over time. The prevalence of electroactive bacteria at the anodes supports the utilization of compost to produce inocula for MFCs potentially fed with other substrates.

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Chapter 10
Applications of Bio-electrochemical Systems in Heavy Metal Removal and Recovery

Bahar Ozbey Unal, Ezgi Bezirhan Arikan, Prasun Kumar, and Nadir Dizge

Abstract Heavy metals which are widely used in many industrial fields such as textile, electronics, metal, cosmetics have toxic and carcinogenic effects against living organisms and cause an increasing environmental and health problems due to their non-biodegradable properties. Therefore, many treatment methods such as membrane separation, precipitation, adsorption, coagulation/flocculation, and ionic exchange can be used for the removal of heavy metals from water/wastewater in order to comply with the strict regulations. Among these methods, bio-electrochemical systems (BESs) is a promising technology for the removal of contaminants as well as heavy metals and can be used for the generation of the energy from wastewater. BESs include anode, cathode, membranes, and external circuit in which microorganisms act as a catalyst on one or both electrodes. During the process, organic matters are biodegraded by electroactive biofilms at the anode to transport electrons to the cathode and heavy metal ions are reduced at the cathode to recover metal(loid)s. Reduced metal(loids) can be deposited on the cathode, precipitated or dissolved in the solution. The key operating parameters in bio-electrochemical processes are the concentration and type of the heavy metals, the amount of the applied voltage, type of the microbial community and membranes, the conductivity of the water/wastewater, the material types of the anode and cathode.

This chapter provides the main definition of the heavy metals and the potential toxicity of heavy metals to the environmental life. It includes conventional heavy metal recovery technologies and the definition of BES as a promising novel

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technology. It also provides the applications of BESs for the recovery of heavy metals which is one of the main challenges and perspectives of BESs.

Keywords Heavy metals · Heavy metals toxicity · Bio-electrochemical systems · Heavy metals recovery

10.1 Introduction

Heavy metals (HMs) have been utilized in many industrial areas such as agriculture, textile, construction, electronics, metal, cosmetics, food processing, etc. In the worldwide, heavy metals are raw materials which have an economic value due to their industrial utilization (Nancharaiah et al. 2015). However, these raw materials are not infinite and they are distributed unevenly. It is also known that the growing world population and consumption as well as changing in the global economy led to increased demand of certain HMs such as lead, copper, and zinc (Hofmann et al. 2018). Hence, certain heavy metals can be included "critical raw materials" group recently (Hofmann et al. 2018). On the other hand, it is known that these valuable metals have toxic and carcinogenic effects to the living organism. After using or during industrial processing, HMs mostly release to the environment and lead to increasing environmental and health problems because of their non-biodegradable properties (Tchounwou et al. 2012; Nagajyoti et al. 2010; Jaishankar et al. 2014). Therefore, certain HMs are acclaimed priority pollutants by the US Environmental Protection Agency (US EPA) (Fairbrother et al. 2007).

Due to environmental concerns and strict regulations, varied treatment methods such as membrane separation, precipitation, adsorption, coagulation/flocculation, and ionic exchange have been used for the removal of HMs from water/wastewater until today (Fu and Wang 2011; Kurniawan et al. 2006; Babel 2003; Al-Rashdi et al. 2013). However, most treatment methods are inadequate, expensive, generating secondary pollution, and need long operational time (Fu and Wang 2011). However, bio-electrochemical systems (BESs) are one of the most innovative technologies used both for the removal of pollutants and simultaneously for energy production from wastewater (Bajracharya et al. 2016). Basically, BES systems have four components: anode, cathode, membranes, and external circuit (Jain and He 2018; Dizge et al. 2019). In the anode, organic substances are oxidized and electrons are formed. Produced electrons are transferred to the cathode by an external circuit and energy is produced. While these reactions happen, the positively charged ions formed in the anode pass through a membrane and migrate to the cathode (Chaudhuri and Lovley 2003).

Recently, BESs have been shown remarkable performance on the removal or recovery of HMs such as chromium (VI), gold (III), lead (II), zinc (II), cadmium (II), mercury (II), and silver (I) (Li et al. 2008; Lu et al. 2015; Nancharaiah et al. 2015). Considering the environmental/economic problems of heavy metals, BESs can be considered as an innovative and one of the best technology to ensure the recovery of

heavy metals. In this review chapter, the situation of the latest technologies and applications of HMs removal/recovery in BESs are discussed.

10.2 Definition of Heavy Metals

Generally, heavy metals (HMs) are known as natural elements that have a high density (>5 g cm^{-3}) and atomic mass (>23) (Appenroth 2010; Koller and Saleh 2018). With the lack of authoritative definition, heavy metals are defined as the elements including both metals and semimetals (metalloids) (Duffus 2002). In a specific approach, they are elements that occupy columns 3–16 of the periods from 4 to 6, including the transition metals, post-transition metals, and lanthanides in the periodic table (Duffus 2002; Koller and Saleh 2018). Heavy metals position in the Periodic Table is shown in Fig. 10.1 (Hoodaji et al. 2012). Therefore, HMs contain transition metals such as chromium (Cr), cadmium (Cd), iron (Fe), cobalt (Co), nickel (Ni), silver (Ag), lead (Pb), zinc (Zn) elements, and the platinum group metals such as ruthenium (Ru), palladium (Pd), platinum (Pt), rhodium (Rh), and metalloids like arsenic (As), tellurium (Te), selenium (Se) (Nagajyoti et al. 2010; Nose and Okabe 2014; Gunn 2013).

Although HMs are found all over the world, they are recently classified as pollutants. It is thought that HMs are originated from two-source; geogenic and anthropogenic (Adriano 1986; Noll 2002). The geogenic source is the earth's crust that contains at low concentrations (ppb) of HMs and they can be released to the environment due to weathering and erosion. Therefore, HMs originated from geogenic sources generate trace level pollutant (Kabata-Pendias 2010). However, volcanic eruption significantly contributes to high level of HMs pollution (Nagajyoti et al. 2010). Conversely, the anthropogenic source is originated from human activities such as domestic, mining, agricultural, and industrial utilizing of HMs. Therefore, these activities lead to the release of HMs in high concentrations and in bioavailable form that can be easily transported and absorbed by living organisms (Adriano et al. 2004).

It is known that living organisms need low concentrations of HMs such as Ni, Cu, Fe, As, and Zn (Singh et al. 2011). Nevertheless, it is also known that all HMs are toxic at high concentrations (Valko et al. 2016). For instance, Zn has significant role for male reproductive activity and Zn deficiency causes anemia as well as inhibits growth and development. On the other hand, excess Zn can damage the living metabolism and cause the same diseases (Nolan 1983; Leah Harris and Gitlin 1996; Rajaganapathy et al. 2011). Furthermore, HMs are utilized in the industrial areas such as agriculture, medical, and technological manufacturing. Excessive use of HMs has led to widespread distribution in the environment (Njati and Maguta 2019). HMs accumulate in the environment because of their non-biodegradability and stability characterization. They accumulate at increasing concentrations in living organisms through the food chain, causing adverse effects (Kazemipour et al. 2008). At higher permissible limits, heavy metals in plants or crops may cause mutations of

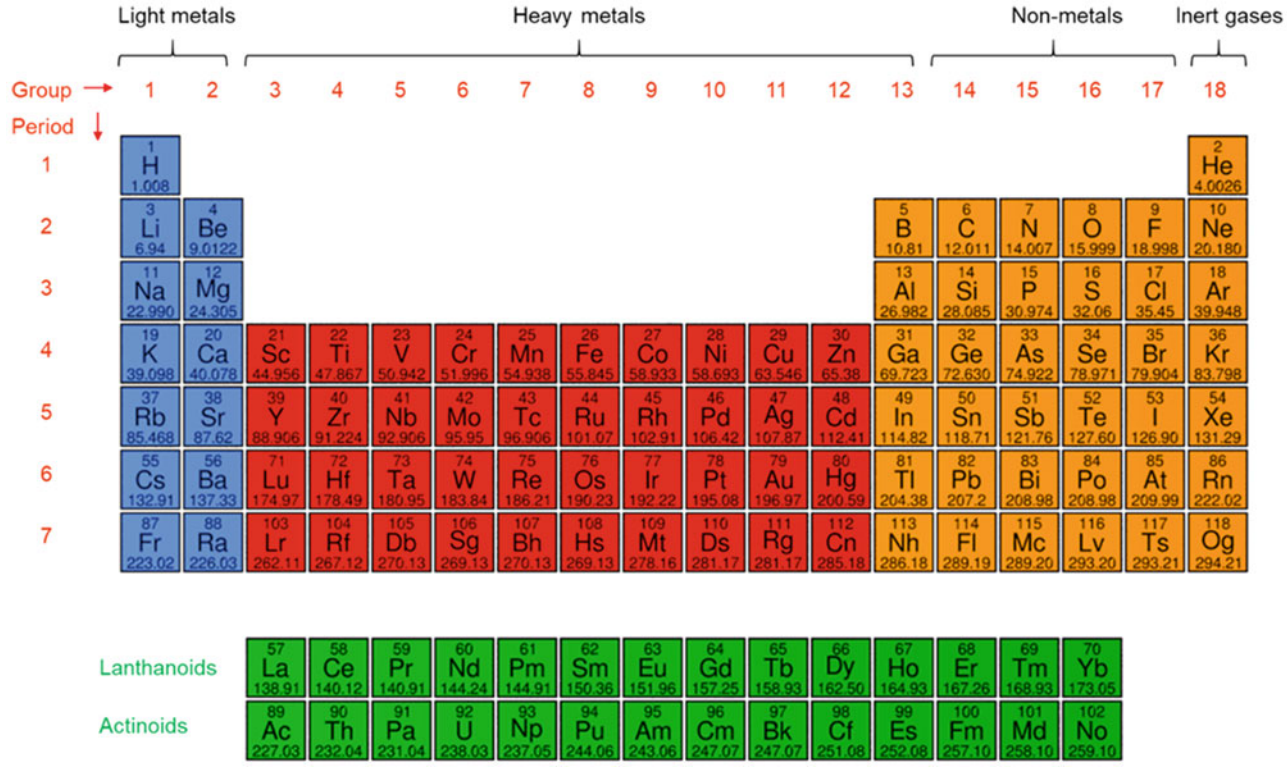

Fig. 10.1 Heavy metals position in the periodic table (www.webelements.com)

genetic materials, functional, and structural membrane disintegration, and may inhibit their growth. HMs can accumulate in fatty tissues/human bones. They are toxic and harmful to humans and other living organisms at higher permissible limits (Carolin et al. 2017; Rai et al. 2019). Hence, in the past decades, HMs and their pollution have been evaluated by the environmental law regulators and agencies in worldwide. Some types of HMs, their industrial usage area, and their permissible limits for humans and plants are demonstrated in Table 10.1.

10.3 Potential Toxicity of Heavy Metals for the Environmental Life

The environment can be defined as a dynamic organism that contains soil, air, water, and living organisms. In recent decades, the environment has witnessed industrial development and unparalleled rapid population growth. Due to these developments, nowadays "the environment" is mentioned along with mostly "pollution." Until today, many harmful pollutants such as polycyclic aromatic hydrocarbons (PAHs), pesticides, polychlorinated biphenyls, synthetic dyestuff, heavy metals, and many others have been released to the environment (Chen et al. 2015). Among these pollutants, heavy metals are known to threaten environmental sustainability (Liu et al. 2019). HMs can enter the environment and can be deposited in the water, air, and soil because of geogenic and anthropogenic sources (Jacob et al. 2018).

The heavy metal pollutants can release into the environment due to industrial wastewater, sewage irrigation, atmospheric deposition, agricultural activities (usage of fertilizer and pesticide), mining activities, metal production, irregular storage of industrial and municipal solid waste (Wuana and Okieimen 2014). Atmospheric deposition seems to be a small factor, but it is one of the major factors causing HMs pollution in the soil (Yan et al. 2018). Combustion of fossil fuel, using Pb-containing fuel for transportation, the production metallurgy, and construction materials lead to producing emissions of HMs and their aerosol forms enter into the atmosphere (Cheung et al. 2011; Duan and Tan 2013). These aerosol forms of HMs are mostly oxidized and condense as fine HM particles in the atmosphere (Wuana and Okieimen 2014). Due to the effect of the wind, they are distributed and adsorbed by the mineral particles and precipitated in the soil (Manafi et al. 2012). When these particles consumed by the microorganisms, it may cause protein denaturation, dysfunction, the destruction of cell membrane integrity in the microorganisms (Chodak et al. 2013). Furthermore, the enzyme activity of soil microorganisms can be decreased. Due to decreasing enzyme activity, organic matter decomposition, and nutrient cycling processes can be affected adversely (Tang et al. 2019). Consequently, the environment ecosystem deteriorates because of heavy metal pollution.

Additionally, HMs can penetrate the soil with mostly poorly treated industrial, agricultural, and domestic wastewater (Vardhan et al. 2019; Chowdhury et al. 2016). Humus in the soil has a high affinity for HM cations and it absorbs HMs from the

Table 10.1 Heavy metals, their industrial usage area, and permissible limits for humans and plants

Heavy metals	Permissible limits for plants[a] (mg/kg)	Permissible limits for humans[b] (mg/L)	Industrial usage area	References
Cd	0.02	0.06	Battery production	Zhang and Reynolds (2019)
			Cement production	Johri et al. (2010)
			Plastic production (as stabilizer)	Zhang and Reynolds (2019)
			Iron and steel production (coating and electroplating process)	Zhang and Reynolds (2019)
			Phosphate fertilizer	Johri et al. (2010)
			Smoking	Kalcher et al. (1993)
Cr	1.3	0.05	Steel and alloy production	Murthy et al. (2011)
			Copy machine toner manufacturing	Chowdhury et al. (2003)
			Textiles, brick lining, chrome plating, leather tanning	Saha et al. (2011)
Cu	10	0.1	Fertilizers and pesticide production	Ameh and Sayes (2019)
			Alloy production	Elshkaki et al. (2016)
			Personal care products	Ameh and Sayes (2019)
			Paper industry	Singh and Chandra (2019)
Pb	2	0.1	Paint and toys production	Njati and Maguta (2019)
			Batteries, machinery manufacturing	Pan et al. (2019)
			Medicine and cosmetic production	Flora et al. (2012)
			Smoking	Kalcher et al. (1993)
Ni	10	–	Stainless steel and alloy production	Cempel and Nikel (2006)
			Chemical and food processing (as catalyst)	Cempel and Nikel (2006)

(continued)

Table 10.1 (continued)

Heavy metals	Permissible limits for plants[a] (mg/kg)	Permissible limits for humans[b] (mg/L)	Industrial usage area	References
			Paper industry	Singh and Chandra (2019)
			Cement production	Cempel and Nikel (2006)
			Battery production	Bansal et al. (2009)
As	–	0.02	Paint production	Sodhi et al. (2019)
			Pesticides production	Bakhat et al. (2019)
			Crop desiccants	Wang et al. (2019)
			Combustion of fossil fuels	Wang et al. (2019)
			Cosmetic products	Sodhi et al. (2019)
Hg	–	0.01	Paper industry	Singh and Chandra (2019)
			Gold extraction	Wang and Wang (2019)
			Pesticides	Singh et al. (2011)
			Battery production	Wang and Wang (2019)

[a]World Health Organization (WHO) (1996)
[b]Singh et al. (2011)

water passing through the soil (Jacob et al. 2018). Generally, about 98% of the HMs in the wastewater are absorbed by the soil and the rest of it are absorbed by the plant (Singh et al. 2011). The roots of plants absorb water containing HMs and they are transported to plant tissues (Ahmad et al. 2016). At higher toxic concentrations of HMs, reactive oxygen species (ROS) such as superoxide ($O^{\bullet 2-}$) and hydroxyl radicals ($^{\bullet}OH$) can be produced and the plant may enter the oxidative stress. As a result, the physiological and genetic structure of the plants change with the carbohydrate and protein content, and HMs inhibit germination or growth (Berni et al. 2019). If these contaminated plants are eaten by animals, heavy metals transfer to other animals and humans. In the case of forest fires, heavy metals return to the atmosphere (Fang et al. 2010).

HMs sorbed by soil mineral particles or plants are not biodegradable by microbial microorganisms. Therefore, they remain permanently in the soil. During this time,

HMs can convert into different chemical forms that have different toxicity, bioavailability, and mobility (Wuana and Okieimen 2014). For instance, chromium is mostly available as Cr (VI) and Cr (III) forms in soil. Whereas Cr (III) is a micronutrient and a nontoxic metal for some microbial species, Cr (VI) is highly toxic (Garnier et al. 2006). However, ionic forms of HMs such as As^{3+}, Hg^{2+}, Pb^{2+}, Cd^{2+}, and Ag^{+} in the soil can be transported to surface water (like the river, lake, and sea) and groundwater reservoir via filtration. It is also possible that atmospheric deposition of HMs can also enter into the aquatic systems by acid rain (Singh et al. 2011). These ionic forms of HMs are mostly bound to particulate matter and settle down to the sediment in aquatic systems (Singh and Kalamdhad 2011). Sediment-bound HMs can be also taken up by aquatic organisms (Peng et al. 2008). For example, fish, as an aquatic organism, can take HMs from food, non-food particles, gills, water consumption, and skin (Singh and Kalamdhad 2011). When HMs (especially Hg) enter into the fish, ROS is produced, which can damage their metabolism (Woo et al. 2009). In addition, HMs can accumulate in fish oils and tissues. The fish containing HMs can be consumed by carnivores and HMs are transported to humans through the food chain (Afshan et al. 2014; Peralta-Videa et al. 2009).

The human body can tolerate trace amounts of heavy metals without serious health problems. But for long-term exposure, HMs may cause to the consuming of essential nutrients in the human body, thereby functional disorder of vital organs such as the brain, heart, kidney, liver, and nervous system (Fig. 10.2) (Sardar et al. 2013; Jacob et al. 2018). Furthermore, some HMs such as Pb, Cd, and Hg have carcinogenic effects (Chowdhury et al. 2016).

Due to the adverse effects of HMs on human health, it is emphasized by the regulatory agencies such as WHO and US EPA that safe drinking water is crucial to human life. Furthermore, these agencies, which protect human life and the creation of healthy generations, have proposed the maximum permissible limit values for some HMs in drinking water (Table 10.2).

Literature survey showed that the main factor for HMs pollutions was the inadequate treatment of industrial, agricultural, and domestic wastewater. It is also considered that the soil and these polluted wastewater act like a distribution system and it is the most important factor in HMs pollutions that threatening living organisms and the environment.

10.4 Conventional Heavy Metal Recovery Technologies

Wastewater produced from industries mentioned above includes a significant amount of heavy metal concentrations which have toxic or harmful effect to surroundings (Carolin et al. 2017). These pollutants can be converted into less toxic substances by sequential conventional heavy metal recovery methods such as adsorption, ion exchange, coagulation/flocculation, chemical precipitation, electrochemical processes, advanced oxidation processes, and membrane filtration systems (Azimi et al. 2017). All these treatment technologies have some advantages and

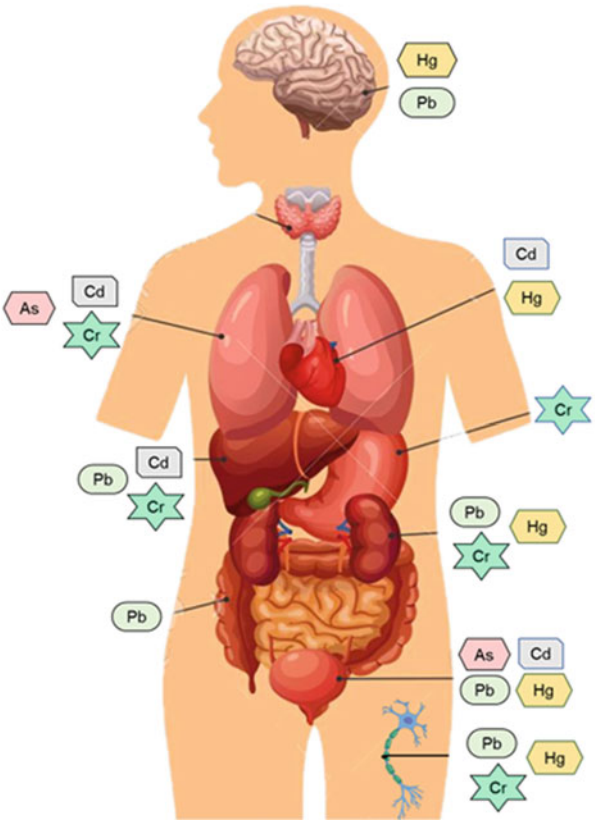

Fig. 10.2 Heavy metal effects on vital organs in the human body

Table 10.2 Recommended permissible limits of some HMs in drinking water (Kumar et al. 2017)

Heavy metals	Permissible limits of US EPA (mg/L)	Permissible limits of WHO (mg/L)
As	0.010	0.010
Cd	0.005	0.003
Cu	1.300	2.000
Hg	0.002	0.001
Ni	0.040	0.070
Pb	0.015	0.010
Zn	5.000	3.000

disadvantages compared to each other such as treatment performance, produced water quality, operational, and maintenance (O & M) cost.

Chemical precipitation is an easily applicable process which can be used for the treatment of HMs from inorganic discharges. In chemical precipitation, the dissolved heavy metal ions react with chemicals and are transformed into the insoluble solid phase at high pH conditions (pH 11) (Azimi et al. 2017). Then, the solid phase is separated from the treated water by filtration or sedimentation. Generally,

precipitated heavy metal ions are in the form of phosphate, sulfide, carbonate, and hydroxide (Nzihou and Sharrock 2010). The chemical precipitation technique is not proper for the low concentrations and high amount of hazardous sludge formation is another problem that is difficult to manage (Kuan et al. 2010). However, the chemical precipitation technique is easy to implement on a large scale due to its low cost.

Coagulation/flocculation is another method to remove HMs from wastewater. In this process, the net surface charge of the colloids is reduced by electrostatic repulsion in the coagulation mechanism and the size of the stabilized colloids are increased by the addition of polymers in the flocculation mechanism. Then, the flocculated particles are separated from wastewater by filtration or sedimentation. The formation of hazardous sludge, a requirement for the high amounts of chemicals are the main disadvantages similar to the chemical precipitation process (Pang et al. 2011; Johnson et al. 2008).

Ion exchange processes have many advantages such as high removal capacity and efficiency for the treatment of HMs from wastewater. Especially, synthetic resins have high efficiency to remove almost all heavy metals. Especially, both strongly and weakly basic resins are the main ion exchangers in which the metal cations are changed with hydrogen ions in the sulfonic groups or carboxylic groups and the processes can be applied to remove HMs (Fu and Wang 2011). Similarly, the negative charged heavy metals can be replaced by the anions in the synthetic resins such as hydroxyl and chloride ions. Natural resins such as zeolites can be used as an alternative to synthetic resins because of their low cost. The less sludge formation makes the ion exchange process advantageous over other processes such as chemical coagulation/flocculation and precipitation.

The adsorption process is a low-cost alternative method for the removal of HMs which supplies high removal efficiency and low fouling problems. In the adsorption process, heavy metals are adsorbed into the active sites of the adsorbents by physically or chemically interactions (Bilal et al. 2013; Ojedokun and Bello 2016). The presence of various adsorbents, being a reversible technology, the repeated use of adsorbents, and the absence of the formations of toxic pollutants are the main advantages of this process (Carolin et al. 2017).

Membrane filtration is another alternative method to remove HMs from wastewater in which both heavy metal removal/recovery and disinfection takes place together. The separation of the contaminants depends on their charge, molecular size, concentration, solution pH, and applied transmembrane pressure (Basaran et al. 2016). Basically, reverse osmosis, nanofiltration, ultrafiltration, and electrodialysis are the main membrane separation processes used for heavy metal removal. Membrane processes have many advantages, for example, better removal efficiency of contaminants, smaller footprint, easy operation, long filtration media life, and some disadvantages, for example, their high capital and O & M costs (Qdais and Moussa 2004; Nadeem et al. 2019).

Heavy metals can be treated by electrochemical processes such as electrocoagulation, electrodeposition, electroflotation, electrodialysis, electrodeionization, and bio-electrochemical systems (Bazrafshan et al. 2015). The

process efficiency depends on the electrode material in the electrochemical reactor, current density, wastewater characterization, etc. Although high removal efficiencies of HMs make this technology advantageous, high capital cost, the short service life of electrodes, and expensive electricity requirement limit its extensive usage (Zhang et al. 2013). However, bio-electrochemical systems (BESs) can overcome these limits for HMs recovery from water/wastewater.

10.5 Definition of Bio-electrochemical Systems

BESs can be called as a microbial electrochemical system (MEC) in which microbes or enzymes are implicated in the at least one of the oxidation or reduction reactions (Kumar et al. 2018). In a microbial fuel cell (MFC), electrical power is obtained by the degradation of organic matter by microorganisms in bioanode. In microbial electrolysis cell (MEC), an exterior electrical power is provided to drive the formation of valuable products. In microbial electrosynthesis (MES), CO_2 or organic compounds are reduced cathodically to high value-added products. Besides, there are other BESs, for example, used for the desalination of water called as microbial desalination cells (MDCs) (Fig. 10.3) (Bajracharya et al. 2016; Dizge et al. 2019).

Bio-electrochemical systems (BESs) have electrochemical cells in which microorganisms act as a catalyst on one or both electrodes (Hamelers et al. 2010). In the BES mechanism, microorganisms catalyze the reactions taking place at the electrodes, and electrons are moved from the oxidized component to the anode for the oxidation reaction or to the cathode for the reduction reaction. Because of the many microorganisms are electrochemically active, BESs have great potential for the formation of energy and chemicals (Sleutels et al. 2012).

In the BES mechanism, a principal electron contributor should be provided to the anode and a final electron acceptor should be supplied to the cathode. The benefit of using two separate compartments is to separate oxidation and reduction products from each other and to facilitate the extraction of valuable products (Hamelers et al. 2010).

The application areas of the BESs have been increased in recent years and they have a high possibility of the usage of various oxidizable components at the bio anode such as municipal wastewater, many industrial wastewater, acetate, starch, etc. Besides, microorganisms could catalyze many reduction reactions at cathode such as oxygen to water, proton to hydrogen, and nitrate to nitrogen gas. The energy efficiency of the BESs relies on the voltage and Coulombic efficiencies of the reactions that occurred at both electrodes. In order to increase the applicability of BESs, advantages of the process (the economic price of the products and treatability of the wastewater) must be greater than capital and operational costs. Generally, an increased current density is a necessity for low capital costs and a high removal efficiency, which lets smaller space demand (Sleutels et al. 2012).

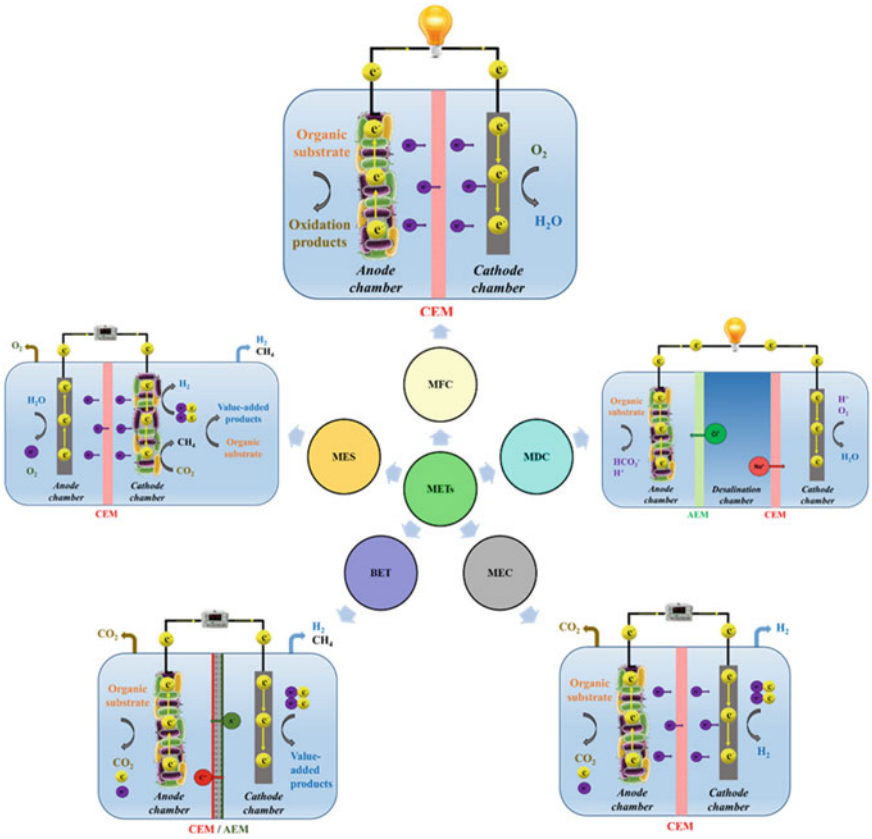

Fig. 10.3 Schematic diagram of microbial electrochemical technologies. *MFC* microbial fuel cell, *MDC* microbial desalination cell, *MEC* microbial electrolysis cell, *BET* bio-electrochemical treatment, *MES* microbial electrosynthesis system (Dizge et al. 2019)

10.6 Applications of Bio-electrochemical Systems in Heavy Metals Recovery

Waste streams containing metals should be considered as a valuable resource for the recovery of worthy and rare elements. Even though conventional biological treatment systems are evaluated as an economical technology for heavy metal-containing wastewater treatment, they remain inadequate for heavy metal recovery. Hence, BESs are considered as an attractive technology for the removal/recovery of HMs from different kinds of wastewater and metallurgical and process wastes recently. The general concept for metal removal using BESs is that organic matters are biodegraded by electroactive biofilms at the anode and produced electrons are transferred to the cathode and heavy metal ions are reduced at the cathode to recover metal(loid)s (Fig. 10.4) (Nancharaiah et al. 2015). Reduced metal(loids) in cathodes

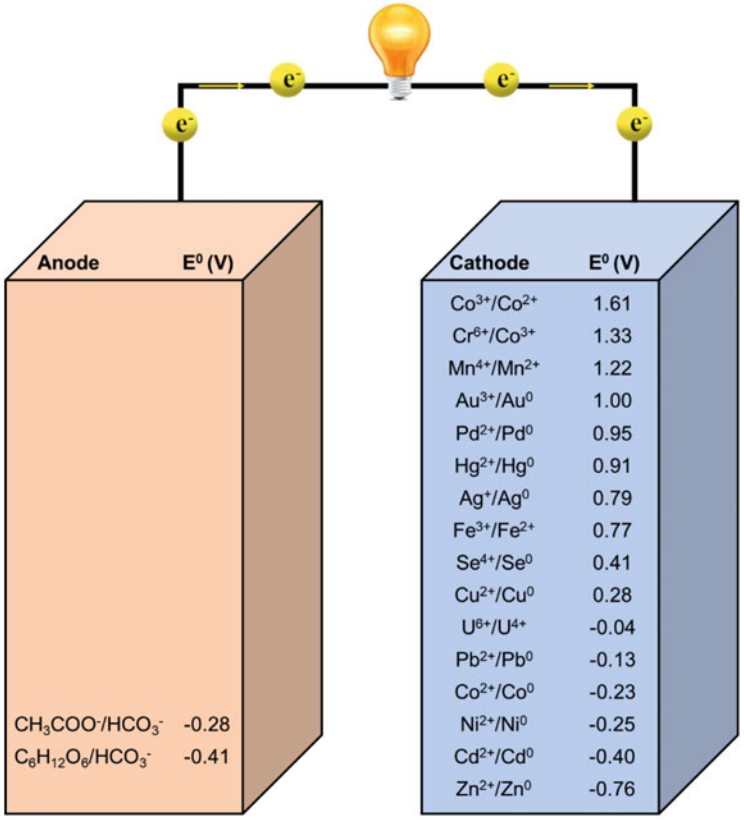

Fig. 10.4 Redox tower of selected metal ions as electron donors and electron acceptors to remove or recover of metal ions in BESs (Nancharaiah et al. 2015; Dominguez-Benetton et al. 2018)

can also follow three ways in the cathode chamber to be removed or recovered in BESs: (1) deposited on the cathode, (2) precipitated in the solution, or (3) dissolved in the solution (Lu et al. 2015).

In BESs, several metal ions can be a representative for oxygen and serve as an effective terminal electron acceptor. For example, when the cathode chamber was fed with a fly ash leachate, copper was removed and recovered in BES without extra energy input and metal copper was deposited on the cathode of an MFC. It was reported that higher than 97.1% of Cu(II) removal efficiency with an initial Cu (II) concentration of 52.1 mg/L was obtained for 36 h operation period in the leachate. Cu(II) was reduced and recovered mainly as metallic Cu on cathodes (Tao et al. 2014). In addition to the recover of copper, metallic Ag recovery and power generation were also achieved by using cathodic reduction in BESs (Tao et al. 2012). An electron donor (acetate) on the anode and an electron acceptor (both Ag^+ ions and Ag(I) thiosulfate complex) on the cathode was used for metallic Ag recovery in dual-chamber BESs. They reported that up to 95% of Ag(I) removal

Table 10.3 Heavy metal recovery efficiency using different BESs

Heavy metal	BES type	Metal removal efficiency (%)	Reaction time (h)	Applied voltage/ maximum power output	References
Cr(VI)	MFC	99.5	25	1.6 W/m^2	Li et al. (2008)
Cr(VI)	MFC	75.4	240	0.97 W/m^2	Zhang et al. (2012)
V(V)	MFC	67.9	240	0.97 W/m^2	Zhang et al. (2012)
Co(0)	MEC	92.0	6	0.2–0.5 V	Jiang et al. (2014)
Cu(0)	MEC	99.2	42	1.0 V	Luo et al. (2014)
Ni(0)	MEC	97.0	62	1.0 V	Luo et al. (2014)
Cu(II) Pb(II) Cd(II) Zn(II)	MEC	Metals were recovered	–	0 V for Cu 0.3 V for Pb 0.5 V for Cd 1.7 V for Zn	Modin et al. (2012)

was succeeded and metallic Ag with >91% purity was electrodeposited on the cathode (Tao et al. 2012).

The reduction of copper ions in a cathode with simultaneous electricity generation with glucose as a substrate in MFCs was also proposed by Tao et al. 2011 and metallic copper and cuprous oxide (Cu_2O) were recovered. Different concentrations of $CuSO_4$ solution from 50.3 to 6412.5 mg Cu^{2+}/L as the catholyte solution at pH 4.7 and different resistors from 0 to 1000 Ω as external load were examined by using dual-chamber MFCs. High Cu^{2+} removal efficiency (>99%) with 1.3 mg/L final Cu^{2+} concentration was obtained at an initial 196.2 mg Cu^{2+}/L concentration with an external resistor of 15 Ω, or without an external resistor. Cu^{2+} was reduced to cuprous oxide and metallic copper on the cathodes according to X-ray diffraction (XRD) analysis (Tao et al. 2011).

Cobalt, which is a rare metal, was recovered from the aqueous solution after leaching of Co(II) from $LiCoO_2$ using BES (Huang et al. 2014a). $LiCoO_2$ used in Li-ion batteries was the source of Co(II) ions that were reduced to Co(0) on the cathode of a MEC. A sequential MFC–MEC (MFC-Co(III)/Co(II)–MEC-Co(II)/Co(0)) process was suggested for leaching and recovery of cobalt from waste lithium-ion batteries (Huang et al. 2014b). The cobalt leaching and Co(II) reduction were obtained 46 and 7 mg/L/h in MFCs and in MECs, respectively, with an overall system cobalt yield of 0.15 g Co/g Co. The results showed that cobalt was completely recovered and recycled to spent lithium-ion batteries with no external energy consumption using the sequential MFC–MEC system. Other studies on the recovery of HMs using different BESs from solutions or wastewaters are presented in Table 10.3.

Metallic copper and nickel recovery from acid mine drainage (AMD) and simultaneous H_2 production on the cathode were carried out by using two-chamber MEC

with an externally applied voltage of 1.0 V (Luo et al. 2014). The recovery efficiency of Cu^{2+} reached 99.2% within 42 h. However, the Ni^{2+} recovery efficiency reached 97% at the end of batch operation for 62 h. It can be concluded that the MEC was successfully used to separate metals from the AMD, to recover value-added products of metallic copper and nickel, and to produce H_2 gas (Luo et al. 2014).

Uranium was also recovered from the groundwater with a pure culture of *Geobacter sulfurreducens* using BES and graphite electrodes were used as the electron donor (Gregory and Lovley 2005). Under optimized conditions, 80 μM of U(VI) was deposited on the cathode and 87% of the uranium was recovered from the electrode surface.

10.7 Challenges and Perspectives

Developments in BESs are promising technology for the recovery of heavy metal ions in practical applications and commercialization. However, the biggest challenge facing BESs is their inability to treat all metal ions and required external power supply (Ezziat et al. 2019). The strategic factors influencing the BESs performance for recovery of heavy metal ions are biocatalysts, electrodes, electrolytes, and membranes (Jadhav et al. 2017). The development of heavy metals resistance microbial community is essential for efficient recovery. Moreover, adsorption and diffusion of heavy metals from the cathode chamber to the anode chamber should be reduced to obtain a high recovery. Valuable metal catalysts such as Pt should be abolished to decrease the operating costs for real applications. For this purpose, cheaper materials with large surface areas, such as stainless steel wool, activated carbon cloth, and foam may be used as an alternative to Pt catalyst. There are still major deficiencies and difficulties for recovering valuable and rare metals from real waste/wastewaters, although BESs have been performed successfully using different metal ions as electron acceptors (Nancharaiah et al. 2016). Membrane biofouling is another important challenge. This undesired event occurs in MFCs, as biofilm will unsurprisingly grow on and inside chambers during long-term operation (Ezziat et al. 2019). The use of new polymers such as polybenzimidazole in membrane synthesis can be a solution to prevent membrane biofouling because of inhibited the adhesion of bacteria on the membrane surface by this polymer (Angioni et al. 2017). From a broader perspective, BES technologies should be evaluated not only by the economic feasibility but also by the need to meet biotechnological expectations for large scale applications. In addition, there is a necessity to explain the behavior for metal removal under non-ideal conditions from real streams (Pant et al. 2012; Yu 2016).

