Chapter 12
Rewiring Extremophilic Electrocatalytic Processes for Production of Biofuels and Value-Added Compounds from Lignocellulosic Biomass

Navanietha Krishnaraj Rathinam, Rajesh K. Sani, and David Salem

What Will You Learn from This Chapter?

This chapter will introduce the basic concepts of bioelectrocatalysis and the advantages of extremophiles for bioelectrochemical systems. The chapter will discuss electrogenic activity and electron transfer characteristics of extremophiles and their applications in microbial fuel cells, microbial electrolytic cells, microbial desalination cells, and

N. K. Rathinam (✉)
Department of Chemical and Biological Engineering, South Dakota School of Mines and Technology, Rapid City, South Dakota, USA

BuG ReMeDEE Consortia, South Dakota School of Mines and Technology, Rapid City, SD, USA

Composite and Nanocomposite Advanced Manufacturing-Biomaterials Center (CNAM-Bio Center), Rapid City, SD, USA
e-mail: Navanietha.Rathinam@sdsmt.edu

R. K. Sani (✉)
Department of Chemical and Biological Engineering, South Dakota School of Mines and Technology, Rapid City, South Dakota, USA

BuG ReMeDEE Consortia, South Dakota School of Mines and Technology, Rapid City, SD, USA

Composite and Nanocomposite Advanced Manufacturing-Biomaterials Center (CNAM-Bio Center), Rapid City, SD, USA

Chemistry and Applied Biological Sciences, South Dakota School of Mines and Technology, Rapid City, South Dakota, USA
e-mail: Rajesh.Sani@sdsmt.edu

D. Salem
Composite and Nanocomposite Advanced Manufacturing-Biomaterials Center (CNAM-Bio Center), Rapid City, SD, USA

R. K. Sani, N. K. Rathinam (eds.), *Extremophilic Microbial Processing of Lignocellulosic Feedstocks to Biofuels, Value-Added Products, and Usable Power*,
https://doi.org/10.1007/978-3-319-74459-9_12

microbial electrosynthesis. The use of extremophilic bioprocesses for production of bioenergy and value-added products from lignocellulosic biomass will also be discussed.

12.1 Introduction

Tremendous advancements in science, technology, and medicine all over the world have led to remarkable growth in industrialization, supporting an ever-growing population, which in turn threatens the supply of commodities, including electricity from non-renewable resources. The primary energy consumption in the United States was nearly 96 quadrillion BTU in 2016 (U.S. Energy Information Administration, Monthly Energy Review, April 2017). For electricity generation, most countries, including the United States, mainly depend on non-renewable sources of energy, such as coal, and on nuclear sources. Nuclear power plants can have drastic negative impacts on mankind and the environment (Kyne et al. 2016). Coal power plants emit radionucleotides which cause respiratory disorders and other deleterious effects (Pandit et al. 2011). Although non-renewable sources provide maximum power output, they are depleting rapidly, have grave environmental consequences, and cause major health hazards to biota, including humans (Navanietha Krishnaraj and Yu 2015).

On the other hand, several countries are moving towards predominant use of renewable energy sources, and this trend is likely to grow. While most renewable sources of energy—such as solar, wind, and tidal energy—are ecofriendly and help to mitigate the issues of global warming/climate change (Panwar et al. 2011), they demand huge costs for installation, operation, and maintenance.

Bioelectrochemical systems (BES) are a promising approach to producing bioelectricity (Pant et al. 2012). BES are electrochemical devices that make use of the complex enzymatic machinery of electroactive microorganisms and its electron transfer characteristics for mediating bioelectrocatalysis. The microorganisms or enzymes act as electrocatalysts in these bioelectrocatalytic systems and are referred to as microbial electrocatalysts and enzymatic electrocatalysts, respectively (Schröder and Harnisch 2013). In addition to bioelectricity generation, the BES make use of the electrocatalytic activity of the electroactive microorganisms, which have a wide range of applications such as production of biofuels (bioelectricity, biohydrogen, methanol, biodiesel), water treatment (including desalination), biosensing of analytes, and production of value added compounds (Sleutels et al. 2012).

BES help to convert chemical energy (wastes) into electrical energy and vice versa. They have several advantages over conventional energy systems because of their low cost, eco-friendly nature, high conversion efficiency, and mild operating conditions. Different configurations of bioelectrochemical systems such as a Biological Fuel Cell, Microbial Electrolytic Cells, Microbial Desalination Cells, Microbial Electrosynthesis, and Electrochemical Biosensors have been reported in the literature (Logan et al. 2006, 2015; Navanietha Krishnaraj et al. 2015).

The use of microorganisms in electrochemical systems, or any other bioprocessing operation, has limitations in that they can thrive and mediate electrocatalytic reactions only in a narrow range of operating conditions (temperature, pH, pressure, etc.). Enzymes have

high catalytic rates and could confer better sensitivity and selectivity when compared with microbial catalysis. On the other hand, they are fragile and become denatured at elevated conditions. These limitations of the microorganisms/enzymes can be circumvented by the use of extremophilic systems. The use of extremophilic bioelectrocatalysts in electrochemical systems has the advantage that they can catalyze a wide range of substrates, including the recalcitrant lignocellulosic biomass (Turner et al. 2007; Bhalla et al. 2014a, b). Lignocellulosic biomass is generated at very large volumes from agricultural and municipal wastes, and the use of lignocellulosic biomass in extremophilic electrocatalytic process will greatly help in cutting down BES operating costs. This chapter will provide the basic concepts of BES and will discuss the scope of using extremophiles as electrocatalysts in lignocellulosic biomass fed Bioelectrochemical Systems.

12.2 Electroactive Extremophiles

Electroactive microorganisms are those organisms that can exhibit electrocatalytic activity. They can produce/consume electrons upon oxidation/reduction of electron donor and electron acceptor, respectively, and transfer the electrons across the electrode–electrolyte interfaces. They are the key players in any bioelectrochemical system and can serve as electrocatalysts in electrochemical reactions. The electron transfer in microbial electrocatalysis becomes difficult if the electrocatalytic reactions occur deep within the cell. However, wiring the redox sites of the enzymes/microorganisms to the electrode surface is difficult, as the respiratory proteins in the Gram-positive bacteria are covered by a thick peptidoglycan layer and a periplasmic space. This problem can be circumvented by careful selection of electroactive microorganisms. A good electroactive microorganism should contain the conductive proteins on the surface of the cell wall and, besides having good oxidation ability, must have good electron transfer characteristics from the microorganism to the electrode at the electrode–electrolyte interface. Beside these features, other properties are also advantageous, such as electrochemical activity over a wide range of pH and temperature, resistance to substrate/product inhibition, and resistance to toxins. Microorganisms can transfer electrons either by the direct electron transfer mechanism or using electron shuttling compounds. Direct electron transfer is carried out by the microorganisms using c-type cytochromes, pili (commonly referred as microbial nanowires), or extracellular minerals. Reguera et al. (2005) reported the pili-mediated electron transfer in *Geobacter sulfurreducens*.

