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Principle and Product Overview of Bioelectrosynthesis

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1.1 Introduction

With the pressing crisis of depletion of fossil fuels, the past decade has seen the significant growth in the use of renewable energy, which leads to the growing research efforts toward electricity production from solar, wind, wave, or biomass energy (as opposed to petroleum, coal, or gas) in a sustainable way [1]. As electricity produced based on these renewable sources is usually intermittent and off-grit, electrosynthesis has been considered as an effective strategy to store electrical energy from renewable sources in the forms of chemical compounds [2]. Adequate electrocatalysts are necessary to catalyze the electrode-driven chemical reactions, yet these chemical catalysts are usually too expensive to be scaled up for practical applications. As a result, biocatalysts, which can be an enzyme, an organelle, or even a whole cell, have drawn increasing attention in electrosynthetic processes because of their higher specificity and versatility [3]. Moreover, microbes as catalysts are inexpensive to grow and, if the microbes catalyzing the reactions gain enough energy for cell maintenance, are self-sustaining and long-lived. Therefore, bioelectrosynthesis represents a promising approach to store renewable energy or produce target chemicals in an energy-sustainable and low-cost way.

Bioelectrosynthesis has emerged that electrical energy can be combined with biosynthesis to drive CO_2 fixation as a means to directly produce the target compound or lead to the formation of acetyl-CoA and its derivatives for further synthesis. There are many assumptions for bioproduction in different pathways (Figure 1.1), which require inputs of solar energy or electrical energy (as an indirect solar derivative). One could speculate that instead of the Wood–Ljungdahl pathway (which would produce acetyl-CoA), the Calvin–Benson–Bassham cycle (which yields triose phosphates) can be driven on electrical current, leading to the formation of fermentable substrate from electricity and CO_2. This fermentable substrate could then further be used for bioproduction purposes.

Bioelectrosynthesis: Principles and Technologies for Value-Added Products,
First Edition. Edited by Aijie Wang, Wenzong Liu, Bo Zhang, and Weiwei Cai.

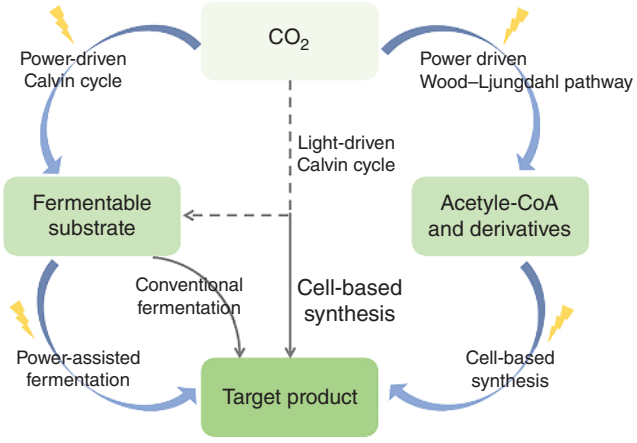

Figure 1.1 Overview of different routes toward bioproduction from CO_2. Source: Rabaey et al. 2011 [1]. Reproduced with permission of Elsevier.

Lastly, the fermentation itself can be complemented by electrical current to provide reducing equivalents to the cell. This can be considered as a hybrid metabolism when effective charge transfer occurs toward the cell. The major assumptions are summarized in Table 1.1. The theoretically achievable bioproduction densities for bioelectrosynthesis (product–carbon per hectare per annum) appear excessive at first glance. However, it is crucial to point out that photovoltaic panels are relatively efficient in capturing solar energy and that a first study producing acetate from CO_2 has indicated high electron yields. Other factors such as CO_2 and nutrient supply are likely to become limiting before these theoretical values are achieved. Therefore, electrosynthesis of organic compounds via abiotic or enzymatic catalysis of carbon dioxide reduction at electrode surfaces has been evaluated as a strategy for converting electricity into useful organic products for some time [7–9].

Bioelectrosynthesis relies on the use of biocatalysts on the electrode surfaces to achieve electricity-driven synthesis. For biocatalyst, it has the following advantages in the bioelectrosynthetic processes: (i) the high reaction specificity and controllability of enzymes and organelles, (ii) self-regeneration of the whole microorganisms as the catalyst, (iii) adaptation of the microbial (catalyst's) quantity to the required conversion activity, (iv) flexibility in substrate use, (v) high versatility for product formation or conversion pathways, and (vi) improving the performance by decreasing the overpotentials at both anodes and cathodes [10–12]. However, microorganisms as biocatalysts are still far from perfect. Unlike true catalyst, microbes have been shown to consume part of the substrate or donor for growth albeit possibly only intermittently and are hard to keep a steady function or phenotype in different microenvironments.