10.8 Conclusions

Recently, BES technologies which discovered through the search for alternative energy sources, have been remarkably attracted attention due to their functionality and performance. Besides the production of hydrogen and electricity, BESs are a useful stage and they have great potential for recovering heavy metal ions from wastewater, groundwater, aqueous streams, and wastes. The cathodic reduction of metal ions coupled to organic substrate oxidation can be used for the recovery of several heavy metals. Heavy metal ion concentration, heavy metal type, applied voltage, microbial community, membrane type, conductivity, anode or cathode materials, and system configurations will affect heavy metal recovery efficiency. Further researches at the molecular level are needed to understand deeply the mechanisms between heavy metals and biocatalysts.

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Chapter 11
The Role of Denitrifying Bacteria Within the Bioelectrochemical System for Nitrate-Containing Wastewater Treatment

Xiaojun Jin and Hong Liu

Abstract Bioelectrochemical systems (BESs) with the coexistence of denitrifiers and electricigens were generally observed for simultaneous nitrogen removal and electricity production. As the increasing of nitrate, the percentage of denitrifiers increased and the percentage of electricigens relatively decreased until it lost its dominant position. In denitrifying BES, anodic heterotrophic denitrification could improve organics removal and energy recovery efficiency during the treatment of nitrate-containing wastewater. In this chapter, the developments of denitrifying BES as well as the evolution of the microbial community were comprehensively introduced. Furthermore, a special type of bacteria, denitrifying electricigens, was also introduced and utilized in BES for the treatment of nitrate-contaminated waters.

11.1 Introduction

Nitrogen pollution has become an increasing problem in the environment, especially the excessive emission of nitrate. Nitrate is generally found in the effluent of the aerobic ammonium oxidation process during the wastewater treatment. The discharge of excessive nitrate poses a growing threat to public health around the world, especially causes water eutrophication in the environment (Manassaram et al. 2006). Biological treatment seems a less costly technology for nitrogen removal in terms of operation and maintenance costs, comparing to the physical and chemical processes in traditional methods (Butz and Jackson 1977).

During a complete denitrification process, liquid nitrate is firstly reduced to liquid nitrite by the nitrate reductase. Next, nitrite was gradually reduced to gaseous nitric oxide, gaseous nitrous oxide, and nitrogen gas by the enzyme catalyst of nitrite

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Table 11.1 The distribution of the denitrifying genera in taxonomy

Archaea	*Haloarcula*, *Halobacterium*, *Haloferax*, *Ferrogiobus*, *Pyrobaculum*	
Bacteria	Not Proteobacter	*Gram-positive*: *Bacillus*, *Corynebacterium*, *Prankia*, *Dactylosporangium*, *Dermatophilus*, *Gemella*, *Listeria*, *Kineasporia*, *Micromonospora*, *Microtetraspora*, *Nocardia*, *Pilimelia*, *Propionibacterium*, *Saccharomonospora*, *Saccharothrix*, *Spirrilospora*, *Streptomyces*, *Streptoverticillium*
		Gram-negative: *Aquifex*, *Flexibacter*, *Empedobacter*, *Flavobacterium*, *Sphingobacterium*, *Synechocystis* sp. PCCC 6803
	Proteobacter	*α-proteobacteria*: *Agrobacterium*, *Aquaspirillum*, *Azospirillum*, *Blastobacter*, *Bradyrhizobium*, *Gluconobacter*, *Hyphomicrobium*, *Magnetospirillum*, *Nitrobacter*, *Paracoccus*, *Pseudomonas*, *Rhizobium*, *Rhodobacter*, *Rhodoplanes*, *Rhodopseudomonas*, *Roseobacter*, *Sinorhizobium*, *Thiobacillus*
		β-proteobacteria: *Achromobacter*, *Acidovorax*, *Alcaligenes*, *Azoarcus*, *Brachymonas*, *Burkholderia*, *Chromobacterium*, *Comabacter*, *Eikenella*, *Hydrogenophaga*, *Janthinobacterium*, *Kingella*, *Microcirgula*, *Neisseria*, *Nitrosomonas*, *Ochrobactrum*, *Oligella*, *Ralstonia*, *Rubrivivax*, *Thauera*, *Thermothrix*, *Thiobacillus*, *Vogesella*, *Zoogloea*
		γ-proteobacteria: *Acinetobacter*, *Alteromonas*, *Azomonas*, *Beggiatoa*, *Deleya*, *Halomonas*, *Marinobacter*, *Moraxella*, *Pseudoalteromonas*, *Pseudomonas*, *Rugamonas*, *Shewanella*, *Thioploca*, *Thiomargarita*, *Xanthomonas*
		ε-proteobacteria: *Wolinella*, *Campylobacter*, *Thiomicrospiro*

reductase, nitric oxide reductase, and nitrous oxide reductase, respectively (Knowles 1982). And these genes of oxidoreductases are correspondingly encoded by the narGHI, nirK or nirS, norBC, and nosZ, respectively. The genes of these enzymes were also used as molecular markers for the cultivation-independent analysis of denitrifying bacteria in the environment. Denitrifying bacteria, as the carriers of denitrifying genes, are widely appeared at the natural or contaminated environment. These microorganisms are taxonomically and biochemically very diverse (Table 11.1). Most are heterotrophic bacteria, and even some utilize one-carbon compounds, whereas others can spontaneously grow on hydrogen and carbon dioxide or reduced sulfur compounds (Hwang et al. 2009). One group is photosynthetic (Kim et al. 1999). Most of them possess the complete reductases for reducing nitrate to nitrogen gas. But some are termed nitrite dependent because there is no nitrate reductase or nitrous oxide reductase in cells. Sometimes microorganisms cannot produce nitrous oxide from nitrate or nitrite, though they possess nitrous oxide reductase (Knowles 1982). Therefore, nitrate removal is closely related to the microbial characterization of both biofilm and activated sludge in bioreactors.

Biological denitrification includes autotrophic denitrification and heterotrophic denitrification. The former is generally suitable for polluted groundwater treatment, due to the carbon source does not need to be externally added. However, the removal efficiency of nitrogen is commonly limited. The latter seems to have higher efficiency with the wastewater treatment than the former. But, to increase the removal

efficiency of heterotrophic denitrification, sufficient organic carbon matter is required, which produces a large amount of excess sludge. Electrochemical technique (Bioelectrochemical system, BES) has been considered an alternative strategy because of the lack of additional chemical reagent, active sludge decrement, and high efficiency (Park et al. 2005; Chandrasekhar and Ahn 2017). Besides the nitrate reduction at anode, biocathode inoculated with either autotrophic or heterotrophic denitrifiers could also be adopted for nitrate removal in BES. Many researchers have reported the microbial communities of BES for denitrification. However, further analysis and comparison between the denitrifying bacteria in BES and bioreactor have been not investigated. This chapter aimed to review the microbial communities of BES for nitrate removal, and the electricity performance was also conjointly analyzed.

11.2 Main Text

Recent progress in wastewater treatment has led to the development of BES which uses microorganisms capable of electrochemically active and extracellular electron transfer, that facilitates the electron transfer to the anode where oxidation of pollutants (Debabov 2008; Chandrasekhar et al. 2014a). BES contains microbial electrolysis cell (MEC) and microbial fuel cell (MFC). Both of them use microorganisms as a biological catalyst on the electrode. MEC also needs to connect a counter electrode and an external power source (Abudukeremu et al. 2015a, b, 2016b). MEC seems a sustainable and energy-saving technology for H_2 generation and contaminant degradation. MFC is generally used to treat wastewater as well as harvest energy. Recent progress has led to the rapid development of BES for the treatment of various waste waters (Animesh et al. 2016; Rijuta et al. 2017). Furthermore, substantial advancement has been made in enhancing BES as a potential technology towards industrial applications for wastewater treatment (Ghafari et al. 2008). Combination of electrochemical method along with biological denitrification accelerates the denitrification process, and simultaneous declines the cost (Chandrasekhar et al. 2015). To date, it has received an increasing attention in denitrifying BESs.

Since nitrate can be reduced to nitrogen, it can be used as a potential electron acceptor at the cathode in BESs. Thus, using BES for nitrate-contained wastewater treatment can achieve simultaneous electricity generation and nitrate removal. Comparing to the activated sludge from the bioreactor and BES with different cathodes, the overall 8–97% higher nitrate removal rate could be obtained in BES with the bioelectrode (Animesh et al. 2016). The denitrifying MFC using biocathode showed high efficient nitrate removal and current density (Jin et al. 2018).

To date, the enhancing mechanism is still unclear, and which seriously block the development of the denitrifying BES. According to the previous researches, three assumptions about the enhancement of electrochemical denitrification were proposed, containing micro-surrounding pathway, H_2 pathway, and directly electron pathway. The first two pathways were proposed basing on the traditional mechanism

of biological denitrification. As known to all, most denitrifying microorganisms are anaerobic or facultative bacteria. In the cathode of BES system, ORR consumes oxygen and thus contributes to the anaerobic microenvironment for biologically denitrification. As known, denitrifiers are common facultative anaerobic or strictly anaerobic bacteria. However, even oxygen exposes on the headspace, most denitrifiers can still reduce nitrate once in a while. The H_2 pathway is based on the speculation of H_2 could be generated through cathode, and next utilized by autotrophic denitrifiers as an electron donor in BESs (Abudukeremu et al. 2017, 2016b; Gopalakrishnan et al. 2017). The third directly electron pathway means the electrons from cathode are directly transferred to the denitrifying bacteria in the cathode chamber. The development of extracellular electron transfer (EET), especially the direct electron transfer (DET), greatly promoted the attention of the directly electron pathway. Currently, more and more studies about the DET had elaborated the pathway of electron transfer.

The recent development of biocathode MFC can provide final alternative electron acceptor (nitrate instand of oxygen) and it can also produce bioelectricity generation. Clauwaert et al. (2007) first realized simultaneous organics oxidation by biological anodic and nitrate reduction by denitrifiers on the cathode of a two-chambered MFC without external power supply (Clauwaert et al. 2007). This biocathode MFC could simultaneously achieve the maximum power density (MPD) of 8 W/m^3 and a nitrate removal rate of 0.146 kg NO_3^--N/d/m^3, demonstrating that the feasibility of combining biological denitrification with organics removal in biocathode MFC for nitrate-contaminated wastewater treatment. Bioelectrochemical technology can be utilized for nitrogen removal mainly originated from groundwater, surface water, and waste water. Up to now, nitrate removal using biocathode BES has been realized through either heterotrophic denitrification or autotrophic denitrification. Denitrifiers on the cathode directly use the electrons from the anode for nitrate reduction. Gregory et al. (2004) reported that autotrophic denitrifying bacteria preferentially utilized an electrode as the electron donor for nitrate reduction. Then several researchers have confirmed that nitrate can be treated using biocathodes in either an autonomous MFC or a MEC with an external power source (Virdis et al. 2009). These methods using BESs are usually useful for in situ remediation of nitrate-contaminating groundwater (Knoche et al. 2016). For example, Zhang and Angelidaki (2013) assembled a novel bioreactor, named submerged microbial desalination denitrification cell (SMDDC) for nitrate-contaminated groundwater treatment. This special reactor could simultaneously realize electricity generation and biological desalination in continuous mode. Nitrate from the groundwater was transferred into the anode chamber through the separator (cation exchange membrane, CEM) and the anolyte then was directly flowed to the cathode chamber for nitrate removal. Pous et al. (2015) reported a cost-effective strategy using BES with nitrate as electron acceptor and organic matter or water as electron donor for groundwater treatment. This technology imposed an extra power source for enhancing the nitrate reduction. Furthermore, some factors were also reported to evaluate the performance of denitrifying MFCs (Zhao et al. 2016). Also, ammonia could be oxidized in the cathode and then nitrate was in situ reduced via biological or

electrochemical process. The usefulness of coupling short-cut nitrification and bioelectrochemical denitrification in the cathode chamber of MFC was reported for nitrogen removal and obtained a removal rate of 0.0125 kg N/m^3 (Li et al. 2016).

Generally, a complete BES contains physicochemical and biological processes. And the latter requires the electrogenic microorganism (e.g., *Geobacter* species) to support its work (Kashima and Regan 2015). For denitrifying BES, the electrogenic denitrifiers could rapidly adapt and then enrich on the surface of the anode. In single-chambered MFCs (SCMFCs), nitrate reduction not only occurred in the cathode with bioelectrochemical denitrification but also in the anode with heterotrophic denitrification (Huang et al. 2018; Drewnowski and Fernandez-Morales 2016). Researchers fabricated an air cathode SCMFC coupling heterotrophic denitrification with anodic respiring and obtained a nitrate reduction rate of 60 mg/L/h (Drewnowski and Fernandez-Morales 2016). Unfortunately, some conditions, like the original construction, the type of substrates, and nitrate concentration, inevitably affect the dominated genera of anodic microbial community. For example, with the initial nitrate concentration increasing (from 0 to 800 mg/L), the percentage of denitrifying bacteria increased from 11.2% to 79.5%, while the percentage of electricigens decreased from 71% to 8.1% in SCMFC (Huang et al. 2018). The genera of *Thauera* and *Geobacter* were, respectively, considered as the dominant genus of denitrifiers and electricigens. The proportion of electricigens in anodic biofilm was obviously decreased when the addition of initial nitrate concentrations to the anode of SCMFC was increased. Although the construction of the biological community is obviously different, MPDs had little affected by nitrate in SCMFC. It is speculated that the amount of electricigens were not a limiting factor in MFCs. A cooperation mode (e.g., direct interspecies electron transfer, DIET) between denitrifiers and electricigens was used to improve the electron transfer from bacteria to the solid electrode (Kumar et al. 2018). Another reason suggested that the denitrifying bacteria might have the capability of extracellular electron transfer. Yang et al. reported a denitrifying SCMFC with 74.5% *Thauera*, demonstrating that *Thauera* has both the capability of extracellular electron transfer and nitrate reduction (Yang et al. 2019). Pous et al. fabricated a dominated—*Thiobacillus* (involved in NO_2 and N_2O reduction) biocathode BES for denitrification, and the bioelectrochemical reduction of nitrate was realized (Pous et al. 2015). Although a wider number of sub communities were involved in denitrification, *Thiobacillus* was enriched from 0% to 33% in the biocathode.

In our research, acetate as an electron donor was cultured in MFCs, and nitrate as an alternative substrate was added to the anode chamber. The electricity performance and anodic microbial communities of MFCs with nitrate or not were analyzed and compared (Jin et al. 2019). Results showed that nitrate significantly affected the genus of anodic microbial communities. As shown in Fig. 11.1, the proportion of denitrifying bacteria increased significantly from 16.2% in MFC without nitrate (Fig. 11.1A) to 37.0% in MFC with nitrate (Fig. 11.1B), whereas the exoelectronic bacteria decreased from 73.3% to 39.6%. Furthermore, the type of electricigens also increased from *Geobacter* in MFC without nitrate (MFC-C) to the combination of *Geobacter* and *Pseudomonas* in MFC with nitrate (MFC-D). *Geobacter* has been

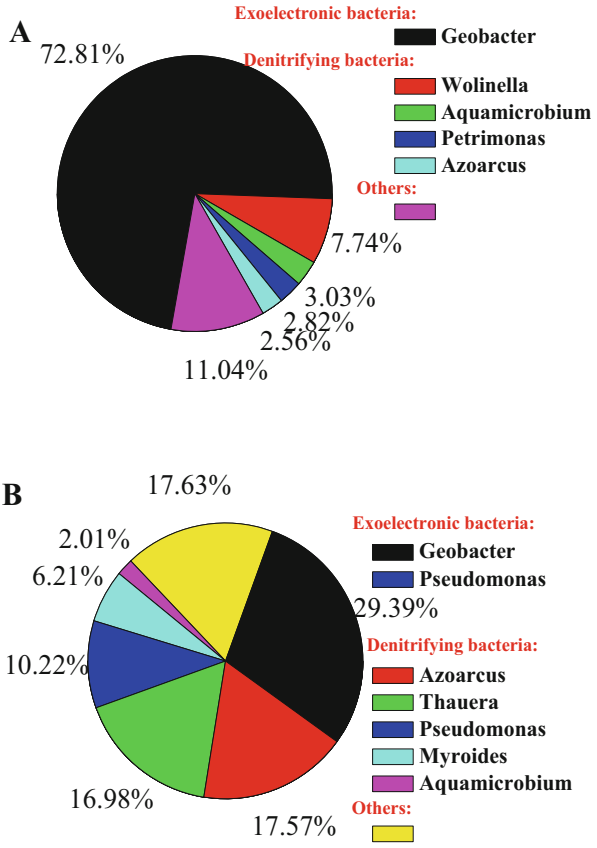

Fig. 11.1 Composition of bacterial community in the anodic biofilms of MFC without or with nitrate. (Referenced by Jin et al. 2019)

confirmed its ability of extracellular electron transfer and plays a crucial role in anodic biofilms of BESs. Another genus *Pseudomonas* was accounted for 1.5% in MFC without nitrate. However, the proportion was significantly increased to 10.2% in MFC with nitrate. *Pseudomonas* species have been verified the capacity of extracellular electrons transfer from bacteria to the solid electrode in BES or reduce nitrate to nitrogen in bioreaction. Further analysis about the characteristic sequences (*NirS*) of denitrifiers showed that the denitrifying bacteria was composed of *Azoarcus*, *Pseudomonas*, and *Thauera*, which were accounted for 40.3%, 36.3%, and 21.2%, respectively (Fig. 11.2). Importantly, with a long-term operation, the proportion of *Pseudomonas* continuously increased to 26.3%, until considering as a dominant genus. In this system, Geobacter could not use nitrate as an electron acceptor, and no negative effect of nitrate on power production was detected. In a word, *Pseudomonas* was considered the denitrifying electricigens in this system (Jin et al. 2019).

Based on the difference in microbial communities, the performance of nitrate removal and electricity generation was also changed. Though no significant changes in voltage output, the cycles of power generation sharply shorted in the presence of nitrate (Fig. 11.3a). The MPD increased by 14.1% (Fig. 11.3b) and the internal

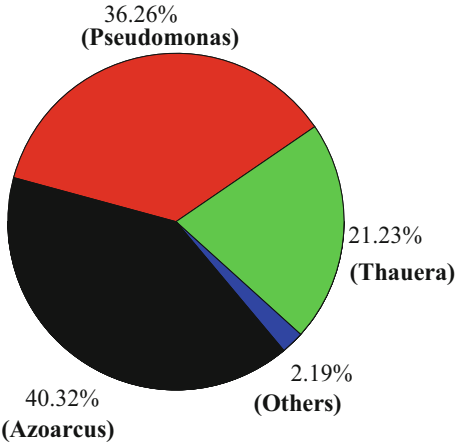

Fig. 11.2 The relative abundance of the denitrification genes (*NirS*) in the anodic biofilm of MFC. (Referenced by Jin et al. 2019)

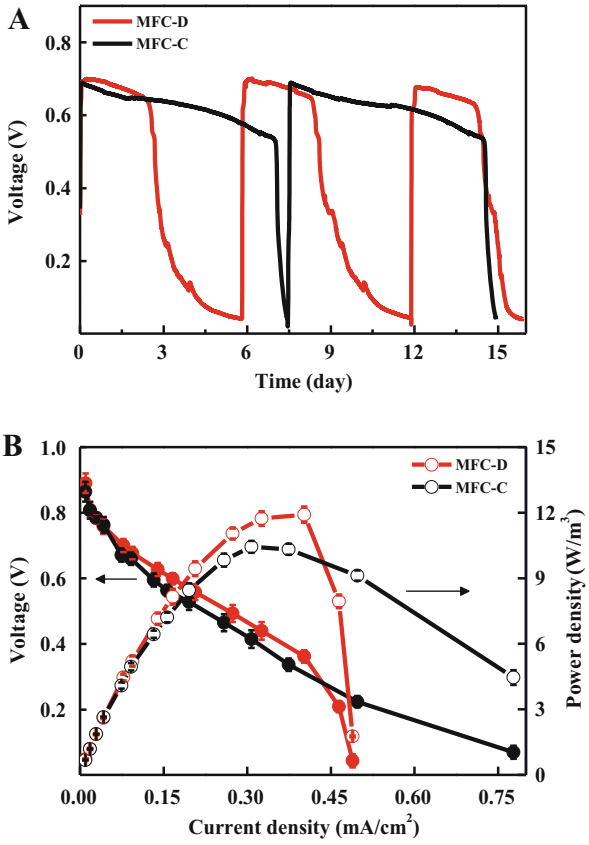

Fig. 11.3 Voltages (**a**) and power densities (**b**) of MFC without nitrate (MFC-C) and with nitrate (MFC-D). (Referenced by Jin et al. 2019)

Table 11.2 The denitrifying electricigens in BESs in the literature

Genus	Electron donor	Power density (mW/m^2)	Ref.
Shewanella oneidensis	–	–	Cruz-Garcia et al. (2007)
Ochrobactrum anthropi	Acetate	89	Zuo et al. (2008)
Comamonas denitrificans	Acetate	35	Xing et al. (2010)
Calditerrivibrio nitroreducens	Acetate	823	Fu et al. (2013)
Pseudomonas aeruginosa	Wastewater	173.3	Manogari and Daniel (2015)
Geobacter metallireducens	Acetate	–	Kashima and Regan (2015)

resistance (R_{in}) relatively decreased from 150 to 100 Ω. The changes of MPD and R_{in} could be explained by alleviating anolyte acidification and DIET between denitrifiers and electricigens. Therefore, the extracellular electron transfer by electricigens was stimulated. With a low nitrate concentration, MFC performance has not obvious negative influence as previous reports (Fu et al. 2013). Compare to single chamber MFCs, the effect of nitrate on electricity performance was more sensitive in dual chamber MFCs when nitrate was added to the anode chamber. For example, nitrate nitrogen of 20 mg/L could make current output decreased in a dual-chamber MFC. The inhibition concentration is also related to the configuration of the reactor. In a micro-scale dual-chamber MFC, only nitrate nitrogen of 4 mg/L could also make current output decreased too. It seems that dual-chamber MFC was more sensitive to nitrate than single-chamber MFCs. This discrepancy was mainly attributed to the characterization of functional microbes. Generally, nitrate can be removed by anodic denitrifiers and electrons can be transferred to anode by electricigens for generating electricity in MFCs. Once these two processes happen independently, denitrification will no negative effect on power generation. Interestingly, specific functional bacteria with simultaneous denitrification and electricity performance must be considered in denitrifying MFCs. To date, the mechanism of denitrifying electricigens is still unclear and resulted in the optimal conditions in denitrifying MFCs are still uncontrolled.

Up to date, researchers have reported a few denitrifying electricigens possessing the denitrification capacity and anodic respiration in BESs (Table 11.2). Fu et al. (2013) reported a current output of MFCs with *Comamonas denitrificans* or *Calditerrivibrio nitroreducens* was negative effected by nitrate, suggesting that the possible change in electron transfer mechanism and resulted in the electricity performance negatively (Fu et al. 2013). The similar conclusion about a shift between anodic denitrification and anode respiration was also reported by Kashima and Regan (2015) when *Geobacter metallireducens* as a denitrifying electricigen was inoculated in a BES. The nitrate concentration determined the electricity performance and there existed a critical level in this system. Once the addition of nitrate was higher than the critical concentration, the electron flowed to the anode

was severely restrained. Besides the above mentioned, other electricigens containing *Shewanella oneidensis* (Cruz-Garcia et al. 2007), *Pseudomonas aeruginosa* (Manogari and Daniel 2015), and *Ochrobactrum anthropi* (Zuo et al. 2008) also have the capacity of heterotrophic denitrification, but the comprehensive performance of denitrification and electricity generation is still unclear.

In our experiment, a novel denitrifying electricigen (named *Mycobacterium* sp. EB-1) was isolated and inoculated into a dual-chambered air cathode MFC (Jin et al. 2018). The MPD of 0.84 W/m^2 could be achieved for simultaneous electricity generation and nitrate removal. And further research suggested that no mediator referred to the extracellular electron transfer in MFC. The concurrent processes of anode respiration and anodic denitrification is a limitation rather than an inhibition of the electron donors in this system. The conclusion was greatly different from the previous reports. For instance, the isolated strain Yu37-1 as a denitrifying electricigen, belonged to *Calditerrivibrio nitroreducens*, was strongly inhibited when 20 mM nitrate was added to the anode chamber of MFC (Fu et al. 2013). Similarly, Xing et al. (2010) presented a MFC with *Comamonas denitrificans* DX-4, and the voltage output obviously decreased when 10 mM nitrate was added to the anolyte. Though it is confirmed that nitrate has a negative effect on the current generation in BES, the cooperation mode between anodic respiration and anodic denitrification was realized in our study (Jin et al. 2019). As known, organics oxidized by microbes and generated a large mass of electrons, which were, respectively, flowed into anodic respiration, anodic denitrification, and others contained electron losses for the overpotential and biomass synthesis in denitrifying MFC. The electron flux has a great association with the concurrent metabolism of anodic respiration and anodic denitrification (Virdis et al. 2009; Chandrasekhar et al. 2014b). With an increasing amount of nitrate, electrons for denitrification increased and electrons for anodic respiration relatively decreased, indicating the electron consumption rate for anodic denitrification was much faster than that for anodic respiration rate. When the sum of above rates is bigger than electron production rate, inhibition of the current generation will occur. Otherwise, the electron production rate can satisfy the sum of the rate for anodic denitrification and anodic respiration, electricity performance will not be influenced. It is indicated that a critical condition existed for the symbiotic metabolisms of denitrification and anode respiration in MFCs inoculated with exoelectrogenic denitrifying bacteria. Therefore, whether or not to inhibit the electricity performance mainly depends on the ability of organics metabolism and electron transportation by bacteria in a BES. Generally, the maximum metabolic rate of organic matters and anodic respiration rate are fixed in a stable system. However, nitrate concentration plays a key role in the anodic denitrification rate. In MFC with mixed culture, the amount and composition of the microbial community changed with the presence of nitrate, and the rates also changed relatively. Besides, DIET between bacteria interferes the analysis of experiment results. In a word, the complex condition makes the mechanism analysis of the biological community difficult.

11.3 Conclusion

This chapter reviews the development of denitrifying bacteria and electricigenic bacteria in BESs with nitrate-containing wastewater treatment in the previous study especially introduced the key role of denitrifying electricigens. Denitrifying bacteria was easily involved in denitrification with BESs for nitrate removal. BESs incorporating heterotrophic denitrification could improve electricity recovery and carbon removal efficiencies. In MFC with mixed culture, the proportion of electricigens decreased and the proportion of denitrifiers relatively increased with the increasing nitrate concentration. Within the anodic biofilm of denitrifying BES, denitrifying electricigens, capable of simultaneous denitrification and electricity generation, would finally occupy as dominant bacteria.

11.4 Opinion

Denitrifying electricigens are the amazing genera in denitrifying BESs for nitrogen removal. However, the electron fluxes for denitrification and electricity and the electron transfer mechanism are unclear. Further researches should pay attention to these special bacteria for enlarging the isolating scope. If we could extract certain genes like nitrate reductases, the performance of electrochemical activity bacteria might be analyzed by considering certain functional genes as molecular markers.

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Chapter 12
Bio-electrochemical Remediation of Petroleum Hydrocarbons

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Abstract Bioelectrochemistry and, more specifically, microbial electrochemistry are research fields that establish their fundaments on the molecular and electrochemical link between microbes (also known as exoelectrogens or, focusing only on bacteria, electrochemically active bacteria) and electrodes. Bioelectrochemistry can be used as a strategy in bioremediation when traditional bioremediation is not an option due to the lack of suitable electron acceptors, and in which bioelectrochemical systems (BESs) are used for the removal of pollutants from the environment. For example, in subsurface hydrocarbon-polluted water, the absence of final electron acceptors may limit the biodegradation rate. Therefore, bioelectrochemical systems can be used as a sustainable remediation technology. Moreover, microbial metabolism can be stimulated in a BES when overpotential is applied, increasing the rate of pollutant degradation. BES has been studied for the remediation at laboratory and pilot scale of water, soil, and sediments affected by organic pollutants, such as hydrocarbons (aliphatic, aromatic) and chlorinated compounds. In addition, BES can be exploited as biosensors to detect organic pollutants in environmental matrices and remote sites. One of the main challenges in this field is to scale up the technology towards the commercial BES remediation applications.

12.1 Introduction

12.1.1 Petroleum Hydrocarbons as Pollutants

During the last century, the world economy has been based on petroleum and its refined products, using it as the main manufacturing and energy source for industry and people (Varjani 2017). Due to a growing economy, during 2015, the increase of

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oil requests in the world was 1.54 million barrels per day higher than the previous year, especially in non-OECD countries (OPEC 2015). Environmental petroleum release frequently occurs when oil is extracted or during the processes of refining, transportation, and storage (Okoh 2006; Das and Chandran 2011; Fuentes et al. 2014; Varjani 2017). Spills in marine environments constitute less than 10% of total hydrocarbon releases. Ninety percent of total discharge to the environment is represented by routine activities (Ivshina et al. 2015). A review on polluted areas in Europe identified around 1,170,000 possible contaminated sites (PCSs) and 127,000 contaminated sites of which around 45% have already been remediated (Panagos et al. 2013).

Benzene, toluene, ethylbenzene, and xylenes (BTEX), polycyclic aromatic hydrocarbons (PAHs), phenols, minerals, oil, and chlorinated hydrocarbons (CHC) are petroleum components or derivatives. The distribution of contaminants in groundwater shows two main classes of pollutants: hydrocarbons and heavy metals, where petroleum pollution contributes jointly to 54.4% of groundwater contamination (Fig. 12.1b) (Panagos et al. 2013). The discharge of these compounds into the environment is the principal reason for water and soil contamination (Holliger et al. 1997; Das and Chandran 2011). Even small oil spills into surface and subsurface waters can cause high concentrations of hydrocarbons that often overpass the limits dictated by the law (Spence et al. 2005).

The fate and distribution of hydrocarbons in the environment depend on several biotic (Acton and Barker 1992) and abiotic factors as physical processes related to weathering (Galt et al. 1991). It has been reported that petroleum components cause mutations and death of water and soil biota (Couillard et al. 2005) due to their high toxicity (Tang et al. 2011). Specific oil components have carcinogenic and neurotoxic properties, such as benzene, toluene, xylenes, naphthalene and *n*-hexane (Ritchie et al. 2010). Petroleum spills in water that prevents sunlight to pass through it affect not only the biota but also physical and chemical processes. Hydrocarbon-polluted waters, soils, and sediments should not be used for agriculture, urbanization, and as water source for people and animals. The removal of hydrocarbon components from the environment involves physical, chemical, and biological processes (Okoh 2006; Fuentes et al. 2014).

12.1.2 Remediation of Petroleum Hydrocarbon Contaminated Sites

The removal of pollutants from the environment is a requirement for sustainable development. Remediation technologies are applied in situ or ex situ. Physicochemical and biological processes have been applied to the clean-up of contaminated environments (Tyagi et al. 2011; Fuentes et al. 2014; Daghio et al. 2017). Physical strategies include extraction, thermal desorption, soil washing, and filtration techniques; while chemical treatments involve the addition of strong oxidant or reducing

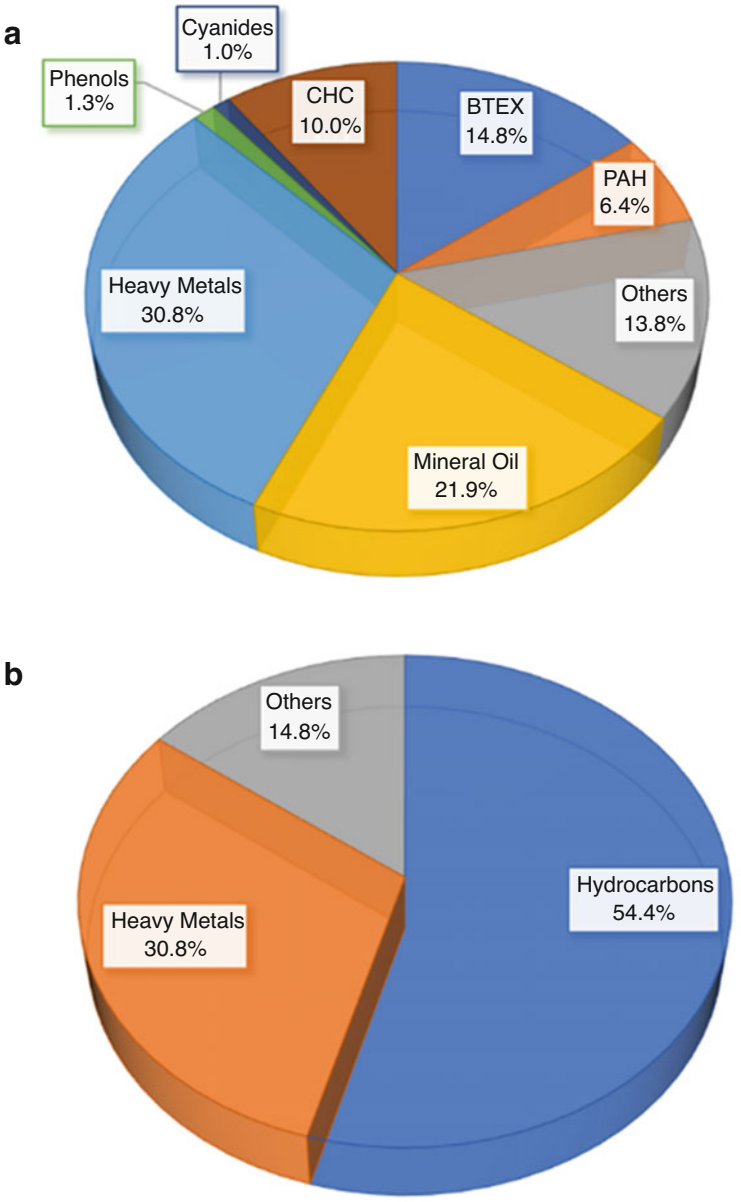

Fig. 12.1 General distribution of contaminants affecting soil and groundwater in Europe (**a**) and grouped by type of contaminants (**b**). Adapted from Panagos et al. (2013)

agents to lower the toxicity of the pollutants. Bioremediation is an attractive technology for the restoration of polluted waters and soils (Rojas et al. 2011; Fuentes et al. 2014; Orellana et al. 2018). Bioremediation is an efficient, cost-effective, and

eco-friendly technique that relies on the microbial capabilities to metabolize the pollutants into harmless or less-toxic compounds, causing minimal ecological effects (Atlas 1995; Morgante et al. 2010; Saavedra et al. 2010; Méndez et al. 2017; Orellana et al. 2018; Durán et al. 2019). The most common strategies in bioremediation are biostimulation and bioaugmentation. Biostimulation consists in the stimulation of indigenous microorganisms with degradative capabilities through the addition of nutrients and/or electron acceptors. Bioaugmentation is the application of microorganisms that possess selective metabolic capabilities (Mrozik and Piotrowska-Seget 2010; Fuentes et al. 2014). To bioremediate hydrocarbon contaminated water, the most common biostimulation approaches are bioventing, water circulation systems, air sparging, and biobarriers. Bioventing is used mainly to stimulate aerobic degradation processes by pulling air above the watercourse. In water circulation systems, water is extracted and amended with electron acceptors and nutrients and back injected into groundwater. During air sparging, compressed air is injected, and oxygen is provided to enhance the natural aerobic microbial degradation of pollutants. Biobarriers involve a permeable and biologically active fence located perpendicularly to the plume, creating a zone of high microbial activity (Alvarez and Illman 2005). Microorganisms have been vastly used to bioremediate hydrocarbon-polluted environments (Tyagi et al. 2011), including soils (Rivelli et al. 2013; Fuentes et al. 2016), sediments (Militon et al. 2015), and water (Acton and Barker 1992; Farhadian et al. 2008). Pollutants can be used by microorganisms as carbon and energy sources, leading to their complete degradation (mineralization) or are converted through detoxification processes into harmless compounds (Rivelli et al. 2013). Microorganisms are the main biocatalysts for hydrocarbon bioremediation (Fuentes et al. 2014). Diverse microorganisms are capable to metabolize a wide range of hydrocarbons through evolved mechanisms that activate these compounds and generate metabolic intermediates that are funneled into central catabolic pathways (Méndez et al. 2011; Fuentes et al. 2014; Agulló et al. 2017; Durán et al. 2019; Espinoza-Tofalos et al. 2020).

12.2 BES for the Remediation of Hydrocarbons

Biological strategies for the remediation of environmental matrices have several advantages in comparison with physicochemical technologies. However, biological techniques may have also drawbacks. For example, in bioaugmentation the fate of added microorganisms is difficult to predict and in biostimulation, the addition of nutrients and electron acceptors might present some disadvantages (e.g., the formation of toxic intermediates, the elevated cost of continuously insufflating air). Moreover, when air is injected in soil or underground water, the probability that most volatile hydrocarbons will be stripped is high. Thus, to trap the volatile pollutants filters that may imply high cost should be used.