Recently, reports also revealed that these microbial nanowires have metallic-like conductivity (contrary to the previous assumption that electron transfer in biological system is via electron tunneling), which helps in direct interspecies electron transfer between syntrophic organisms, in addition to having the ability to transfer electrons between the electron donors/electron acceptors (Malvankar and Lovley 2012). Organisms such as *Shewanella oneidensis* MR-1 perform direct electron transfer with the c-type cytochromes located on the periplasmic membrane (Schuetz et al. 2009). Extracellular polymeric substances are also shown to contain redox proteins such as c-type cytochromes and biofilm promoting proteins, thereby mediating direct electron

transfer reactions at biofilm–electrode (electron acceptor) interfaces. Certain microorganisms produce mediators or electron shuttling compounds such as flavin, quinone, and phenazine for mediating the electron transfer reactions (Schuetz et al. 2009; Rabaey et al. 2004). Genome analysis and microarray have been used to study the regulation of electron transfer genes in the electroactive biofilm grown onto the electrode surface (Holmes et al. 2006). Reports are also available on the morphological characteristics as well as the basis for conductivity in pili nanowires of certain electroactive microorganisms (Malvankar et al. 2015). Vargas et al. (2013) showed that aromatic amino acids are essential to confer conductivity to pili in *Geobacter sulfurreducens*.

The use of extremophiles as electrocatalysts in bioelectrochemical systems will aid in improving the electrocatalytic activity and the overall performance of the bioelectrochemical system. Extremophilic organisms are promising candidates for developing electrochemical systems that can operate at extreme environments such as high/low temperatures, high/low pH, high/low pressures, saline environments, etc. In addition, the extremophiles can mediate the oxidation/reduction of a wide range of electron donors/acceptors at very high rates. They can also oxidize recalcitrant materials such as lignocellulosic biomass. The use of extremophiles will have the added advantage of developing a robust system for cost-effective commercialization, which has been a major limitation of any microbial/enzymatic systems. For instance, Rastogi et al. (2010) isolated different thermophiles from compost samples using a cellulose-degrading enrichment culture technique. The results of the 16S rRNA analysis of the isolated cultures showed that the sequences were related to *Actinobacteria*, *Bacteroidetes*, *Chloroflexi*, *Deinococcus-Thermus*, *Firmicutes*, and *Proteobacteria*. Different isolates belonging to the *Geobacillus*, *Thermobacillus*, *Cohnella*, and *Thermus* that displayed potential to degrade cellulose, carboxymethyl cellulose (CMC), or ponderosa pine sawdust were identified. Among the different isolates, *Geobacillus* sp. WSUCF1 was selected based on its higher growth rate and cellulase activity. The optimal pH and temperature for carboxymethyl cellulase (CMCase) activity of WSUCFI was reported as 5.0 and 70 °C. The WSUCF1 CMCases had a km value of 1.08 mg/mL and retained 89% of the initial CMCase activities after incubation at 70 °C for 1 day. The whole genome sequence of this *Geobacillus* sp. strain WSUCF1 revealed several genes encoding lignocellulose degradation (Bhalla et al. 2013). The results of the genome annotation showed that among the 865 ORFs that are responsible for carbohydrate metabolism, 70 open reading frames (ORFs) were related to polysaccharide degradation, 3 ORFs for cellulose degradation, and 13 ORFs were annotated as xylan-degrading enzymes. This strain was also shown to produce endoglucanase, xylanase, and β-xylosidase (Bhalla et al. 2014a, b, 2015). Recent investigations have shown that the thermophilic strain WSUCF1 can respire onto the carbon felt electrode and perform direct electron transfer reactions (our unpublished data) indicating the value of this strain for applications in bioelectrochemical systems with lignocellulosic substrates as feedstocks. The use of an inexpensive and abundant lignocellulosic biomass in any bioelectrochemical system will greatly help to decrease the cost of operation as well as to provide safe disposal of these wastes, generated in huge volumes from different sources of environment.

12.3 Biological Fuel Cells

Biological fuel cells are electrochemical systems that aid in converting chemical energy into electrical energy with the aid of either electroactive microorganisms or isolated enzymes (Rathinam et al. 2018; Shrestha et al. 2018). These devices operate on the principles of microbial or enzymatic electrocatalysis and are referred to as Microbial Fuel Cells (MFC) and Enzymatic Fuel Cells, respectively. In a biological fuel cell, the microorganism/enzymes are used to oxidize the electron donor and transfer the electrons onto the anode. The electrons received by the anode travel across the external circuit and are transferred to the cathode where microorganism/enzymes are used to reduce the electron acceptor. The anode and cathode compartments are separated by proton exchange membrane. A scheme showing the construction and operation of MFC is shown in Fig. 12.1. In a biological fuel cell, microorganisms/enzymes can be used as electrocatalyst for oxidation, reduction or both. If the electroactive microorganisms used in the microbial fuel cell are capable of performing direct electron transfer onto the electrode, then it is referred to as mediator-less MFC. The use of robust catalysts such as extremophiles in MFC help in the oxidization/reduction of a wide range of electron donor/electron acceptor at accelerated rates. In the case of lignocellulose based bioelectrochemical systems, the electroactive microorganism must be able to oxidize lignin using polyphenol oxidase, laccase, lignin and peroxidase, and hydrolyze cellulose with the help of endoglucanase and cellobiohydrolase. In addition, they must be able to oxidize the