Microbial electrosynthesis (MES) is a form of microbial electrocatalysis, which is an emerging area in microbial electrochemical research and development. The concept of MES was used to describe the process when a microbial catalyst reduces CO_2 into multicarbon chemical commodities with electrons derived

Table 1.1 Assumptions regarding the theoretical production rates as well as expected substrate requirements for bioelectrosynthesis, conventional fermentations, and algal bioproduction systems (c$ refers to dollar cent) [1, 4–6].

	Aerobic fermentations	Anaerobic fermentations	Current-driven lithoautotrophy – aerobic	Current-driven lithoautotrophy – anaerobic	Algal production
Carbon source (cost c$/mol C)[a)]	Glucose (c$0.6)	Glucose (c$0.6)	CO_2 ($0)	CO_2 ($0)	CO_2 ($0)
Electron donor (cost c$/mol C)[a)]	Glucose (c$0.15)	Glucose (c$0.15)	Electricity (c$0.16)	Electricity (c$0.16)	Water (c$ 0)
Growth yield (mol C/mol C)	0.57	0.14	0.13	0.015	0.04–0.10
Maximal production density per hectare[b)]	<3 tonnes C (as glucose)	<3 tonnes C (as glucose)	<1121 tonnes C (as butanol)	<1121 tonnes C (as butanol)	<25 tonnes C (as biodiesel)

a) Assumptions: Cost of fermentable substrate c$20 per kilogram and at 24 electrons per glucose molecule; electrical power delivered at 1 V and 0.06 $/kWh.

b) Assumes 100% conversion of raw materials to product. For glucose, annual average production per hectare from sugarcane is assumed. For lithoautotrophy, case calculations assume photovoltaic electricity productions at 500 W/m^2 averaged irradiation and 20% efficiency of the photovoltaic panel. The bioelectrochemical system is assumed to have a 1 V cell voltage. For algal production, assumed annual production 50 tonnes biomass dry weight per hectare, of which <50% biodiesel-C.

from the cathode of a bioelectrochemical system (BES) by applying an electric current designed primarily to perform biological reductive reactions. Generally, the electric current would ideally be produced by a renewable source of power. To be corresponding to the definition of conventional electrosynthesis and the microbial versatility found in different MES-based systems, it was expanded to mean "an alternative bioenergy strategy to use electrical energy as a source of reducing/oxidizing power for biochemical production, wherein the microorganisms facilitate the transfer of electrons from the cathode of a electrochemical system and production of desirable liquid transportation fuels and value-added chemicals." Thus, in addition to the electricity-driven reduction of CO_2, bioelectrosynthesis also includes the electricity-driven reduction or oxidation of other organic feedstocks [1, 8, 12–14].

MES relies on electrical current as a driver, which can allow on-site conversion of electrical energy (current) to chemical energy (a fuel). When coupled with a renewable source of electricity, the process will not only avoid the use of fossil fuels but utilize CO_2 from waste streams, which would not compete for food crops or arable land and would only use small amounts of water and nutrients compared with the agricultural production of biofuels and chemicals. Therefore, the MES has several advantages, including (i) the double benefits of carbon sequestration and organics production, (ii) the feedstock mainly coming from wastes, (iii) high electricity efficiency to chemical commodities (c. 80–90%), and (iv) the potential that it could address the harvesting, storage, and distribution problems associated with energy crops, solar and wind farms, and natural gas exploration because the electricity can be from any renewable source, no matter how it is supplied, intermittent, stranded, or curtailed, and microbes may harvest it as power, especially utilizing solar energy in a 100-fold higher efficiency than biomass-based chemical production [1, 8, 13–18].

These characteristics demonstrate that a better understanding of the processes and mechanisms of bioelectrosynthesis would highly likely help address the need of energy and carbon storage as well as chemical production. As more is learned, additional applications will probably emerge. This chapter provides an overview of the principles and products of bioelectrosynthesis. This also includes a history of bioelectrosynthesis usage together with the properties of microbial catalysts and electrochemical hardware that affect their productivity, stability, long-term efficiency, and versatility in the environment and related topics are discussed in greater detail in subsequent chapters.