These limitations might be overcome by the application of bioelectrochemical systems (BESs) for the remediation of hydrocarbons from underground water, soils,

and sediments (Table 12.1). Figure 12.2 illustrates a BES for hydrocarbon bioremediation. BES uses the redox gradient between a buried electrode and the hydrocarbons. Microorganisms that colonize the electrode surface oxidize these organic pollutants in absence of oxygen using the electrode as a non-exhaustible electron acceptor. Then, electrons are remotely transferred by the electrode to oxygen or other thermodynamically favorable electron acceptor (Lovley 2011; Morris and Jin 2012; Lu et al. 2014a, b).

BES-based technologies are advantageous compared with traditional bioremediation methods: (1) the electrode acts as an inexhaustible electron acceptor/donor and (2) the co-localization of pollutants, microbes and electron acceptor, enhance the removal of the contaminants (Lovley 2011; Wang et al. 2015).

Morris and Jin (2008) used a BES to couple hydrocarbons removal with electric power production. BESs have been applied to study the electrochemical-driven biodegradation of hydrocarbons in water (Morris et al. 2009; Franzetti et al. 2017; Espinoza-Tofalos et al. 2018; Palma et al. 2018a, b, 2019), soils (Wang et al. 2012, 2019), and sediments (Morris and Jin 2012; Cruz Viggi et al. 2015).

12.2.1 BES for the Remediation of Hydrocarbon-Polluted Water

The remediation of several classes of hydrocarbons has been studied in BES: single compounds (Rakoczy et al. 2013; Wei et al. 2015; Palma et al. 2018a, b), mixtures (Adelaja et al. 2017; Palma et al. 2019), and wastewater (Morris et al. 2009; Majumder et al. 2014; Srikanth et al. 2016; Daghio et al. 2017; Roustazadeh Sheikhyousefi et al. 2017; Mohanakrishna et al. 2018; Espinoza-Tofalos et al. 2020).

Benzene degradation by microbial communities has been studied in microbial fuel cells (MFC) and polarized BES. The limitations of these systems have been studied, providing special attention to the cathodic abiotic reaction. Oxygen is the most studied and used electron acceptor on the cathodic chamber (Rakoczy et al. 2013; Wei et al. 2015; Liu et al. 2018). However, also ferricyanide (Wu et al. 2013) and anoxic cathodes have been employed in BES for the removal of aromatic hydrocarbons (Daghio et al. 2018; Palma et al. 2018a, b, 2019). Air-cathodes in MFC configuration often lead to oxygen diffusion through the cation exchange membrane (Rabaey and Verstraete 2005; Morris et al. 2009; Adelaja et al. 2015). Oxygen diffusion from the cathodic chamber to the anodic one in BES for benzene removal has been reported by compound-specific isotope analysis, revealing that monohydroxylation is the benzene activation step (Rakoczy et al. 2013; Wei et al. 2015). To study the BES-based technology for in situ remediation, a mixed culture from a polluted site or refinery wastewater should be used as inoculum due to the high abundance of hydrocarbon-degrading microorganisms. A novel bioelectrochemical reactor configuration, "the bioelectric well," revealed higher phenol removal when the bioelectrochemical reactor was re-inoculated with refinery

Table 12.1 BES-based technologies applied in hydrocarbon bioremediation

	Hydrocarbon source (ppm)	Environmental matrix	Microbial inoculum	BES system (L)	Poised potential (V)	Removal efficiency (%)		Study
						Closed circuit	Control/ open circuit	
Water	*n*-Alkanes and aromatics mixture (203.5)	Refinery groundwater	Native microbial community	Dual chamber MFC (0.01–246.5)		[a]		Morris and Jin (2008)
	Diesel (300)	Refinery groundwater	Native microbial community	Dual chamber MFC (0.9)		82	31	Morris et al. (2009)
	Benzene (11.7–19.5)	Sulfide contaminated groundwater	Native microbial community	Dual chamber MFC (1)		18–80		Rakoczy et al. (2013)
	Benzene (10.9–21.7)	Artificial polluted water	Oil cracking wastewater	Dual chamber MFC (1.3)		100		Wu et al. (2013)
	Toluene (69)	Artificial polluted water	Oil cracking wastewater	Dual chamber MFC (0.5)		100		Lu et al. (2014a, b)
	PAH (2213)	Refinery wastewater	*Pseudomonas putida* BCRC 1059	Dual chamber MFC (0.25)		30		Majumder et al. (2014)
	Phenanthrene and benzene (20–30)	Artificial polluted water	Sewage treatment anaerobic sludge	Dual chamber MFC (0.25)		92		Adelaja et al. (2015)
	Benzene (15)	Polluted groundwater	Native microbial community	Dual chamber MFC (0.32)		80		Wei et al. (2015)
	Aliphatics and phenol (60–90)	Refinery wastewater	Enriched culture	Dual chamber MFC (0.25)		81		Srikanth et al. (2016)
	Aliphatics (2000)	Refinery wastewater	Native microbial community	Single chamber MFC (0.125)		97		Roustazadeh Sheikhyousefi et al. (2017)

	Phenol (25)	Artificial polluted water	Refinery wastewater	Bioelectric well (0.25)	0.2	99.5		Palma et al. (2018b)
	BTEX mixture (8–20)	Artificial polluted water	Refinery wastewater	Polarized BES (0.12)	0.8	~100		Daghio et al. (2018)
	TPH (1090)	Refinery wastewater	Domestic wastewater	Single chamber MFC (0.35)	0.5	92	45	Mohanakrishna et al. (2018)
	Benzene (60)	Polluted groundwater	Petrochemical wastewater consortium	Tubular MFC (5.7)		100		Liu et al. (2018)
	Toluene (45)	Artificial polluted water	*Cupriavidus metallidurans* CH34	Dual chamber MFC (0.32)		87	10	Espinoza Tofalos et al. (2018)
	BTEX (25)	Artificial polluted water	Native microbial community	Bioelectric well (0.25)	0.2	94		Palma et al. (2019)
Sediments	Toluene (140)	Artificial polluted marine sediment	*Geobacter metallireducens* ATCC 53774 and DSM 7210	Dual chamber MFC (0.94)	0.3	~100	14–77	Zhang et al. (2010)
	TPH (16000)	Beach sediment	Native microbial community	Sediment MFC (0.05)		24	2	Morris and Jin (2012)
	IFO180 (11.9)	Artificial polluted marine sediment	Native microbial community	Oil spill snorkel (0.12)		80		Cruz-Varjani (2017)
	Toluene (~35)	Artificial polluted marine sediment	Refinery wastewater	Bioelectric well (0.25)	0.2	~100		Palma et al. (2018a)
	TPH (~14,000)	Marine sediment	Sandy marine sediments	Sediment MFC (1)	2	58–59	36–44	Bellagamba et al. (2017)
	PAH (50)	Artificial polluted sediment	Native microbial community	Sediment MFC[a]		31–36		Sherafatmand and Ng (2015)
	PAH (0.2–0.5)	Marine sediment	Native microbial community	Sediment MFC (0.9)		94		Hamdan et al. (2017)
	PAH (54–420)	River sediment	Native microbial community	Single chamber MFC (390)		74		Li et al. (2017)

(continued)

Table 12.1 (continued)

	Hydrocarbon source (ppm)	Environmental matrix	Microbial inoculum	BES system (L)	Poised potential (V)	Removal efficiency (%)		Study
						Closed circuit	Control/ open circuit	
	Toluene (40)	Artificial polluted marine sediment	Native microbial community	Polarized BES (0.25)	0.3 V	100		Daghio et al. (2016)
Soils	Phenol (80)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC (0.25)		90		Huang et al. (2011)
	TPH (28,300)	Hydrocarbon contaminated soil	Native microbial community	U tube MFC (2.736)		a		Xin et al. (2012)
	TPH (114,600)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC (3)		48–79	38–45	Lu et al. (2014a)
	TPH (12,250)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC (50)		82–90	68	Lu et al. (2014b)
	PAH (82–103)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC[a]		27–54	24–34	Yu et al. (2017)
	n-Alkanes (23–48,207)	Hydrocarbon contaminated soil	Native microbial community	Soil MFC (0.324)		13		Zhang et al. (2014)

BTEX benzene, toluene, ethylbenzene, xylenes; *PAH* polyaromatic hydrocarbons, *IFO180* intermediate fuel oil, *TPH* total petroleum hydrocarbons
[a]Data non-reported

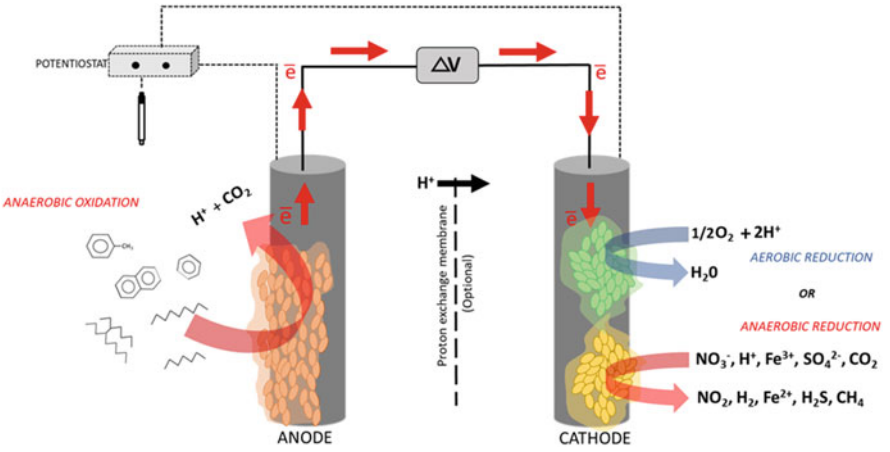

Fig. 12.2 General scheme of BES for petroleum hydrocarbons remediation. BES remediation of hydrocarbons uses the redox gradient between electrodes. Under anaerobic conditions, electroactive microorganisms use hydrocarbons as electron donors towards an anode that acts as a virtually inexhaustible electron acceptor. In ex situ systems, electroneutrality is maintained by ions transport through an ion-permeable medium or membrane. Electrons travel via an external circuit to the cathode, where they are finally transferred to a suitable electron acceptor. Electricity can be an output product of this process. In addition microbial metabolism may be stimulated by applying an external overpotential through a power source (two electrodes configuration; a voltage difference is applied between electrodes) or with a potentiostat (three electrodes configuration illustrated in this figure; a selected voltage may be imposed on the working electrode)

wastewater compared with municipal activated sludge inoculation (Palma et al. 2018b). In this study, the anode was potentiostatically set at +0.2 V versus SHE and the cathode was maintained anoxic. The application of an external voltage may be advantageous because it stimulates microbial metabolism (Wagner et al. 2010). This is related to the fact that the main factor that determines the optimal metabolic conditions in a reactor is the potential of the terminal respiratory proteins used by exoelectrogenic bacteria (Wagner et al. 2010). Therefore, the optimal imposed voltage should be tested depending on the inoculum and the type of pollutants. Two and three electrodes configurations have been studied. When the anode was potentiostatically polarized, applied voltages ranged between +200 mV and +500 mV in studies for the removal of toluene, phenol, and BTEX (Zhang et al. 2010; Daghio et al. 2016; Palma et al. 2018a, b). However, microbial metabolism can be stimulated also by applying a voltage difference between anode and cathode (two electrodes configuration). This set-up presents the advantage to require less sophisticated instrumentation (especially if an in situ application is required), because just a power supply is needed but not a potentiostat. However, the disadvantage is that the working potential is no longer controlled and varies depending on the redox conditions of the medium.

12.2.2 BES for the Remediation of Hydrocarbon-Polluted Sediments

Sediments are environmental matrixes particularly suitable to be treated with microbial electrochemical technologies for two main reasons: (1) sediments are anoxic, thus optimal for bioelectrochemical oxidation on the anode surface, and (2) sediments are water-saturated, hence electrolytic conditions are guaranteed (especially marine sediments). Due to the favorable conditions for their development, sediment microbial fuel cells (SMFC) have been studied for the degradation of hydrocarbons.

Different configurations have been tested, from the double chamber where processes can be well controlled (Zhang et al. 2010; Daghio et al. 2016; Bellagamba et al. 2017), to the single chamber that reproduces better an in situ application (Morris and Jin 2012; Cruz Viggi et al. 2015; Sherafatmand and Ng 2015; Hamdan et al. 2017; Li et al. 2017; Palma et al. 2018b). In single chamber SMFC, the anode is buried into the sediment and the cathode can be placed on the overlying aerobic water or completely submerged (which does not guarantee oxic reactions on the cathode surface). In studies with aerated cathodes, phenanthrene removal reached 89% (Hamdan et al. 2017), whereas PAHs (including benzo(a)pyrene, benzo(k)-fluoranthene, and benzo(a)fluoranthene) were efficiently removed up to 94% (Li et al. 2017). Interestingly, in a study that compared aerated vs anoxic cathodes, SMFCs achieved 42% naphthalene, 31% acenaphthene, and 36% phenanthrene removal when an aerobic cathode was operated, and 77%, 53%, and 37% removal, respectively, when the cathode was placed under anaerobic conditions (Sherafatmand and Ng 2015). This demonstrates that both configurations can be used, depending on the overlying water oxygen concentrations and/or operational requirements. An innovative set-up, the so-called "oil-spill snorkel" simplifies the system set-up, by burring part of a single conductive material (the snorkel) in the sediment (that acted as an anode) and leaving the other half on the overlying O_2-containing water (oxic zone) (Cruz Viggi et al. 2015). Even if this design showed lower performances than other similar studies (21% TPH removal within 22 days), it is an inexpensive and simple alternative for the removal of hydrocarbons from sediments.

12.2.3 BES for the Remediation of Hydrocarbon-Polluted Soils

The remediation of hydrocarbon-polluted soils with BES-based technologies has not been extensively investigated. However, since Huang et al. (2011) proposed this technology to remediate phenol-contaminated soil in a MFC, the use of this technology has found a new field of application.

Soils polluted with phenol (Huang et al. 2011) but mainly soils contaminated by petroleum hydrocarbon were studied in soil MFC (Xin et al. 2012; Lu et al. 2014a, b;

Zhang et al. 2014; Yu et al. 2017; Wang et al. 2019). Unlike BES technologies for water remediation, soil studies have been focused in MFC-based designs that stimulate the microbial metabolism without external polarization, by using potentiostatically controlled buried anodes with air-cathodes, the so-called soil microbial fuel cells. Water content (Xin et al. 2012; Wang et al. 2019), the distance between anodes (Lu et al. 2014a, b; Yu et al. 2017), or both factors (Xin et al. 2012; Wang et al. 2019) are the most studied variables, but also reactor design (e.g., U-shape) (Xin et al. 2012), electrodes arrangement (horizontal or vertical) (Zhang et al. 2014), electrodes materials (Lu et al. 2014a), and soil texture (Lu et al. 2014a; Wang et al. 2019).

Water content is indeed a key parameter for the successful remediation of hydrocarbon-polluted soils. High water contents (possibly up to saturation) are needed to favor mass transport phenomena and to lower the internal resistance (Xin et al. 2012). In a study with saturated vs unsaturated conditions, at the end of the experiment (248 days) a maximum of 59% and 45% TPH were removed in saturated sandy and clay soils, respectively, which was approximately 48% (sandy soil) and 55% (clay soil) higher than under unsaturated conditions.

Most studies indicate that the radius of influence (ROI) is a key factor and that TPH removal rates decrease with the distance from the anode (Yu et al. 2017) due to less microbial electrochemical activities and mass transfer phenomena. However Lu et al. (2014a) concluded that the TPH degradation rates in BESs were higher than those in control reactors operated at open circuits, suggesting that bioelectrochemical stimulation had a positive influence on the pollutants removal, even at a certain distance from the electrodes. In any case, water content and distance from electrodes are highly linked. Wang et al. (2019) reported that TPH removal was not enhanced when measured 35 cm far from the anode (in comparison with open circuit controls) when the soil was unsaturated. However, under saturation conditions, at 35 cm of distance, an increase in toluene removal (11%) was observed in saturated sandy soils. Interestingly, saturated soils may inhibit classical aerobic bioremediation of hydrocarbons but enhance bioelectrochemical bioremediation. In a report focused on the study of ROI, the authors concluded that the TPH degradation rate was highly dependent on the radius of influence during the first samplings (days 5 and 15) but became a less significant variable with longer incubation times. On day 120, a maximum of 90% (68% in control) of TPH was removed from soil, and the soil TPH fraction was independent on the distance from the anodes (Lu et al. 2014b). By correlating the amount of TPH degradation and the radial distance from the BES anodes, it was possible to predict the ROI after a specific time of treatment. However, the ROIs may be influenced by some soil characteristics such as water content, matrix permeability, and porosity, besides the type of pollutant.

The structure of microbial communities is indeed influenced by the use of an electrode as an electron acceptor. Lu et al. (2014a) reported that *Proteobacteria* was the most abundant phylum in the polluted soils treated with MFC technology. However, *Actinobacteria*, *Bacteroidetes*, *Firmicutes*, and *Acidobacteria* were also observed. Differences in composition of bacterial communities were observed in non-contaminated (mainly *Proteobacteria* and *Actinobacteria*) and hydrocarbon-

contaminated soils (higher levels of *Proteobacteria*). *Proteobacteria* increased by conventional and BES bioremediation. Interestingly, an increase of *Firmicutes* was observed specifically in BES hydrocarbon remediation. Mainly *Proteobacteria* were observed at the carbon cloth anode, whereas *Firmicutes* showed an increase in the biochar anode. Wang et al. (2019) reported that the dominant genus on the bioanodes was *Geobacter* (~27%), which is a model electroactive and hydrocarbon-degrading bacterium.

12.3 Challenges

Remediation using BES has been studied at laboratory and pilot scale. One of the main challenges is to scale up remediation technology using BES towards commercial applications. To improve the remediation efficiency by BES, critical physicochemical parameters should be determined and modulated, and the radius of influence of electrodes in matrices should be increased. Proteobacteria and specifically *Geobacter* genus have been associated with BES. Nevertheless, most of the electrode organisms in BES are unknown and have not yet been cultivated. Therefore, the microbial communities involved in BES remediation processes should be further characterized. Next-generation sequencing technologies and metagenomic approaches will be useful to determine the main microbial players in BES involved in the removal of the petroleum hydrocarbons in different matrixes, and the mechanisms involved in the degradation and extracellular electron transfer. Main microbes should be cultivated, and their metabolism characterized. These studies will be useful to increase the knowledge of the process for the design of improved remediation processes using BES towards knowledge-driven engineering for commercial applications.

12.4 Conclusions and Future Perspectives

BES has been applied for the remediation of several classes of hydrocarbons from water, soil, and sediments in different polluted scenarios. Degradation of hydrocarbons by microbial communities has been studied in MFC and polarized BES. Oxygen is the most used electron acceptor on the cathodic chamber, but also anoxic cathodes have been employed in BES for hydrocarbon bioremediation. The application of an external voltage through polarization by a potentiostat of the anode (from +200 to +500 mV vs SHE) could stimulate microbial degradation of diverse hydrocarbons, but other voltages can be studied depending on the characteristics of the matrix including the microbial community. MFC in different configurations (double and single chambers) have been used for the degradation of hydrocarbons in sediments. In addition, few studies reported bioelectrochemical treatment of polluted soils. The saturation of soil with water is critical for successful

bioelectrochemical remediation of hydrocarbon-polluted soils. The microbial communities are key players for BES bioremediation, but the degrading microorganisms are largely unknown. The main challenge of remediation using BES is to scale up the process for commercial applications and in situ bioremediation. BES remediation processes represent an attractive alternative to develop a robust and sustainable technology for the clean-up of petroleum hydrocarbon-polluted waters, sediments, and soils for a circular economy.

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Chapter 13
Rice Paddy-Field Microbial Fuel Cells: Fundamental and Recent Progress

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Abstract Microbial fuel cells (MFCs) are devices that exploit living microbes for the conversion of organics into electricity. Bioreactor-type MFCs have been extensively examined in the laboratory for applying them to processes that convert organic wastes into electric power. It is also possible to exploit MFCs at the interface between water and sediment in the aquatic environment, and these MFCs are termed sediment MFCs (S-MFCs). One option of S-MFCs is rice paddy field MFCs (RP-MFCs), in which anodes are set in the rhizosphere of rice plants, whereas cathodes are placed in flooded water. Studies have attempted to optimize electrode structures to enhance power outputs, and recent work has reported power outputs as high as 140 mW m^{-2} (based on the projected area of the anode). In addition, studies have been conducted to gain insights into microbes involved in power generation in RP-MFCs, and analyses using metabarcoding of PCR products and metagenomics have suggested that *Geobacter* relatives occur at anodes and contribute to the current generation. This chapter describes the fundamentals and recent signs of progress of RP-MFCs that are expected to serve as on-site power sources contributing to sustainable agriculture.

Keywords Energy harvest · Microbial fuel cell · Microbial solar cell · Exoelectrogen · Electrochemically active bacteria · Extracellular electron transfer

13.1 Introduction

Electric power is essential for human society, and a large portion of electric power is currently generated in power plants with the expense of fossil fuels. Two concerns are associated with this power generation; first, fossil fuels are finite resources, and it has been predicted that most of these will be depleted within the twenty-first century.

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Second, the combustion of fossil fuels emits carbon dioxide, the major causative agent in global warming. For the sustainable development of human society, renewable resources, such as solar energy, water energy, and biomass, must be more extensively used for power generation. Furthermore, it is eagerly anticipated that as yet unexploited energy sources will be discovered.

Recently, it has been suggested that the natural environment harbors massive amounts of as-yet unexploited energy sources, and researchers in the field of sustainable developments search for technologies that harvest unexploited natural energy for electricity generation (Priya and Inman 2009). A variety of technologies have been proposed for this purpose (Priya and Inman 2009), and these include rice paddy field microbial fuel cells (RP-MFCs). RP-MFCs are on-site power generators that can serve as energy sources for wireless monitors for measuring environmental parameters in paddy fields, such as, water and atmospheric temperatures, humidity, and solar irradiation (Kouzuma et al. 2014).

Microbial fuel cells (MFCs) are devices that use living microbes for the conversion of organics into electricity (Logan et al. 2006; Watanabe 2008). Bioreactor-type MFCs have been extensively examined in the laboratory for their application to processes that convert organic wastes onto electric power (Santoro et al. 2017). It is also possible to set up MFCs at the interface between water and sediment in the aquatic environment (termed sediment MFCs, S-MFCs), and researchers have examined S-MFCs at marine sediments and riverbeds (Tender et al. 2002; Reimers et al. 2006; Donovan et al. 2008). In S-MFCs, anodes are buried in sediments, while cathodes are floated in water immediately above the sediments, and electricity is generated primarily with the aid of microbes at anodes. RP-MFC is a type of S-MFC that is set in a rice paddy field (Kaku et al. 2008). Rice is one of the major agricultural crops in the world, in particular, in Asian countries, including Japan, and paddy fields reach over 2 million hectares in Japan, sharing over 50% of the total cultivated field (Ministry of Agriculture, Forestry and Fisheries 2018). It is therefore suggested that RP-MFC has a large potential, while further research is necessary for practical application.

In this chapter, we describe fundamentals and recent progresses for RP-MFCs and other S-MFCs with focuses on structures, performances, and microbes involved in power generation. Based on current knowledge, we discuss future directions of the research on RP-MFCs.

13.2 Structures and Performances of RP-MFCs and Other Plant MFCs

13.2.1 RP-MFCs

The use of MFC systems for electricity generation in rice paddy fields was first reported by Kaku et al. (2008), and, since then, such systems are termed RP-MFCs. A similar idea of MFC was also examined in pot cultures of rice plants

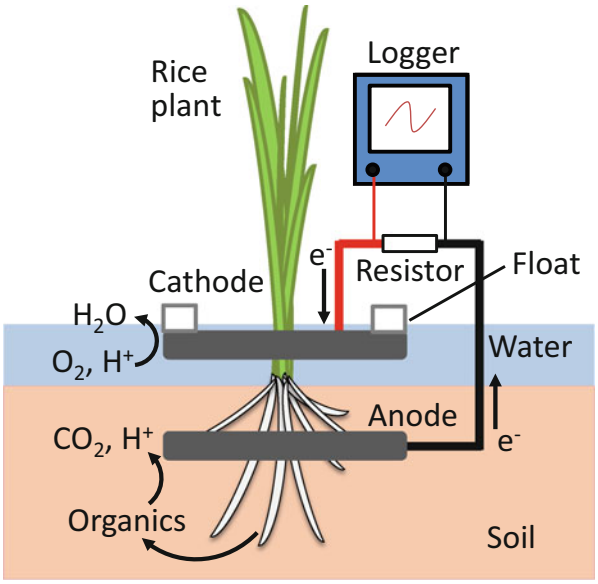

Fig. 13.1 Schematic diagram for RP-MFC

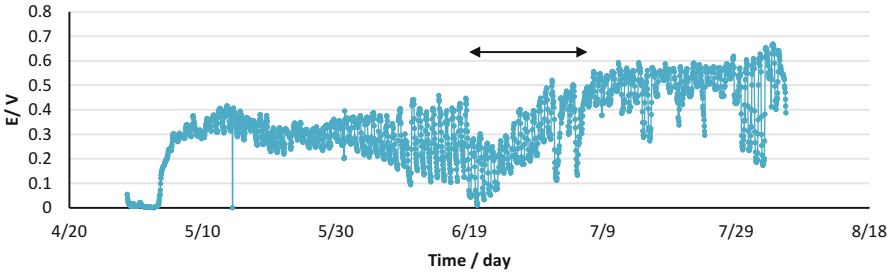

Fig. 13.2 Time course of cell voltage of RP-MFC. The operation was started at the end of April and terminated in August. An arrow indicates a rainy period

(de Schamphelaire et al. 2008). In RP-MFCs, anodes are set in rhizospheres of rice plants, while cathodes are placed in flooded water (Fig. 13.1). These electrodes were made of graphite, a material most widely used for electrodes in MFCs. In the rhizosphere, rice plants excrete photosynthesized organics from roots, and these organics serve as substrates (electron donors) for exoelectrogenic bacteria and other microbes. Electrons released from organics by oxidative catabolic reactions of exoelectrogens are captured by anodes, transferred to cathodes via external circuits, and used for the reduction of oxygens at the cathode surface (Fig. 13.1). According to the electromotive force between organics oxidation and oxygen reduction, electrons are transferred from the anode to cathode, resulting in the power generation at the external circuit. A characteristic feature of RP-MFC is the circadian oscillation of the electric output that increases in the day time and drops at night (Fig. 13.2). In

order to investigate mechanisms behind the oscillated electric output, Kaku et al. (2008) conducted two experiments, namely, measurement of electric outputs after rice plants were shaded with black clothes, and detection of root exudates in the light and dark. They have found that the shading of rice plants substantially decreases the electric output, and rice roots excrete organics, e.g., organic acids, only in the light. From these results, they suggest that the oscillated electric output from RP-MFC is attributable to the daily oscillation of sun irradiation and resultant oscillated organics excretion from rice roots. It is therefore considered that the oscillated electric output is an evidence for the plant/microbe cooperation for the conversion of light energy (sun irradiation) into electric energy (i.e., microbial solar cell).

The above work has provided the concept of RP-MFC, while the maximum power density reported in that work was 6 mW m^{-2} (normalized to the projection area of the anode) at the maximum (Kaku et al. 2008). Subsequent studies have been conducted for examining technical breakthroughs to improve power outputs from RP-MFCs. To cite an instance, Takanezawa et al. (2010) examined several factors affecting electric outputs from RP-MFCs, including anode thickness, anode depth, modification of cathodes with oxygen-reduction catalysts, and external resister used during the start up. The study has shown that anode depth, oxygen-reduction catalysts, and external resister largely affect the resultant power output. Another study has optimized sizes of anodes and cathodes and reported the maximum power density of 140 mW m^{-2} (based on the projected area of the anode) using relatively small anodes (Ueoka et al. 2016). It should also be noted that outputs from RP-MFCs are largely influenced by weather (temperature and sun irradiation); for instance, Japan has a rainy season in June and July, during which the output from RP-MFC drops substantially (Fig. 13.2).

13.2.2 Other Plant MFCs

In addition to RP-MFC, S-MFCs are also operated in association with other plants, such as *Typha latifolia*, a perennial herbaceous plant, and *Spartina anglica*, species of cordgrass (Strik et al. 2008). These MFCs are termed plant MFCs (P-MFCs), and Table 13.1 summarizes representative studies on P-MFCs. In P-MFCs, as has been reported for RP-MFCs, plants provide rhizosphere exoelectrogenic microbes with organic substrates (Strik et al. 2008). Liu et al. (2013) examined P-MFCs planted with *Ipomoea aquatica* (water spinach), showing that their power densities were twice as high as those of unplanted MFCs. In addition, the study has also shown that nitrogen-removal efficiencies of the planted MFCs are much better than those of unplanted MFCs (e.g., 90.8% vs. 54.4%), and it has been deduced that untreated nitrate in unplanted MFCs lowered electricity generation (Liu et al. 2013).

Saz et al. (2018) operated P-MFCs using four different plants, *Typha latifolia*, *Typha angustifolia*, *Juncus gerardii*, and *Carex divisa*, for examining wastewater treatment in experimental wetlands. It has been shown that P-MFCs with *T. angustifolia* exhibit the best performances in terms of ammonia removal and

Table 13.1 Representative P-MFC studies

Study	Support medium	Anode material	Cathode material	Plant	P_{max} (mW m^{-2})[a]
Helder et al. (2010)	Graphite granule	Graphite rod	Graphite felt	*Spartina anglica*	222[b]
				Arundinella anomala	22[b]
Liu et al. (2013)	Gravel	Granular achieved carbon	Stainless steel mesh	*Ipomoea aquatica*	12
Villaseñor et al. (2013)	Gravel	Graphite	Graphite	*Phragmites australis*	43
Oon et al. (2015)	Gravel	Carbon felt	Carbon felt	*Typha latifolia*	6
Lu et al. (2015)	Gravel	Graphite disk	Carbon cloth	*Canna indica*	18[c]
Liu et al. (2017)	Gravel	Granular activated carbon	Granular activated carbon	*Spartina alterniflora*	60
Saz et al. (2018)	Gravel	Graphite	Magnesium	*Typha latifolia*	13
				Typha. angustifolia	18
				Juncus gerardii	8
				Carex divisa	9
Rathour et al. (2019)	Gravel	Stainless steel	Stainless steel	*Fimbristylis dichotoma*	199
Xu et al. (2019)	Ceramics/sand	Titanium cylinder	Titanium mesh	*Phragmites australis*	16

[a]Normalized to the anode projected area unless otherwise stated
[b]Normalized to the planting surface area
[c]Normalized to the cathode projected area

power output among the examined P-MFCs, and the authors have considered that *T. angustifolia* is able to provide rhizosphere microbes with appropriate environments for organics degradation and electricity generation. From these results, the authors recommend *T. angustifolia* for fueling P-MFCs that are installed in wetlands (Saz et al. 2018). In addition to plant species, plant growth phases are also considered to affect MFC performances (Moqsud et al. 2015); in that study, authors have revealed that electricity generation of P-MFCs is high during the vegetative growth phase compared to that during the reproductive phase.

Another study has shown that salinity affects the performance of P-MFCs (Xu et al. 2019). In that study, authors constructed wetlands for treating wastewater, in which P-MFCs were operated. As a result, P-MFCs treating saline wastewater exhibited high power densities compared to those treating non-saline wastewater (16.4 mW m^{-2} vs. 3.9 mW m^{-2}, for example). Microbiome analyses have shown that putative exoelectrogens were more abundantly detected at anodes in the presence of saline wastewater than those in non-saline wastewater, and it has been

suggested a relatively high ionic strength of the electrolyte may be necessary for exoelectrogens to actively grow in P-MFCs (Xu et al. 2019). It is therefore likely that P-MFCs exhibit good performances in coastal areas and salt-damaged areas.

In summary, P-MFCs, including RP-MFCs, have been operated in rice paddy fields, wetlands, and experimental pod cultures. While comparisons of these MFCs in terms of power output suggest that rice is one of the best plants used for P-MFCs, plants that naturally occur in wetlands, such as *T. angustifolia*, are also useful for efficient power generation.

13.3 Microbes at Work in RP-MFCs and Other P-MFCs

13.3.1 Anode-Associated Microbes

In order to characterize microbiomes that occur around anodes of P-MFCs, including RP-MFCs, studies have used metabarcoding of 16S rRNA gene amplicons and shotgun metagenomics (Kouzuma et al. 2014). It is well known that microbiomes occurring in the rhizosphere are highly diverse (Berg 2009). Similar trends have also been observed in anode-associated microbiomes in RP-MFCs, in which bacterial species related to exoelectrogens, such as members of the genera *Geobacter*, *Clostridium*, *Bacillus*, and *Desulfobulbus*, have frequently been detected (Kouzuma et al. 2013; Wang et al. 2015; Lu et al. 2015; Abbas et al. 2019; Gustave et al. 2019). Among them, members of the genus *Geobacter* have been considered to be the major exoelectrogens in many natural and engineered ecosystems, owing to the widespread distribution in anaerobic environments and the high capability of extracellular electron transfer to electrodes via outer membrane-localized cytochromes (Kouzuma et al. 2018; Logan et al. 2019). On the other hand, Wang et al. (2019) have suggested that physicochemical properties of soil, including, water content, electrical conductivity, total sulfur, and iron content, significantly affect the abundance and species of exoelectrogens. The authors have reported that among the genera related to *Desulfobulbus* and *Bacillus* are the most abundant in coastal and arid-land soils, respectively, while *Geobacter*, *Clostridium*, and *Anaeromyxobacter* are abundant in paddy and lakeshore soils. Other studies have however reported that members of *Desulfobulbus* and *Bacillus* occur in anode-associated microbiomes in RP-MFCs (Wang et al. 2015; Lu et al. 2015). These differences may be ascribable to salinity; a study on MFCs using brackish sediments as inocula has shown that *Geobacter* occurs only when NaCl concentrations in electrolytes are lower than 0.1 M, while *Desulfuromonas* occurs at higher concentrations (Miyahara et al. 2016). It is hence concluded that dominant exoelectrogens can vary depending on environmental conditions.

In the rhizosphere, plant-root exudates, such as carbohydrates, fatty acids, and amino acids, are utilized as carbon and energy sources of microbial residents, thereby significantly affecting the structure of rhizosphere microbiomes (Smalla et al. 2001; Berg and Smalla 2009). Plant species are therefore considered important

factors that determine the structure of exoelectrogenic microbiomes in P-MFCs. Timmers et al. (2012) characterized anode-associated microbiomes in P-MFCs planted with reed mannagrass (*Glyceria maxima*), in which members of *Geobacter* were abundantly detected along with anaerobic cellulolytic bacteria affiliated with the families *Clostridiaceae* and *Ruminococcaceae*. From these observations, it has been suggested that electricity generation in *G. maxima*-planted P-MFCs is based on syntrophic interactions among exoelectrogenic and fermentative bacteria.

In RP-MFCs, members of *Anaeromyxobacter*, as well as those of *Geobacter*, have been abundantly detected in association with anodes (Kouzuma et al. 2013; Cabezas et al. 2015; Lu et al. 2019). *Anaeromyxobacter* is regarded as a genus that includes exoelectrogens (Wang et al. 2019), and an isolate of *Anaeromyxobacter dehalogenans* has been reported to be capable of dissimilatory metal reduction (Marshall et al. 2009). These studies suggest that members of *Anaeromyxobacter* play a particularly important role in electricity generation in RP-MFCs. Studies have also reported that members of the class *Anaerolineae*, which includes a fermentative isolate of *Anaerolinea thermolimosa* (Yamada et al. 2006), are relatively abundant in anode-associated microbiomes in RP-MFCs (Kouzuma et al. 2013; Cabezas et al. 2015). It is likely that members of this class contribute to the fermentative degradation of rice root exudates and supply electron donors to exoelectrogens, such as members of *Geobacter* and *Anaeromyxobacter*.

The studies introduced herein suggest that ecological interactions between fermentative and exoelectrogenic microbes work at anodes of P-MFCs, thereby facilitating the current generation with the expense of root exudates. Further studies will be conducted to address how such ecological interactions can be managed to improve electric outputs from P-MFCs.

13.3.2 Cathode-Associated Microbes

In MFCs, including RP-MFCs, cathodes are made of graphite/carbon, and, in many cases, doped with oxygen-reduction catalysts (Takanezawa et al. 2010). This is because the oxygen-reduction activity at the graphite/carbon surface is generally low, and it is necessary to enhance the activity to circumvent cathode-limited electric outputs from MFCs. Most of these catalysts, e.g., platinum catalysts, however, are expensive, and studies have been conducted to develop cheap and sustainable alternatives and/or technologies to reduce amounts of catalysts used at the cathode.

One approach to tackle this requirement would be the use of microbes present at cathode surfaces. It has been known that a variety of microbes occur at the cathode, and some of them contribute to the cathodic reaction in MFCs (Rabaey and Rozendal 2010; Kracke et al. 2015). Cathodes using microbes as catalysts are termed biocathodes, which have been examined in various bioelectrochemical systems (Marshall et al. 2012). A distinctive feature of cathodes in S-MFCs, including RP-MFCs, would be that these are exposed to ever-changing and, in some cases,

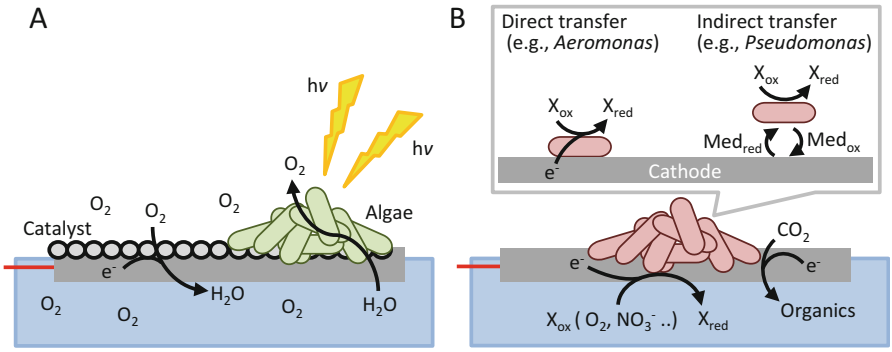

Fig. 13.3 Schematics for microbe-stimulated cathode reactions in RP-MFCs. (**a**) Oxygenic photosynthesis-assisted cathode reaction. (**b**) Biocathode reaction. X_{ox}, oxidized form; X_{red}, reduced form. *Med* electron mediator

harsh environments, e.g., the direct exposure to air, which may cause the development of distinctive microbiomes at cathodes of S-MFCs and RP-MFCs.