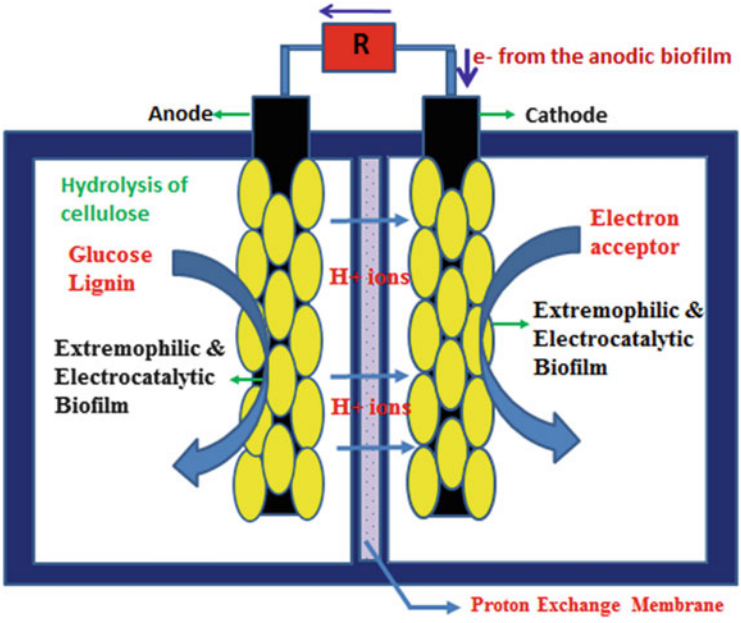

Fig. 12.1 Construction and operation of MFC

hydrolyzed cellulose to produce electrons and efficiently transfer the electrons onto the anode with the help of direct electron transfer or mediated electron transfer mechanisms. MFC have been shown to be promising for treatment of different wastewaters containing glucose, ethanol, cellulose, acetate, and soak-liquor (Navanietha Krishnaraj et al. 2013; Bhuvaneswari et al. 2013; Selvaraj et al. 2016). Different configurations of MFC, electrode materials, electrode functionalization strategies, and membranes have been reported in the literature (Navanietha Krishnaraj et al. 2014; Karthikeyan et al. 2016; Bella et al. 2016).

Different electroactive microorganisms have been harnessed as electrocatalysts in lignocellulose fed bioelectrochemical systems. For example, Ren et al. (2007) reported a two-chamber MFC with the co-culture of *Clostridium cellulolyticum* and *Geobacter sulfurreducens* as electrocatalyst and cellulose as substrate for the generation of bioelectricity. It was reported that *C. cellulolyticum* were found attached to cellulose particles in suspension, whereas *G. sulfurreducens* adhered to the electrode. This showed that the *C. cellulolyticum* was involved in the hydrolysis of lignocellulosic biomass whereas *G. sulfurreducens* performed electrocatalysis of glucose. The bacterial distribution and biofilm architecture of *C. cellulolyticum* and *G. sulfurreducens* fed in a two-chamber MFC containing cellulose have been investigated in detail (Ren et al. 2007, 2008). Fluorescence in situ hybridization (FISH) and quantitative reverse transcription-polymerase chain reaction (QRT-PCR) analyses were performed to analyze the ecology of the electroactive microorganisms in MFC. This investigation suggested that there is a distinct function-related distribution of these two bacteria in the MFC.

Rezaei et al. (2009) designed a U-tube MFC with *Enterobacter cloacae* as a catalyst for the simultaneous degradation of cellulose with and without the use of an exogenous mediator. Two different strains of *E. cloacae* were used for the electrocatalysts of cellulose in a MFC. *E. cloacae*, isolated from a waste water treatment plant, produced a current density of 127 ± 14 mA/m^2 at 1.8 ± 0.02 mW/m^2. The electrocatalytic activity of *E. cloacae* with different carbon sources such as glycerol, glucose, and *N*-acetyl-D-glucosamine showed that these substrates generated a higher power output than cellulose. These results suggest that the hydrolysis of cellulose is the limiting factor in the bioelectricity generation with *E. cloacae*.

A new bacterial strain from the cellulose fed bioelectrochemical system was identified (Kodama and Watanabe 2011). The isolated strain was found to be Gram-negative, non-spore-forming, straight or slightly curved rods. The cells had one or two polar prosthecae, and reproduced by binary fission or by budding. They oxidized a wide range of sugars and produced lactate, acetate, and fumarate. They could reduce nitrate, ferric iron, oxygen, and fumarate, but not sulfate and malate. The DNA G+C content of the newly isolated strain was found to be 64.7 mol%. Phylogenetic analysis based on the 16S rRNA gene sequence revealed that the new strain belonged to the genus *Rhizomicrobium* and named as a *Rhizomicrobium electricum* sp. nov.

There are a few reports in the literature on the bioelectricity generation from complex polysaccharides using pathogenic microorganisms. MFC with *Clostridium butyricum* as electrocatalyst for the bioelectricity generation from molasses and starch was developed (Niessen et al. 2004). The mean current density of 1.1 mA/cm^2 was

generated with polytetrafluoroaniline modified platinum electrodes. However, the use of pathogenic microorganisms as the entire cell bioelectrocatalyst in MFC is very risky and might pose several ethical issues. Hassan et al. (2012) demonstrated cellulose fed MFC with potassium ferricyanide as a catholyte using the mixed and pure cultures of *Nocardiopsis* sp. KNU (S strain) or *Streptomyces enissocaesilis* KNU (K strain). MFCs with pure cultures of *Nocardiopsis* sp. KNU and *Streptomyces enissocaesilis* KNU were supplemented with cellobioase enzyme for the hydrolysis of cellulose and they produced a power output of 162 mW/m^2 and 145 mW/m^2 respectively. MFC with the mixed culture produced a power output of 188 mW/m^2 at a current of 0.5 mA with 1 g/L cellulose as substrate without the use of cellobiase enzyme. The use of enzymes in MFC is not economically feasible and will not be suitable for practical applications.

Wang et al. (2009) produced bioelectricity with a single-chamber, air-cathode MFC using corn stover as a substrate. The MFC with the mixed culture and corn stover produced a maximum power of 331 mW/m^2. The MFC was operated for over 60 days, and the denaturing gradient gel electrophoresis showed that the presence of *Rhodopseudomonas palustris* was involved in the electrochemical reaction. The major limitation with the mesophiles in any bioelectrochemical system is their limited potential to perform both hydrolysis of lignocellulosic biomass as well as electrocatalysis of the hydrolyzed sugars. Most of the reports either rely on more than one species of microorganisms, or consortium, to mediate these two reactions simultaneously. Alternatively, there have also been reports on engineering new configurations of bioelectrochemical systems for bioelectricity generation from lignocellulosic biomass.

The use of *Canna indica* (canna), a lignocellulosic aquatic plant, was reported as a substrate for MFC without pretreatment (Zang et al. 2010). Rumen microorganisms were used as MFC bioelectrocatalysts. A novel three chambered MFC design for the simultaneous degradation of lignocellulosic materials for bioelectricity generation and pigment production, coupling the catalytic activities of a lignocellulolytic cyanobacterium and an electrogenic acetic acid bacterium has been reported (Navanietha Krishnaraj et al. 2015). The three-chamber MFC comprises a first compartment for pretreatment; a second compartment as the anode, and a third compartment as the cathode. *Oscillatoria annae* was used for the hydrolysis of cellulose in the pretreatment compartment, acetic acid bacteria were used for the electrooxidation of sugars in the anode compartment and ferricyanide was used as electron acceptor in the cathode compartment. Gregoire and Becker (2012) designed an integrated reactor coupling the tubular air cathode MFCs and leach-bed bioreactors to develop a new solid-substrate MFC with a single chamber wherein monomerization of cellulose, fermentation, and anode respiration occurs. The solid-substrate MFC with corncob pellets yielded continuous power output for more than 60 days. Exposure to oxygen at regular intervals limited methanogenesis leading to the enhanced generation of bioelectricity. Furthermore, the use of *Geobacter metallireducens* for bioaugmentation improved the power output of the MFC.