1.2 Evolution of Bioelectrosynthesis

In the context of electricity-driven bioproduction, reducing power provided by means of an electrode can either redirect fermentation pathways (sometimes called electrofermentation) or drive respiration. Electrofermentation has evolved since the report in 1979 that current supply through a mediator could increase L-glutamic acid yields [19, 20]. A significant advance was the finding in 2004 that direct electron transfer happened from cathodes to an attached biofilm

Geobacter sulfurreducens with reducing fumarate to succinate [21]. In the past 15 years, microbial electrochemical systems developed rapidly as a key environmental technology at the nexus of water and energy research interests, particularly in the concept of microbial electrolysis cells (MECs) to produce H_2 at the cathode through the reduction of protons, which could be used as fuel in turbines, internal combustion engines, fuel cells, as well as ovens and heaters [12, 14, 22–24]. For example, it was reported that anodic biofilms can be converted to cathodic biofilms for H_2 production [23]. Meanwhile, bioelectrochemical synthesis of H_2 can also be achieved by cathodically generating H_2 accompanied with methane production in a bioanode [22]. It should be noted that the elimination of membranes or separators converted dual-chamber MECs to single-chamber reactors and significantly increased H_2 generation rate, but the increase in H_2 was more likely inhibited by methanogenesis to generate CH_4 [25, 26]. Moreover, other inorganic chemicals have been produced in the cathode chamber of MECs. Rozendal et al. reported that hydrogen peroxide can be produced by reducing oxygen through the two electron reduction [27].

However, the concept of MES was only introduced in 2009–2010, with the initial findings related to the conversion of electrical current into methane from carbon dioxide by the *Methanobacterium palustre* on the biocathode at a set cathode potential less than −0.7 V (vs. Ag/AgCl) [28]. It was recognized by 2010 that biofilms of *Sporomusa ovata* growing on graphite cathode surfaces using pure cultures could use electrons derived from an electrode for the reduction of carbon dioxide to acetate and small amounts of 2-oxobutyrate at a high coulombic efficiencies (CEs) of acetate production (over 85%) [13]. However, considering that hydrogen is typically produced at the low potentials that were required for active methanogenesis, subsequent studies have questioned whether hydrogen produced at the cathode was the actual electron donor [29, 30]. Studies showed that a wide diversity of microorganisms, such as *Clostridium ljungdahlii*, *Clostridium aceticum*, *Sporomusa sphaeroides*, and *Moorella thermoacetica*, are capable of reducing carbon dioxide to produce organic acids with electrons derived from an electrode as the sole electron donor without using hydrogen [14, 16]. The mixed cultures were reported to generate acetate from a biocathode poised at −590 mV (vs. standard hydrogen electrode [SHE]) with CO_2 as the only carbon source over 150 days, which further demonstrated the stability, resilience, and improved performance of electrosynthetic biocathodes following long-term operation [31, 32]. It was also recognized that 13.5 mM of alcohols as well as C4 compounds can be produced by reducing acetate at the cathode, but some processes required addition of mediators, such as methyl viologen (MV) [33]. In a similar way, the use of a cathode potential at −0.9 V vs. SHE in a BES without addition of an external mediator leads to the cathodic formation of medium chain fatty acids including caproate, butyrate, and smaller fractions of caprylate as the main products from acetate [34], which can be harvested as a valuable chemical. Notably, butyrate is an industrial feedstock with many applications in the pharmaceutical and chemical industries and can be converted into fuels through esterification [35]. Recently, a study has shown the bioelectrochemical transformation of CO_2 as a sole carbon source to butyrate using mixed microbial

cultures for the first time, but the products were a mixture of acetate, butyrate, ethanol, and butanol, and CO_2 reduction to butyrate was hydrogen driven [36].

Furthermore, research in the area of metabolic engineering attempted to optimize the cellular metabolism of an organism to satisfy the desired process objectives mainly including significantly facilitating electron uptake and improving organic synthesis by modifying microorganisms. Typically, this is achieved by introducing exogenous metabolic pathways and manipulating native metabolic pathways or by manipulating cellular redox and energy reactions in order to overproduce desired metabolites [14, 37, 38]. Bioelectrochemical techniques are also used to manipulate the redox metabolism, such as supplying reducing power by generating reduced NADH within the cell through interactions with an electrode, which are effective to increase the synthesis or biotransformation of several products including ethanol, *n*-butanol, and succinate in a variety of hosts including *Saccharomyces cerevisiae*, *Clostridium acetobutylicum*, and *Actinobacillus succinogenes* [37, 39, 40]. It is widely known that acetyl-CoA, the central intermediate in acetate production in acetogens, is the building block for microbial synthesis of a wide diversity of desirable organic products, which should be possible with genetic engineering to divert carbon and electron flow in acetogenic microbes toward the production of butanol [41, 42].