Studies have suggested two possible mechanisms underlying microbe-stimulated cathode reactions in S-MFCs. First, several studies have shown that microalgae are present in biofilms formed on cathodes of RP-MFCs, and they increase dissolved oxygen concentrations around cathodes due to oxygenic photosynthesis, resulting in improved cathode activities (Fig. 13.3a) (Chen et al. 2012; Srivastava et al. 2019). In another study, S-MFCs were subjected to light/dark cycles, also showing that light irradiation promotes oxygen production by phototrophs and lowers mass transport limitations for the oxygen-reduction reaction at cathodes (Bardarov et al. 2018).

On the other hand, it has been reported that there exist microbes in the natural environment that can uptake electrons from cathodes and utilize them for their intracellular metabolism, such as, oxygen respiration and CO_2 fixation (Fig. 13.3b) (Huang et al. 2011). Microbes exhibiting such activities are the focus of recent studies on microbial electrosynthesis (Claassens et al. 2016), in which anaerobes, such as those affiliated with the genera *Sporomusa*, *Clostridium*, *Methanosarcina*, have been reported to use electrons from cathodes for fixing carbon dioxide to produce acetate or methane (Rabaey and Rozendal 2010; Karthikeyan et al. 2019). However, microbes that grow at the cathode surface of RP-MFCs may be different from these anaerobes, since oxygen concentrations around cathodes are substantially high (Rago et al. 2017).

Biofilm microbiomes formed on cathodes of S-MFCs have been analyzed (Reimers et al. 2006; de Schamphelaire et al. 2010). These studies have shown that gammaproteobacteria, such as members of the genera *Pseudomonas* and *Aeromonas*, are abundantly present. These genera include facultative aerobes that exhibit electrochemical activities (Logan et al. 2019); in particular, *Aeromonas hydrophila*, a close relative of the genus *Shewanella*, is known to possess an extracellular electron transport pathway (Conley et al. 2018), thereby performing direct electron transfer for taking electrons from cathodes (Fig. 13.3b). On the other

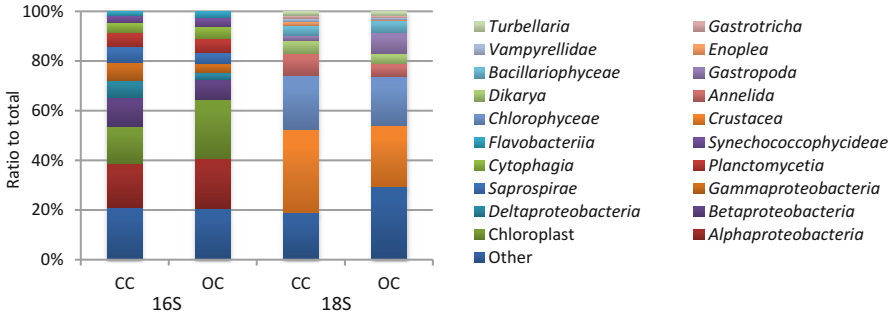

Fig. 13.4 Microbiome structures in cathode biofilms in RP-MFCs as assessed by metabarcoding of rRNA gene amplicons. Analyses were conducted as described by Vamshi Krishna and Venkata Mohan (2016). Bacterial (16S) and eukaryotic (18S) communities established under open-circuit (OC) and closed-circuit (CC) conditions were compared, indicating that some bacteria, including those affiliated with *Gammaproteobacteria* and *Deltaproteobacteria* were more abundantly detected under the CC condition than those under the OC condition

hand, *Pseudomonas aeruginosa* is known to secret electron mediators and perform indirect electron transfer (Wang et al. 2010) (Fig. 13.3b).

Our recent study has found that *Gammaproteobacteria* (e.g., members of the family *Sinobacteraceae*) and *Deltaproteobacteria* (e.g., members of the family *Geobacteraceae*) are more abundantly detected at the surface of cathodes in RP-MFCs under closed-circuit conditions than those under open-circuit conditions (Fig. 13.4), suggesting that these bacteria would grow by using electrons taken from cathodes (Hirose et al. unpublished results). The occurrence of *Geobacter* relatives in cathode biofilms is unexpected since oxygen is abundantly present around cathodes. A possible explanation would be that thick biofilms are formed on cathodes, in which anaerobic regions are formed at the bottom (immediately above the cathode surface). Among eukaryotes, members of *Chlorophyceae* (algae) and *Crustacea* (arthropods) were abundantly detected by metabarcoding of 18S rRNA gene amplicons from RP-MFC cathodes (Fig. 13.4). Further studies would be necessary for identifying the functions of these microbes.

13.4 Future Perspectives

It has been shown that RP-MFCs are devices that convert light energy into electricity under the cooperation between rice plants and microbes. As described above, studies have shown that microbes present not only around anodes but also around cathodes play important roles for electricity generation. It has therefore been considered that a deeper understanding of these microbes is necessary for further improving RP-MFCs. In particular, although two possible roles of cathode microbes in RP-MFCs have been proposed, information is limited as to how much these microbes contribute to electricity generation relative to the activity of chemical

catalysts. In addition, studies may also be necessary for enhancing the performance of anode microbes for transferring electrons to electrodes. We expect that achievements to be obtained in these studies will provide us with reliable on-site power sources that are applicable to wireless sensors useful for smart agriculture.

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Chapter 14
Current Applications and Future Perspectives of Microbial Fuel Cell Technology

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Abstract This chapter aims to discuss the current and potential applications of microbial fuel cells (MFCs), a suitable technique for energy harvesting. In the last few years, MFC technology has been extensively investigated due to its capacity for wastewater treatment with simultaneous generation of bioelectricity, with many efforts devoted to increasing the efficiency of these devices. Although their practical implementation remains a challenge, the scope of application has been expanded to several fields in which they have achieved encouraging results. Among the drawbacks, the scaling-up of MFC systems poses issues for large installations. The strengths and limitations of these bioelectrochemical devices are analyzed for their potential application in terms of electricity generation at large scale and power supply to small electronic devices, municipal and industrial wastewater treatment, metal removal, water decolorization, added-value chemical production, and biosensing. Several strategies proposed in the literature for the scaling-up of the technology are also analyzed.

Keywords Microbial fuel cells · Bioenergy · Wastewater treatment · Scaling-up · Persistent pollutant removal · Chemical production · Sensing

14.1 Introduction

Bioelectrochemical systems (BESs) have gained great attention for multiple applications in the last few years, including power generation, water treatment, production of chemicals, sensing, and desalination. BESs can be classified according to the biocatalysts employed into microbial and enzymatic systems. The first type can be

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further categorized into microbial fuel cells (MFCs), microbial electrolysis cells (MECs), microbial solar cells (MSC), and microbial desalination cells (MDCs) (Shemfe et al. 2018; Bajracharya et al. 2016). Among them, MFCs have been widely studied over the last decade, which has translated into an exponential rise in the number of scientific publications on these devices. Although the concept was presented for the first time in 1911 by Potter (1911), it was only until the early 2000s when the interest for these green devices really began to spread among the scientific community (Santoro et al. 2017; Zhou et al. 2013).

MFC devices exploit microbial metabolism to transform the chemical energy of a given substrate into electricity. In this respect, the nature of substrates that can be employed for power generation varies from pure organic compounds to waste materials, which enables obtaining a twofold benefit, waste treatment or valorization with simultaneous electricity generation. Bacteria oxidize the organic matter in the anode chamber, generally under anaerobic conditions, which implies a low potential reaction, releasing protons and electrons in the process. The separator allows protons to be selectively transferred to the cathode, while electrons are led through an external circuit in order to obtain an electrical current. In addition, the cathodic reaction consists of the reduction of oxygen, a high redox potential reaction in which oxygen combines with the protons and electrons resealed in the anode (Bajracharya et al. 2016). Nevertheless, the configurations of MFCs reported in the literature are very diverse, with multiple set-up options. These include separator-less devices, double-chamber systems, with anodic and cathodic compartments and single-chambered fuel cells with the cathode exposed to the air. Moreover, they can be set up in a wide variety of designs, including cylindrical and rectangular shapes and self-stack and multitubular assemblies (Zhou et al. 2013). Other specific configurations are intended for certain functionality requirements such as submersibility (Hernandez-Fernandez et al. 2015). Figure 14.1 displays the two general configurations of MFC systems.

Despite the progress made in the last few years and the potential of MFCs, the real implementation of the technology is still very limited. In this regard, there are still remaining challenges needing to be addressed to overcome important limitations and encourage its deployment. Among them, it is necessary to develop high-efficiency anodes to enhance microorganism kinetics, optimize biofilm development, and improve electron transference processes between biocatalysts and the electrodes, especially when waste substrates are employed (Santoro et al. 2017). In such cases, Coulombic efficiencies and power densities are generally low. Another bottleneck of the technology is the cathodic oxygen reduction reaction (ORR) due to the low kinetics and high overpotential. While noble materials such as platinum offer high catalytic activity, its high cost has promoted the search for other efficient and affordable catalysts that can be used as an alternative at the cathode. In this sense, many options including carbon nanomaterials and transition metal oxides have been investigated (Ben Liew et al. 2014).

Apart from electrode components, the separator is another key component with a central role in determining MFC performance. Its optimization is very important and poses a challenge when the system has to be scaled up. As already mentioned,

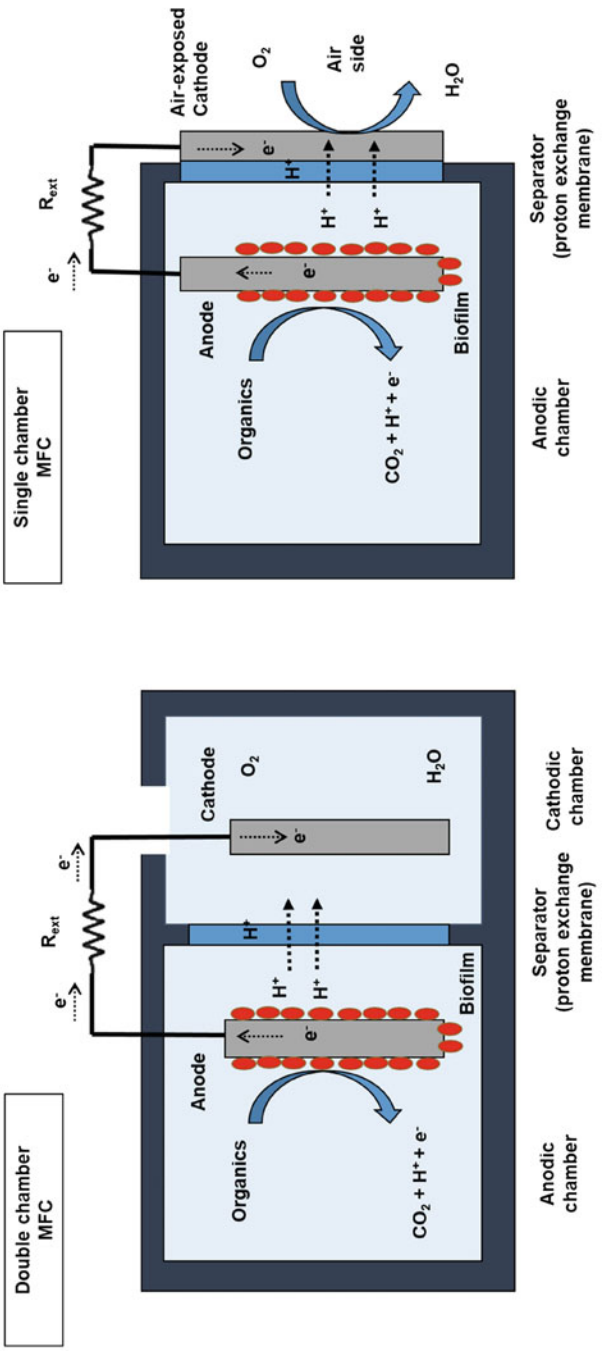

Fig. 14.1 Schematic representation of single- and double-chamber MFC systems

separator-less configurations have also been developed but, in such cases, and due to undesirable diffusion phenomena between anode and cathode compartments, the efficiencies are even lower than in standard configurations containing a separator. Nafion® is the most often used separator in MFCs, however, as in the case of platinum as a standard catalyst, Nafion® membranes offer several disadvantages such as high cost and oxygen crossover issues. Thus, a large number of potential alternative options have been studied to replace Nafion® membranes, including cation, anion, and bipolar membranes, glass fibers, ultrafiltration membranes, porous fabrics materials, ionic liquid-based membranes, and ceramic separators, among others (Santoro et al. 2019; Logan 2010).

As seen, most of the components and the processes in MFCs are being subjected to intensive research in order to improve their performance with the ultimate objective of bringing this technology into the market for real implementation and commercialization. Nevertheless, the potential of the technology is very high and this has resulted in a diversification of the fields in which it can be applied. This chapter reviews the applications of MFC technology, which in turn depends on the scale of the devices. These applications range from urban and industrial wastewater treatment with simultaneous bioelectricity generation, to sensors development and chemical production.

14.2 Microbial Fuel Cell Applications

MFCs constitute a versatile technology with multiple applications. The most obvious is the generation of bioelectricity by exploiting organisms' metabolism from a suitable substrate. However, these devices offer an ideal framework for other uses including sensing, chemical recovery, feedstock treatment or, more specifically, removal of persistent pollutants (Greenman et al. 2019; Sawasdee and Pisutpaisal 2014). Also, in terms of versatility, there are different strategies when scaling up these devices according to the required applications. MFCs can be employed for large-scale purposes or, alternatively, can be miniaturized for small-scale uses. In both cases, multiple units can be stacked, for instance, to increase the level of power density, water treatment capacity, or voltage response. In addition, they offer modularity in terms of component design and connections between units (Logan et al. 2015). As it is to be expected, the design and configuration of MFC devices will have to be selected according to the end-use requirements. Following, the main applications reported so far for MFC technology are described.

14.2.1 Power Generation and Supply

As mentioned above, protons and electrons are generated at the anode as a result of the metabolism of substrates performed by electroactive microorganisms. While a

final high-potential electron acceptor is needed at the cathode in order to complete the circuit, a suitable electron carrier is required at the anode chamber to transfer the electrons released in the oxidation process to the anodic electrode. Certain bacteria such as *Shewanella, Geobacter*, and *Rhodoferax* species are capable of generating electricity from several organic compounds. Electrons can be released in these bacteria through nanowire-shape pili, cell membranes, or by molecular carriers (Yamasaki et al. 2018). At the anode, bacteria attach to the electrode material and colonize the available surface developing a biofilm. Thus, in principle, electrons can be directly transferred from the substrate to the anodic electrode through the conductive biofilm, in the absence of a redox chemical mediator, which can pose toxic issues to bacteria populations and can increase the operational costs (Hernandez-Fernandez et al. 2015).

One of the advantages of MFCs is the great variety of organic substrates that can be effectively exploited to generate electricity. Especially, the use of urban and industrial wastewater effluents underlines the benefits of the technology due to the possibility of waste valorization for internal energy recovery and organic matter removal. Many works have already shown the possibility of using a considerable range of industrial wastewaters from different sources as feedstock. They include food processing, brewery, distillery, dairy, and agro wastewaters, among other options (Chen et al. 2014; Tharali et al. 2016; Ogugbue et al. 2015; Pandey et al. 2016). Apart from wastewater coming from urban or industrial facilities, other types of waste feedstocks such as leachate landfill (Li and Chen 2018) and human urine (Ieropoulos et al. 2012) have shown their potentiality for energy generation in MFCs.

The power densities achieved in MFCs can greatly depend on the type of materials that form the devices, on the substrate and on the scale employed. Conversely, it is necessary to differentiate from single units and stack MFC devices, which are formed by several units. The miniaturization of MFC devices can lead to higher power densities; however, the net power in a single unit would be very low due to the limited substrate capacity and the assembly of multiple units would be required for higher power supply. Generally, the highest power densities have been obtained with volume capacities below 30 mL. For example, optimized configurations with advanced strategies have reached maximum power densities of almost 7 W m^{-2} (normalized to anode surface) with an anode volume of 2.5 mL (Fan et al. 2007) and of over 1.5 kW m^{-3} (normalized to volume) working with 12 mL of anode capacity (Fan et al. 2008). When it comes to the order of liters, power densities are usually lower than 35 W m^{-3}. For instance, a system with a capacity of 250 L formed by two MFC modules outputted 0.5 W m^3, which can be considered relatively low (Feng et al. 2014). This would suggest that the scaling-up of MFCs is problematic and challenging, and thus the shift of the capacity from the order of millimeters to the order of liters does not necessarily translate into an increase in power density.

The main limitations regarding the scaling-up of MFCs are associated with the rise in internal resistance because of (1) potential losses given by the increasing distance between electrodes, (2) increasing separator resistance, and (3) the

resistances of the solutions in the anodic and cathodic compartments. With the objective of enhancing power performance, it is required to maximize the ratio between available electrode surface area and MFC volume capacity and to minimize the spacing between the anode and the cathode while developing high efficiency and innovative materials for the components (Santoro et al. 2017; Logan 2010; Wei et al. 2011).

The recent trends for the design of large-scale reactors are based on the stacking and modularization of single MFC units. Considering voltage output, a single reactor is limited by a pair of electrodes, and the voltage is generally lower than 0.8 V. MFC units can be assembled in parallel or in series mode in order to increase current density or voltage output, respectively. Nevertheless, several issues have been also reported for the operation of MFC stacks, including voltage reversal, additional contact voltage losses, or fuel starvation. Under continuous operation, other problems can arise due to electrical and hydraulic connections. The connection of multiple MFCs in continuous mode requires to share in-flow and out-flow streams. Some works have addressed this aspect by measuring the voltage loss for different connection modes in an MFC stack, finding that when the MFCs are connected both electrically and hydraulically at the same time, the voltage output is up to 36% lower than when they are electrically connected but hydraulically isolated (Zhuang and Zhou 2009). This implies that it would be preferable to separately feed the MFC of a stack, with single recirculating loops for individual units, which is not considered a feasible option towards the large-scale implementation of MFCs in terms of wastewater treatment. In fact, it has been reported that cascade operation with the outlet of a unit being connected to the inlet of the following unit can provide higher COD removal rates. To sum up, even the modularization of MFC can pose several challenges for large-scale plants.

There are several attempts of large-scale MFC operation. One of the recent examples consisted of a plant with a total capacity of 1 m^3, which was set up with 50 modules (Liang et al. 2018). When artificial wastewater was employed, the power output was as high as 125 W m^{-3} or equivalently 7.6 W m^{-2}. In the case of municipal wastewater, the maximum power decreased down to 60 W m^{-3}, equivalent to 3.6 W m^{-2}. As it can be inferred, the use of real and complex substrates can cause a decrease in power performance. Other examples include a 96-module MFC configuration of 200 L of capacity that was capable of supplying enough power for a 12 V water pump (Ge and He 2016). Other pioneering attempts to set up modularized MFCs of 1 m^3 fed with brewery wastewater were unsatisfactory due to the limited current generated and problematic operation (Logan 2010).

Apart from large-scale applications, MFC has proven to be capable of supplying power to small electronic devices. The energy generated in MFCs can be employed for powering electrochemical sensors and small-scale telemonitoring systems such as wireless sensor networks (WSNs) to transmit data gathered by sensors to a remote location. If the sensing systems are intended for environmental monitoring, MFCs can represent a green opportunity to power these systems with no impact (Yang et al. 2014). Usually, WSN devices require a minimum of 3 V and given the low outputs of MFCs in terms of voltage and currents, it is necessary to store the energy

generated in components such as capacitors that can then provide power at the required amount. This approach has been proved successful for wireless data transmission (Shantaram et al. 2005).

Floating MFCs are an attractive option to harvest energy in natural water bodies. This configuration usually includes a floating cell structure with the electrodes physically separated by an electrically isolating material (Huang et al. 2012). These floating devices can be placed, for example, in lakes or rivers to power remote environmental sensors. Recently, Schievano et al. (2017) operated floating MFCs in Italian ponds for over a year. Each of the devices installed generated a cell potential of up to 0.8 V and power outputs of up to 3.5 mW. The floating systems, displaying a simple design, were capable of powering LED lights and remote data transmission devices, showing the viability of using low power density from MFCs (40 mWh/day) to power environment sensors.

In addition to using external devices coupled to MFCs for energy storage, it has been proved that the anode and the cathode in MFCs can be utilized as negative and positive electrodes for the design of an inner supercapacitor to increase power. This type of design is known as supercapacitive MFCs and is capable of generating higher current pulse discharges and power than conventional devices (Santoro et al. 2019, 2016). The highest performances provided by single MFCs have been achieved in these supercapacitive designs. For example, high-voltage cathodes made of bilirubin oxidase outputted a power performance of 19 mW with pulse currents of up to 45 mA (or 84 W m^2 per cathodic surface area) (Santoro et al. 2016). Supercapacitive operation has also been used to increase the power of ceramic MFCs. These MFC types represent a promising option for scaling-up, due to the properties of ceramic separators, which include very low cost, structural strength, significant stability, and modularity, in comparison to other separator options (Yousefi et al. 2017). Very recently, a supercapacitive MFC stack constructed with low cost ceramic separators and using a Fe-N-C catalyst based cathode was capable of generating around 37 mW (stack based on 28 MFCs and 1 L of total capacity, power density equivalent to 36.9 W m^{-3}) (Santoro et al. 2019).

Several proof-of-concept works have employed MFCs to power robots. Artificial agents (called EcoBots I and II (Ieropoulos et al. 2005)) have been directly powered by MFCs. Advanced designs presented by Leropoulos et al. (Ieropoulos et al. 2010) comprise self-sustainable robots that can complete a thermodynamic cycle of ingestion–digestion–egestion (called EcoBot III). The design of this artificial agent demonstrated the autonomy of MFCs as a power source and showed that miniaturization of MFCs could represent a real option for scaling-up. This research group has also intensively investigated the use of urine as a substrate in MFCs. As mentioned, the versatility of MFCs allows them to be operated under a wide range of waste types, including human urine, which has shown to be capable of generating electricity enough to power mobile phones and for LED internal lighting (Sawasdee and Pisutpaisal 2014; Walter et al. 2017).

14.2.2 Wastewater Treatment

MFCs can be fed with biodegradable substrates ranging from municipal and industrial wastewater to landfill leachates and other effluents of waste nature. In the first studies on the technology, synthetic wastewaters have been usually employed to investigate MFC performances with the objective of understanding the working principles in these devices and optimizing the operational variables. Real waste effluents can present complex matrixes and greatly disfavor efficiency in comparison to model effluents. However, in order to reach the practical implementation of MFCs, many works have already addressed the operation with waste substrates (Gude 2016; Ortiz-Martínez et al. 2015).

The removal percentage of organic matter from the feeding substrate is one of the main parameters that are usually monitored in MFCs. Removal rates of over 90% can be achieved depending on the hydraulic retention time (HTR), substrate concentration, temperature, and fuel cell design. The nature of the substrate has a strong influence on bacterial populations and biofilm development, and thus on power and current density and Coulombic efficiency. This last parameter is of special relevance when electrical generation is the main purpose since it is calculated as the rate between the real charge transferred to anode surface and the theoretical maximum charge considering the complete substrate oxidation into electricity. In this sense, the total COD removal in MFCs can be due to electrogenic pathways that generate electricity from the organic matter or due to alternative fermentative mechanisms not contributing to the generation of energy, since exoelectrogenic bacteria compete with other microorganisms for the substrate. As a representative case, the work of Jahdav et al. (2009) achieved a 90% of COD removal in MFCs after 200 h of operation with synthetic wastewater, but with very low Coulombic efficiencies ($\leq$1.5%).

Conventional organics such as acetate or glucose have been usually employed to feed MFCs inoculated with electrogenic microorganisms. There are many examples in the literature employing these compounds, and they can also provide different efficiencies. Chae et al. (2009) compared the performance of acetate, glucose, butyrate, and propionate in two-chambered MFCs inoculated with anaerobic sludge for over a year of operation. Among them, acetate yielded the highest Coulombic efficiency CE (over 72%), while butyrate, propionate, and glucose offered efficiencies below 40%. In the case of glucose, the yield was as high as 15%, due to its fermentation by non-electricity generating bacteria that were present in the inoculation sludge. In general, it has been proved that the performance of MFCs when using complex waste substrates can be reduced fivefold when compared to the use of simple substrates (Gude 2016).

In addition to wastewater treatment in terms of COD reduction, MFCs are investigated as a means of removing different types of pollutants in wastewater streams, such as metals and persistent pollutants like xenobiotics.

14.2.2.1 Municipal and Industrial Wastewater Treatment

The average energy consumption in activated sludge processes for domestic wastewater treatment amounts to 0.3–0.6 kWh m^3. In wastewater treatment municipal plants, energy is mostly consumed in aeration (40%), sludge treatment (30%), and other processes such as additional pumping (30%) (Ali et al. 2018). Municipal wastewaters can contain over 9 times more energy needed for their treatment (Heidrich et al. 2011). The possibility of electricity recovery with simultaneous wastewater treatment in MFCs has raised high expectations on this technology to replace conventional wastewater treatments to reduce energy costs (Li et al. 2014). Another benefit of MFCs is that these systems produce much lower sludge in comparison to activated-sludge reactors (ASR). In a recent work, Asai et al. (2017) specifically addressed the comparison between the total amount of waste sludge generated in MFC systems and that produced in an ASR (respective volume capacities of 1.5 L). The daily production of waste sludge in MFCs was found to be much lower in comparison to the ASR when both systems were fed with artificial domestic wastewater and also with artificial industrial wastewater, with respective COD values of 500 and 1500 mg L^{-1}. Between 80 and 100 operation days, the daily production of waste sludge domestic wastewater yielded around 10 mg L^{-1} D^{-1} in the MFC and 50 mg L^{-1} D^{-1} in the ASR. In the case of industrial wastewater, the yields were about 35 mg L^{-1} D^{-1} for the MFC and 90 mg L^{-1} D^{-1} for the ASR. Despite these promising advantages, MFCs are yet to be further studied to address the limitations that hinder its practical implementation, mainly low power densities, material costs, and scaling-up for high treatment capacity. As already noted in the previous section, the maximum power outputs achieved in MFCs (with volume capacity in the order of liters) are several watts per cubic meter.

Many works have addressed the treatment of municipal wastewater in MFC devices, including some pilot-scale plants in real urban treatment plants. Zhang et al. (2013) constructed an MFC of 4 L of treatment capacity to be installed in a real plant in Milwaukee (USA) without temperature control (temperatures varying from −10 °C to 36 °C) and fed with primary effluent without further pretreatment. The total maximum COD removal was 70%, but at high HRTs (11 h) and in favorable conditions of warm temperatures. MFC operation and COD removal were severely affected by cold temperatures. In terms of normalized energy recovery (NER), the maximum output achieved was 0.003 kWh kg^{-1}-COD. Jiang et al. (2011) constructed a reactor of 20 L of capacity using primary effluent in continuous mode characterized by COD values in the interval of 100–500 mg/L. In this case, COD removal efficiency was 66% for an HRT of 5 h with a concomitant power density of up to 0.17 W m^{-3}, equivalent to 0.003 kWh kg^{-1}-COD. As mentioned before, a modularized pilot plant with a total capacity of 1 m^3 was set up and run for 1 year (Liang et al. 2018). In terms of wastewater treatment, COD removal rates of 70–90% were achieved for municipal wastewater with COD concentrations below 50 mg L^{-1} and with maximum energy recovery of 0.005 kWh m^{-3}. The total construction costs of the plant amounted to 36 kUSD.

Also recently, an MFC formed by four individual cells with 45 L of total volume capacity was installed in a full-scale plant in Bottrop (Germany) (Hiegemann et al. 2016). The most favorable results were attained for 22 h of HRT, with respective removals rates for TSS, nitrogen, and COD of 40, 28, and 24% and a Coulombic efficiency of almost 25% (the initial value of COD was 118 mg/L). While the removal rate of COD achieved can be considered lower in comparison to other studies, the removal of nitrogen was unexpectedly high. Conversely, TSS removal was due to hydrolysis processes that convert the particulate matter into a soluble substrate. The NER was 0.36 kWh kg^{-1}-COD. With these results, simulated scenarios for full-scale MFCs implemented in the plant provided over 4% of total energy savings.

While the scaling-up of MFC systems still remains a challenge for their integration in wastewater treatment plants, many attempts have been made to study the possibility of treating wastewater originated in industrial facilities, which would require smaller scale installations. A wide range of industrial wastewater types has been assessed in MFCs, including effluents coming from brewery, distillery, food, dairy, cheese whey, starch processing, swine, paper, pharmaceutical, and refinery industrial facilities (Gude 2016). In comparison to municipal wastewater, the use of this type of effluent can offer significantly higher amounts of organic matter.

Food processing wastewaters are rich in organic constituents such as carbohydrates or organic acids, which can be easily biodegraded by bacteria. With this type of wastewater, significant COD removal rates and Coulombic efficiencies (up to 70%) have been reported (Mohamed et al. 2017). Mansoorian et al. (2016) evaluated the treatment of industrial wastewater from a protein food industry in dual-chamber MFCs of 1.5 L of capacity (Mansoorian et al. 2013). This type of effluent was characterized by an initial COD loading of around 1900 mg L^{-1} and a BOD_5 loading of around 1300 mg L^{-1}. The system was operated with feeding rates from 0.2 to 1.6 mL min^{-1}. In this case, it was possible to achieve removals rates of COD and BOD_5 of up to 86 and 79%, respectively. They also monitored other common parameters in water treatment, observing removal efficiencies for TSS, VSS, and SO_4 of 68, 62, and 30%, respectively, and removal rates for NH_3 and P of 73 and 18%. Some of these elimination mechanisms in MFCs have not been fully understood yet. According to the authors, the decrease in the content of TSS and VSS could be explained by the action of biologically catalyzed mechanisms that degrade complex organic matter in colloidal form. Conversely, some microorganisms are capable of employing sulfate as the final electron acceptor, converting it into sulfide. Interestingly, the removal of ammonium was very high in a system operated in anaerobic conditions, implying that anaerobic oxidation, denitrification mechanism, and other removal pathways can occur in MFCs with bioelectricity generation. The reduction of the content of phosphorus was not as impressive. In this case, the conversion of organic phosphorus into orthophosphate can also take place (Mansoorian et al. 2013; Luo et al. 2002).

Brewery wastewaters have shown to be a very suitable feeding effluent for MFCs. They usually display high levels of COD (1000–5000 mg L^{-1}) (Gude 2016; Wen et al. 2010a). High removal efficiencies from 79% to 85% have been achieved with

Coulombic efficiencies of up to 38% and power densities of 11 W m^3 in small capacity MFCs (Wang et al. 2008). The removal efficiencies reported for this type of wastewater (95%) can be even higher when using high HTRs (>14 h) (Wen et al. 2010b). In distillery industries, spent wash is produced in the alcohol production process, generating recalcitrant wastes with high COD and BOD contents that can be used in MFCs. In this case, COD removal efficiencies of 60–80% have been reached (Pallavi and Udayashankara 2016).

Other types of substrates can present more complex compositions. For example, livestock industry wastewater can offer extremely high contents of organic material (up to COD loadings of 10^5 mg L^{-1}), but they can also contain high amounts of nitrogen-rich compounds and other organic materials that are difficult to be biodegraded (e.g. cellulose). On its part, dairy wastewaters are composed of carbohydrates, proteins, and fats, and they have also been effectively used in MFC devices. Mohan et al. (2010) achieved high substrate degradation (COD removal of over 95%) together with high removal of proteins (78%) and turbidity (99%) in batch mode.

MFCs are also capable of treating wastewater from the petroleum refinery industry, which can contain a wide variety of chemicals (e.g. sulfides, cyanides, benzenes, ammonium compounds, among others). Along with significant COD removal, Guo et al. (2016) reported oil removal rates of 66–84% from refinery wastewater in MFC using granule graphite and activated carbon in the anodic chamber. Aromatic organics, saturated hydrocarbons, and volatile phenols were the main pollutants present in the wastewater employed. The overall pollutant removal mechanism was described as a combination of adsorption on the carbon materials and by the action of microbial metabolism.

Table 14.1 summarizes the main results obtained with different types of industrial wastewater (COD removal rates and power density).

14.2.2.2 Specific Compound Treatment

Under the perspective of water treatment, the applications of MFCs have been extended to the recovery or removal of heavy metals. These species can be frequently present in effluents coming from industries devoted to petroleum refinery, metal plating, or the production of pesticides and paint. Moreover, ligands such as ammonia can form stable complexes with many metal ions, which make them more difficult to be recovered. The conventional techniques based on chemical, physical, and biological methods for the treatment of heavy metals require high energy consumption and can be ineffective when metal concentrations are low (from 1 to 100 mg L^{-1}). MFCs have shown to be an alternative for metal energy removal. In the anode, bacteria can reduce ion metals, which are then deposited. Moreover, it is possible to use compounds with high redox potential as electron acceptors instead of oxygen at the cathode; several ion metals can be used for this purpose (Ezziat et al. 2019; Mathuriya and Yakhmi 2014). This last approach is the most frequent, generally implying the use of double-chamber MFCs. The possibility of removing

Table 14.1 Wastewater types used in MFCs for treatment and energy generation (power output included as reported, i.e. normalized to anode area or volume)

Wastewater type	Maximum COD removal (%)	Maximum power performance	Ref.
Food processing	60	338 mW m^{-2}	Mohamed et al. (2017)
Brewery	85	12 W m^{-3}	Wang et al. (2008)
Distillery spent wash	80	26 mW m^{-2}	Liu et al. (2004)
Dairy	95	1.10 W m^{-3}	Venkata Mohan et al. (2010)
Refinery	84	330 mW m^{-3}	Guo et al. (2016)
Paper production	80	60 mW m^{-2}	Velasquez-Orta et al. (2011)
Cheese production	80	3.2 W m^{-3}	Kelly and He (2014)
Slaughter house	93	578 mW m^{-2}	Katuri et al. (2012)
Swine wastewater	83	45 mW m^{-2}	Min et al. (2005)
Pharmaceutical	78	2.2 W m^{-3}	Velvizhi and Venkata Mohan (2011)

heavy metals including chromium, copper, iron, cobalt, vanadium, or manganese, among others, has been already proved in MFCs (Ezziat et al. 2019).

When using the anode for metal removal, heavy metals pose toxic issues to the bacterial populations inoculated. Thus, maximum tolerable concentrations need to be determined to ensure bacterial activity. For instance, high removal rates have been achieved for heavy metals such as Cd and Zn (90% and 97%, respectively) in the anode of single-chamber MFCs with simultaneous energy production (3.6 W m^{-2}) (Abourached et al. 2014). In this case, the maximum tolerable concentrations were in the order of 200–400 μM. The strategy involved the use of a low amount of sulfate and lactate in the electrolyte to remove metals via sulfide precipitation and biosorption. It is worth noting that heavy metals removal can be accomplished with simultaneous COD removal and power generation. Recently, yields of ion removal of 72% from synthetic hydraulic fracturing flowback water have been reported along with a COD decrease of over 90% and power generation of around 2600 mW m^{-3}.

As mentioned above, the principle of metal removal when treated in the cathode is based on their use as electron acceptors. Several examples include the reduction of Cu(II) into Cu(0) with very high efficiencies (Ter Heijne et al. 2010), the removal of Cr(IV) at concentration of 200 mg L^{-1} with simultaneous energy production (Sahinkaya et al. 2017), or the removal of mercury (Hg^{2+}) through precipitation in the presence of Cl^- followed by electroreduction at the cathode (Wu et al. 2017). The number of works addressing the removal of heavy metals in MFCs at the cathode is relatively high and reveals the prospects of the technology to accomplish this objective (Ezziat et al. 2019).

Other works have addressed the in situ remediation of rivers to remove organic matter or heavy metal species. The operation of lab-scale sediment MFCs with anoxic cathodes showed the possibility of reducing Ag(I), Cu(II), and Hg(II) with efficiencies higher than 90% after 60 days operation (Wu et al. 2017). Metal complexes can be even more stable and difficult to be removed, but Zhang et al. (2012) demonstrated the possibility of eliminating copper-ammonia with an efficiency of 96% after 12 h from solutions in the cathode chamber containing concentrations of this compound of 13.55 mg L^{-1}. In the process, Cu_2O and Cu crystals were deposited over the surface of the cathode.

While many studies have proven the feasibility of MFC metal removal from aqueous effluents, very few have actually dealt with the further separation of the reduced metallic products that can be deposited over the MFC components, such as the electrodes. This can be a challenge when using electrodic materials like carbon cloth, graphite, or activated carbon, from which metal separation is difficult to be performed. Conversely, the use of alternative electron acceptor species to oxygen can lead to increasing power generation and ultimately to expanding the scope of application of MFC technology

Not only metal ions can be treated in MFCs, but other pollutants such as nitrates have also been used as terminal electron acceptors. For example, nitrate can be reduced to N_2 via denitrification (Jia et al. 2008). This strategy can be extended for the removal of other compounds like permanganate (You et al. 2006) and persulfate (Li et al. 2009).