The extremophilic electrochemical systems have several advantages such as operation at robust conditions, stability of the bioelectrocatalysts for a longer time, and better electrocatalytic activity. However, these systems suffer from several limitations. The use of thermophilic or psychrophilic systems demands suitable

electrolytes which can mediate electron transfer at high or low temperatures. Evaporation will be a major issue in the case of thermophilic systems, and it demands external energy to maintain the high temperature. The choice of membrane is also a major factor in these electrochemical systems. A suitable membrane should have good proton conductivity even at extreme conditions such as high or low temperatures, acidic/alkaline environments, and high pressure. The membrane must resist the high pressures, and should be able to resist the entry of oxygen and hydrogen gases. The configuration of the biofuel cell for operation in an extreme environment is a major challenge and materials that resist high/low temperatures/pressures/pH are required for the fabrication of an electrochemical system.

The use of extremozymes will have several advantages over normal enzymes in developing enzymatic fuel cells. The use of extremozymes will help to oxidize the electrons at much faster rates compared to microbial systems. They will be more promising in developing implantable fuel cells to power low energy devices such as pacemakers. Different types of extremophiles have been reported in the literature for biological fuel cell applications. The use of thermozyme based biological fuel cells have several advantages such as better mixing, high substrate solubility, good mass transfer rate, and decreased risk of contamination. Several reports are also available on the use of different sugars as substrates in extremophilic microbial fuel cells. However, the cellulose fed microbial fuel cell systems are limited.

Thermophilic microbial fuel cells with Firmicutes as electrocatalyst and 10-mm acetate as the sole electron donor have been developed (Wrighton et al. 2008). The thermophilic MFC were operated at 55 °C over 100 days of operation and produced a power density of 37 mW/m^2 and a coulombic efficiency of 89%. Choi et al. (2004) developed a microbial fuel cell with thermophilic microorganisms such as *Bacillus licheniformis* and *Bacillus thermoglucosidasius* operated at 50–70 °C. It was shown that the developed microbial fuel cell could oxidize a wide range of electron donors such as fructose, galactose, glucose, lactose, maltose, mannitol, mannose, sorbitol, starch, sucrose, and trehalose. Abramov et al. (2013) reported a thermophilic process using *Thermoanaerobacterium* (including *T. thermosaccharolyticum* and *T. aotearoense*) and *Clostridium* genus including *C. cellulosi* and *C. thermocellum* for hydrogen production from cellulosic wastes coupled with bioelectricity production using membrane-bound [NiFe]-hydrogenase from *Thiocapsa roseopersicina* strain BBS. Different wastes such as paper (filter paper, newsprint or magazine paper, 15.0 g/L), wheat bran (10.0 g/L), wood sawdust (15.0 g/L), kitchen waste (15.0 g/L), straw (15.0 g/L), and different wastes from the brewing industry (yeast, 15.0 g/L and spent grains, 15.0 g/L) were treated by this process. The use of psychrophilic microorganisms as electrocatalysts have also been reported in the literature. Catal et al. (2011) reported single-chamber air-cathode mediator-less microbial fuel cells with the samples obtained during anaerobic digestion of grass silage as substrate. The psychrophilic system (15 °C) produced a power output of 31 ± 1 Wm^3, and removed the chemical oxygen demand (COD) and total phenolics over 90% and 30–75%, respectively.

In summary, the electroactive microorganisms are promising candidates for a lignocellulose fed microbial fuel cell. They should be able to hydrolyze cellulose as

well as oxidize glucose to generate electrons. The extremophilic microorganisms can also be used as electrocatalysts for the reduction of electron acceptors in the cathode compartment of the MFCs. Suraniti et al. (2013) reported the immobilization of thermostable bilirubin oxidase (BOD) from *Bacillus pumilus* onto the electrode for developing a bioelectrode for bioelectrochemical applications. The thermostable BOD was immobilized in a cross-linked redox-active hydrogel film having pendant osmium moieties grafted on a polyvinylimidazole backbone of the electrode. They displayed high electrocatalytic activity in the electron shuttling compounds at a broad pH range of 7–10 and temperature of 70 °C. Similar reports for the reduction of CO_2, a thermophilic biocathode, were also made based on electromethanogenesis. The biocathodes containing thermophiles (55 °C) such as *Methanothermobacter*-related methanogen and *synergistetes*- and *thermotogae*-related bacteria mediated the electrocatalysis to produce CH_4 at high rates of 1103 mmol m^{-2} day^{-1} at an applied voltage of 0.8 V (Fu et al. 2015). Kobayashi et al. (2017) analyzed the draft genome of a novel *Coriobacteriaceae* sp. strain EMTCatB1, isolated from the metagenome of a thermophilic electromethanogenic biocathode that actively catalyzes electromethanogenesis.

12.4 Microbial Electrolytic Cells

Microbial electrolytic cells (MECs) are electrochemical devices that operate on the principles of bioelectrocatalysis, as in the case of microbial fuel cells. However, the MECs differ from MFCs in the way that the MECs convert electrical energy to chemical energy whereas the MFCs convert chemical energy to electrical energy. The operational principle of an MEC is the reverse of an MFC. The MEC produces hydrogen with the aid of the external voltage, as in the case of electrolytic cells, but the MEC makes use of the voltage produced by the microorganism in addition to the external voltage. In the anode compartment, the substrate (electron donor) is oxidized by the electroactive microorganism and it produces electrons and protons (H^+ ions). The electrons are collected by the anode and reach the cathode through an external circuit. The protons generated by the anodic reaction reach the cathode through the electrolyte. The cathodic reaction mediates the generation of hydrogen by combining the H^+ ions. Figure 12.2 depicts the operational principle of MEC. The electrochemical potential produced by the bioelectrocatalytic oxidation reaction in the anode compartment is insufficient to provide the reducing power required for the hydrogen evolution reaction (HER) at the cathodic site. It requires an additional voltage (normally 0.2 V–1.0 V) for the hydrogen evolution reaction (Logan et al. 2008). MEC requires a very small supplementary external voltage when compared to the much higher voltage (>1.2 V) needed in the case of conventional water electrolysis processes. MEC for biohydrogen production is therefore an energy-efficient option.