Until now, most of the studies based on the technology of microbiology and molecular biology in the bioelectrochemical areas focused on the mechanisms for electron exchange from microbe, such as the model *Shewanella oneidensis* or *G. sulfurreducens*, to electrode, while electron transfer from electrodes to microbes may not be a simple reversal of electron transfer from cells to electrodes. For example, deletion of the genes for pili or OmcZ production essential for optimal current production of *G. sulfurreducens* had no impact on the capacity for current uptake [8, 43]. As discussed in several conceptual review articles [8, 12, 24, 30, 44, 45], it is likely that different pathways exist for electron uptake in microorganisms attached to cathodes, that is, either accepting electrons directly from electrodes (direct electron transfer) or alternatively using electron shuttles and cathodic H_2 as electron carriers for the reduction of carbon dioxide (indirect electron transfer). Even though there is considerable progress in understanding the pathways for how electrons may be transported from the electrode to the cell, the complex interactions between microorganisms and cathode as well as interspecies for extracellular electron transfer (EET) toward microorganisms are not yet known, which is one of the key challenges to scale bioelectrosynthesis to practical applications. Besides, microbial attachment, biofilm development, electron transfer rate at the cathode surface, chemical production rate as well as biocathode materials, selective microbial consortia, and efficient reactor designs are all crucial elements to be optimized toward this objective.

To our knowledge, the study interests of bioelectrosynthesis have blossomed in the past decade, resulting in an exponential growth in the number of journal articles (Figure 1.2), but it is still in its infancy and there are also many technological and economic challenges, especially the significant engineering of the microbes and the reactors to be solved before it can be applied practically in large scale.

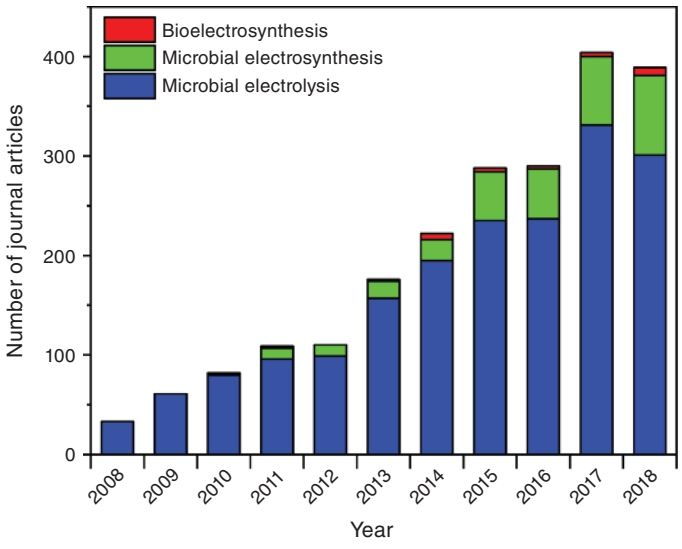

Figure 1.2 Number of published journal articles on bioelectrosynthesis containing the phrases "bioelectrosynthesis," "microbial electrosynthesis," or "microbial electrolysis."

1.3 Fundamental Principles of Bioelectrosynthesis

Microbial fuel cells (MFCs) and MECs are examples of recent biotechnologies known as BESs that combine biological and electrochemical processes to produce electricity, hydrogen, or other useful chemicals [8, 12, 24, 46]. MFCs deliver electrical power from nearly any source of biodegradable organic or inorganic matter in waste streams by exoelectrogenic microorganisms on the anode, which have attracted extensive attentions at the early stage of BESs research [47–51]. MECs needs a small external power to make the reaction thermodynamically feasible, which can enable the generation of many different more value-added chemical products from biomass, such as hydrogen production [24, 46, 52, 53]. Energy is added into an MEC by either using an external power source or setting an electrode potential using potentiostat. Over the past decade, it has been known that MECs not only further store electricity as the desired commodities for conserving energy and reducing the dependency on fossil fuels but also capture/fix carbon dioxide while alleviating the greenhouse effect [12, 13, 16, 24, 54], which leads to the emergence of bioelectrosynthesis.