Finally, MFCs can serve as a means for azo dye decolorization with simultaneous energy recovery. This process can be performed in the anodic or the cathodic chamber. Dyes are xenobiotic compounds whose colors are due to the presence of the azote bond (–N=N–). Wastewater effluents from textile industries present this type of persistent disposal causing serious toxicity issues. The degradation of dye in MFC anodes has been linked to oxidative mechanisms performed by bacteria instead of sorption by dead and live cells. Alternatively, if dyes are used in the cathode, they can play the role of final electron acceptors while water is decolorized (Ilamathi and Jayapriya 2018). The interest in this application of MFC technology has greatly increased in the last years. Azo dyes such as acid orange 7, Congo red, Alizarin yellow R, thionine based textile dyes, or reactive blue 160 have been successfully removed from water in MFC devices, with removal efficiencies between 73 and 96% (Ilamathi and Jayapriya 2018; Ding et al. 2010; Cui et al. 2014), depending on the dye type, initial concentration, and operation time, with simultaneous power generation.

14.2.3 Biohydrogen and Other Chemicals Production

The bioelectrochemical reactions taking place in MFCs offer a framework to perform different reaction pathways for the production of value-added chemicals. This approach usually requires the use of hybrid systems in which MFCs are integrated.

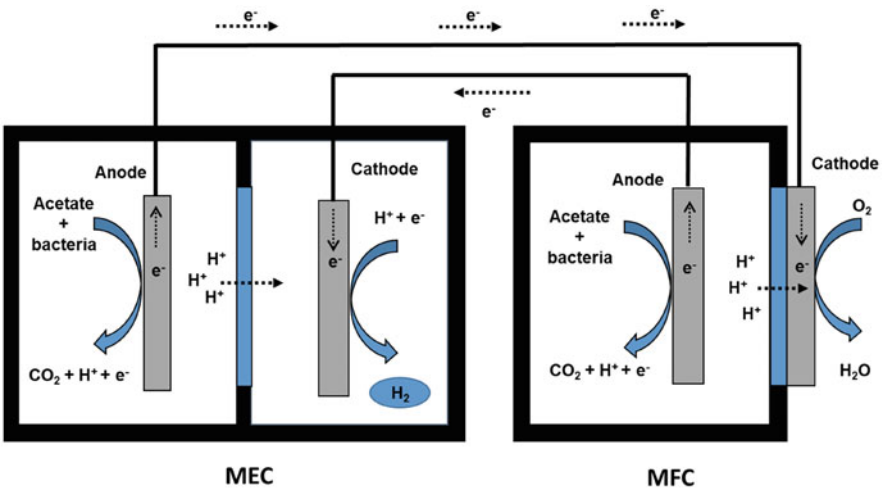

Fig. 14.2 MFC-MEC coupled system for hydrogen production

Microbial electrolysis cells (MECs) consist of a modification of MFC systems in which the objective is to produce hydrogen, requiring an external voltage source to perform the process. Liu et al. (2005) showed that it is feasible to produce hydrogen at the cathode by supplying additional potential (>0.2 V) to that naturally generated by bacteria. The external voltage requirement in an MEC device is notably lower in comparison to that required in electrolysis processes (1.8 V in principle). These authors achieved production rates of up to 9 mol-H_2 per mol-glucose by applying external voltage.

However, the coupling of pure MFCs with MECs devices allows avoiding the necessity of using external power supply for H_2 production as described in Fig. 14.2. In this hybrid system, an MFC unit is utilized as an energy source for the second one. Sun et al. (2008) reported a coupled MEC-MFC configuration to produce H_2 with no other external electric power source, achieving 1.60 mol-H_2 per mol-acetate.

Another route for hydrogen production consists of the promotion of acidogenic bacteria in the anaerobic digestion taking place in MFC anodes (Chandrasekhar et al. 2015). In the anaerobic digestion, the first stage comprises hydrolysis of large organic substrate to be transformed into small molecules. Subsequent acidogenesis converts them into short-chain compounds (e.g. ketones, fatty acids, alcohols), which can be transformed into H_2, CO_2, and CH_3COOH (acetic acid) by the action of acidogenic bacteria. If not avoided, methanogenic bacteria can transform hydrogen into methane, which would be undesirable for this purpose. Thus, for hydrogen production, the boost of acidogenesis while inhibiting methanogenesis stage can help to produce hydrogen. This can be accomplished by heating and acid treatment to reduce the growth of methanogens while enriching acidogenic bacterial populations (Lao-Atiman et al. 2017).

Further efforts have been addressed to develop hybrid systems combining dark fermentation with MFCs and MECs. With this strategy, Wang et al. (2011) achieved to generate 0.48 m^3-H_2 m^{-3} day^{-1} (normalized to MEC volume). Further research efforts are required to enhance the efficiency of coupled MFC-MEC units by optimizing operational conditions (e.g. initial organic concentration, buffer conditioning for increasing conductivity, etc.) and through the development of highly efficient and low-cost component materials (Zhang et al. 2019).

In addition to hydrogen production, other chemicals production processes have been implemented in hybrid MFC-MEC systems. Zhao et al. (2012) electrochemically reduced CO_2 into formic acid in the cathode of an MEC assisted by an MEC for external voltage supply, in a similar way as was described in Fig. 14.2 for hydrogen production. In this case, it is necessary to supply CO_2 to saturate the cathodic solution. Formic acid was produced at a rate of 21 mg L^{-1} h^{-1}. The MEC system included a cathode electrode covered with multi-wall carbon nanotubes (CNTs) and tetraamino functionalized cobalt phthalocyanine with the purpose of reducing the overpotential of the CO_2 conversion into formic acid.

Other chemicals can be produced in MEC systems such as hydrogen peroxide and ethanol, in which extra voltage could be provided by MFCs. For instance, to produce hydrogen peroxide via the bioelectrochemical oxidation of organic matter in the anodic chamber coupled to the reduction of oxygen to hydrogen peroxide at the cathode, it is necessary an additional voltage supply of 0.5 V (Steinbusch et al. 2010). Also, Steinbusch et al. (2015) showed the possibility to reduce acetate into ethanol with biocathodes based on mixed cultures and by applying 0.55 V as external voltage. New chemical production routes may be developed in the future for hybrid MFC-MEC systems.

14.2.4 Biosensing

Previously in this chapter, the use of MFCs to power wireless sensor networks (WSNs) has been mentioned. Nevertheless, these bioelectrochemical devices can also be employed as biosensors themselves. They have been employed for sensing water quality and toxicity, as well as for pH and temperature monitoring. MFCs could be implemented in natural environments and be operated online with minimum impact, offering a simple and fast response in testing assays for target water analytes. The principles of biosensing in MFCs lie on the fact that any disturbances of the metabolic pathways of the microorganisms can cause a change in the electricity generated in a single device, and therefore anodic biofilm can act as a bioreceptor, while the anode electrode would play the role of a transducer. Thus, changes in operational parameters such as the presence of specific compounds, pH, temperature, or conductivity can be correlated with changes in the MFC response. If needed, an amplifier can be added to obtain an easily measurable signal as displayed in Fig. 14.3 (Chouler and Di Lorenzo 2015; Pietrelli et al. 2016).

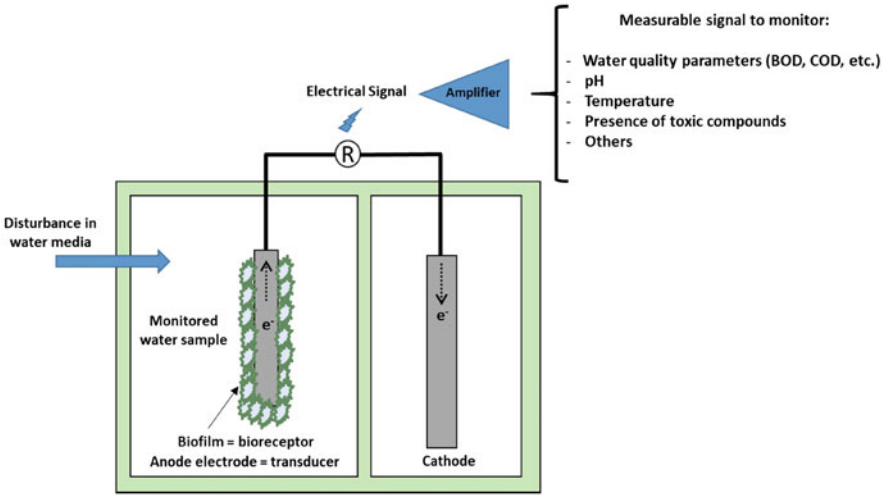

Fig. 14.3 MFC systems as biosensor for water sample monitoring

The determination of biochemical oxygen demand (BOD) is usually performed through incubation tests, which can last up to 5 days. Within a certain range of concentration, the electrical signal obtained in an MFC can be used to determine the BOD in a feeding solution. In fact, the first application of MFCs as biosensor for BOD was already reported in 1977 before the interest for this technology would outburst in the earliest 2000s (Karube et al. 1977). In this early work, it was possible to measure the current generated as a proportional response to glucose-glutamic concentration up to 400 mg L^{-1}, which as the saturation value (corresponding to a current of 100 μA). In this regard, it is important to obtain a stable response from the system that can be correlated to the substrate concentration. Further efforts have been devoted to MFCs as biosensors for BOD determination within the 3–500 mg L^{-1} range (Chouler and Di Lorenzo 2015; Di Lorenzo et al. 2009, 2014).

MFCs can also be applied for the detection of compounds of toxic nature in water media. The first attempt was reported for pollutants like pesticides, heavy metals (e.g. Pb and Hg), and polychlorinated biphenyl (PCB), with lower detection limits set at 1 mg L^{-1} (Kim et al. 2007). The application of MFCs as biosensors was extended for the detection of other heavy metals (Liu et al. 2014) as well as for sulfonamides (sulfamethoxazole), antibiotics (sulfadiazine), and ammonia derivatives (chloramine B) (Patil et al. 2010). Depending on the specific compounds, the lowest concentration limits can vary from 0.01 μg L^{-1} (for antibiotics such as sulfadiazine) to 0.16 mg L^{-1} (for chloramine B).

Finally, MFCs can be utilized for the analysis of common parameters such as pH and temperature. The influence of pH in MFC performance is significant, since it can greatly affect ion concentrations, biofilm development, and membrane potential (Yang et al. 2014). Changes in current output of several orders have been correlated for pH shifts of up to 1 unit. By means of buffer solutions (e.g. phosphate, borax,

bicarbonate) it is possible to maintain pH and obtain stable current production. Thus, variations in pH can be correlated to MFC performance (Pietrelli et al. 2016; Ivars-Barceló et al. 2018). In the same way, another important factor influencing MFC responses is temperature, to which MFCs are very sensitive, especially in terms of compartment conductivities and microbial activity. This influence could be exploited for temperature sensing with MFC devices (Pietrelli et al. 2016).

14.3 Outlook

MFCs can convert chemical energy directly into electricity through oxidative and reductive reaction pathways with concomitant wastewater remediation. Intensive research on the technology in the last few years has opened the way to different applications in several fields. However, in order to advance towards large-scale applications and practical implementation, multiple limitations need to be overcome in the near future.

The main applications of MFC devices discussed in this chapter comprise power production at different scales, wastewater treatment, including municipal and industrial effluents, removal of specific and persistent pollutants and heavy metals, hydrogen production, and biosensing. Thus, one of the strengths of this technology is its versatility. Moreover, MFCs can be fed with many waste types ranging from urine to landfill leachate. Although the Coulombic efficiencies with this type of complex substrates are low, the removal rates of oxygen chemical demand that can be achieved are significant.

Among the biggest challenges for the deployment of MFC technology is the scaling-up for practical applications, with the objective of increasing the capacity of water treatment. Recent works have shown that modularization is one of the most plausible options. Moreover, in comparison to conventional wastewater treatment, the amount of sludge produced is significantly lower. Yet, the power densities obtained in large-scale installations are relatively low. New strategies should be developed to enhance the reaction kinetics and electron-transfer rates in MFCs. The scaling-up cannot be limited to the optimal enlargement of the anode but to the improvement for electrodes, membranes, and catalysts.

Conversely, the use of MFCs to power small devices offers promising prospects and has already been proved. One of the most interesting applications in this regard consists of the use of MFC to harness energy in natural environments to power sensing platforms and remote transmission data. The integration of MFCs in small devices may be approached in the future.

Another advantage of the technology is the capacity to remove heavy metals and azo dyes from water effluents. Traditional methods to remove heavy metals can be energy intensive and ineffective for low metal concentrations. MFCs have shown to be successful to reduce several metal ions when acting as electron acceptors in the cathode at a wide range of concentrations. One limitation may be given by the further

separation of metal deposited onto the materials that form the MFC components, such as carbon materials.

The sensitivity of the technology to many factors such as substrate concentration, ion species, pH, or temperature can be also exploited for sensing purposes with online mode operation. Finally, it can be expected that the next research efforts will further focus on the development of hybrid systems for biohydrogen and chemical production. These pathways have been less explored in comparison to wastewater treatment and can bring new opportunities in the future, also as a result of the combination of MFC with other technologies.

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Chapter 15
The Role of Microbial Electrolysis Cell in Bioenergy Production: Current Applications and Pilot Plant Experiences

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Abstract Microbial electrolysis cell (MEC) is a promising and relatively newer bioelectrochemical process for sustainable H_2, value-added compounds production, and simultaneous wastewater treatment. As compared to other alternative technologies, the MEC has shown numerous strengths: primarily, the MECs pledge the feasibility to generate H_2 at proportionately less energy consumption than the usual energy demand for water electrolysis. In addition, MECs are not restricted by dark fermentation (DF) barriers; H_2 could be completely extracted from the spent medium in MECs, achieving remarkable H_2 recovery than all kinds of processes.

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What is more, significantly pure H_2 is generated in the MECs cathodes. Besides, MECs incorporate both treatment of waste and bioenergy generation, thereby the MEC possesses the benefits of being environmentally friendly, efficient, and waste disposal. Also, MEC has the ability to produce a wide variety of value-added products, such as methane, hydrogen peroxide, ethanol, and so on, and all these attracted enormous attention in both academia and industry. The MEC must be robust enough to be used in the field for bioremediation or energy production.

Keywords Bio-H_2 generation · Microbial electrolysis cell · Electrode materials · Electrochemically active bacteria · Electron transfer · Wastewater · MEC reactor design

15.1 Introduction

Growing world energy requirements are an inevitable challenge by virtue of the high population growth rate and rapid industrial development. At present, the huge part of world energy requirement is fulfilled through traditional fossil fuel (FF) sources. However, it would appear from a large number of studies that depletion and adverse impacts on the environment are two major issues associated with carbon-based FF sources (Abdeshahian et al. 2014; Kadier et al. 2016a, 2018a; Yılmaz and Balta 2017). Thus, it is imperative to explore alternative and renewable sources of energy to meet the power requirements (Cheng and Logan 2011; Watson et al. 2015; Azman et al. 2016; Kadier et al. 2019; Safari and Dincer 2019). In this regard, bioelectrochemical systems (BES) are rapidly developing bioenergy technologies that incorporate biological and electrochemical processes to convert organic materials or biomass into useable forms of bioenergy or resources, including bioelectricity, bio-H_2, nutrients, heavy metals, minerals, and other valuable chemicals (Rozendal et al. 2008; Shahgaldi et al. 2014; Zhang and Angelidaki 2014; Shamsuddin et al. 2019). Among all, so far reported BESs, microbial fuel cells (MFCs) and MECs have gained widespread attention and researched extensively on account of their ability to generate bioelectricity and bio-H_2, respectively (Kundu et al. 2013; Deval et al. 2017; Beegle and Borole 2017; Kumar et al. 2017a). MECs

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attracted great attention for H_2 generation utilizing organic wastes, such as wastewater and other renewable feedstocks (Kadier et al. 2014; Kumar et al. 2017b; Kadier et al. 2017a; Huang et al. 2020). Requiring less energy input than water electrolysis and with greater efficiency than fermentative H_2 production make the MEC technology very promising.

15.2 Working Principles and Thermodynamics of MEC

In general, a standard MEC consists of anode chamber (anaerobic), cathode chamber, ion exchange membrane or separator, and an external power source or supply. Figure 15.1 represents a schematic overview of a two-chamber MEC and its operation. In the MEC, electrochemically active bacteria (EAB) (Saratale et al. 2017) such as *Geobacter, Shewanella*, *Desulfuromonas*, *Pseudomonas*, *Escherichia*, and *Klebsiella* are colonized on the anode surface under anaerobic (without O_2) conditions and break down the organic matters in the feedstocks or wastewater and produce electrons (e^-, negatively charged particles), protons (H^+, positively charged hydrogen ions), and CO_2 (Eq. (15.1)). The EAB pass the generated e^- to the anode electrode, while protons are distributed or migrated freely into the cathode of MEC to take part in the reduction reactions (Eq. (15.2)).

In the meantime, the e^- passed through uninterruptedly via an external circuit to the cathode with aid of external power sources, and where the e^- are combined with the free H^+ and produced H_2 (Eq. (15.2)). During this electrochemical process, both the anodic and cathodic chambers are kept at anaerobic conditions. The CO_2 can be

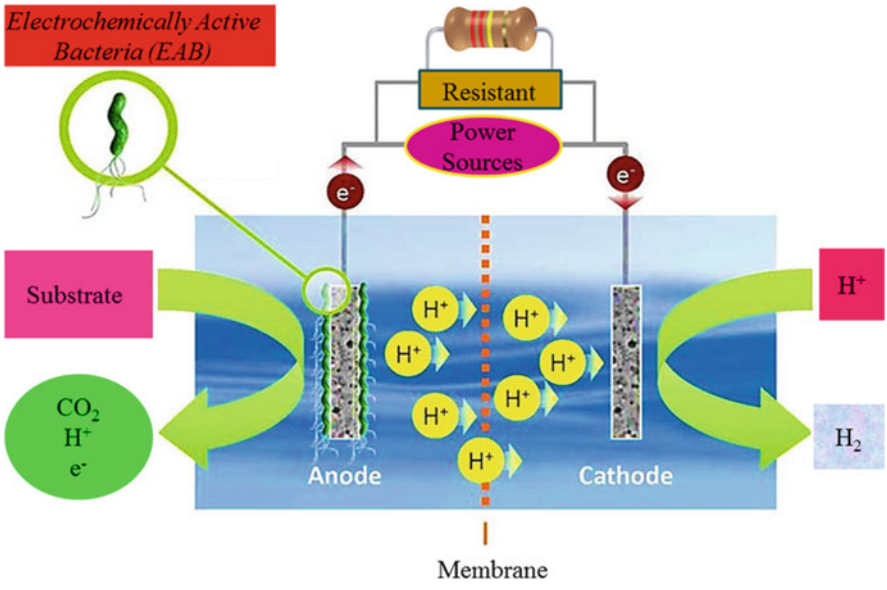

Fig. 15.1 Diagram of a common double-chambered MEC and working principles

captured and reused. For acetate as a model substrate, the MEC process could be presented as below electrochemical reactions or equations:

$$\text{Anode}: CH_3COO^- + 4H_2O \rightarrow 2HCO_3{}^- + 9H^+ + 8e \left(E^{\circ}_{an} = 0.187\ \text{V}\right) \quad (15.1)$$

$$\text{Cathode}: 8H^+ + 8e^- \rightarrow 4H_2 \left(E_{H+/H_2} = -0.414\ \text{V vs.NHE}\right) \quad (15.2)$$

$$\text{Overall Reaction}: CH_3COO^- + 4H_2O \rightarrow 2HCO_3{}^- + H^+ + 4H_2 \quad (15.3)$$

However, thermodynamically, production of H_2 via reduction of H^+ is not a spontaneous process. For that reason, to generate H_2 at the MEC cathode, at least a cathode potential of −0.414 V vs NHE (normal hydrogen electrode) is required (Rozendal et al. 2006; Kadier et al. 2015, 2016b; Lu and Ren 2016). The electrode potentials in MEC are computed in accordance with the Nernst equation. The electrode potential for the cathode reaction may be written according to Eq. (15.4):

$$E_{cat} = E^{\circ}_{cat} - \frac{RT}{2F} \text{In} \frac{P_{H_2}}{[H^+]^8} \quad (15.4)$$

$$= 0 - \frac{8.314 \times 298.15}{2 \times 96,485} \text{In} \frac{1}{\left[10^{-7}\right]^8} = -0.414\ \text{V}$$

where $E^{\circ}_{cat}$is the electrode potential for H_2 (0 V), R universal gas constant (8.314 J/K/mol), T temperature (K), and F Faraday's constant (96,485 C/mol e^-). The electrode potential for the anode reaction may be written as (Eq. (15.5)):

$$E_{an} = E^{\circ}_{an} - \frac{RT}{8F} \text{In} \frac{[CH_3COO^-]}{\left[HCO^{3-}\right]^2 [H^+]^9} \quad (15.5)$$

$$= 0.187 - \frac{8.314 \times 298.15}{8 \times 96,485} \text{In} \frac{0.0169}{[0.005]^2 \left[10^{-7}\right]^9} = -0.3000\ \text{V}$$

where E°_{an} is electrode potential (0.187 V) for acetate oxidation, for a solution with HCO^{3-} = 0.005M, CH_3COO^- = 0.0169M, pH = 7 (Logan 2008; Kadier et al. 2016a). Accordingly, the cell voltage (E_{cell}) essential for an MEC to generate H_2 at the cathode with these terms is as Eq. (15.6):

$$E_{cell} = E_{cat} - E_{an} = (-0.414\ \text{V}) - (-0.300\ \text{V}) = -0.114\ \text{V} \quad (15.6)$$

The negative E_{cell} indicated that H_2 may not be generated from acetate naturally, and in order to make the HER favorable and generate H_2, an applied input voltage ($E_{ap} > 0.114$ V) has to be provided. In practical terms, E_{ap} should be greater than the theoretical E_{cell} because of activation loss, ohmic loss, and mass transport loss (Kadier et al. 2016a). Earlier MEC studies reported that $E_{ap} \geq 0.2$ is required to yield quantifiable current and H_2 generation in MEC (Rozendal et al. 2006; Cheng and Logan 2007; Lu and Ren 2016; Khan et al. 2017). Nonetheless, this voltage

(0.2 V) is greatly smaller than the voltages required for water electrolysis (generally 1.8–2.0 V) (Rozendal et al. 2008; Zhang and Angelidaki 2014).

15.3 The Main Operational Modes of MECs

The operation modes could instantly impact on the performance of MEC in regard to HPR, organic matter removal, and the energy efficiency (Kadier et al. 2017b, 2018b). In this section, the main operational modes of MECs are comprehensively reviewed and discussed.

15.3.1 Batch Modes

The batch modes were frequently adopted in MEC studies, as a concept of proof, to evaluate the catalytic activity of novel cathode materials (Su et al. 2016), the viability of using novel substrates in MEC (Selembo et al. 2009; Liu et al. 2012b; Rani et al. 2020), the efficiency of methanogen inhibition (Hu et al. 2008; Kadier et al. 2018b), and the possibility of producing other value-added chemicals (Cusick and Logan 2012). For instance, the exposure of cathodes to air was frequently adopted in fed-batch modes, in order to suppress the growth of methanogens. A novel SS fiber felt cathode was recently tested in MEC, and it was found that the H_2 production increases via the increase of the fed-batch circles due to a decline in overpotential caused by corrosion (Su et al. 2016). The batch mode was also applied in MEC for more precise quantitative evaluation of the H_2 yield (Y_{H_2}) and electron flows (Lee et al. 2009). Moreover, the research experience obtained in fed-batch could be served as a reference for continuous-flow modes.

15.3.2 Fed-Batch Modes

The fed-batch mode, in classical fermentation theory, was termed as "During fed-batch, one or more nutrients are delivered to the fermenter, while the products remain in the fermenter till the end of operation" (Lee et al. 1999). According to this definition, the substrate in the fed-batch operated MEC, e.g., acetate should be added periodically without the replace of the solution, to control the substrate at a certain concentration and to avoid the potential inhibition of EAB caused by the high concentrations of substrates (Sharma and Li 2010). However, in MEC studies, people are apt to confuse the two issues of fed-batch mode and batch mode. Most of the MECs claimed operated in fed-batch modes, where the MEC reactors were emptied, refueled with substrate, and purged with ultra-high purity (UHP) N_2 after each fed-batch cycle (Wang et al. 2009; Selembo et al. 2010; Call et al. 2009).

15.3.3 Continuous-Flow Modes

The performance of MECs operated in continuous-flow modes, where mixed cultures were generally used, was significantly affected by the following parameters: the hydraulic retention time (HRT), the organic loading rate (OLR), the catalysis ability of the cathode, and the way of H_2 collection. For example, the HRT has shown considerable impacts on H_2 generation and energy intake in a tubular semi-pilot MEC fed via DWW (Gil-Carrera et al. 2013a, b, c). A membrane-free continuous-flow MEC with a gas-phase cathode was constructed, and a volumetric HPR of 6.3 m^3 H_2/m^3 day was obtained under substrate non-limiting conditions (Tartakovsky et al. 2009). The biggest challenge of MECs in continuous-flow mode was the H_2 consumption caused by the re-oxidizing of bioanode and the conversion to methane with CO_2. For instance, a single-chamber MEC using the carbon fibers, lacking metal catalysts, as the cathode was proposed for H_2 generation. However, the H_2 recycling between the cathode and the anode reached 62–76% of obtained current density, and it was revealed Coulombic efficiency (C_E) as high as 190–310%, and the cathodic conversion efficiency was 16–24% (Lee and Rittmann 2009). Furthermore, a continuous-flow MEC (1000 L) was fabricated and used for H_2 production from the winery wastewater treatment. However, most of the generated gas was turned to CH_4 (86 ± 6%), with a maximum HPR of 0.19 ± 0.04 m^3 H_2/m^3 day (Cusick et al. 2011). Similarly, the multi-electrode system based MEC was constructed and the results demonstrated that it can be scaled-up predominantly on the basis of surface area of cathode, hence that H_2 can be entirely used up in a continuous-flow MEC if methanogens are fully suppressed (Rader and Logan 2010; Kadier et al. 2018a).

15.4 Diverse Applications of MEC Beyond Hydrogen Production

By the use of different E_{ap} or set potentials, changing the microbial population, and amending the system configurations, MECs can be rechannelled to offer the desired applications. As a result of the discovery of bio-cathodes, numerous new brunch applications of MECs have been studied and which have expanded the application scopes of MEC-related processes (Fig. 15.2).

15.4.1 Value-Added Chemicals Production

It is well known that the most often application of the MEC technology is the bio-H_2 production (Call and Logan 2008). Apart from bio-H_2, in the past years, numerous MEC researches have also concentrated on producing different kinds of value-added

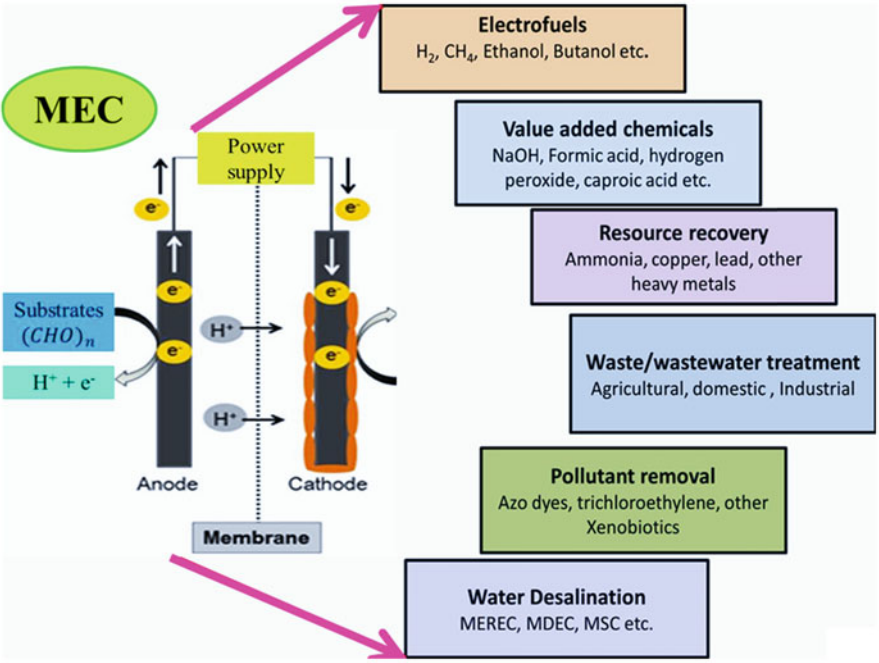

Fig. 15.2 Various applications of MEC technology

chemicals (Kadier et al. 2016a, 2018a; Kumar et al. 2017b; Hua et al. 2019) such as methane (Cheng et al. 2009), hydrogen peroxide (Rozendal et al. 2009; Arends et al. 2014; Sim et al. 2018), ethanol (C_2H_5OH) (Steinbusch et al. 2009), and so on. Table 15.1 illustrates some previously reported valuable bioproducts from MECs, such Bio-CH_4, CH_3COO^-, H_2O_2, ethanol (C_2H_5OH), and formic acid (CH_2O_2).

15.4.2 Resources Restoration in MECs: Heavy Metal, Nutrient, Ammonium (NH_4^+), and P

Acid mine drainage (AMD) is a major issue for mankind because of the low pH, huge amount of heavy metals, and additional poisonous elements. If it is not appropriately managed, it could seriously pollute surface and ground water as well as land. Luo et al. (2014a, b) reported the extraction of metals from AMD and concurrently H_2 generation by using MEC (Table 15.2). They used a dual-chamber MEC to remove various metals from AMD and simultaneously produce H_2. Throughout the test, it was found that Cu^{2+} restored at cathode, followed by Ni^{2+} and Fe^{2+}. Interestingly, the AMD having only Cu^{2+} resulted in highest HPR of 1.1 H_2 m^3/m^3 day. The highest restoration of 89% was obtained in AMD having a

Table 15.1 Overview of the value-added bio-chemicals produced via MEC technology

Value-added bioproducts	Input voltage (V)	MEC reactor configuration	Cathodes	Electron acceptor in the cathode	Production rate (mmol/L/h)[a]	References
Acetate/$C_2H_3O_2^-$	0.4	Dual-chamber	Graphite sticks	CO_2	–[b]	Nevin et al. (2010)
CH_4	0.7–1.0	Single/two-chamber	Biocathode	CO_2	0.06	Cheng et al. (2009)
CH_4	−0.55[c]	Dual-chamber	Biocathode	CO_2	0.006 CH_4 m^3/m^3 day	Van Eerten-Jansen et al. (2011)
Ethanol/C_2H_6O	−0.55[c]	Dual-chamber	Biocathode	Acetate	0.00003	Steinbusch et al. (2010)
Formic acid/CH_2O_2	1.13[d]	Dual-chamber	Pb	CO_2	0.09	Zhao et al. (2012)
Hydrogen peroxide/H_2O_2	0.5	Dual-chamber	CGE	O_2	(1.9 ± 0.2 kg H_2O_2/m^3 day)	Rozendal et al. (2009)
H_2O_2	−0.4[e]	Dual-chamber	(CGE)	O_2	(141 ± 3 mg H_2O_2/L-h)	Sim et al. (2015)

[a]Calculated based on total reactor volume with the available data
[b]Not stated; CGE-carbon gas diffusion electrode
[c]Cathode potential
[d]The power source is MFC stack
[e]Anode potential

Table 15.2 Performance of MEC: (a) ammonium and P recovery and (b) heavy metals recovery and H_2 production reported in recent literature

Influent type	MEC reactor type	Applied voltage (V)	HPR (H_2 m^3/m^3day)	TAN recovery or removal rate	References
Real human urine	Dual-chamber MFC	1.0	0.50 A/m^2 (I_V)	3.29 g N/m^2 day	Kuntke et al. (2012)
5 × diluted urine	Dual-chamber MEC	1.0	48.6 ± 7.47	173.4 ± 18.1 g N/m^2 day	Kuntke et al. (2014)
Synthetic urine	Dual-chamber MEC		61 ± 1% (current efficiency)	384 ± 8 g N/m^2 day	Luther et al. (2015)
Urine	MEC-TMCS	0.5	1.6 A/m^2 (I_V)	46% (TAN)	Kuntke et al. (2016)
Pig slurry	Dual-chamber MFC		3.5 A/m^2 (I_V)	60% (TAN recovery)	Cerrillo et al. (2016)
Landfill leachate	MEC-FO system	0.8	0.760 A/m^2 (I_V)	63.7 ± 6.6 (TAN)	Qin et al. (2016)
Sidestream centrate	MEC-FO system	0.8	–	99.7 ± 13 (TAN)	Zou et al. (2017)
2 × diluted and undiluted urine	MEC-TMCS system	0.5	1.7 ± 0.2 A/m^2 (I_V)	31 ± 59% (TAN recovery)	Zamora et al. (2017)

Note: I_V current density, *FO* forward osmosis, *TMCS* transmembrane chemisorption (TMCS) reactor, *TAN* total ammonia-nitrogen recovery

mixture of metals; it was possibly resulting from the use of the electrons for H_2 generation and metal reduction. Another group, Qin et al. (2012) reported Ni^{2+} removal with wastewater in MEC. During their study, impacts of initial pH, Ni^{2+} concentrations, and E_{ap} on the performance of MEC were investigated. Obtained results illustrated that the advantage of MECs over both electrolysis cell and MFC as removal efficiency (RE) peaked threefold. With the raise in the initial Ni^{2+} from 50 to 1000 mg/L, the RE was decreased. Nevertheless, in respect of mass, removal of Ni^{2+} enhanced continuously via the initial concentrations. Moreover, when E_{ap} raised from 0.5 to 1.1 V, RE had increased from 51 ± 4.6% to 67 ± 5.3%. The results obtained in this study established MEC capable of removing Ni^{2+} from wastewater. Furthermore, Colantonio and Kim (2016a) studied Pb^{2+} removal in a laboratory scale MEC. They demonstrated membrane-free Pb^{2+} extraction with applying different E_{ap}. The results showed that Pb^{2+} removal could be gained by MECs. Moreover, traditional approaches to remove toxic cadmium from wastewater are costly and also have serious environment and health problems. Colantonio and Kim (2016b) reported wastewater treatment and simultaneous H_2 generation using MEC. They showed fast removal of cadmium (50–67% in 24 h). They decipher three

Influent type	MEC reactor type	Applied potential	Metal recovered	Purity/ removal efficiency	HPR (H_2 m^3/m^3 day)	References
Soil	Two chamber	NA	Copper	99.9%		Fedje et al. (2015)
Synthetic media	Two chamber	0.5	Cd(II)	5.86 ±- 0.25 mg/L h	0.35 ± 0.07	Wang et al. (2016)
Synthetic media	Two-chamber	1.4	Sulfate	sulfate removal rate: 60%		Coma et al. (2013)
Domestic wastewater	Bioelectro-dialysis (four chambers)	1.2	Sulfate	18.9gSO_4^{2-}/m^2 day	0.29 L/ L day	Zhang and Angelidaki (2015)
Fly ash leachate	Two-chamber BES-ER	6.0	Cu(II)	98.5%		Tao et al. (2012)
		6.0	Zn(II)	95.4%		
		6.0	Pb(II)	98.1%		
AMD	Two-chamber MEC	1.0	Cu^{2+}	99.2 ±- 0.1%/ 560 mg/L day	1.1	Luo et al. (2014a, b)
		1.0	Ni^{2+}	97 ± 1.3%/ 530 mg/ L day	0.6	
		1.0	Fe^{2+}	97 ± 1.8%/ 168 mg/L day	0.4	

Note: *AMD* acid mine drainage, *BES* bioelectrochemical system, *ER* electrolysis reactors

different Cd removal mechanisms, namely cathodic reduction; $Cd(OH)_2$ precipitation; and $CdCO_3$ precipitation. Additionally, Chen et al. (2016) tried to enhance the Cd(II) removal in MECs utilizing acetate as a feedstock. They also reported the significance of long period bacterial population adaptation at raised Cd (II) concentrations. However, the identical paramount species of microorganism was observed in various ranges in the acetate and $NaHCO_3$. These results revealed that the importance of both longer bacterial community acclimation and fuel source to enhance Cd(II) removal and H_2generation in MECs.

As for ammonium and P recovery (Table 15.2), Kuntke et al. (2014) used an MEC for ammonium and COD removal and H_2 generation from diluted urine. The function of voltage generation in MECs on N portion and the prospective of CEM were taken into consideration in an investigation carried out by Haddadi et al. (2014).

More recently, Yuan and Kim (2017) reported an enhanced phosphorus recovery by testing numerous cathode arrangements and applied voltage conditions in a

laboratory size MECs fed with dewatering centrate collected from wastewater treatment plant (WWTP).

15.4.3 MECs for Treatment of Different Types of Wastewaters

With respect to wastewater treatment applications, it is well known that wastewater often contains a complex mixture of organics that can be utilized by EAB in MEC reactors. In recent years, different kinds of wastewater such as synthetic, domestic, agricultural, and industrial wastewaters have been used as substrates for feeding EAB in MECs to produce H_2 (Kadier et al. 2014; Escapa et al. 2016; Hua et al. 2019). This drives the MEC platform a promising and renewable source of bioenergy and biofuels. Besides, the production of a beneficial and value-added product in the course of wastewater treatment in MECs, including H_2, CH_4 could assist to lower the operating costs (Hua et al. 2019).