Extremophilic microorganisms can be used for the oxidation of substrate in the anode compartment or hydrogen evolution reaction in the cathode compartment. Lignocellulosic biomass would be the feedstock of choice in the anode

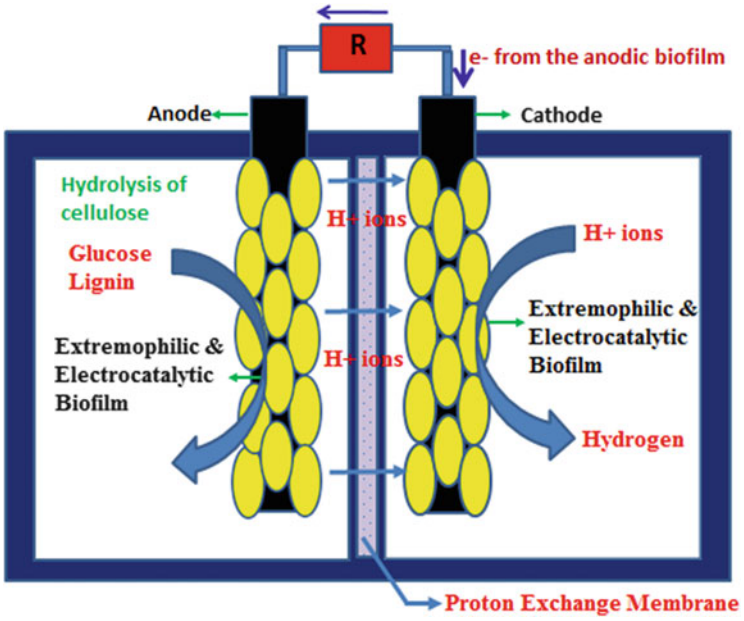

Fig. 12.2 Construction and operation of MEC

compartment. As in the case of MFC, individual extremophilic microorganism co-cultures/consortia having both hydrolytic activity and electrogenic activity will be useful to produce electrons from the lignocellulosic biomass. Different organisms have been reported in the literature for use as electrocatalysts for the electrocatalysis of lignocellulose in the anode compartment as well as reduction of protons to hydrogen in the cathode compartment of MECs. Recently Shehab et al. (2017) reported the use of brine pools from three different locations of the Red Sea, namely, Valdivia, Atlantis II, and Kebrit for the enrichment of the anodic compartment of MECs. The developed MEC operated under thermophilic (70 °C) and hypersaline (25% salinity) conditions and produced a high current of 6.8 ± 2.1 A/m^2 in MECs operated at a set anode potential of +0.2 V vs. Ag/AgCl (+0.405 V vs. standard hydrogen electrode). Fu et al. (2013) reported a thermophilic biocathode containing six different phyla (predominantly Firmicutes) for hydrogen production in a two compartment MEC. The developed biocathode produced a current density of 1.28 ± 0.15 A/m^2 and hydrogen production rate of 376.5 ± 73.42 mmol day^{-1} m^{-2}. Wang et al. (2014) developed a psychrophilic biocathode for hydrogen production in molasses wastewater fed MEC. The developed MFC with biocathode (operated at a low temperature of 9 °C) produced an overall hydrogen recovery of 45.4% with an applied voltage of 0.6 V. Lu et al. (2011) reported that the psychrophilic single-chamber MECs operated at low temperatures of 4 °C or 9 °C with anodic biofilm containing *Geobacter psychrophilus*. The rates of hydrogen production in the acetate fed MEC ranged from 0.23 ± 0.03 to 0.53 ± 0.04 m^3 H_2 m^{-3} d^{-1}, and it produced the

maximum hydrogen yield of 2.94 ± 0.02 mol H_2 mol^{-1} acetate. Lu et al. (2012) reported the synergistic effect of methanogenesis and homoacetogenesis for hydrogen production in a MEC at 25 °C. The hydrogen yield of the single-chamber MEC operated at 4 °C amounted to 6 mol H_2 mol^{-1} glucose and reached a maximum rate of around 0.37 ± 0.04 m^3 H_2 m^{-3} d^{-1}.

12.5 Microbial Desalination Cells

Microbial desalination cells (MDCs) are those electrochemical systems that make use of the electrocatalytic activity of the microorganisms for simultaneous bioelectricity generation and desalination of water. Basically, the operation principle of MDCs is similar to electrosmosis and electrodialysis (Cao et al. 2009; Qu et al. 2012). In MDC, the anodic and cathodic compartments are separated by a desalination chamber and the electrochemical potential of the microorganisms is utilized to drive the transport of ions. The anode and desalination chambers are partitioned by an anion exchange membrane whereas the cathode and the desalination chamber are partitioned by a cation exchange membrane. The schematic diagram showing the principle of MDC is shown in Fig. 12.3. Microorganisms are utilized for the oxidation of electron donors in the anode compartment and reduction of electron

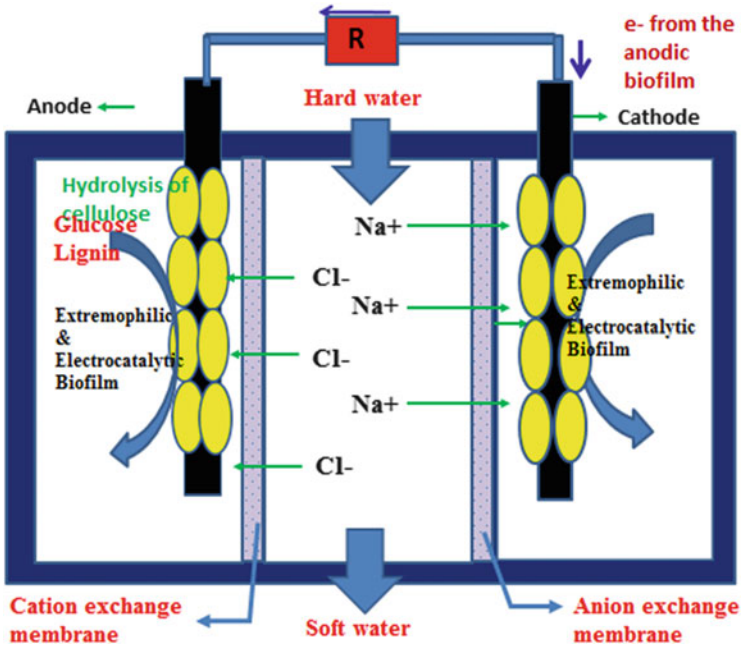

Fig. 12.3 Construction and operation of MDC

acceptors in the cathode compartment, as in the case of MFCs. The potential gradient developed in this process is used for driving the transport of dissolved Na^+ and Cl^- ions through the selective ion exchange membranes toward the cathode and anode, respectively. The larger the potential difference between the anode and the cathode, the higher is the rate of desalination. Hence, for any bioelectrochemical systems, the ideal choice of bioanode should have more negative anodic potential, and the biocathode should have more positive cathodic potential.