Microbial electrosynthetic processes are conducted in so-called BESs, which consist of an anode, a cathode, and, typically, a membrane separating the two chambers. An oxidation process occurs at the anode, whereas a reduction process occurs at the cathode, and the electrodes are surrounded by an electrolyte, which is generally an aqueous solution or wastewater (as a feed source) and contains the reactants and/or products. Microorganisms utilize electrons derived from the cathode to directly (via electron transfer) or indirectly (through evolved chemicals) catalyze the production of chemicals, including hydrogen, methane,

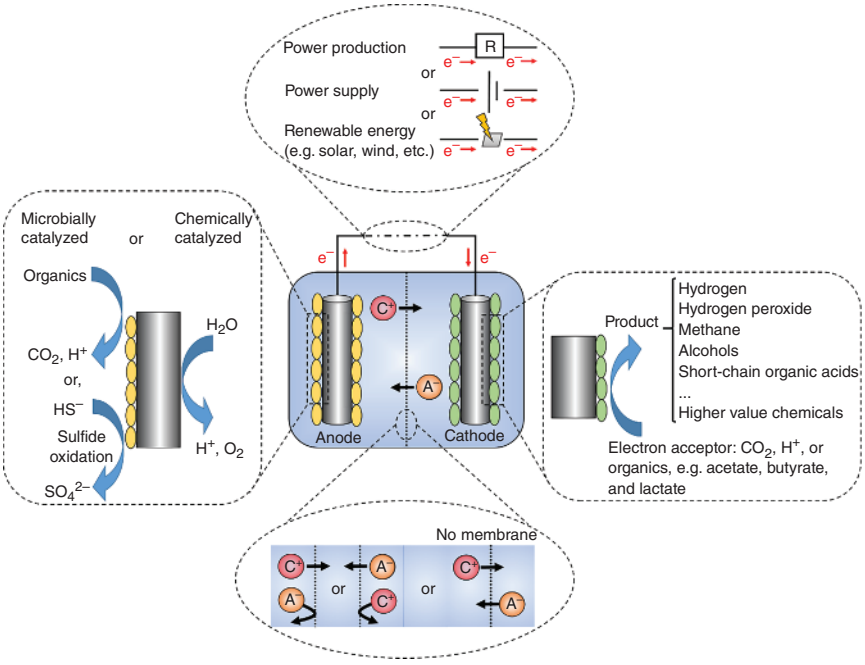

Figure 1.3 Basic principles of MES [12, 14, 24, 55]. A plethora of choices can be made regarding the membrane, the nature of the catalysts at both the anode and the cathode, and the source of the reducing power. This leads to a highly versatile technology that can carry out a diverse range of processes. MES can also be coupled to environment-friendly anodic processes.

short-chain organic acids, alcohols, etc., with only electricity and CO_2 as feedstock (Figure 1.3) [12, 15, 36, 55]. Moreover, MES can also start from basic organic compounds, such as acetate, butyrate, and lactate, which are ubiquitously present in wastewaters and fermenter effluents, and then produce more attractive higher value end products (Figure 1.3) [12, 33]. Therefore, bioelectrosynthesis as a new platform technology that could produce the versatile fuels has gained increasing concerns [29, 31, 56, 57].

There are several challenges in bioelectrosynthetic systems, such as the internal losses, which lead to the considerably less energy gained or more invested in reality, similar to the other systems of BESs [12, 58–60]. Firstly, the oxidation or reduction reaction at the electrode will incur the so-called activation overpotential, causing a voltage loss because of imperfect catalysis at the electrode. Secondly, when electrons flow through an electrical circuit, the resistance of electrolyte together with losses in the electrodes and the electrical circuit will lead to an ohmic loss. Thirdly, at higher current densities (or low mixing), the supply of substrate to the electrode or the discharge of protons or hydroxyl ions may cause diffusion limitations. Multiple mixed communities or pure cultures have shown the ability to catalyze CO_2 reduction by using electricity as donor. Multicarbon compound production rates by MES have been increased substantially over the past five years; for example, the acetate production rate has been increased 433-fold (c. 282 $mM/d/m^2$), whereas the

electron transfer rate enhanced 521-fold (c. 475 mA/m^2) [17, 18, 24, 55]. Yet the obstacles of low microbial productivity, poor stability, and low efficiency of CO_2 to multicarbon compounds still stood out. Ongoing efforts conducted on the development of MES as an economically viable technology mainly include optimizing microbial catalysts and electrochemical hardware and characterizing the electron transfer mechanisms from cathode to microbes.

1.4 Plethora of Applications for Chemical Production

Currently, as the focus has shifted to microbial reductive processes at the cathode, bioelectrosynthesis, which has a better energy efficiency than MFCs alone and can couple chemical oxygen demand (COD) removal and energy recovery from waste with chemical synthesis, is being explored for a number of applications [46, 61, 62]. For example, microorganisms can catalyze electrochemical reactions such as proton reduction to molecular hydrogen or the reduction of carbon dioxide to organics such as methane, acetate, etc. (the process of MES), which holds strong promises for a new concept for biofuel generation. The following mainly focuses on the known function of bioelectrosynthesis for different valuable extracellular chemical end products because of the electrocatalyzed reduction reaction in the cathode. Individual applications are described and discussed in detail in various chapters of this book.