15.4.3.1 Domestic and Synthetic Wastewater Treatment

Ditzig et al. (2007) were the first to demonstrate H_2 production in an MEC using DWW as the inoculum and the substrate and evaluated performance and the viability of treatment. Besides, to assess the feasibilities of DWW treatment and H_2 production, a pilot-scale MEC was manufactured. The reactor generated great purity H_2 (100%) at a HPR of 0.015 H_2 m^3/m^3 day (Heidrich et al. 2013). Likewise, another MEC was constructed and operated for 12 months with raw DWW as feed. The MEC generated an average HPR of 0.007 H_2 m^3/m^3 day (Heidrich et al. 2014). In addition, Ivanov et al. (2013) reported a basic quantitative technique for comparing the efficiency of MECs operated with various wastewaters in fed-batch mode. Moreover, Montpart et al. (2015) used synthetic wastewater (carbon sources such as glycerol, milk, and starch) in single-chamber MEC. They reported greater outcomes in terms of I_V 150 A/m^3, HPR 0.94 m^3 H_2/m^3 day, and $r_{cat\ (H_2)}$ 91%.

15.4.3.2 Treatment of Industrial Wastewater (IWW)

In other studies, it was revealed that a membrane-less MEC using GFB anode could be operated with full-strength or diluted swine wastewater (Wagner et al. 2009). The results established MEC as an efficient process for H_2 generation and swine wastewater treatment. Likewise, Cusick et al. (2010) studied H_2 generation efficiency of MECs fed via DWW and winery wastewater. H_2 generation expenses were higher for winery wastewater as compared to DWW (Cusick et al. 2010). In consideration of these results, the early pilot-scale MEC with a reactor size of 1000 L was

manufactured for the current generation and COD removal by treating winery wastewater (Cusick et al. 2011). In the study, the maximum volumetric current density of 7.4 A/m^3 was obtained after 100 days (Cusick et al. 2011). Kiely et al. (2011) treated potato processing and dairy manure wastewater in an MEC. Prior to use, potato processing wastewater was diluted via ultrapure water for the purpose of lowering the OLR. With $E_{ap} = 0.9$ V, the MEC generated 4.5 mA of current and 0.74 m^3 H_2/m^3 day of HPR, the $r_{H_2}$was 80%, with COD removal of 79%. Along the same lines, for the first time Ren et al. (2013) utilized refinery wastewaters as fuel in MEC. The outcomes were alike to those in DWW. Besides, Tenca et al. (2013) tested IWW (methanol rich) and food processing wastewater (FPWW) as substrate in MEC. Wang et al. (2014) investigated the Pt/CC and biocathode for H_2 production performance using molasses wastewater under low temperature (9 °C). In their study, at $E_{ap} = 0.6$ V, the overall r_{H_2} of 72.2% was gained when the Pt/CC was employed. Mahmoud et al. (2014) studied the pre-fermentation of less biodegradable landfill leachate with BOD_5/COD ratio of 0.32 in MECs. The results showed that semi-continuously fermented leachate notably improved its performance. In addition, waste-activated sludge (WAS) which comprises a considerable quantity of carbohydrates was employed as feedstocks in MECs (Liu et al. 2012a, b; Sun et al. 2014). An integrated DF-MEC was analyzed for H_2 production from sugar beet juice (Wu et al. 2013), the overall H_2 generation was 25% of initial COD, and 57% more energy was recovered via the integrated bio-H_2. Spent yeast (SY) is a major issue for the brewing industry and could be utilized in MECs to regain energy (Sosa-Hernández et al. 2016). SY from both bench alcoholic fermentation and ethanol was employed in varied concentrations. The best result was found at 750 mg COD/L SY + 1200 mg COD/L ethanol.

15.5 Initial Pilot-Scale and Real-World Applications of MECs

The new direction of study in the MEC platform provides diverse future real-world applications as, for example, H_2 generation, treatment of the wastes and wastewaters, and synthesis of value-added chemicals. The advancements for the commercial viability of this technology depend on various factors such as range of the microorganisms, electrode materials, separators or membranes, feedstock categorizations, operating parameters (Escapa et al. 2016; Katuri et al. 2019). Although the H_2 and power production from MEC platform via several types of organic waste and wastewaters, was achieved, but the maximum Y_{H_2} and power was gained from the acetate (Jeremiasse et al. 2010). As summarized in Table 15.3, a small number of studies were dedicated to the implementation of the MEC platform in semi-pilot or large scale applications. The values reported are comparatively smaller than the small-size bioreactors (Heidrich et al. 2013, 2014). Other than that, other concerns

Table 15.3 Pilot-scale experience on MEC platform for simultaneous treatment of wastewater and H_2 production

Feedstock	Bacteria source	Reactor size (L)	HPR (m^3 H_2/m^3 day)	References
Domestic WW	Wastewater	120	0.015	Heidrich et al. (2013)
Domestic WW	Wastewater	88	0.007	Heidrich et al. (2014)
Winery WW	Mixed anaerobic culture	1000	0.19	Cusick et al. (2011)
Raw municipal WW	Domestic WW	10	COD removal: 60–76% Energy intake: 0.9 kWh/kg COD	Gil-Carrera et al. (2013a)
Domestic WW	Domestic WW	4	COD removal: 85% Energy intake: 1.6 kWh/kg COD	Gil-Carrera et al. (2013b)
Domestic WW	Domestic WW	4	COD removal: 80% Energy intake: 0.3–1.1 Wh/g COD	Gil-Carrera et al. (2013c)
WW	WW	16	COD removal: 67%	Brown et al. (2014)
Domestic WW	Domestic WW	175	0.005	Cotterill et al. (2017)
Urban WW	Urban WW	130	0.031	Baeza et al. (2017)
Acetate medium	Effluent from lab-scale MECs	110	The highest H_2O_2 conversion efficiency was only 7.2 ± 0.09%, the maximum I_V was 0.94–0.96 A/m^2	Sim et al. (2018)
Urban WW	Urban WW	167	r_{H_2} and η_{E+S} were 82% and 55.1%, respectively	Chen et al. (2019)

are uptake of H_2 by H_2 consuming populations eventually decreasing the H_2 and electricity production capacity of MECs.

As an example, Cusick et al. (2011) revealed that the pilot-scale experiment utilizing winery wastewater in a continuous-flow MEC reactor of 1000 L indicated that longer start-up time is the great barrier of the large scale MEC, as a consequence of the deprivation of acetate in the initial start-up, non-familiarized EAB, alterations in the pH and temperature. Likewise, semi pilot-scale MEC implementation

employing planar and tubular reactor configurations emphasized the impact of up-scaling on reactor performance while assisting to determine and optimize manifold vital operating parameters, namely HRT, E_{ap}, reactor configuration, anodic size (Gil-Carrera et al. 2013a, b, c).

In recent years, Brown et al. (2014) configured an MEC reactor to study the impact of technical enhancements in the geometry and the flow regimes in MECs/BESs, and it was revealed that introduction of a model for evaluation of the MECs performance is a very beneficial tool for comparison of various systems. In a recent study, Heidrich et al. (2014) constructed a 120 L MEC reactor and utilized DWW at varied temperatures for over a period of 365 days. The results revealed that the H_2 generation was active at upper temperature with a peak HPR of 1.2 m^3 H_2/m^3 day under an average temperature of 16.5 °C. Whereas throughout the functioning of MEC, the CH_4 was found in insignificant amount ($\leq$0.8%) in the anode of MECs. Accordingly, it was an indication that preservation of optimum temperature is crucial for efficient H_2 generation from complex wastewaters. On top of that, for the purpose of promoting the scale-up of MEC platform to achieve commercial scale application, the long-term operation, robustness, and suitability of this technology must be evaluated. Nevertheless, further improvement in the reactor configuration did not enhance the HPR (0.005 m^3 H_2/m^3 day) with C_E less than 10% (Cotterill et al. 2017). More recently, an enhanced HPR (0.031 m^3 H_2/m^3 day) with cathodic C_E of 82% was reported (Baeza et al. 2017), employing the same reactor design as Heidrich et al. (2013).

15.6 Conclusions

MEC is an electricity-mediated BES, primarily developed for high-performance bio-H_2 generation from waste and wastewaters. Compared to conventional biological H_2 generation technologies, MECs can address thermodynamic boundaries and attain high-H_2 yield from a broad range of organic matters at comparatively mild conditions. Over the last decade, the MEC technology has been regarded as a potentially attractive and eco-friendly technology to combat the global climate change and energy emergency. Currently, most of the MEC studies have been conducted at a greatly small size with synthetic substrates, and the goal of scaling up MEC for the real-world application in biofuels production and wastewater treatment is far from success. Thus, to specify gaps in the knowledge and examine how MECs can be progressed in respect of performance and commercial level applications, this chapter was aimed to provide a comprehensive literature review related to the MEC technology, main working principles, operation modes, present applications, and the most recent pilot-scale MEC research regarding bio-H_2 production and wastewater treatment.

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Chapter 16
Surface Modification Approaches for Methane Oxidation in Bioelectrochemical Systems

Jamil Islam, Namita Shrestha, Jejal Reddy Bathi, Rajesh K. Sani, and Venkataramana Gadhamshetty

Abstract Methane (CH_4) is not easily amenable to typical chemical and electrochemical oxidation processes, primarily due to its stable and inert nature. However, given the vast reserves of CH_4, it is important to develop robust and sustainable processes for converting CH_4 into value-added products. This chapter explores the use of bioelectrochemical systems (BESs) for converting CH_4 directly into electricity. Colonization of methanotrophs on the electrode surfaces is a key to implement CH_4-based BESs. We present potential chemical functionalization approaches for tuning surficial properties of the electrodes, with a goal of promoting the colonization of methanotrophic cells and their growth into electrogenic biofilms on the electrode surfaces. This chapter presents potential solutions for addressing technical challenges of high overpotential, low CH_4 solubility, and slow oxidation kinetics, which represent an Achilles' heel to CH_4-based BESs.

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16.1 Introduction

Natural gas, a form of methane (CH_4) supports 21.4% of the world's total energy demand (Energy_Agency 2015). Ironically, CH_4 is also the second most profuse greenhouse gas (Shindell et al. 2012). The oil and gas industry alone contributes to 40% of the total CH_4 emissions in the United States, equivalent to 4% of the greenhouse gas emissions (Gadhamshetty et al. 2015). Considering an exponential increase in the oil as well as gas production in the United States, the CH_4 emissions can also be expected to increase. Being a non-polar substance, CH_4 rarely interacts with other compounds. Its C-H links are strongest among all of the alkanes, rendering it difficult to oxidize CH_4 ($\Delta H_{298} = 105$ kcal/mol) (Shindell et al. 2012; Blanksby and Ellison 2003) in both leaner systems (atmosphere) and chemical reactors.

A set of expensive cleaver molecules and catalysts are often used to make the C-H bonds of CH_4 functionable. Higher temperatures needed to debond the C-H strength will eventually deactivate the catalysts. Lower temperatures can avoid deactivation issues, but they reduce the overall CH_4 oxidation kinetics. Considering these issues, it is desirable to develop robust microorganisms for converting CH_4 directly into value-added products in BESs. On a positive note, microorganisms that oxidize CH_4 ("methanotrophs") freely exist in many natural systems including the ocean's bed. For example, archaeal methanotrophs syntrophically coexist with sulfate-reducing bacteria (SRB), where the surplus electrons from CH_4 oxidation are transferred to SRB (Valentine 2002). Methanotrophs use methane monooxygenase (MMO) enzymes to oxidize CH_4 under ambient conditions. It is thus plausible to use robust methanotrophs for oxidizing CH_4 in BESs (Sirajuddin and Rosenzweig 2015).

Biofilms growth begins with the colonization of methanotrophic cells on the surface of the electrodes, both on the anode or cathode in BESs. The electrogenic biofilms then transfer electrons from CH_4 oxidation through the outer cell wall to the electrode surface. This chapter will discuss a key chemical functionalization techniques to grow biofilms of methanotrophs on the electrode surfaces. Considering that the extracellular electron transfer process is a surface phenomenon, functionalization strategies provide a means to tune the surface properties and control the biofilms growth. This chapter focuses on the surface properties of commercial electrodes based on carbonaceous materials (Carbon paper, graphite felt, graphite brush, carbon fibers, polymers) and metals (nickel, copper, and steel). This chapter also provides a background information on BES principles, methanotrophs and its biofilms, soluble MMO (sMMO), and particulate MMO (pMMO) enzymes required to carry out CH_4 oxidation, and surface modification techniques to enhance biofilms growth.

16.2 Methanotrophs

Methanotrophs represent a dispersed consortium of aerobic microorganisms that can utilize CH_4 as the source of carbon and energy (Wang et al. 2017). They synthesize sMMO or pMMO enzymes to oxidize CH_4. To facilitate CH_4 in BESs, it is crucial to understand the concepts of sMMO and pMMO activity (Sirajuddin and Rosenzweig 2015; Wang et al. 2017).

16.2.1 Soluble Methane Monooxygenase (sMMO)

sMMO is a multivariate enzyme that uses methane monooxygenase hydroxylase (MMOH) to carry out the oxidation of the substrate (Fig. 16.1). The sMMO reductase (MMOR) facilitates the transport of electrons from CH_4 oxidation to the working site. A sMMO regulatory protein (MMOB), MMOH and MMOR are the essential three proteins which are required for the activity of sMMO (Sirajuddin and Rosenzweig 2015; Hanson and Hanson 1996). A $\alpha_2\beta_2\gamma_2$ homodimer, 251-kDa MMOH ends with three subunits (α,β,γ) and catalyzing portion. Diiron active site accounts for hydroxylation of the CH_4 deeply hidden in the α subunit (Elango et al. 1997; Rosenzweig et al. 1993). 38-kDa MMOR holds the flavin adenine dinucleotide and ferredoxin domains that facilitate the transfer of two-electron from nicotinamide adenine dinucleotide to the diiron active center (Lund and Dalton 1985). The 16-kDa MMOB strongly influences the ability of the active center to couple O_2 activation with CH_4 oxidation (Merkx et al. 2001; Sazinsky and Lippard 2006). Both MMOB and MMOR are encrypted by *mmoB* and *mmoC* genes, respectively.

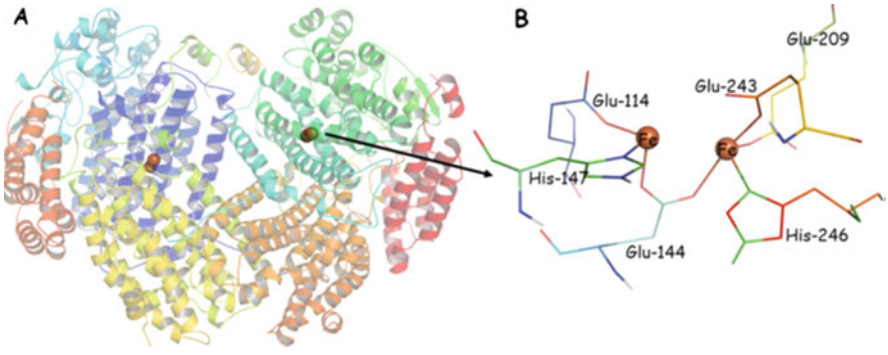

Fig. 16.1 The overall structure of sMMO hydroxylase of Methylococcus capsulatus (**a**) the iron atoms (sphere shape), active sites, dirron center, fenced by four helices have been shown in dimer structure of hydroxylase. This figure was made by the protein data bank (PDB) file 1MTY. (**b**) The diiron center is synchronized by unequivocally residues among MMOHs in the BMM family that is Ball-and-Stick illustration (Adapted from Wang et al. 2017)

sMMO is a well-categorized representative of the bacterial multicomponent monooxygenase (BMM) family. Among the BMM family members including alkene as well as aromatic monooxygenases, tetrahydrofuran monooxygenases, phenol hydroxylases, alkene monooxygenases, and hyperthermophilic aromatic/alkene monooxygenases, sMMO enzyme exhibit a unique ability to hydroxylate CH_4. Certain methanotrophs express only sMMO while others possess subset encoding genes for both pMMO and sMMO. To express sMMO, methanotrophs require at least 1 μM copper species in the growth medium (Stanley et al. 1983; Choi et al. 2003). The sMMO expression also depends on the transcriptional activator, *mmoR*, and a GroEL-like chaperone protein, *mmoG* (Scanlan et al. 2009; Csaki et al. 2003). sMMO offers flexibility to work with a broad range of hydrocarbons inclusive of C1-C8 n-alkanes, alkenes, and larger molecules including benzene, styrene, naphthalene, ethylbenzene, and cyclohexane (Burrows et al. 1984; Green and Dalton 1989).

16.2.2 Particulate Methane Monooxygenase (pMMO)

Unlike sMMO, pMMO is a membrane-bound protein that cannot be easily separated from the plasma membrane. An assembly of an $(\alpha\beta\gamma)_3$ trimeric structure exists in the membrane of pMMO (Lieberman and Rosenzweig 2005; Myronova et al. 2006). One copy of each of the three subunits namely α, β, and γ compose the functional enzyme that corresponds to *PmoB* (45 kDa), *PmoC* (27 kDa), and *PmoA* (23 kDa), respectively (Chan and Yu 2008; Lieberman et al. 2003). The molecular masses of *PmoA, PmoB,* and *PmoC* are 28,302.25, 42,664.66, and 29,690.34 amu, respectively (Pham et al. 2013; Wendeborn 2019). pMMO can oxidize only C1-C5 n-alkanes and terminal alkanes to 2-alcohols and 1,2-epoxides (Miyaji et al. 2011; Jiang et al. 2010). To stimulate pMMO expression by methanotrophs, a minimum of 5 μM copper is required (Stanley et al. 1983; Choi et al. 2003; Prior and Dalton 1985; Sirajuddin and Rosenzweig 2015) (Fig. 16.2).

16.3 Grand Challenges for CH_4 Oxidation

16.3.1 Low Aqueous Solubility

The solubility diagram of CH_4 provides (Fig. 16.3) (Wilhelm and Battino 1985; Rettich et al. 1981) indicate that CH_4 does not easily dissolve in water under ambient conditions (Ma and Huang 2017).

At low pressures, the solubility of CH_4 is proportional to its partial pressure. Thus, CH_4 solubility can be increased by increasing its partial pressure in the headspace. As shown in Fig. 16.3, CH_4 solubility changes with both temperature and pressure. Lower temperatures and higher pressures will improve the dispersion

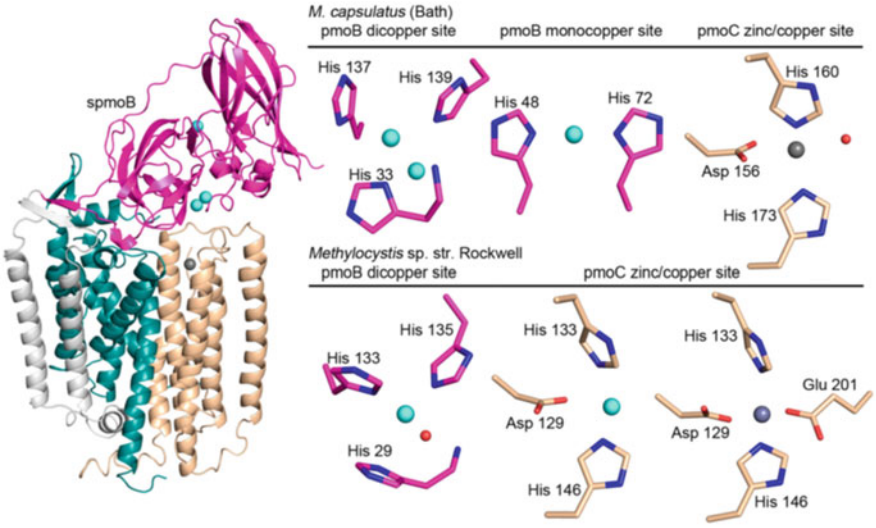

Fig. 16.2 The illustration of the metal centers within the pMMO crystal structures. The dicopper, monocopper, and zinc/copper sites as cyan and gray spheres inside a protomer of *M. capsulatus* pMMO for methane oxidation (Adapted from Sirajuddin and Rosenzweig 2015)

of CH_4 in aqueous electrolytes (Pruteanu et al. 2017; Wilhelm et al. 1977). At constant pressure of 30 MPa and lower temperatures (353–393 K), the CH_4 solubility increases at a rate of 0.0095 $m^3/(m^3\ K)$. At higher temperatures (413–453 K), the incremental factor is only 0.005 $m^3/(m^3\ K)$ (Ma and Huang 2017). The preferable temperature range for methanotrophs is 25 °C–35 °C (Mohanty et al. 2007). This information can be used to optimize reactor conditions in BESs and maximize CH_4 solubility.

16.3.2 Overpotential

The overpotential is the additional potential required to push the reaction at a specific rate and beyond the thermodynamic limits. It is the difference between an equilibrium potential and the potential at which the catalyst functions at a specific current under given environmental conditions. For example, Eq. (16.1) represents the overpotential required to produce hydrogen via the proton reduction.

$$2H^+ + 2e^- \rightleftharpoons H_2 \quad \text{overpotential} = \left| E_{H+} - E_{cat/2} \right| \tag{16.1}$$

The Tafel equation can be used to determine the correlation between the overpotential and catalytic rate and subsequently determine the overpotential of a molecular catalyst (Bard and Faulkner 2001). The catalytic response of a molecular

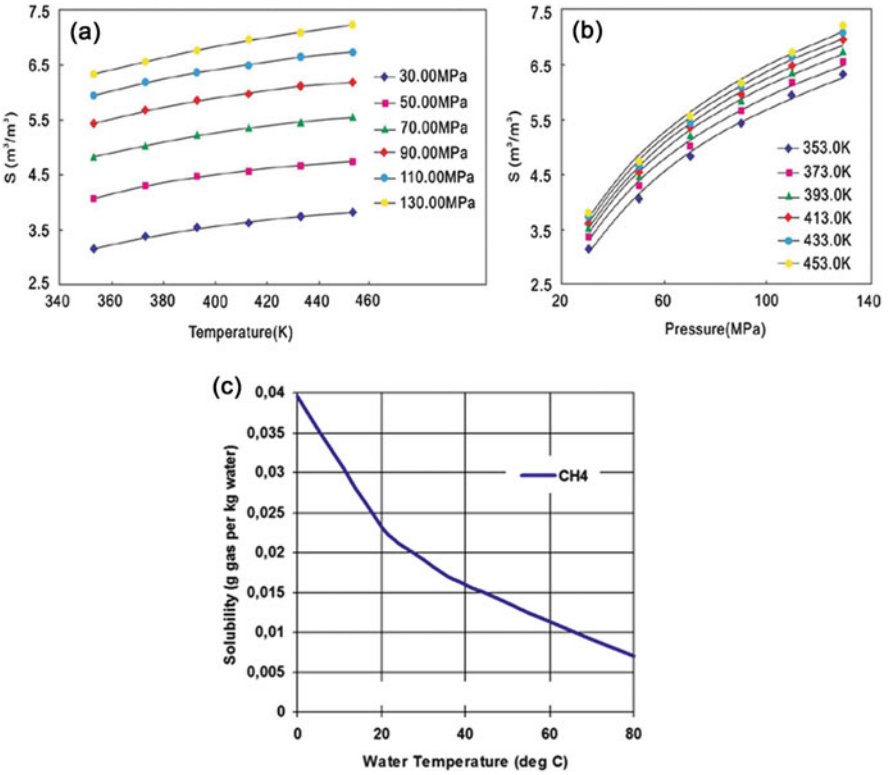

Fig. 16.3 The temperature and pressure effect on the solubility of CH_4 (Adapted from Ma and Huang 2017)

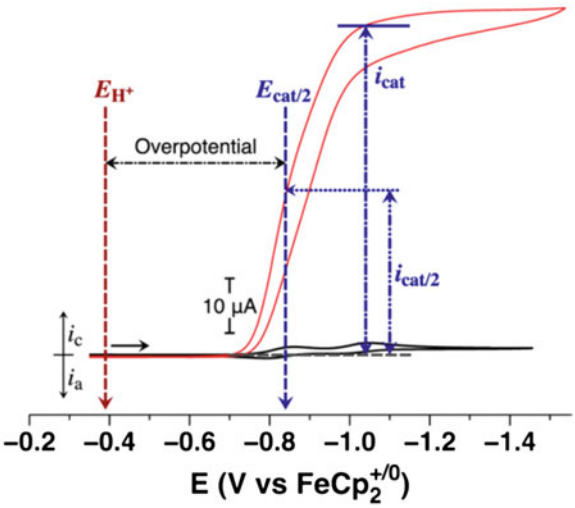

Fig. 16.4 The cyclic voltammogram showing the $E_{cat/2}$ and the overpotential for H_2 production. The $Ni(P^{Ph}{}_2N^{Ph}{}_2)_2(BF_4)_2$[14] of 1 mM conc in 0.2M NBu_4PF_6 solution, dissolved in acetonitrile. The scan rate 50 mV/s and the diameter were maintained 1 mm. The red line indicates the presence of 0.26M DMF and 0.26M $[(DMF)H]^+$ (Adapted from Appel and Helm 2014)

catalyst is prompted by a specific redox reaction (Fig. 16.4). The catalytic current typically follows an exponential trend and exhibits an increasing overpotential characteristic (Appel and Helm 2014). Section 16.5 will discuss surface modification techniques for reducing overpotential associated with CH_4 oxidation in BESs (Kim and Surendranath 2019).

16.3.3 Challenges with Activation of C–H Bonds

CH_4 oxidation is unfavorable from both kinetic and thermodynamic perspectives (Lee and Dempsey 2017). Due to the high pKa value of CH_4, acid-base chemistry approaches alone cannot activating C-H bonds effectively under ambient conditions. CH_4 oxidation is also challenged by its low solubility. CH_4 bioelectrochemcial conversion can be enhanced by the use of robust methanotrophs, process intensification techniques, and metabolic engineering approaches (high-turnover enzymes and high catalyst loading) (Haynes and Gonzalez 2014) and surface modification techniques (a focus of this study).

16.4 Bioelectrochemical Systems

A microbial fuel cell (MFC) is a classic example of BESs that uses exoelectrogenic biofilms to oxidize carbon sources on the anode surface and generate electric current (Rao et al. 1976; Davis and Yarbrough 1962). Methanotrophs can catalyze CH_4 oxidation, generate electric current, and transport the current to the anode (negative terminal), the external electric load, and finally the cathode (positive terminal) (Fig. 16.5).

Table 16.1 provides a summary of the studies on CH_4 oxidation in MFCs. In the mid-nineteenth century, a bacterial fuel cell was used by Hees that contained

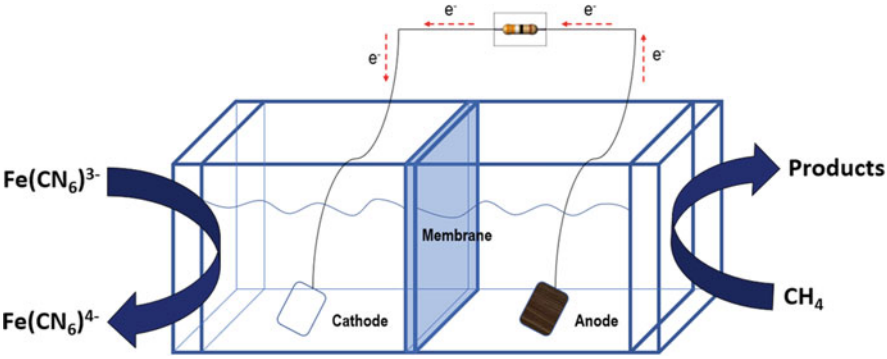

Fig. 16.5 A schematic of a microbial fuel cell

Table 16.1 Critical overview of studies on CH_4-fed BESs

Reference	Goal	Experimental details	Result
McAnulty et al. (2017)	Construct a synthetic microbial consortia that generated electricity from methane	Constructed three types of synthetic consortium of anaerobic methanotrophs, *Geobacter sulfurreducens* and engineered archaeal strain to converts methane directly into electricity	Maximum power density from methane oxidation was 168 mW/m^2
Soo et al. (2016)	Used pure culture of methanogens to convert methane into liquid biofuel precursors	Engineered pure culture of *Methanosarcina acetivorans* (archaeal methanogen) to grow on methane and convert it into the biofuel precursor acetate anaerobically	Anaerobic methanotrophic archaeal population *Methanosarcina acetivorans* cells used 15 ± 2% methane and generated 10.3 ± 0.8 mM acetate
Strong et al. (2015)	A critical overview of methanotrophs that enable the use of methane as a feedstock in biotechnology applications	Reviewed 157 methane related peer-review journal	Demonstrated the potential to use methane as a carbon source for methanotrophs and their consortia
Chen and Smith (2018)	Use dissolved methane from anaerobic effluents in microbial fuel cells	Three reactor configurations (dual-chamber MFC, air cathode, single-chamber MFC) were operated using synthetic, methane-saturated medium in continuous mode. (20 °C, hydraulic retention time 4, 8, and 16 h)	85% dissolved methane was removed, and 0.55 ± 0.06 V potential was generated. Illumina sequencing (16S rRNA and 16 rRNA gene) was used to identify *Geobacter* and methanotrophs in anode biofilm samples
Myung et al. (2018)	Develop a two-staged microbial fuel cells for treating methane	Two-step strategy, firstly conversion of methane to methanol, followed by using methanol as the substrate in the MFC for electricity generation	Maximum power density of 426 ± 17 mW/m^2 was achieved. Microbial community analysis showed acetogens converted methanol into acetate, which is then consumed by exoelectrogens for electricity generation
Ding et al. (2017)	Decouple DAMO archaea from DAMO bacteria in a microbial fuel cell	Methane based MFC was used to investigate the decoupling of denitrifying anaerobic	DAMO MFC achieved 0.653 mW/m^2 power density and 25 mV potential

(continued)

Table 16.1 (continued)

Reference	Goal	Experimental details	Result
		methane oxidation archaea and bacteria	
Cheng and Logan (2007)	Electrosynthesis process for converting electrical current into methane	Two-chamber electrochemical reactor (abiotic anode, biocathode, no metal catalysts) was used for carbon dioxide reduction to methane with a set potential	At set potential (-1.0 V), 96% current capture efficiency was achieved and biocathode was dominated by a single Archaeon, Methanobacterium palustre. When single-chamber MEC was used, energy efficiency was 80% (electrical energy and substrate heat of combustion)
Girguis and Reimers (2011)	Use methane as a carbon substrate in microbial fuel cells	Introduced the concept of harnessing the power of microbial metabolism by using methane as a carbon source	Patented the concept
Promoppatum and Viswanathan (2016)	Quantified performance of a flare gas recovery system that used electrochemical processes to convert methane into methanol	Evaluated the techno-economic analysis of proposed approach in comparison to other gas flare recovery systems	Proposed electrochemical conversion of methane from petroleum gas into methanol for recovery of flare gas
de Klerk (2015)	Engineering analysis for performance evaluation of a process used to convert methane into methanol	Evaluated engineering application of direct conversion of methane to methanol and the potential applications of this process as a platform for small-scale gas-to-liquids	Engineering evaluation showed no potential benefit of application of direct methane to methanol conversion instead of indirect synthesis of methanol (current industry standard)
Khirsariya and Mewada (2013)	Discuss a single-pot process for converting methane to methanol	Discussed thermodynamic feasibility of reaction of direct oxidation of methane	Concluded, that there is still no process that produces a reasonable methanol yield but there has been encouraging process. Suggested that improved catalyst, novel reactor design and operation can enhance methanol selectivity
Tomita et al. (2008)	Develop an electrochemical Fuel Cell for enabling direct oxidation of methane into	Evaluated hydrogen-oxygen fuel cell (Sn0.9In0.1P2O7 as electrolyte) for selective	Demonstrated feasibility of direct conversion of methane to methanol by increasing reaction

(continued)

Table 16.1 (continued)

Reference	Goal	Experimental details	Result
	methanol under ambient conditions	oxidation of methane to methanol	temperature above 80 °C
Montpart et al. (2014)	Use methanol to generate electricity and hydrogen in bioelectrochemical systems	Studied MFC with methanol as sole carbon source using anodic syntrophic consortium (exoelectrogenic plus fermentative bacteria). MFC was tested through three different strategies: direct replacement of acetate instead of methanol, progressive replacement of acetate/methanol, and a two-step consortium development bioaugmenting methanol fermenting bacteria with anode respiring bacteria	Best performance was observed in two-step consortium development bioaugmenting anode respiring bacteria with methanol fermenting bacteria which increased power output (220 mW) by tenfold
van Hees (1965)	Construct a Fuel Cell that uses bacteria to treat methane	Fuel cells with a *Pseudomonas methanica* suspension at the anode and sterile medium at the cathode that used methane and air	Maximum power density was 2.8 μW/cm^2 (2.6 mW/ft^2) at 0.35 V. Developed 0.5–0.6 V on open circuit
Rizzo et al. (2013)	Develop microbial fuel cells technology for mitigating methane from paddy fields	Proposed preliminary investigation one-dimensional process-based MFC model (simulates vertical and temporal dynamics of the chemical compounds) for mitigating CH_4 emissions in the paddy field	Demonstrated potential of this technique, with reductions up to 28.1%, 24.1%, and 26.5% for daily minimum, daily maximum, and total CH_4 emissions, respectively. The current density achieved was 400 mA/m^2
Jeon et al. (2012)	Use microbial fuel cells to control methane emission from lake sediment	Demonstrated the control of methane emission in a hypereutrophic lake using electrochemical techniques (sediment MFC)	Maximum power density achieved was 6.80 mW/m^2. Methane emission decreased by 35-fold in closed circuit condition in comparison to open circuit condition
Kaku et al. (2008)	Study the interactions between plant and microbe to enable electricity generation in a rice paddy field	Installed sediment MFC in a rice paddy field (from May to September) and examined electricity generation	Power density achieved was 5.8 mW/m^2

(continued)

Table 16.1 (continued)

Reference	Goal	Experimental details	Result
Ishii et al. (2008)	Study morphological and phylogenetic comparisons of microbial communities in methanogenesis versus electrogenesis	Inoculated two H-type MFCs, using enriched microbes from rice paddy field soil grown in cellulose fed electrogenic/methanogenic conditions	The reactor operating in closed circuit condition produced 9.8 mW/m^2 of current along with a small amount of methane but the reactor operating in open circuit condition actively produced methane with a very small amount of current

Pseudomonas methanica suspension sparged with CH_4 and air at the anode and sterile medium at the cathode (van Hees 1965). The fuel cell generated an open circuit voltage (OCV) of 0.5–0.6 V. Another group (Girguis and Reimers 2011) used a two-compartment MFC with 200 mL of 0.2-μm filter-sterilized methanotrophic media inoculated with 1 mL of *M. methanica* culture in anode bubbled with a mixture of CH_4 (10 ml min^{-1}) and air (1 ml min^{-1}) and 0.01-μm filter-sterilized air in the cathode compartment. The average current density in their MFCs was recorded 10.5 mA m^{-2}.

MFCs can be used to mitigate CH_4 emissions in wetlands soils. A one-dimensional process was developed by Rizzo et al., to study processes that influence CH_4 oxidation in the sediment MFC (SMFC) that was used to mimic soil systems (Rizzo et al. 2013). The MFC was treated as an additional pathway to simulate the vertical and temporal dynamics of CH_4 oxidation within the paddy soil. A major finding was that the CH_4 oxidation increases with the increasing depth of the anode owing to the higher levels of CH_4 at the bottom of the muddy layers in the soil. The model demonstrated that CH_4 emissions from the soil reduced by 28.1%, 24.1%, and 26.5% for daily minimum, daily maximum, and total CH_4 emissions, respectively. A study by Jeon et al. has demonstrated that SMFC can curb CH_4 emissions from a hypereutrophic lake. After 10 days of SMFCs operation, the maximum power density was recorded 6.80 mW m^{-2} and a 35-fold decrease in CH_4 emission was observed under closed circuit operation in comparison to open circuit operation (Jeon et al. 2012).

A study by McAnulty and coworkers have developed a synthetic consortium based on a mixture of anaerobic methanotrophs, *Geobacter sulfurreducens,* and engineered archaeal strain to facilitate bioelectrochemical oxidation of CH_4. Microorganisms from the CH_4 - acclimated sludge (including *Paracoccus denitrificans*) contribute to facilitating electron transfer by providing electron shuttles. *Geobacter sulfurreducens* oxidizes acetate, to create an MFC that converts CH_4 directly into electrical current. The maximum power density observed from this two-compartment MFC with synthetic consortium was 168 mW m^{-2} (McAnulty et al. 2017).

The poor electrogenic activity of methanotrophs limits the performance of CH_4-fed MFCs (McAnulty et al. 2017; Kadier et al. 2018). The power production in CH_4-fed MFCs can be promoted by a dual-step strategy, involving bioconversion of CH_4 into methanol as the first step, followed by the use of the methanol to fuel electricity generation in MFCs (Myung et al. 2018; Kumar et al. 2018). Myung used aerobic methanotrophs from the activated sludge of an aeration basin in the wastewater treatment plant to oxidize CH_4 to methanol in the first step. In the second step, a single-chamber air cathode MFC (graphite fiber brush anode) was used to produce electricity from the methanol using a mixed-culture community. The two-stage system significantly improved the maximum power density as 426 mW m^{-2} (Myung et al. 2018; Deval et al. 2017; Chandrasekhar and Mohan 2014a; Jawaharraj et al. 2020).

There has been considerable interest over the years to understand the morphological features, characterization, and electrogenicity of methanotrophs. Ding et al., studied the physiological characteristics of denitrifying anaerobic CH_4 oxidizers (DAMO) in MFCs (Ding et al. 2017; Chandrasekhar et al. 2015). DAMO-MFCs demonstrated poor electrogenic capability (25 mV) but successfully showed percentages of DAMO bacteria (initial: 24.4% to final: 2.07%) and archaea (initial: 24.4% to final: 65.77%) based on sequencing. Soo et al., provided insights into archaeal contribution in CH_4 cycling in the environment. They engineered the archaeal methanogen (*Methanosarcina acetivorans)* that converts CH_4 into acetate under anaerobic conditions. *M. acetivorans* cells were incubated at 37 °C in 40-mL bottles with butyl rubber stoppers and crimped with aluminum seals (6 weeks in 8 mL HS medium and 10 mM $FeCl_3$) to maintain anaerobic conditions. *M. acetivorans* cells consumed 15% CH_4 (corresponding to 143 μmol of CH_4) and produced 10.3 mM acetate (Soo et al. 2016; Chandrasekhar and Mohan 2014b). Despite all ongoing researches, the detailed mechanism of AOM and aerobic oxidation of CH_4 is in its infancy. Readers are suggested to review the literature to understand the merits and demerits of the CH_4-to-electricity electrochemical conversion when compared with flare gas recovery techniques (Promoppatum and Viswanathan 2016; de Klerk 2015; Chandrasekhar and Ahn 2017).