MDCs are hybrid strategies that makes use of electrochemical and membrane separation techniques. When compared with the conventional desalination systems, MDC has the advantage that it has minimal energy consumption, accelerated rates of desalination, and minimal damage/fouling to the membrane. In addition, unlike the constant pressure or constant volume filtration systems, MDS is independent of pressure and does not demand special configurations or reactor systems to resist high pressures. However, in terms of electrocatalysis, MDCs are similar to MFCs. MDCs differ from MFC in configuration/construction.

12.6 Bioelectrosynthesis

Bioelectrosynthesis is a bioelectrochemical process by which electroactive microorganisms/enzymes make use of the electrochemical potential for the synthesis of value added products (Rabaey and Rozendal 2010). This is similar to the microbial or enzymatic processes, but differs in that the oxidation or reduction potential is applied to the bioelectrocatalyst (electroactive enzyme/microorganism). The scheme depicting the concept of bioelectrosynthesis is shown in Fig. 12.4. The use of oxidation and reduction potential helps in accelerating the electrooxidation/electroreduction of electron donor/electron acceptor. In a microbial electrosynthesis process, electrical energy is transformed into chemical energy.

Microorganisms have been well explored for the synthesis of several industrially important compounds such as organic acids, amino acids, vitamins, antibiotics, therapeutic compounds, etc. Unlike the enzymatic processes, the microorganisms make use of a series of reactions to synthesize the product. The electrocatalysts aid in mediating the oxidative/reductive synthesis of the desired product from the reactants. Reports are available on the electro organic synthesis approaches for the treatment of waste waters by oxidizing/reducing the toxic electron donors/electron acceptors into nontoxic forms. The bioelectrosynthesis strategy is a hybrid approach making use of catalytic activity of microbial/enzymatic catalysts as well as electrochemical potential. When compared with bioprocesses, the bioelectrosynthetic processes have much higher specificity. The use of specific applied potential on the bioelectrodes also has the additional benefits of decreasing the side reactions/by products which are a major limitation in the conventional microbial systems that greatly demands serious downstream processing strategies.

Photobioelectrocatalysts such as photosynthetic bacteria, algae, or cyanobacteria can also be used as electrocatalysts for the bioelectrosnthesis of value added

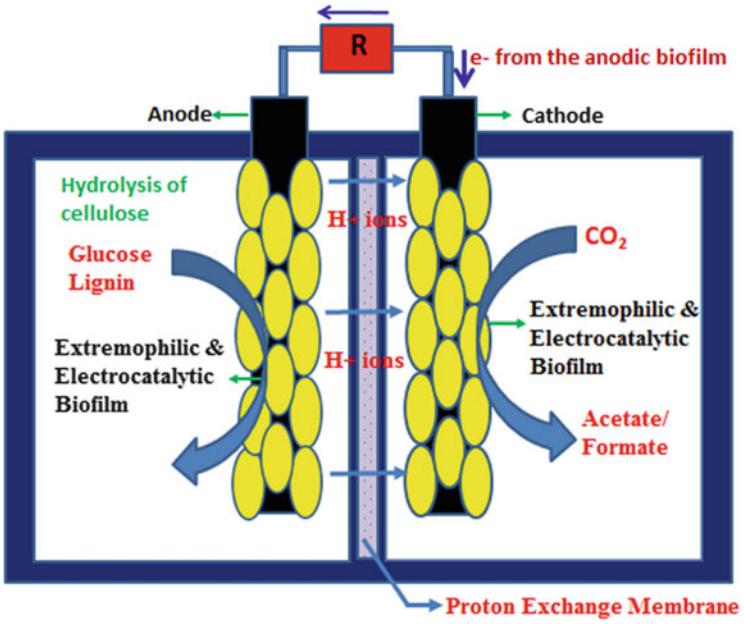

Fig. 12.4 Concept of bioelectrosynthesis

products. In principle, on irradiation with photons, the photosynthetic organisms produce electrons which are used to drive the metabolic machinery. Photomicrobial electrosynthetic processes will likely improve the production of biofuels and value-added products. Several reports are available on the use of acetogenic microorganisms' bioelectrosynthesis of acetate (also known as electroacetogenesis) (May et al. 2016). Reports have also been documented in the literature on the use of thermophiles for enhanced electrosynthesis of acetate. Electrochemical investigations on electron uptake rate of *Moorella thermoautotrophica* at a cathode potential of −0.4 V (vs. standard hydrogen electrode) showed the temperature dependence and demonstrated a maximum current density of 63.47 mA/m^2 at 55 °C. Further, it has been shown that an increase in temperature from 25 to 50 °C increased the electrosynthesis rates of formate and acetate by 23.2- and 2.8-fold, respectively (Yu et al. 2017). In addition, the effect of immobilizing the thermophilic *Moorella thermoautotrophica* along with carbon nanoparticle showed that rates of electrosynthesis of acetate and formate significantly increased by 14- and 7.9-fold reaching to 58.2 and 63.2 mmol m^{-2} day^{-1} with 65% coulombic efficiency.

Take Home Message

- Bioelectrochemical Systems (BES) are a promising strategy for the synthesis of biofuels and value-added products due to their ecofriendly nature and ability to catalyze at normal operating conditions.

- Use of extremophiles in BES can aid in circumventing the limitations of the conventional biological processes.
- The extremophiles/extremozymes can help facilitate immobilization and increase stability/activity leading to improved electron transfer and enhanced electrocatalysis.
- Use of lignocellulosic biomass will help to cut down the cost of the electrochemical process. Limitations from the recalcitrant nature of lignocellulosic biomass can be overcome with the aid of highly efficient extremophilic microorganisms. This seems to be promising for commercial applications in the future.

Acknowledgements Financial support provided by the National Science Foundation in the form of BuG ReMeDEE initiative (Award # 1736255) is gratefully acknowledged. The authors gratefully acknowledge the financial support provided by NASA EPSCoR (Award # 1736255). The authors also gratefully acknowledge Department of Chemical and Biological Engineering at the South Dakota School of Mines and Technology for the support. Funding from the Governor's Office of Economic Development, South Dakota (number) is greatly appreciated.