1.4.1 Hydrogen Production

Hydrogen (H_2) has a high energy content of 121 MJ/kg, which is a clean energy carrier with zero carbon emission. Currently, 96% of commercial H_2 produced today is delivered from fossil fuels via steam reforming, thermochemical conversion (pyrolysis), and gasification. However, the above-mentioned methods are not always environment-friendly. The bioelectrosynthesis of H_2 in MECs, probably the cleanest and the most efficient method, provided completely new avenue for sustainable hydrogen production from renewable biomass and wastewaters [46, 63–65].

In principle, exoelectrogenic microorganisms colonized on the anode surface to form an anode-respiring biofilm and decompose the organic matter or wastes into CO_2, electrons, and protons as a part of its metabolism. Meanwhile, the electrons traveled through an external circuit to a cathode, where the reduction of H^+ to molecular H_2 gas takes place. As this reaction is nonspontaneous (thermodynamically not favored because of the positive Gibbs free energy of the reaction), an external voltage practically at least 0.2–0.25 V must be supplemented to make it happen for the H_2 production in MECs (Figure 1.4).

Crucially, there are several limits that can affect the performance of the MEC toward up-scaling and widespread applications, including low hydrogen production rate (HPR), high internal resistance, complicated architecture, and expensive materials. As for the problem that the hydrogen evolution reaction (HER) on plain carbon electrode is very slow and a high overpotential is needed to generate

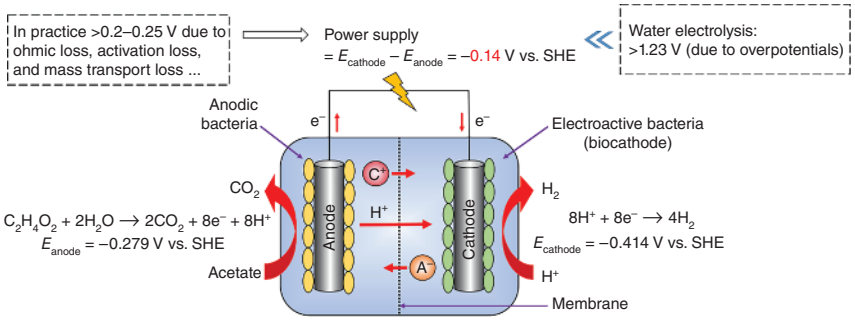

Figure 1.4 Principles of the bioelectrosynthesis of H_2 in microbial electrolysis cell.

H_2, platinum (Pt), an expensive metal catalyst, is usually used as the catalyst at the cathode in MECs, which has two major drawbacks including its high cost and poisoning by chemicals such as sulfide (a common constituent of wastewater). To resolve this problem, several attempts have been made to search for Pt-free cathode materials for HER in MECs. To date, it was found that first-row transition metals are very useful because of their stability, easy availability, low cost, low overpotentials, and low toxicity to living organisms. Considerable research efforts on cathodic material for MEC show that stainless steel (SS) and nickel alloys as well as nanostructured cathode materials represent a good compromise between cost and efficiency [63–67].

Many researchers have studied and explored several metabolic processes present in the cathode, stepping toward a possibility to develop a biocathode. For instance, Rozendal et al. firstly carried out investigation of a bioelectrode for H_2 production from a naturally selected mixed culture of electrochemically active bacteria [23]. Chen et al. attempted to modify biocathodes with polyaniline (PANI)/multiwalled carbon nanotube (MWCNT) composites to improve hydrogen production in single-chamber MECs, which achieved an HPR of 0.67 m^3 H_2/m^3 d at E_{ap} = 0.9 V [68]. Croese et al. demonstrated that a mixed microbial consortium established on graphite felt cathodes of MEC could produce H_2 at a rate of 2.4 $m^3/m^3/d$ [69]. Compared with chemical catalysts, the use of electroactive microorganisms as cathode catalysts to make the biocathode MECs is superior to abiotic cathodes because H_2 could be produced at a similar rate and cheaper biocatalysts can also self-generate without producing secondary pollution (Table 1.2). Therefore, biocathodes are a welcome advancement, i.e. increasing the bioelectrosynthesis of H_2, in the quest to implement MECs for practical applications.