16.5 Surface Modifications of Electrodes

The surface properties of the electrode impact the current generation in MFCs. Typical materials used as electrodes include copper (Kargi and Eker 2007), stainless steel (Dumas et al. 2008), activated carbon (Kalathil et al. 2011), carbon fiber, carbon cloth (Logan et al. 2007), graphite (ter Heijne et al. 2008) and nanostructured materials (Hou et al. 2013). A major challenge with the use of metallic electrodes is that they are vulnerable to corrosion and microbial corrosion. This chapter only provides a general overview of material functionalization techniques that can be used to tune desirable surface properties and control the biofilms growth of any given microorganism including methanotrophs. We focus on wettability, surface

roughness, surface charge, and accessible surface area, all of which influences cell attachment, biofilms growth, and associated extracellular electron transfer abilities (Liu et al. 2004; Chandrasekhar and Mohan 2012).

16.5.1 Wettability

The degree of biofilms growth depends upon the degree of initial cell attachment which again depends on the wettability of the underlying surface. Wettability is the ability of the electrolyte to stay in contact with the electrode. Intermolecular interactions between an electrolyte and electrode promote the force components (adhesion and cohesion), both of which govern the wetting phenomena. In the case of gas-electrode-electrolyte interfaces in CH_4-fed BESs, the wetting phenomena are complex and it is influenced by an interplay between electrode, electrolyte, and CH_4. Table 16.2 provides examples of surface modification approaches using different surfactant templates. The –OH, –COOH, =CO, and –CO groups promote interactions between reactive oxygen and carbon atoms on the carbonaceous electrode. These groups promote attachment of water molecules on the electrode surfaces and enhance the surface wettability (Wan et al. 2013; Tareq et al. 2019).

Figure 16.6 shows the contact angles of bare carbon paper, carbon paper modified with polyethyleneimine (PEI), and carbon paper modified with multiwall carbon nanotubes (MWCNTs). As shown, the PEI renders hydrophilicity (contact angle =15°) while MWCNT renders hydrophobicity (contact angle = 165°) to the carbon paper, respectively (Choudhury et al. 2017). Other functional groups are those based on p-aniline derivatives including $–CH_3$, –OH, $–SO_3H$, $–N^+(CH3)_3I$. Among these functional groups, the $–SO_3H$ group is the most effective group for rendering hydrophobicity (see Tables 16.2, 16.3, and 16.4).

Diels-Alder reaction chemistry is a convenient method for tuning surface properties of carbon nanostructures as well as the metal electrodes including steel (Bian et al. 2013; Seo et al. 2013). Pristine carbon nanostructures can undergo Diels–Alder reactions with a range of functional dienes and dienophiles, and more importantly without the need of a catalyst. The Diels–Alder reaction can be used as a strategy for the synthesis of carbon nanostructures (fullerenes, carbon nanotubes, and graphene) as well as for their subsequent surface functionalization. A recent study by the authors group used the Diels-Alder chemistry to develop a mechanochemical approach to synthesize graphene particles and simultaneously functionalize them with maleic anhydride for subsequent use as fillers in difunctional bisphenol A/epichlorohydrin epoxy. The maleic-anhydride-functionalized graphene epoxy coating (MAGE) was then used to tune the wettability of the mild steel (MS) as well as improve its microbial corrosion resistance. The MAGE-MS displayed higher hydrophobicity (90° ± 2) compared to epoxy-MS (80° ± 2) (Chilkoor et al. 2020). Thus, MAGE-MS can be used in BESs that require hydrophobic electrode surfaces to promote cell attachment and biofilm growth (Liu et al. 2004).

Table 16.2 Critical overview of studies on surface modification of electrodes

Matrix/ substrate	Coating/ modification	Wettability (contact angle)	Surface roughness and porosity	Surface charges	Effects	Applications	Reference
Glassy carbon plate	Electrochemical reduction of p-aniline derivatives ($-CH_3$ $-OH$, $-SO_3H$ $-N(CH_3)_3I$)	$-CH_3$; hydrophobic (80.2°) –OH; hydrophilic (52.9°) $-SO_3H$; hydrophilic (10.0°) $-N^+(CH_3)_3I$); Hydrophilic (15.0°)	NA[a]	Negative Neutral Negative Positive	Positively charged and hydrophilic surfaces demonstrated higher biofilm formation when compared to negatively charged and hydrophobic surfaces. Additionally, positively charged surface and hydrophilic surfaces were shown to be more selective to the electroactive microbes (e.g., *Geobacter*) and more advantageous for the electroactive biofilm formation	Current generation	Guo et al. (2013)
Glassy carbon plate	Electrode surface polished	NA[a]	Roughness (Ra ~100 nm and ~10 nm)	NA[a]	Roughness reduced impedance significantly compared to the smoother surface	Power generation	Ye et al. (2013)
Gold anode	1 mercapto-undecanol (–OH) undecanethiol ($-CH_3$) 1-mercapto undecanoic acid (–COOH) 1-mercaptoundecyl Trimethylamine ($(N(CH_3)_3^+$)	–OH hydrophilic (27°) $-CH_3$ hydrophobic (102°) –COOH hydrophilic (19°) $-N(CH_3)_3^+$ hydrophilic (51°)	NA[a]	Neutral Negative Negative Positive	Positively charged and hydrophilic surfaces boosted biofilm growth when compared to the negatively charged and hydrophobic surfaces	Power generation	Santoro et al. (2015)

Carbon paper	MWCNT PEI	MWCNT hydrophobic (170°) PEI hydrophilic (0°)	NA[a]	NA[a] Negative	MWCNT enhanced hydrophobicity of electrode surfaces along with the greater biofilm formation compared to PEI modified electrode	Current generation	Choudhury et al. (2017)
TORAY Carbon paper SGL carbon paper	PTFE	135° and 155° (20 wt% PTFE and without PTFE respectively) 153° and 148° (20 wt% PTFE and without PTFE respectively)	Low-frequency roughness (Ra ~100–300 μm) and high-frequency roughness (Ra ~5–10 μm)	NA[a]	Carbon papers with high PTFE content demonstrated high level of hydrophobicity which indicates slowdown of biofilm formation	MFC	Santoro et al. (2014)
Carbon cloth	Nitric acid + sulfuric acid (CC-AS) Ammonium nitrate (CC-AN) Ammonium sulfate (CC-AS)	Enhanced wettability	High-frequency roughness (Ra ~20–100 nm) and low-frequency roughness (Ra ~9–5 μm)	NA[a]	The modified electrode surface demonstrated higher content of N, O, S, and oxidized carbon which eventually assisted for the biofilm growth and consequently higher power density generation as reported	MFC	Liu et al. (2004)
Carbon cloth	Reduced graphene oxide (rGO)	Hydrophobic (123°)	Porous	NA[a]	Surfactant rGO provided significant surface area to the electrode substrate and facilitated to create the hydrophilic surface with functional groups (carbonyl, carboxyl, and ammonium) which improved biocompatibility ended with higher biofilm formations	MFC	Chang et al. (2017)

(continued)

Table 16.2 (continued)

Matrix/ substrate	Coating/ modification	Wettability (contact angle)	Surface roughness and porosity	Surface charges	Effects	Applications	Reference
Carbon felt	UV/O_3 treatment	Enhanced hydrophilicity	Porous	NA[a]	UV/O_3 treated carbon felt demonstrated as a hydrophilic surface as a result of high oxygen content on it. Due to the reduction of the amount of C=C, the impedance decreased. Healthier biofilm formation was observed	MFC	Cornejo et al. (2015)
Carbon cloth	Biochar particles	NA[a]	Highly porous	NA[a]	Biochar is inexpensive and it ended the electrode surfaces with higher effective surface area and excellent surface porosity	MFC	Wendeborn (2019)
Ni foam	Reduced graphene oxide (rGO)	Hydrophobic	Highly porous	NA[a]	Coating of rGO on Ni foam demonstrated excellent electrical conductivity, effective enhanced surface area, and higher porosity. The 3D porous Nickel scaffold confirmed higher electron transfer and mass transfer efficiency in power generation applications	MFC	Wan et al. (2013)

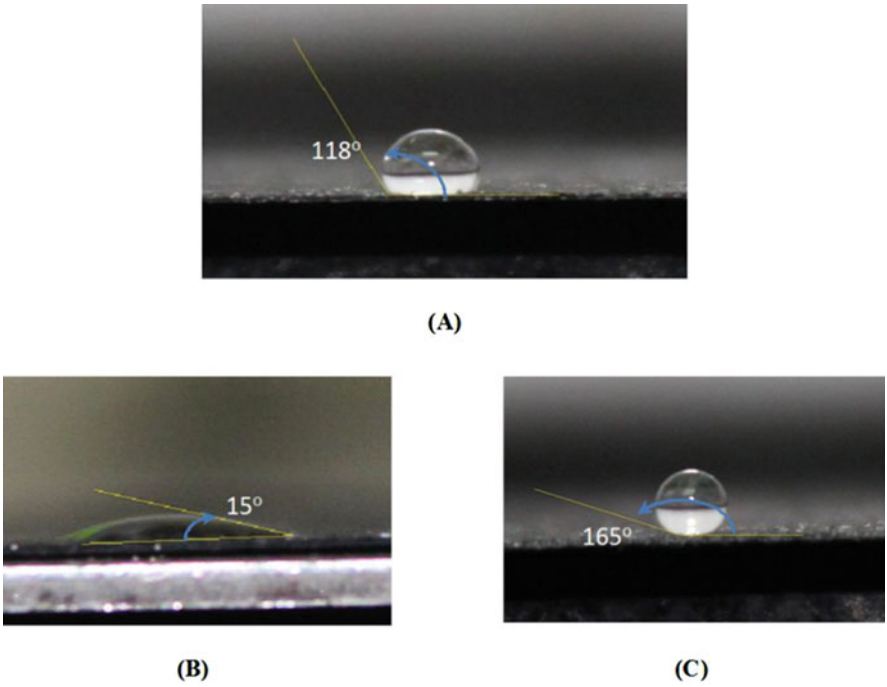

Fig. 16.6 Contact angles of (**a**) Bare carbon paper, (**b**) Carbon paper modified with PEI (0.17 μg/cm^2), (**c**) Carbon paper modified with MWCNTs (0.56 mg/cm^2) (Adapted from Choudhury et al. 2017)

Figure 16.7 depicts results from a non-turnover CV test where *Geobacter* biofilms were grown on gold surfaces modified with –COOH or –OH, containing 1-mercaptoundecanoic acid and 1-mercaptoundecanol. The former modification techniques rendered contact angle of 19° and the latter yielded 27°, respectively (Santoro et al. 2015). Dissimilar surfactants can also be used to create hydrophilic surfaces. For example, TORAY paper modified with 20 wt% polytetrafluoroethylene (PTFE) yields hydrophilic surface (CA = 135°) when compared to the bare TORAY carbon paper (CA =135°). A recent study has reported that carbonaceous electrodes such as SGL (a non-woven carbon paper, brand name SGL) carbon paper modified with 20 wt% PTFE yields hydrophobic surfaces (CA = 153°) (Santoro et al. 2014).

Acid treatment approaches could also be used to add hydrophilic functional groups on carbonaceous electrodes. Many earlier studies have been successfully used to treat carbon cloth with nitric acid, sometimes combined with sulfuric acid treatment to improve hydrophilicity. Chemicals such as ammonium nitrate and ammonium sulfate also yield hydrophilic surfaces. Surface properties of metal electrodes, especially porous electrodes based on metals such as nickel can be treated with reduced graphene oxide (Chang et al. 2017; Islam et al. 2020), as well as UV light and ozone (Cornejo et al. 2015; Sen and Tareq 2016).

Table 16.3 A critical review on anodic surface properties and feasible recommendation for enhancing surface properties for methanotrophs

Reference	Consortia	Anode	Typical surface properties	Comments on Biofilms	Challenges and recommendations
McAnulty et al. (2017)	1. Engineered archaeal strain 2. *Paracoccus denitrificans* 3. *Geobacter sulfurreducens*	Carbon fiber	Porous, hydrophobic surface	*M. acetivorans* develops biofilms	Biofilms growth can be accelerated by turning the hydrophobic surface into hydrophilic surface by soaking carbon fiber into hydrogen peroxide, nitric acid, sodium hypochlorite, etc.
Soo et al. (2016)	*Geobacter*	Ammonium peroxydisulfate treated carbon brush	High specific surface area, hydrophilic surface	Biofilms identified	Positive charged surface enhances bacterial attachment and biofilms growth which can be achieved followed by a coating of electrode with p-aniline derivatives ($-N(CH_3)_3^+$)
Myung et al. (2018)	Methane-oxidizing bacteria (collected from activated sludge)	Graphite fiber	High specific area, high electrical conductivity	Biofilms explored	Catalytic properties and the hydrophilic surface can be upgraded by coating with reduced graphene oxide (rGO)
Baudler et al. (2015)	*Geobacter, Shewanella*	Silver, Nickel, Copper, Gold	Silver and Copper typically antimicrobial metals. All are highly electrically conductive and relatively expensive	Biofilms detected	Silver and Copper can exterminate bacteria, so direct interfacial contact between bacteria and these metals can be avoided by surface coatings. Especially, the concentration level of Copper in the solution originated from corrosion can control methane monooxygenase enzyme expression (*sMMO* or *pMMO*) for the subsequent methane oxidation

Wan et al. (2013)	*Shewanella oneidensis MR-1*	Nickel foam (NF) coated with reduced graphene oxide (rGO/NF)	Highly porous, High conductivity, super hydrophilicity, High specific surface area	Biofilms found	High porosity reduces electrode conductivity and durability for the long term MFC performance
Liu et al. (2004)	Mixed culture	Graphite rod	High conductivity, chemical stability and Cheaper	Biofilms growth observed	Enhanced surface area and optimal surface roughness are recommended for the improved electrocatalytic activity
Ahn and Logan (2010)	Mixed culture	Graphite fiber brush	High specific area and easy to manufacture	NA[a]	Positively charged surfaces are recommended
Ishii et al. (2008)	*Geobacter sulfurreducens*	Carbon cloth	High porosity and relatively expensive	Biofilms growth observed	High porosity can decrease the electrical conductivity of the electrode. High Conductive coating material might be added to decrease porosity and enhance effective surface area
He et al. (2005)	Mixed culture	Reticulated vitreous carbon (RVC)	High electrical conductivity	Mixed-culture biofilms observed	Enhanced catalytic activity and specific area can be achieved by surface coatings

[a]Not available

Table 16.4 Critical overview on wettability property of electrodes and various functional groups

Substrates	Functional groups/coatings materials	Wettability (contact angle)	Reference
Glassy carbon plate	$-CH_3$ –OH $-SO_3H$ $-N^+(CH_3)_3I$)	Hydrophobic (80.2°) Hydrophilic (52.9°) Hydrophilic (10.0°) Hydrophilic (15.0°)	Guo et al. (2013)
Gold anode	–COOH	Hydrophilic (19°)	Santoro et al. (2015)
Carbon paper	MWCNT PEI	Super hydrophobic (170°) Super hydrophilic (0°)	Choudhury et al. (2017)
TORAY Carbon paper SGL carbon paper	135° and 155° (20 wt% PTFE and without PTFE respectively) 153° and 148° (20 wt% PTFE and without PTFE respectively	Hydrophobic Hydrophobic	Santoro et al. (2014)
Carbon cloth	None	Hydrophobic (123°)	Chang et al. (2017)
Ni foam	None	Hydrophobic	Wan et al. (2013)

16.5.2 Surface Roughness and Porosity

Surface modifications techniques can be used to modify the topography of electrode surfaces as well as to modify any attached functional groups on their surfaces. These techniques can be used to tune surface roughness and porosity, providing a route to control the sorption of bacterial cells. A recent study by Zhou et al. has demonstrated that the roughness of a glassy carbon plate electrode can be boosted by polishing it with a grinder-polisher configured with SiC sandpaper (Ye et al. 2013). In general, rough surfaces promote initial bacterial adhesion, subsequent biofilm formation, extracellular electron transfer (Liu et al. 2004; Santoro et al. 2014).

As shown in Fig. 16.8, the electrochemical impedance to the charge transfer reactions on the rougher glassy carbon surface is an order of magnitude lower compared to the smoother surface. The SEM image in Fig. 16.9 confirms that rougher surfaces enhance biofilm growth when compared to smoother surfaces (data reflects finding after 13 days of the exposure in BESs).

16.5.3 Surface Charges

Many researchers have reported that the surface charges (positive or negative) on the electrodes influence the rate of biofilm growth and in turn the start-up time to achieve

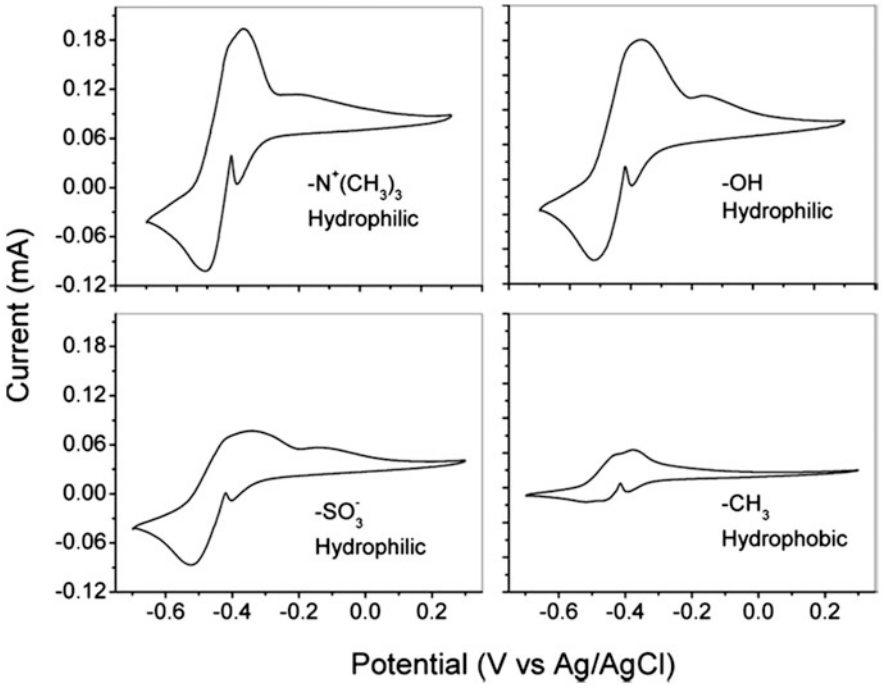

Fig. 16.7 The optical demonstrations of cyclic voltammogram of the different modified substrate (scan rate: 1 mV/s) (Adapted from Guo et al. 2013)

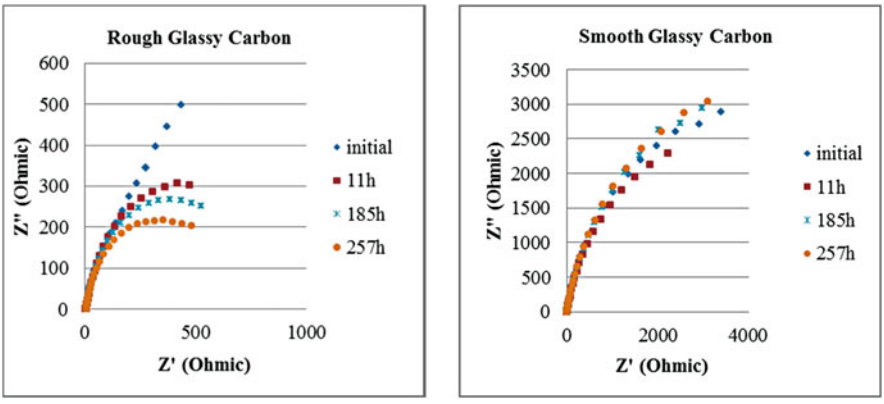

Fig. 16.8 The comparison of electrochemical impedance spectroscopy (EIS) of the anode of MFCs (**a**) rough (Ra ~100 nm) and (**b**) smooth (Ra ~10 nm) glassy carbon electrodes (Adopted from Ye et al. 2013)

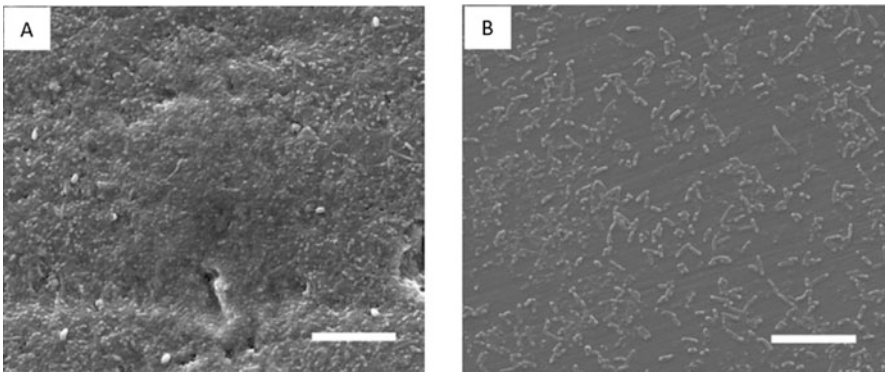

Fig. 16.9 The SEM illustrates the biofilms growth after 315 h on (**a**) rough (Ra ~100 nm) and (**b**) smooth (Ra ~10 nm) electrode surfaces. Scale bars denote 10 μm (Adopted from Ye et al. 2013)

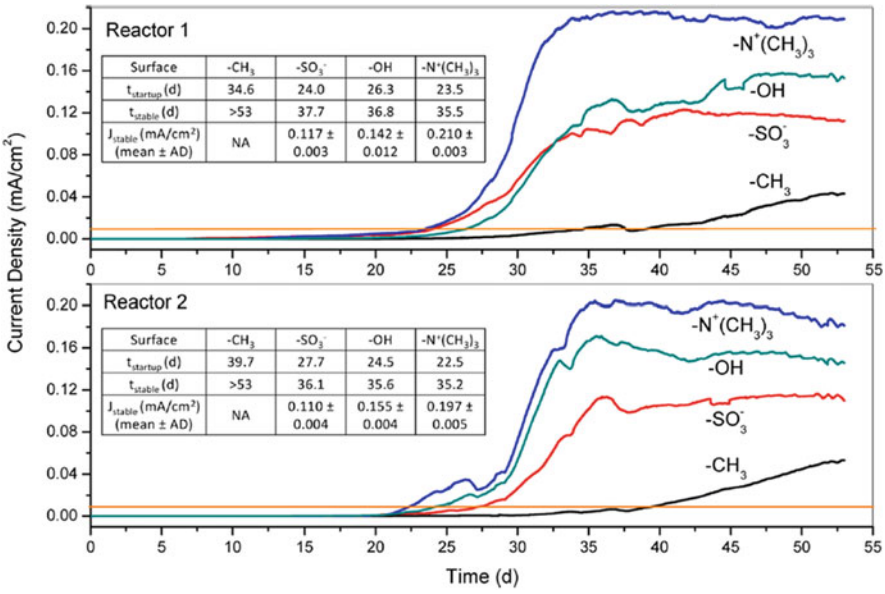

Fig. 16.10 Current density versus time in MFCs with dissimilar modified electrodes. $t_{startup}$ represents the start-up time of current, while t_{stable} is the time required to reach a steady current and average current density at the steady stage (J_{stable}) of each electrode (Adopted from Guo et al. 2013)

a steady-state BES performance (Guo et al. 2013; Santoro et al. 2015). As shown in Figs. 16.10 and 16.11, the time versus current curve can be used to assess the dynamics of biofilm growth. On another note, as a result of teichoic acids in gram-positive bacteria, the bacterial cell wall has a negative charge. In contrast, the teichoic acids are either linked to the peptidoglycan or to the underlying plasma

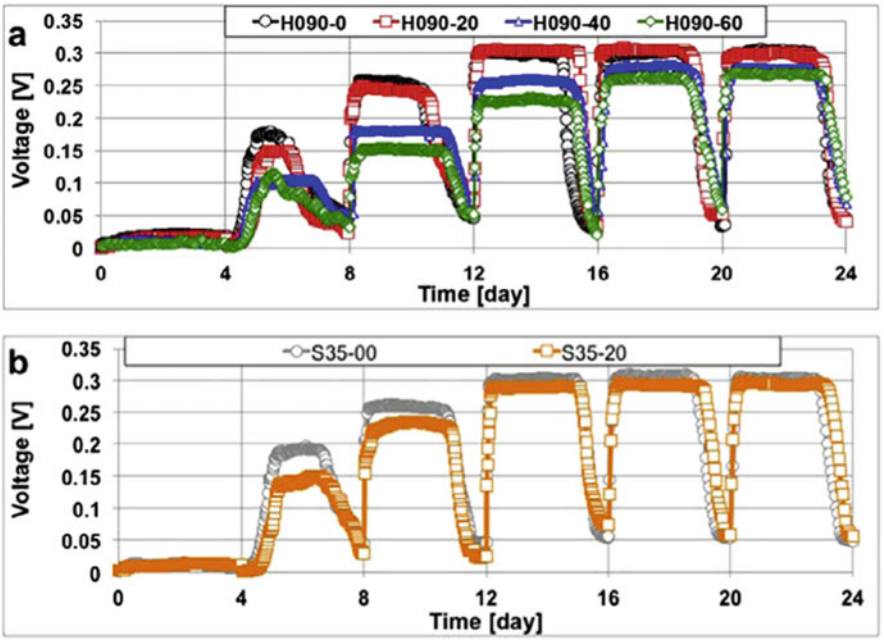

Fig. 16.11 The graphical illustrations of the Start-up voltage in SMFC with TORAY (**a**) and SGL (**b**) carbon papers (Adopted from Santoro et al. 2014)

membrane, attributable to the presence of phosphate in their structure. Similarly, gram-negative bacteria possess phospholipids and lipopolysaccharides in their outer cell, therefore, negatively charged is imparted by lipopolysaccharides (Gottenbos et al. 2001; Swoboda et al. 2010). Thus, positively charged electrode surfaces are attractive when compared with negatively charged bacteria because of the electrostatic forces (Cheng and Logan 2007; Picot et al. 2011). As shown in Fig. 16.10, the positively charged surfaces will produce more current density within the shorter start-up compared to the negatively charged surfaces. As shown in Fig. 16.10, the start-up period of reactors 1 and 2 were influenced by the surface charge and wettability of modified anode.

In both the cases (i.e., Reactors 1 and 2), positively charged surfaces and the hydrophilic surfaces characterized by $-N^{+}(CH_3)_3$ (blue line) demonstrated higher biofilm formation and faster start-up time when compared to negatively charged surface and hydrophobic surface ($-SO_3^{-}$, $-OH$ and $-CH_3$).

16.5.4 Optimal Conditions for Biofilm Growth

An MFC should be designed to promote the growth and proliferation of electrogenic biofilms (Gatti and Milocco 2017; Islam et al. 2020). A matured biofilm especially

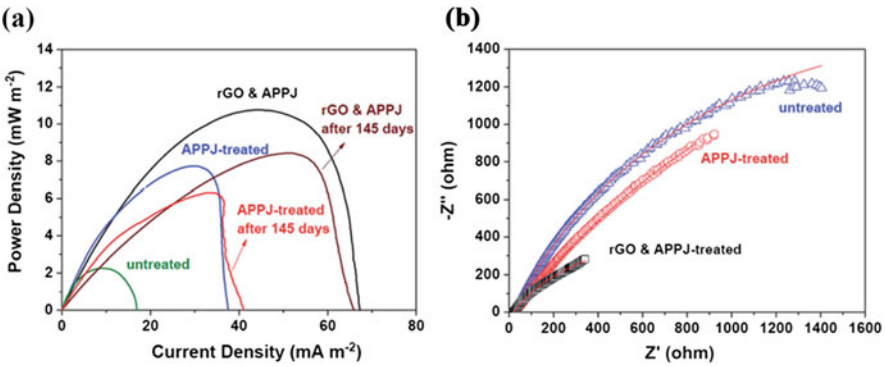

Fig. 16.12 (**a**) Power density curves and (**b**) EIS curves for MFCs with untreated, APPJ-treated, and rGO and APPJ-treated carbon cloths (Adopted from Chang et al. 2017)

increased its ability to participate in extracellular electron transfer typically evolves as the age of culture increases. Considering that activation of overpotential for CH_4 oxidation in MFCs depends upon the specific activity of biocatalysts (i.e., biofilms), it is critical to design electrode surfaces that promote initial colonization of methanotrophs on the electrode surface.

It is also critical to recognize that the formation of dead or inactive cells of an inner lining in the biofilms is characterized by electrochemically inactive nature (Serra et al. 2006). A promising electrode with required surface properties for the methanotrophs is summarized in Table 16.3.

Figure 16.11a, b exposed the effect of PTFE coating on the carbon paper anode in the Start-up period. Particularly, the start-up period was significantly influenced by PTFE content on the anode surface. It was clearly illustrated that without PTFE treatment or low PTFE content (20 wt%) resulted with faster start-up compared to coated one. It means that 20 wt% or without PTFE coating materials facilitated bacterial attachment for easier biofilms formation.

In general, hydrophilic, positively charged, rough and porous surface promote cell adhesion, cell attachment and biofilm formation of methanotrophs. As shown in the power density curves and EIS profiles (Fig. 16.12) and confocal laser scanning microscopy results (Fig. 16.13), the modified surface encourages the growth of *Geobacter* sp.

Surface modification techniques that increase the degree of surface roughness and surface area can be used to enhance the proportion of saturated and unsaturated carbon on the electrode surfaces. As a result, one can expect a decline in the electrode resistance (Fig. 16.12b), promote bacterial attachment, and encourage biofilm formation (Fig. 16.14) (Gatti and Milocco 2017; Mohan and Chandrasekhar 2011). The increase in the coverage of the oxygen groups is a reason for the increased hydrophilicity nature of the electrodes. The decline of C=C bonds is another reason for the decrease in the internal resistance.

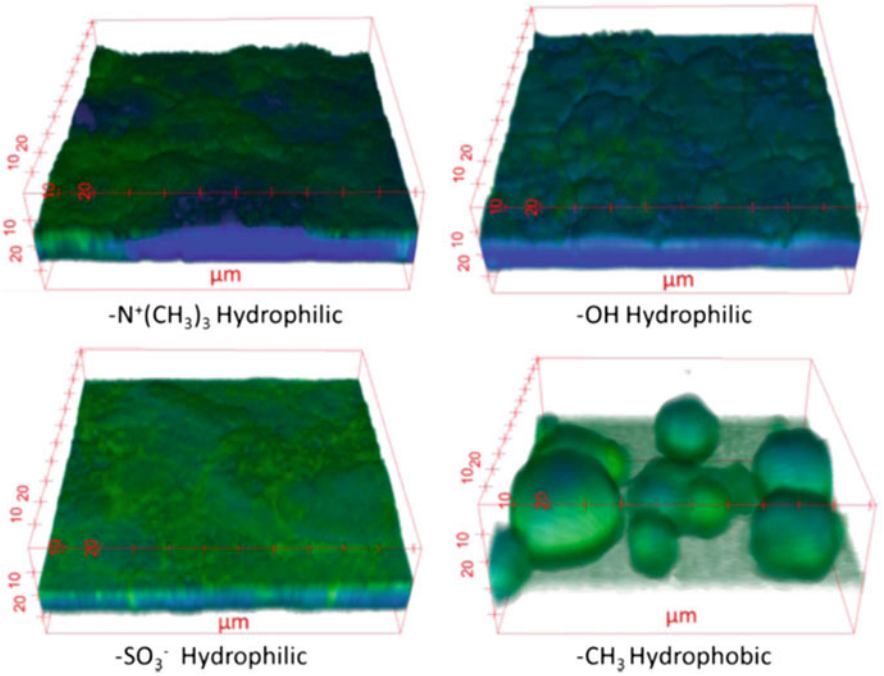

Fig. 16.13 The illustrations of 3D images of biofilms by CLSM on dissimilar modified GC electrodes and done with EUB338mix-FITC (all bacteria, green) and Geo1A-Cy5 (Geobacter, blue) (Adopted from Guo et al. 2013)

16.6 Potential Uses of Methanotrophy

16.6.1 CH_4 Mitigation in Coal Mines

Coal mining activities emit CH_4 into the atmosphere at a rate of 50–500 m^3/s. To reduce CH_4 levels below the explosive limit, the emitted gas is often diluted with fresh air. Thus, CH_4 concentration in the CH_4 ventilation air (MVA) from the mine sites is as low as 0.1–1.0% v/v. The inherent dust in the MVA challenges the design and operation of the typical CH_4 mitigation technologies based on combustion, membrane separation, and adsorption. Despite the fact that these technologies render 98–100% CH_4 removal efficiency, they are not feasible due to the constraints related to the large footprint, complex safety instrumentation, high installation, and operating costs. The BESs could be designed along the lines of biofiltration to enable its operation under ambient conditions and circumvent some of the above disadvantages. Further studies are required to assess if the environmental conditions in the mine (temperature, humidity, nutrients, pH, and toxic impurities) favor the growth of methanotrophs. Low CH_4 solubility may demand higher residence time and oversized reactors.

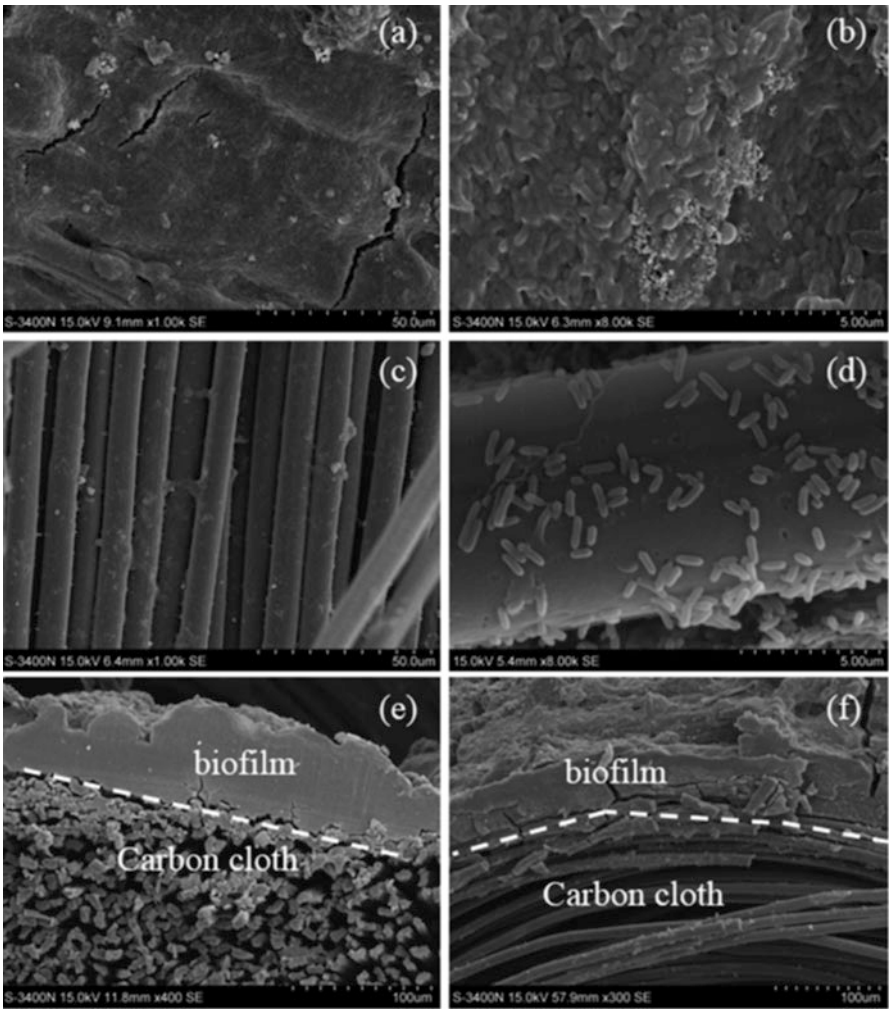

Fig. 16.14 The illustration of SEM images of MFC-10 biofilm on the carbon cloth contrary to flow channel [(**a**) 1000× and (**b**) 8000×], rib [(**c**) 1000× and (**d**) 8000×], and cross-sectional images (**e**, **f**) (Adopted from Zhang et al. 2017)

16.6.2 CH_4 Mitigation in Landfills

Considering the high calorific value of CH_4, the combustion process is an attractive option to control CH_4 emissions from landfills. Flaring is a special case of a combustion technique where the biogas is simply burned in an open flame. While flaring requires only minimal facilities and does not incur energy recuperation, it is effective only when the biogas flowrate reaches 10–15 $m^3\ h^{-1}$ and when CH_4 concentration is greater than 20% v/v. The biogas flow rate is influenced by the

physical parameters of the incoming waste (T and moisture) which again depends upon the type and quantity of the municipal solid waste disposed by the communities on a daily basis. Furthermore, while the gas flow rates from the early phases of a sanitary landfill (establishment phase and fill phase) are typically high, the biogas production drops significantly after 30–50 years.