References

Abramov SM, Sadraddinova ER, Shestakov AI, Voronin OG, Karyakin AA, Zorin NA, Netrusov AI (2013) Turning cellulose waste into electricity: hydrogen conversion by a hydrogenase electrode. https://doi.org/10.1371/journal.pone.0083004

Bella RSD, Hirankumar G, Krishnaraj RN, Prem Anand D (2016) Novel proton conducting polymer electrolyte and its application in microbial fuel cell. Mater Lett 164:551–553

Bhalla A, Kainth AS, Sani RK (2013) Draft genome sequence of lignocellulose-degrading thermophilic bacterium *Geobacillus sp*. strain WSUCF1. Genome Announc 1(4):e00595-13

Bhalla A, Bischoff KM, Sani RK (2014a) Highly thermostable GH39 β-xylosidase from a *Geobacillus* sp. strain WSUCF1. BMC Biotechnol 14:963. https://doi.org/10.1186/s12896-014-0106-8

Bhalla A, Bischoff KM, Uppugundla N, Balan V, Sani RK (2014b) Novel thermostable endoxylanase cloned and expressed from bacterium *Geobacillus* sp. WSUCF1. Bioresour Technol 165:314–318

Bhalla A, Bischoff KM, Sani RK (2015) Highly thermostable xylanase production from a thermophilic *Geobacillus sp.* strain WSUCF1 utilizing lignocellulosic biomass. Front Bioeng Biotechnol 3:84

Bhuvaneswari A, Krishnaraj RN, Berchmans S (2013) Metamorphosis of pathogen to electrigen at the electrode/electrolye interface: direct electron transfer of Staphylococcus aureus leading to superior electrocatalytic activity. Electrochem Commun 34:25–28

Cao X, Huang X, Liang X, Xiao K, Zhou Y, Zhang X, Logan BE (2009) A new method for water desalination using microbial desalination cells. Environ Sci Technol 43:7148–7152

Catal T, Cysneiros D, O'Flaherty V, Leech D (2011) Electricity generation in single chamber microbial fuel cells using a carbon source sampled from anaerobic reactors utilizing grass silage. Bioresour Technol 102(1):404–410

Choi Y, Jung E, Park H, Paik SR, Jung S, Kim S (2004) Construction of microbial fuel cells using Thermophilic microorganisms, *Bacillus licheniformis* and *Bacillus thermoglucosidasius*. Bull Kor Chem Soc 25(6):813–818

Fu Q, Kobayashi H, Kuramochi Y, Xu J, Wakayama T, Maeda H, Sato K (2013) Bioelectrochemical analyses of a thermophilic biocathode catalyzing sustainable hydrogen production. Int J Hydrog Energy 38(35):15638–15645

Fu Q, Kuramochi Y, Fukushima N, Maeda H, Sato K, Kobayashi H (2015) Bioelectrochemical analyses of the development of a thermophilic biocathode catalyzing electromethanogenesis. Environ Sci Technol 49(2):1225–1232

Gregoire KP, Becker JG (2012) Design and characterization of a microbial fuel cell for the crop residue to electricity. Bioresour Technol 119:208–215

Hassan SH, Kim YS, Oh SE (2012) Power generation from cellulose using mixed and pure conversion of a lignocellulosic cultures of cellulose-degrading bacteria in a microbial fuel cell. Enzym Microb Technol 51(5):269–273

Holmes DE et al (2006) Microarray and genetic analysis of electron transfer to electrodes in *Geobacter sulfurreducens*. Environ Microbiol 8(10):1805–1815

https://www.eia.gov/todayinenergy/detail.php?id=30652

Karthikeyan R, Navanietha Krishnaraj R, Selvam A, Woon-Chung Wong J, Lee PKH, Leung MKH, Berchmans S (2016) Effect of composites based nickel foam anode in microbial fuel cell using *Acetobacter aceti* and *Gluconobacter roseus* as a biocatalysts. Bioresour Technol 217:113–120

Kobayashi H, Fu Q, Maeda H, Sato K (2017) Draft genome sequence of a novel *Coriobacteriaceae sp*. strain, EMTCatB1, reconstructed from the metagenome of a thermophilic electromethanogenic biocathode. Genome Announc 5:e00022-17

Kodama Y, Watanabe K (2011) *Rhizomicrobium electricum* sp. nov., a facultatively anaerobic, fermentative, prosthecate bacterium isolated from a cellulose-fed microbial fuel cell. Int J Syst Evol Microbiol 61:1781–1785

Kyne D, Bolin B, Chakraborty J, Grineski SE, Collins TW (2016) Emerging environmental justice issues in nuclear power and radioactive contamination. Int J Environ Res Public Health 13 (7):700. https://doi.org/10.3390/ijerph13070700

Logan BE, Hamelers B, Rozendal R, Schröder U, Keller J, Freguia S, Aelterman P, Verstraete W, Rabaey K (2006) Microbial fuel cells: methodology and technology. Environ Sci Technol 40 (17):5181–5192

Logan BE, Call D, Cheng S, Hamelers HV, Sleutels TH, Jeremiasse AW, Rozendal RA (2008) Microbial electrolysis cells for high yield hydrogen gas production from organic matter. Environ Sci Technol 42(23):8630–8640

Logan BE, Wallack MJ, Kim K-Y, He W, Feng Y, Saikaly P (2015) Assessment of microbial fuel cell configurations and power densities. Environ Sci Technol Lett 2(8):206–214

Lu L, Ren NQ, Zhao X et al (2011) Hydrogen production, methanogen inhibition and microbial community structures in psychrophilic single-chamber microbial electrolysis cells. Energy Environ Sci 4:1329–1336

Lu L, Xing D, Ren N, Logan BE (2012) Syntrophic interactions drive the hydrogen production from glucose at low temperature in microbial electrolysis cells. Bioresour Technol 124:68–76

Malvankar NS, Lovley DR (2012) Microbial nanowires: a new paradigm for biological electron transfer and bioelectronics. ChemSusChem 5(6):1039–1046

Malvankar NS et al (2015) Structural basis for metallic-like conductivity in microbial nanowires. MBio 6(2):e00084

May HD, Evans PJ, LaBelle EV (2016) The bioelectrosynthesis of acetate. Curr Opin Biotechnol 42:225–233

Navanietha Krishnaraj R, Yu JS (2015) Systems biology approaches for microbial fuel cell applications. Bioenergy: Opportunities and Challenges. ISBN-10: 1771881097. Apple Academic Press, USA

Navanietha Krishnaraj R, Karthikeyan R, Berchmans S, Chandran S, Pal P (2013) Functionalisation of electrochemically deposited chitosan films with alginate and Prussian blue for enhanced performance of Microbial fuel cells. Electrochim Acta 112:465–472