1.4.2 Methane Production

Methane is an excellent fuel and is being widely used all over the world. The production of methane has been the most common aim for respiratory bioproduction in dark conditions. Meanwhile, it is commonly detected in the MECs during hydrogen production because of the growth of methanogens, which was considered to be the result of diffusion from the anode to cathode at the early

Table 1.2 A selection of H_2 production rates normalized to the reactor volume ($m^3/m^3/d$) and cathode surface area ($m^3/m^2/d$) in MEC experiments.

Substrate (mg/l)	Inoculum	H_2 production rate ($m^3/m^3/d$)	H_2 production rate ($m^3/m^2/d$)	Cathode, *E* vs. Ag/AgCl/V	Mode of operation	MEC configuration	Cathode	Anode	References
Acetate (60)	Mixed (enriched)	2.2	0.010	−0.7	Continuous (0.01 h)	Dual chamber with cation exchange membrane	Biocathode, graphite paper	Graphite	[70]
Acetate (300)	*Geobacter sulfurreducens*	0.31	0.005	−0.8	Continuous (0.04 h)	Single chamber	Biocathode, nanoporous graphite	Nanoporous graphite	[71]
Acetate (600)	Mixed (enriched)	2.4	0.024	−0.9	Continuous (0.64 h)	Dual chamber with cation exchange membrane	Biocathode, graphite felt	Graphite felt	[69]
Acetate (100)	Mixed (enriched)	0.02	0.002	0.5	Batch	Dual chamber with cation exchange membrane	Titanium mesh disk	Graphite felt disk	[66]
Acetate (2720)	Mixed (enriched from prior MEC)	50	50	1	Continuous (0.003 h)	Dual chamber with anion exchange membrane	Ni foam	Graphite felt	[72]
Ammonia (510)	Mixed (enriched)	0.01	0.001	0.6	Batch	Dual chamber with anion exchange membrane	Carbon felt	Carbon felt	[73]

(Continued)

Table 1.2 (Continued)

Substrate (mg/l)	Inoculum	H_2 production rate ($m^3/m^3/d$)	H_2 production rate ($m^3/m^2/d$)	Cathode, *E* vs. Ag/AgCl/V	Mode of operation	MEC configuration	Cathode	Anode	References
Lactate (910)	*Shewanella oneidensis* MR-1	0.25	3.068	0.6	Batch	Aerated H-type reactor with anion exchange membrane	Graphite (modified with 5% Pt in activated carbon powder)	Carbon fiber fabric	[74]
Methanol (1600)	Mixed (enriched MFC culture)	0.1	0.004	0.8	Batch	Dual-chamber reactor with anion exchange membrane	Graphite fiber cloth (coated with Pt)	Graphite fiber brush	[75]
Sodium bicarbonate (2500)	Mixed (enriched)	45.27	Surface area not reported	−0.8 (vs. SHE)	Batch	Dual chamber with cation exchange membrane	Biocathode, graphite granules	Graphite granules	[76]
Sodium bicarbonate (5000)	Mixed (enriched)	1.15	Surface area not reported	−0.85 (vs. SHE)	Batch	Dual chamber with cation exchange membrane	Biocathode, vitreous carbon (doped with CNT)	Graphite plates	[77]

Hydrogen production rates normalized to electrode surface area were calculated using the projected surface area or the total surface area based on the working electrode.
Source: Kitching et al. 2010 [65]. Reproduced with permission of Elsevier.

stage [53, 78]. Substantial reports in the literature have suggested that cathodic methane production in the MECs was expected by the catalytic conversion of hydrogen to methane (e.g. $4H_2 + CO_2 \rightarrow CH_4 + 2H_2O$) with abiotic cathode [46, 56]. Although methane is sometimes considered a nuisance by-product in hydrogen-producing MECs as it increases the energy and economical cost for purification, several studies have made the production of methane a key objective [79–81]. Cheng et al. for the first time described the production of methane from carbon dioxide reduction in a two-chamber MEC with a methanogen-attached biocathode at a methane production rate about 0.06 mmol/l/h at a voltage of 1.2 V [28]. The authors of this study suggested direct EET as the core mechanisms that the conversion of an electrical current toward methanogens was direct and did not proceed through H_2 (e.g. $CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$). Likewise, Villano et al. utilized both the electrons and CO_2 released at the anode during the microbial oxidation of the organic matter contained in a waste stream for the cathodic generation of methane (Figure 1.5) and obtained a methane production ratio of 0.055 ± 0.002 mmol/D mg volatile suspended solids (VSSs) from CO_2 in a two-chamber MECs with a biocathode incubated with a hydrogenophilic methanogenic culture [29]. Nowadays, research regarding direct EET for methane bioelectrosynthesis is still highly needed to fully explore electron transfer occurring at the cathode surface.