For the smaller landfills, especially when they are devoid of biogas collection systems, biological processes are an attractive option for mitigating CH_4 emissions. Microbial fuel cells designed along the lines of a three-phase biofilter could potentially treat CH_4 to generate electricity. The electrically conducting filter bed (electrode) can be colonized with methylotrophic biofilm to oxidize the gaseous pollutants in the electrolyte. The efficiency of this process may depend upon local climate conditions and the physicochemical conditions of the landfill site. The indigenous methylotrophic consortia from the upper layers of landfills (in constant exposure to the escaping CH_4 from landfills) could potentially serve as the robust biocatalyst in BESs. The prior experience with the landfill-based biofilters could be used to design BESs for mitigating CH_4 from the landfills.

16.7 Conclusions

This chapter highlights potential opportunities for using methanotrophs to drive the next CH_4-BESs. We briefly discussed the bottlenecks involved in developing such systems. Considering that research on CH_4-BESs is still in its embryonic stages, a series of further studies are warranted for identifying optimal surface properties needed to promote the initial cell adhesion state and subsequently other biofilm phenotypes. On a positive note, surface modification techniques are readily available for addressing challenges of low CH_4 solubility, high activation overpotential, and mass transfer limitations. Along with electrode surface modification techniques, advanced bioelectrochemical reactor design guided by a deeper understanding of metabolic preferences including extracellular electron transfer capabilities of methanotrophs can pave a path for turning CH_4 into value-added products under ambient conditions.

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Chapter 17
Microbial Fuel Cells: The Microbial Route for Bioelectricity

Mridul Umesh and Hanish Mohammed Coppath Hamza

Abstract The quest for sustainable energy sources serves as the essential pillar for development of humans since the dawn of civilization. The alarming increase in demand of energy, especially electricity propelled the need to screen for alternative sources of energy over the conventional fossil based non-renewable counterparts. Electricity generation through microbial route functions by the fundamental phenomena of electron transport chain and the microbes operate as the source of energy production utilizing the substrate. Since its initiation, microbial fuel cell has gained a lot of research focus from all over the world. The integration of waste treatment with power generation was highlighted as the most productive and sustainable part of microbial fuel cells. Over the past few decades, a lot of research and development was done on improving the design of fuel cells, searching for cost-effective electrodes and membranes for commercialization. Despite tremendous research done on this domain, its commercialization still faces a lot of hurdles especially once it comes to the overall maintenance and production cost. This chapter summarizes the basic architecture of different microbial fuel cells and the challenges that need to be addressed for making microbial fuel cells a sustainable route for the bioelectricity generation from microorganisms.

Keywords Bioelectricity · Energy · Electrodes · Membranes · Microbial fuel cells

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17.1 Introduction

Energy is the basic element for the functioning of every single action pursuit, need for energy is multiplying with the advancement of life and population increase. Energy is gained from different sources broadly from renewable and non-renewable sources. In 2018, the demand for energy hiked by 2.3% globally. Forty-five percent demand for natural gas and seventy percent for fossil fuel and energy from the renewable sources also escalated (EIA Industrial Sector Energy Consumption 2016). Non-renewable energy sources are mainly from coal, petroleum, and natural gas; they do not replenish and are relatively inexpensive to extract but contribute largely for CO_2 emission. Renewable energy sources are from the renewable sources, namely from sun, wind, hydropower, geothermal and biomass. They are replenishable, less polluting but relatively expensive to extract. A productive economy utilizes more resources and has greater energy output as an influencing factor for the fulfilment of energy requirement by the growing population (Tang et al. 2018). Global energy consumption the energy extracted from all the resources are consumed by mankind through industrial and economy sectors and depends upon the consumerism in each country it varies. As the population increases energy consumption also increases consequently population turns as the major consumer of energy with a great importance in the socio-economic and political spheres since world energy consumption is the measure of civilization. Manufacturing sectors and industries consume the major portion of total consumption of energy in developing countries (Farjana et al. 2018). The economical development activity and technological development intensify fuel consumption; it varies over countries and regions (IEA 2019). According to International Energy Agency (IEA) global energy demand from non-renewable sources augmented to 4.6%, 1.3%, 0.7% and 3.3% by gas, oil, coal and nuclear energy respectively. The renewable energy from all these resources contributed to a 4% growth from the previous years by 2018 (IEA 2018). This implies the need of energy from any resources to meet the demand for the future but the growing global warming concerns demand much from the alternative renewable resources.

17.1.1 Energy from Renewable Resources

Globally energy crisis and global warming are the critical issues concerned with the multiplying population and depleting the non-renewable energy sources (Kadier et al. 2016a). Excessive energy consumption by humans augments environmental pollution, climate change impacts and the greenhouse effect (Saratale et al. 2017). Industrialization and technological developments raising the demand for energy ever and pressurize the environment with climate change and pollution issues (Kumar et al. 2017). The large-scale industrial developments are based on fossil fuel depending energy leading to the depletion of the natural resources and impacting

the global climate change emitting greenhouse gases (Chandrasekhar et al. 2015a). The effect of pollution damaging the environment has accelerated the concern for energy from alternative source (Enamala et al. 2018). It increased the interest of using renewable energy from different sources (Kadier et al. 2017) so energy from renewable resources is given high preference that has lower environmental issues (Kadier et al. 2016b). Also production of energy from renewable resources is gaining more attraction internationally due to the depletion of fossil fuel and overcoming global warming problems (Kadier et al. 2015). Attaining WHO guidelines and Paris agreement for clean air and stable climate requires swift phase change. Energy from renewable sources is gaining importance worldwide for attaining a sustainable development and positive environmental quality. Renewable energy decreases the dependence on the non-renewable energy and sustaining economic condition in energy prices from volatility (Zafar et al. 2019). Renewable energy can profit the public health and climate replacing emissions caused by electricity generation using fossil fuels (Buonocore et al. 2016). Globally less carbon emission and sustainable development energy system are focusing on a sustainable future (Zhang et al. 2018). Study on CO_2 emission nexus between renewable energy and non-renewable energy of 128 countries from 1990 to 2014 ensued that renewable energy can reduce CO_2 emission (Zhang et al. 2018). Petroleum formation in nature is 105 times lagging behind its current consumption. Thus natural petroleum cannot meet the future energy demands and the greenhouse gas emission from their combustion leading to global warming has become more challenging, thus urging for development of green clean energy alternatives (Shuba and Kifle 2018). Hydropower, solar, wind, biomass and geothermal such renewable energy sources do have certain barriers to pass with environmental impacts and few are consequential. The potency of environmental impact variants rely upon the geographical location, technology employed and other factors, but understanding the issues associates with renewable energy effective measures are taken to avoid, to upkeep the supply. Escalating global energy consumption and depletion of conventional energy resources are addressing the insufficiency to meet the energy demand igniting energy crisis where renewable energy utilization becomes significant (Guo et al. 2018). Thermal, photovoltaic and wind generated energy require special medium or storage facility for later use (Gude 2015). Despite the few flaws renewable can substantiate the need of the green and cleaner energy for a better tomorrow.

17.1.2 Waste Management

Waste generation and disposal are an integral part of the society (Reddy et al. 2011a, b). The management of waste becoming a great environmental and public health concern due to rapid urbanization (Mohan and Chandrasekhar 2011b). Waste generation is gravely a global problem; the degree of waste generation depended on economic development, reducing, reusing and recycling efficacious tool for solving

the waste issue (Minelgaitė and Liobikienė 2019). As the population increases the waste generation increases and waste management become a difficult process (Ayeleru et al. 2018). Municipal solid waste projected to reach over 2.2 billion tons per year by 2025 globally; landfilling and incineration are the most commonly used conventional techniques impacting public health negatively (Indrawan et al. 2018). In developing countries more than 70% of the municipal waste consist of degradable materials; these play a considerable part in greenhouse gas production. Only 60% of the municipal wastes are disposed at authorized sites and the remaining disposed at unauthorized sites (Ramachandra et al. 2018). A low carbon energy system of alternative resources and novel technologies to improve efficiency of the energy sector for a resource endowed future are the need of population (Kumar and Pandey 2019). Achieving greener growth efficient waste management and waste treatment are essential (Ghasemi et al. 2013). Waste collection, transport and disposal are the challenges in developing countries and developed countries are generating electricity, heat, biofuel and compost as by-products with the emerging technologies (Moya et al. 2017). Rapid growth of industries generates a vast amount of waste as solids and liquids such as food processing, distillery, dairy, tannery, slaughter houses, Sugar, poultries, sago paper and pulp industries, etc. Composting, recycling and energy recovery in waste management implementation have a great scope minimizing the waste disposed as landfill (Palanivel and Sulaiman 2014). Limitation in availability of land area for waste disposal along with infections associated with careless discharge of waste trigerred the waste management organizations to focus on technologies for recovering sustainable energy alternatives through waste valorisation (Fetanat et al. 2019). The current scenario vitalizes researchers to thrive new different waste to energy alternatives (Beyene et al. 2018). Food and beverage industries are huge consumers of energy and produces substantial amount of biowaste. These biowaste generated offer a promising potential for the recovery of sustainable energy alternatives there by enhancing the overall efficiency of integrated production process (Siqueiros et al. 2019). Paper and pulp industries every year utilize a large quantity of resources such as wood and water, creating a huge amount of solid waste and wastewater; these wastes are not treated properly and discharged, eco-friendly treatment and extracting energy from these wastes are the necessity of the day (Gopal et al. 2019). Slaughterhouse, agriculture and livestock produce large quantities of waste and are potential sources for generating electricity (Shirzad et al. 2019). Waste to energy technologies provide scope to recycle organic waste materials into renewable energy counterbalancing the disposal and environmental costs (Milbrandt et al. 2018). Bioelectrical systems and anaerobic digesters are expanding technologies as renewable energy from waste (Beegle and Borole 2018). Domestic and industrial wastewaters are generated hugely across the world, causing water crisis and environmental downturn, hence sustainable and energy efficient wastewater system is the solution for the issue (Rathour et al. 2019). Wastewater with a high organic load is contemplated as a valuable energy resource (Chandrasekhar et al. 2015b).

17.1.3 Microbial Fuel Cell

Trending shift from "waste to wealth" in the past few decades' vests considerable interest in organic biotic waste due to its high organic content, consequently comparing to conventional treatment technique electro-fermentation gains much interest (Kumar and Pandey 2019). The microbial fuel cell (MFC) is a promising technique for wastewater treatment and simultaneous electricity generation. MFC technology has gained the attention of researchers in the past two decades due to the possibility of utilizing organic waste as a cost effective substrate for energy production through microbial metabolism (Santoro et al. 2017). Climatic change and need for alternative energy are increasing concern and MFC qualifies as a solution of both the need (Slate et al. 2019). MFC is attaining scientific and technological significance considering the global scenario of meeting renewable energy and treatment of waste, the major advantage of MFC (Neto et al. 2018). The increasing demand for elctricity, scarcity of renewable resources for power generation coupled with high cost associated with waste water treatment propelled the need for integrating these domains for the sustainable production of electicity from MFCs using waste water as substrate for microbial growth (He et al. 2017). MFC generates bioenergy from waste reducing environmental pollution and the treatment cost (Gajda et al. 2018). The ability of MFC to utilize broad range of substrates makes MFC a promising and interesting fuel presently (Marks et al. 2019). Bioenergy generation and wastewater treatment advantages of MFC are comprised of energy saving and sludge volume reduction (Zhang et al. 2019). MFC as an alternative energy generation provides sustainable energy from biodegradable compounds; its applications include electricity generation, wastewater treatment, biohydrogen production and biosensors (Goswami and Mishra 2018). Hamza et al. (2017) unveiled the promising potential of MFC in application of heavy metal reduction from the wastewater distillery effluent apart from wastewater treatment and electricity generation. MFC is a susatinable and ecofriendly aternative for generation of energy in tune with wastewater treatment (Chandrasekhar and Mohan 2014b).

17.1.4 Types of MFC

Depending on the design and functioning facility of MFC, different types of MFCs are applied in studies. They are dual chamber MFC, single chamber MFC, up-flow MFC and stacked MFC.

17.1.4.1 Dual Chamber MFC

Double-chamber MFC (see Fig. 17.2) is the simplest design among all MFCs (Niessen et al. 2004; Phung et al. 2004; Kumar et al. 2016). In a typical design,

one bottle (can be of different designs) is used as anode while the other one as cathode, separated by PEM. Usually in a two-chamber MFC, defined medium (or substrate) in the anode and defined catholyte solution are used to generate energy. In other words, the double-chamber MFC is often operated in batch mode. The double-chamber MFC may be in the shape of bottles or cube. The choice of catholyte in the MFC can define the nomenclature of the design. For example, if the air is used in the cathode to provide the electron acceptor, i.e. oxygen, then the MFC can be called as a two-chamber air cathode MFC (Ringeisen et al. 2006; Shantaram et al. 2005). Such MFCs may prove valuable to generate electricity in remote sensing regions. Dual chamber MFC is one of the simplest designs in MFCs (Niessen et al. 2004; Phung et al. 2004). Dual chamber MFC consists of two chambers; they are anode and cathode separated either using salt bridge or proton exchange membrane (PEM). The substrate is used in anode chamber and catholyte water or other catholytes are used in cathode chamber, salt bridge or proton exchange membrane separates the anode and cathode chamber, which helps in the proton transport between the two chambers (Fig. 17.1). The appellation of MFC is also defined by the catholyte or cathode chamber configuration. If air (oxygen) is the electron acceptor for the cathode in the cathode chamber, MFC will be termed as air cathode MFC (Ringeisen et al. 2006; Shantaram et al. 2005).

This design although applied for basic research generally produces low power output due to the intricate design, high internal resistance and electrode based losses

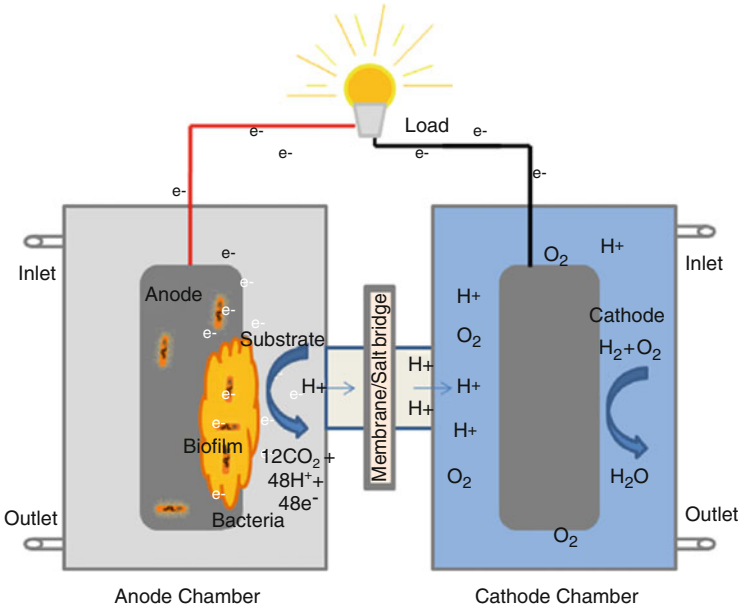

Fig. 17.1 Dual chamber microbial fuel

(Du et al. 2007; Logan and Regan 2006a, b). Hamza et al. (2017) reduced the distance between the anode and cathode as two distinct chambers from the conventional "H" type reactor even using the salt bridge as a separator.

17.1.4.2 Single Chamber MFC (SCMFC)

Single chamber MFC (SCMFC) consists of a single chamber incorporating both the anode and cathode configuring to a single chamber by design, introduced by Park and Zeikus (2003). The anode is placed close or afar to the cathode separated by PEM, decreasing the electrodes spacing aids the reduction in internal Ohmic resistance of the MFC. Combining the two chambers by avoiding catholyte increases the power density (Fig. 17.2). Such MFC is simple, economical and produces much power in rival to double-chamber MFC (Chaudhuri and Lovley 2003; Ringeisen et al. 2006). The major problems such as microbial adulteration and reverse passage of oxygen from cathode to anode occur normally. SCMFCs propose simpler and economic designs. Such MFCs generally have simply an anodic chamber with no requisite of air in a cathodic chamber (Rabaey et al. 2004, 2005).

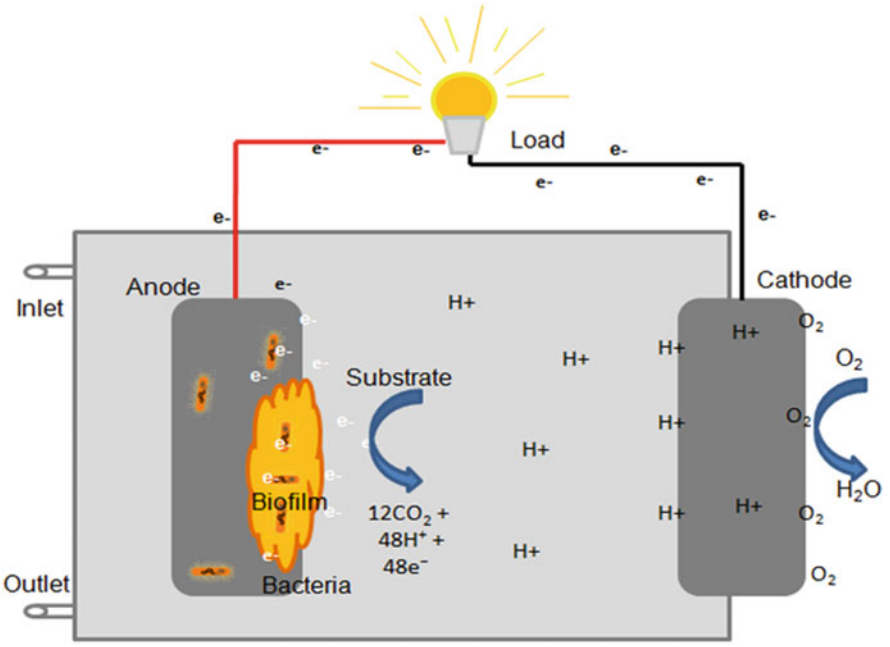

Fig. 17.2 Single chamber microbial fuel cell

17.1.4.3 UP-Flow Tubular MFC

Jang et al. (2004) advanced MFC design working in continuous flow mode. The up-flow MFC is cylindrical shaped (He et al. 2006); assembling the cathode chamber on top above the anode chamber and the anode chamber as the bottom chamber (Fig. 17.3); both the chambers are allocated with glass wool and glass bead layers as separator. Tartakovsky and Guiot (2006) devised a rectangular up-flow MFC separation using polyester pad apart from glass wool and glass beads. The substrate provided from the bottom of the anode that moves upward to the cathode and leaves at the top (Moon et al. 2005). Gradient formed between the electrodes which also help in the favourable action of the fuel cell (Cheng et al. 2006). In up-flow MFC design the anolyte and catholyte are not distinct and lack physical parting consequently the proton transmission associated impediments are reduced (Mohan et al. 2014).

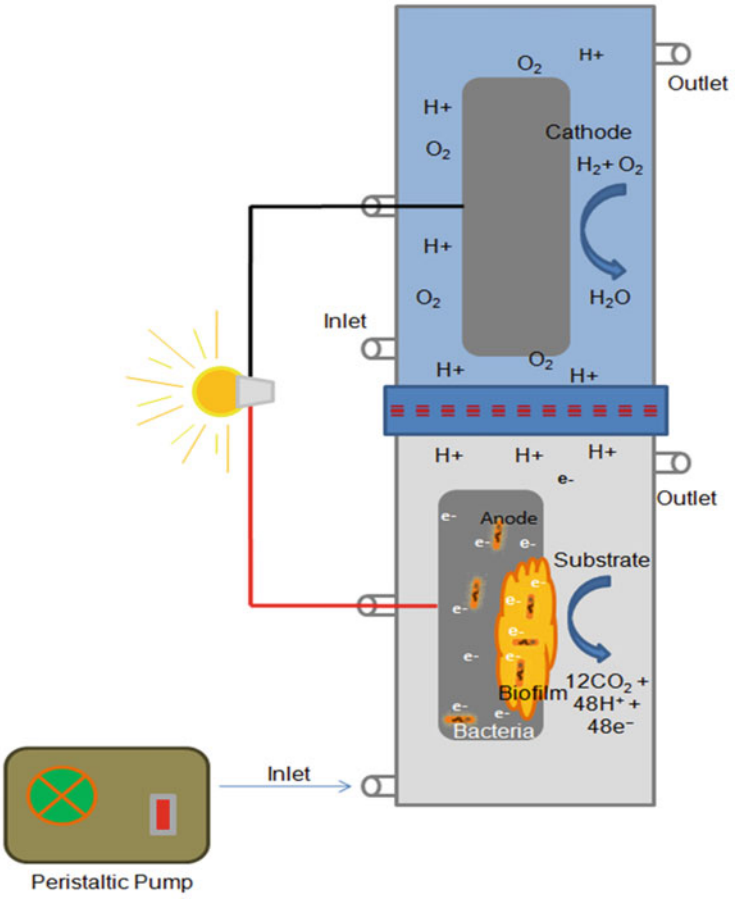

Fig. 17.3 Up-flow MFC

Up-flow MFC's scaling up is ease comparatively with other MFC designs. Substrate pumping is the prime disadvantage of up-flow MFC. Substrate pumping from the anode to cathode assembled at the top requires higher power than the power output generated by the MFC (Zhou et al. 2013). Wastewater treatment is the typical purpose apart from electricity generation from up-flow MFC (Brutinel and Gralnick 2012).

17.1.4.4 Stacked MFC

A stacked MFC comprises several MFCs connected in series or parallel (Fig 17.4) (Logan and Regan 2006a, b; Sun et al. 2012). Stacking increases the MFC output by multiplying individual MFC units power or current output (Logan et al. 2005). This design was observed to enhance the voltage/current output. MFCs stacked in parallel connection do not influence the single unit MFC maximum power output adversely and give six times higher efficiency than the series, parallel-connected stack has higher short circuit current than the series connected stack. The maximum bioelectrochemical reaction rate was recorded in the connection of MFCs in parallel than in the series. Maximizing the chemical oxygen demand (COD) removal, a

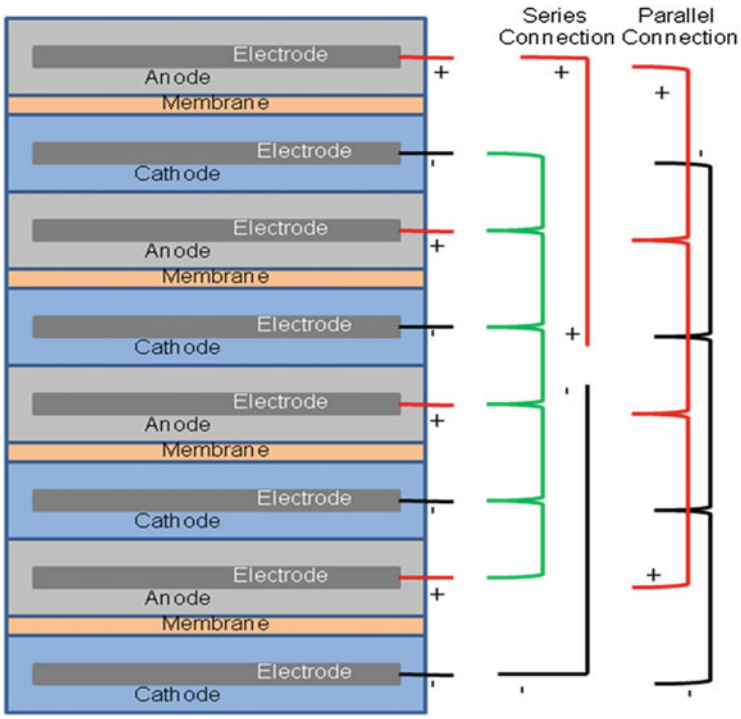

Fig. 17.4 Stack MFC (connecting MFC series/parallel)

parallel connection is preferred if MFC units are not independently operated (Aelterman et al. 2006).

17.1.5 Factors Affecting MFC Performance

The success of MFC depends on the efficiency of the microbial population to transfer electrons generated through the catabolism of organic matter towards the anode portion. This in turn depends on the electrochemical reactions taking place in cathode (Rabaey and Verstraete 2005). The chief factors influencing MFC performance are the type of substrate used, electrode material, nature of the microorganism, proton exchanger, resistance, catholyte, pH and temperature (Du et al. 2007).

17.1.5.1 Substrates Used in MFC

Substrate is regarded as an important factor affecting electricity generation in MFC (Liu et al. 2009). Simple substrates like acetate are effective in more electricity generation due to simpler degradation pathways that could require less energy in breaking the compound by microbes (Ge et al. 2014). The immense potential of MFC in using different wastewater as substrate made researchers to experiment with different streams of wastewater and waste. Wastewaters used in the MFCs are acetate, glucose, lignocellulosic biomass, brewery wastewater, starch processing wastewater, synthetic/chemical wastewater, dye wastewater, landfill leachates, cellulose, chitin, distillery effluent, inorganic and other substrates.

17.1.5.2 Electrode Material

Electrode material oxidation and thereby release of electrons takes place in the anode. In the cathode electrons enter the cell and reduction occurs. Electrodes are alternative electron acceptor promoting the organic contaminants degradation (Mohan and Chandrasekhar 2011a).

Anode

An anode used in MFC should be conductive, non-corrosive, non-fouling, high porosity, high surface area, less expensive and offers provision for an easy scale of the process. Usually carbonaceous materials are preferred as anodes due to their low internal resistance. In dual chambered MFC increasing the size of anode observed to accelerate the power generation substantially as surface area increases with the increase in size of anode (Oh and Logan 2006). The commonly used anode materials and their characteristics are summarized in Table 17.1.

Table 17.1 Anode materials and its characteristics

Anode material	Characteristics	Reference
Carbon paper	Brittle and plain paper	Yuan and Kim (2008)
Carbon cloth	Flexible, high porosity	Cheng and Logan (2007)
Carbon foam	Thick, confers more space for bacterial growth	Reimers et al. (2006)
Reticulated vitrified carbon (RVC)	High porosity and conductivity, effective pore size control	Yuan and Kim (2008)
Graphite rods	Highly conductive, defined surface area and high electrochemical properties	Hamza et al. (2017)
Graphite sheet	Less porous, low power generation	Gao et al. (2013)
Graphite granules	Effective to be used for packed bed reactors	Feng et al. (2010)
Graphite fibres and brushes	High surface area, highly effective	Logan et al. (2007)

Cathode

The scalability of MFC depends on cathode design, this serves as the important factor for its commercialization. The chemical reaction occurring in cathode is quite complex as it involves a tri phase reaction between solid catalyst, air and water. The catalyst should be coated on to the conductive surface and must be exposed to water and air. The most commonly used cathode material includes carbon paper coated with the platinum catalyst in such a way that the catalyst faces water whereas uncoated side faces air (Cheng et al. 2006). Non-precious metals like iron based catalyst were used as alternative to platinum catalyst and observed to produce 3.8 times as much power as graphite cathodes (Park and Zeikus 2003). Plain carbon cathodes without catalyst can also be used in MFC but their power production was found to be greatly reduced. The power generation in catalyst-less MFC can be accelerated by increasing the surface area of catalyst (Reimers et al. 2006). The use of tubular carbon coated cathodes has gained much attention for MFC construction using due to their high porosities and the improved surface area (Zuo et al. 2007). Cathode limitations also include expensive precious metal as cathode to yield higher voltage and current density (Sivagurunathan et al. 2018).

17.1.5.3 Microbes in MFC

Microbes play a vital role in MFC generating electricity and simultaneous wastewater treatment. Microbes convert renewable biomass and waste organic matter into electricity by oxidation of organic compounds and transfer electrons generated by them to electrodes (Lovley 2006). Electrons generated by the microbes in the anode are utilized in cathode as electron acceptors passing through the external circuit (Rahimnejad et al. 2015). Electrogenic microbes are electrochemically active microbes that are capable of accepting and donating electrons from an external

source to an external source like electrodes (Loan and Regan 2006b). The conductive nanowires, C type cytochromes connected with the bacterial outer membrane and pili like functional organelle help in the direct electron transfer to the anode electrode surface through a direct physical contact (Xu and Gu 2011). Some microbes require mediators to transfer electrons to the anode electrode, some microbes are capable to exude mediators by themselves and for some microbes mediators are need to be provided. These mediators' help in shuttling the electron transfer between the microbes and electrodes (Rabaey et al, 2005; Freguia et al. 2009; Deng et al. 2010; Keck et al. 2002). Fermentation process boosts the microbial metabolism (Kumar et al. 2018), elutriation helps in production of readily biodegradable organic acids used directly by anodic biocatalysts for bioelectrogenesis (Chandrasekhar and Ahn 2017) and anaerobic culture bioaugmentation to anodic microflora enhances the electrogenic activity of microbes (Chandrasekhar and Mohan 2012). Substrate degradation consists of oxidation process by bacterial metabolic activity, obtaining energy from the substrate as carbon source (Kumar et al. 2012). Enzymes are involved in the oxidation reduction process releasing protons and electrons during substrate degradation. A mixed microbial consortium is preferred in wastewater and biomass because of its lower cost (Chandrasekhar and Mohan 2014a).

17.1.5.4 Proton Exchange

Oxidation of organic material produces both protons and electrons, the electrons are removed instantaneously via biofilm and the electrical circuit of the MFC. The larger protons have to migrate out of the biofilm to the cathode. This occurs at a much slower rate and may cause a bottleneck inhibiting power production. Every electron produced in the form of current, a proton is also produced within the biofilm (Franks and Nevin 2010).

Salt Bridge

Salt bridge and selective permeable membranes are used for proton exchange in MFCs between the anode and cathode. Salt bridges are the one of the cheapest and easiest proton exchangers in MFC. High salt concentration facilitated the transfer of more protons from the anode to the cathode chamber which reduced the activation loss (Sevda and Sreekrishnan 2012).

Membranes

A membrane in MFC acts as an integral component as it serves as a separator and promotes the transfer of protons between the anode and cathode, hence called as the proton exchange membrane. Membranes can be either cation exchange type or anion

exchange type. The use of membranes in MFC, although widely reporeted in literature, has raised some controversies as well, especially with regard to net power generation. In some of the research studies membrane-less MFC was observed to produce more power than an MFC with membrane bound, to indicating membrane can adversely affect power generation (Liu and Logan 2004). Over the last decade the use of bipolar membrane consisting of an anion and cation membrane joined in a series has gained significant attention. The use of salt bridge instead of the membrane-based system was also devised in certain research works. Impedance spectroscopy studies revealed a low power output in MFC with the salt bridge and that could be correlated directly to their higher internal resistance (Min et al. 2005).

The ability of the membranes to help in better performance than conventional salt bridge makes membranes as an advanced version of proton exchangers in MFCs. Proton exchange membrane fuel cells (PEMFCs) are considered to be a promising technology for a clean and efficient power generation in the twenty-first century. Proton exchange membranes (PEMs) are one of the key components in a fuel cell system. Researchers are focusing to reach the proton exchange membrane with high proton conductivity, low electronic conductivity, low permeability to fuel, low electro-osmotic drag coefficient, good chemical/thermal stability, good mechanical properties and low cost. Table 17.2 describes the performance of different types of membranes used in MFCs.

17.1.5.5 Resistance

Internal resistance is the fundamental element limiting the power output of the microbial fuel cell. Ohmic resistance, charge transfer and diffusion resistance are the factors causing internal resistance in MFC like in any other electrochemical cell (Larminie et al. 2003). Internal resistance includes the anode, cathode, electrolyte and membrane resistances are the other limiting factors influencing the MFC power output performance (Logan and Regan 2006a, b). Resolving them will enhance the performance of MFC. Increasing the surface area of anode and cathode helps in the reduction of internal resistance (Logan et al. 2007; Oh and Logan 2006) as well as increasing the proton exchange membrane surface area and electrolyte ionic strength can help in the internal resistance reduction bound to membrane and electrolyte limiting factor (Oh et al. 2004; Liu et al. 2012, 2018).

17.1.5.6 Catholyte

The electrons generated at the anode through oxidation or breaking the wastewater contents are transferred to cathode and oxidized by electron acceptors eventually (Logan and Regan 2006a, b). Oxygen is predominantly used cathodic terminal acceptor for proton reduction enabling the production of water (Kadier et al. 2018). Oxygen is a quintessential terminal acceptor in MFC cathode due to its strong oxidation potential, low cost and formation of water as the end product

Table 17.2 Membranes used in the MFC and its performance

Membrane types	Type of fuel cell	MFC performance	Reactor volume	Substrate	Cathode	References
Cation exchange membranes (Nafion 117 (Dupont Corp.)	Dual chamber MFC	395 mV	180 mL	Acetate	Phosphate buffer	Choi et al. (2011)
Anion exchange membranes Ralex AEM	Dual chamber MFC	0.729 V	225 mL	Luria broth	Phosphate buffer	Pandit et al. (2012)
Bipolar membrane (anion and a cation membrane joined)	Flat pate MFC	679 mV	785 mL	Acetate	Ferric iron chloride	Ter Heijne et al. (2006)
Ultrafiltration membrane (DIVFLO ultrafiltration)	Cube shaped MFC	320 mV	14 mL	Acetate	Air cathode	Kim et al. (2007)
Microporous filtration membranes (polysulphone membrane on a composite polyester carrier)	Tubular MFC	403 mW/m^2	32 mL	Domestic wastewater	Phosphate buffer	Zuo et al. (2007)
Nanocomposite membrane Activated carbon nanofibre and nafion Sulphonated Tio2	H Shaped MFC Cylindrical Single Chamber MFC	57.64 mW/m^2 1345 mW/m^2	1690 mL 28 mL	Synthetic Wastewater Sewage wastewater	Phosphate buffer Air cathode	Ghasemi et al. (2012) Ayyaru and Dharmalingam (2015)
SPEEK (sulphonated poly(ether ether ketone) ion exchange membrane)	Cube shaped MFC Single chamber MFC	77.3 mW/m^2 670 mW/m^2	420 mL 28 mL	Palm oil mill effluent Dairy wastewater	Phosphate buffer solution Air cathode	Ghasemi et al. (2013) Ayyaru and Dharmalingam (2015)
SPSEBS (sulphonated form of polystyrene–ethylene–butylene–polystyrene tri-block polymer. polystyrene-ethylene-butylene-polystyrene)	Single chamber MFC	600 mW/m^2	28 mL	Glucose	Air cathode	Ayyaru and Dharmalingam (2011)

(Jang et al. 2004; Franks and Nevin 2010). Catholyte impacts in the enhancement of MFC power output, but the nature of catholyte does not influence in the substrate degradation efficiency of MFC (Raghavulu et al. 2009). In order to improve the MFC performance different catholytes are experimented, such as ferricyanide (Oh et al. 2004; Venkata Mohan et al. 2008), permanganate (You et al. 2006), phosphate buffer (Sangeetha and Muthukumar 2011) regardless the higher power production achieved from the catholytes experimented they are considered to be unsustainable in practicability after all it require chemicals and cause environmental related issues (Logan and Regan 2006a, b).

17.1.5.7 pH

pH has a direct correlation with MFC performance. Generally, neutral pH is ideal for the MFC performance. Pre-fermentation of wastewater can make the pH to near neutrality and can speed up the MFC power generation (Guerrini et al. 2013). Alkaline pH condition 8.3 is optimal for anodic reaction in MFC (Deval et al. 2017), MFC performed better at anodic pH 8 compared with pH 6 (Yuan et al. 2011). Anolyte pH in acidic condition influences the bacterial activity at the anode inhibiting the power production thereby affecting the overall performance of the MFC (Behera and Ghangrekar 2009; Puig et al. 2010). The standard procedure in anaerobic digestion for the development of methanogenic bacteria cannot improve the performance of MFC (Jannelli et al. 2017). Anode feed acidification and catholyte alkalization result in the overall poor performance of MFC (Zhuang et al. 2010).

17.1.5.8 Temperature

Temperature also plays a vital role in the performance of MFC in COD removal and electricity generation (Behera et al. 2011; Larrosa-Guerrero et al. 2010) Temperature controls the metabolic activity of the microorganism of the anodic chamber in the MFC through enzymatic reactions (Clauwaert et al. 2008). Higher temperature contributes to higher power density in MFC (Min et al. 2005). Increase in temperature lowers the current generation and columbic efficiency whereas lowering of temperature increases the current and columbic efficiency during the MFC performance (Jadhav and Ghangrekar 2009). MFC, the anodic biofilm development establishes at lower temperature and aids yielding constant voltage from MFC (Liu et al. 2009). The performance of MFC varies depending on the microbial consortium and type of wastewater used. It renders the optimum performance at room temperature.

17.1.6 Challenges in Commercialization

Despite the rapid progress of electromicrobiology emphasizing the development of electricity using the microorganism as sustainable approach to meet the increasing demand for power globally, widespread commercialization of MFCs still faces a lot of constraints once it comes to a scale up study (Chandrasekhar et al. 2018). The major concerns to be addressed while scaling up MFC to a commercial scale bioelectricity unit include:

- Cost effectiveness and molecular design that can be effectively and safely handled should be developed (He et al. 2017; Do et al. 2018).
- Provisions for high power densities and energy efficiencies are limited in existing models and require development.
- Improving catalytic properties of electrode materials while maintaining their performance (Santoro et al. 2017).
- The voltage generated in MFC is generally less and needs to be accelerated to be applied for commercial scale.
- Proper time dependent study on MFC performance should be done to ensure their reproducibility for commercial scale application due to constraints associated with long time changes in enzyme activity, electrode fouling, membrane blockage, build up of metabolites and break down of products (Choi et al. 2011; Xu et al. 2012).
- Maintaining a steady state of electron transport from bacteria to the electrode through the mediator is crucial.
- The development of immobilization technique for microbial enzymes used in MFC and using nano structured substrates although found effective in improving MFC performance should tackle out the issues associated with practicality and cost effectiveness.

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