Navanietha Krishnaraj R, Berchmans S, Pal P (2014) Symbiosis of photosynthetic microorganisms with non-photosynthetic ones for the conversion of cellulosic mass into electrical energy and pigments. Cellulose 21:2349–2355

Navanietha Krishnaraj R, Berchmans S, Pal P (2015) The three-compartment microbial fuel cell: a new sustainable approach to bioelectricity generation from lignocellulosic biomass. Cellulose 22:655–662

Niessen J, Schröder U, Scholz F (2004) Exploiting complex carbohydrates for microbial electricity generation – a bacterial fuel cell operating on starch. Electrochem Commun 6(9):955–958

Pandit GG, Sahu Sk, Puranik VD (2011) Natural radionuclides from coal fired thermal power plants-estimation of atmospheric release and inhalation risk. Radioprotection 46:S173–S179

Pant D, Singh A, Van Bogaert G, Olsen SI, Nigam PS, Diels L, Vanbroekhoven K (2012) Bioelectrochemical systems (BES) for sustainable energy production and product recovery from organic wastes and industrial wastewaters. RSC Adv 2:1248–1263

Panwar NL, Kaushik SC, Kothari S (2011) Role of renewable energy sources in environmental protection: a review. Renew Sust Energ Rev 15(3):1513–1524

Qu Y, Feng Y, Wang X, Liu J, Lv J, He W, Logan BE (2012) Simultaneous water desalination and electricity generation in a microbial desalination cell with electrolyte recirculation for pH control. Bioresour Technol 106:89–94

Rabaey K, Rozendal RA (2010) Microbial electrosynthesis – revisiting the electrical route for microbial production. Nat Rev Microbiol 8(10):706–716

Rabaey K et al (2004) Biofuel cells select for microbial consortia that self-mediate electron transfer. Appl Environ Microbiol 70(9):5373–5382

Rathinam NK, Berchmans S, Salem D, Sani RK (2018) Rewiring the microbe-electrode interfaces with biologically reduced graphene oxide for improved bioelectrocatalysis. Bioresour Technol 256:195–200. https://doi.org/10.1016/j.biortech.2018.02.001

Rastogi G, Bhalla A, Adhikari A, Bischoff KM, Hughes SR, Christopher LP, Sani RK (2010) Characterization of thermostable cellulases produced by *Bacillus* and *Geobacillus* strains. Bioresour Technol 101(22):8798–8806

Reguera G et al (2005) Extracellular electron transfer via microbial nanowires. Nature 435 (7045):1098–1101

Ren Z, Ward TE, Regan JM (2007) Electricity production from cellulose in a microbial fuel cell using a defined binary culture. Environ Sci Technol 41(13):4781–4786

Ren Z, Steinberg LM, Regan JM (2008) Electricity production and microbial biofilm characterization in cellulose-fed microbial fuel cells. Water Sci Technol 58(3):617–622

Rezaei F, Xing D, Wagner R, Regan JM, Richard TL, Logan BE (2009) Simultaneous cellulose degradation and electricity production by *Enterobacter cloacae* in a microbial fuel cell. Appl Environ Microbiol 75(11):3673–3678

Schröder U, Harnisch F (2013) From microbial bioelectrocatalysis to microbial bioelectrochemical systems. In: Alkire RC, Kolb DM, Kibler LA, Lipkowski J (eds) Electrocatalysis, vol 14. Wiley-VCH, Weinheim. https://doi.org/10.1002/9783527680436.ch4

Schuetz B et al (2009) Periplasmic electron transfer via the c-type cytochromes MtrA and FccA of *Shewanella oneidensis* MR-1. Appl Environ Microbiol 75(24):7789–7796

Selvaraj R, Vidhya S, Navanietha Krishnaraj R, Perumal S, Sundaramoorthy S, Maruthamuthu S, Ponmariappan S, Vijayan M (2016) Utilization of soak liquor in microbial fuel cell. Fuel 181:148–156

Shehab NA, Ortiz-Medina JF, Katuri KP, Hari AR, Amy G, Logan BE, Saikaly PE (2017) Enrichment of extremophilic exoelectrogens in microbial electrolysis cells using Red Sea brine pools as inocula. Bioresour Technol 239:82–86

Shrestha N, Chilkoor G, Vemuri B, Rathinam NK, Sani RK, Gadhamshetty VR (2018) Extremophiles for microbial-electrochemistry applications: a critical review. Bioresour Technol. https://doi.org/10.1016/j.biortech.2018.01.151

Sleutels TH, Ter Heijne A, Buisman CJ, Hamelers HV (2012) Bioelectrochemical systems: an outlook for practical applications. ChemSusChem 5(6):1012–1019. https://doi.org/10.1002/cssc.201100732

Suraniti E, Tsujimura S, Durand F, Mano N (2013) Thermophilic biocathode with bilirubin oxidase from *Bacillus pumilus*. Electrochem Commun 26:41–44

Turner P, Mamo G, Karlsson EN (2007) Potential and utilization of thermophiles and thermostable enzymes in biorefining. Microb Cell Factories 6:9. https://doi.org/10.1186/1475-2859-6-9

Vargas M et al (2013) Aromatic amino acids required for pili conductivity and long-range extracellular electron transport in *Geobacter sulfurreducens*. MBio 4(2):e00105-13

Wang X, Feng Y, Wang H, Qu Y, Yu Y, Ren N, Li N, Wang E, Lee H, Logan BE (2009) Bioaugmentation for electricity generation from corn stover biomass using microbial fuel cells. Environ Sci Technol 43(15):6088–6093

Wang Y, Guo WQ, Xing DF et al (2014) Hydrogen production using biocathode single-chamber microbial electrolysis cells fed by molasses wastewater at low temperature. Int J Hydrogen Energ 39:19369–19375

Wrighton KC, Agbo P, Warnecke F, Weber KA, Brodie EL, DeSantis TZ, Hugenholtz P, Andersen GL, Coates JD (2008) A novel ecological role of the Firmicutes identified in thermophilic microbial fuel cells. ISME J 2:1146–1156

Yu L, Yuan Y, Tang J, Zhou S (2017) Thermophilic *Moorella thermoautotrophica*-immobilized cathode enhanced microbial electrosynthesis of acetate and formate from CO_2. Bioelectrochemistry 117:23–28

Zang GL, Sheng GP, Tong ZH, Liu XW, Teng SX, Li WW, Yu HQ (2010) Direct electricity recovery from *Canna indica* by an air-cathode microbial fuel cell inoculated with rumen microorganisms. Environ Sci Technol 44(7):2715–2720