Generally, the bioelectrosynthesis of methane in MECs holds several advantages over conventional biogas, including (i) the possibility to store electricity or H_2 as methane with a high content, (ii) saving energy because the process occurs at ambient temperature and heating is not required, and (iii) the limited sensitivity of the process to toxic compounds such as ammonia, which can be present in

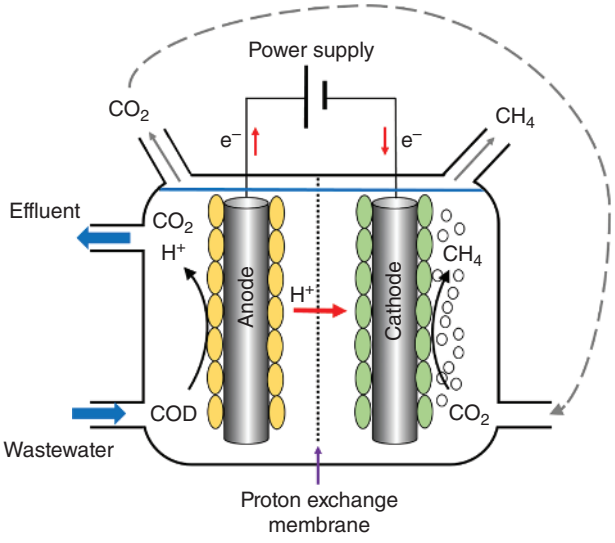

Figure 1.5 Schematic drawing of a bioelectrochemical system for wastewater treatment and simultaneous CH_4 bioelectrosynthesis based on CO_2 reduction. Source: Villano et al. 2010 [29]. Reproduced with permission of Elsevier.

the feedstock (this relates to the sensitivity of methanogens to ammonia, which is formed at high pH values) [22, 46, 64]. Even though the disadvantages, i.e. the low value of methane as a product, the energy investment that is required to produce the methane and the cost of pressurizing such a gas for transport, need to be considered, understanding the underlying mechanisms for methane bioelectrosynthesis is highly attractive from an engineering standpoint.

1.4.3 Alcohol Production

In the process of the bioelectrochemical reduction of CO_2 to acetate, acetyl-CoA is the key central intermediate, which could be a versatile building block for a range of useful organic chemicals and potential biofuels such as ethanol, *n*-butanol, and alcohols, or longer chain fatty acids.

Ethanol as a liquid fuel has been produced by microbial reduction of acetate as the main intermediate of anaerobic digestion with hydrogen as electron donor, whereas the feasibility of ethanol production by using electrode instead of hydrogen as electron donor has been demonstrated in a two-chamber MEC for biological acetate reduction by mixed cultures, which obtained 1.82 mM ethanol production and 49% of CE at best via the assistance of electron mediator such as MV [33, 82]. This suggested that BES aiming to bioelectrosynthesis provides a new way to overcome the limitation of traditional biological ethanol production. However, there are underlying challenges that need to be addressed for making the technology industrially applicable, including whether hydrogen was involved in the mechanism of acetate reduction, how to decrease the operation cost (e.g. irreversibly electron acceptors and energy for distillation), selecting electroactive microorganisms that can accept electrons directly from cathode rather than via mediator for ethanol production, improving the ethanol production rate and the final concentration, and increasing the efficiency of systems by reduction in electrode overpotential, system internal resistance, and energy losses [46, 64].

Butanol is an important chemical intermediate for the precursor of many industrial chemicals in food, chemical, and pharmaceutical industries. Bio-based butanol produced from acetone–butanol–ethanol (ABE) fermentation is preferred as its green renewable feature. Bioelectrosynthesis based on microbial BESs is another method to supply electrons for microbial metabolism, which works at a biocathode by external power input for butanol production [83]. Compared to conventional fermentation method or microbial synthesis, the production rate, efficiency, and concentration of different chemicals were enhanced by assistance of bioelectrosynthesis (Figure 1.6) [84].

Acetogenic microorganisms are an attractive catalyst for the conversion of carbon dioxide to a diversity of multicarbon organic products [86]. Some acetogens will produce high titers of ethanol rather than acetate under the appropriate conditions, and in some instances, 2,3-butanediol and butanol are also produced in wild-type cells [87, 88]. Besides, it has been reported that the final products of MES included acetate, butanol, propanol, and ethanol by use of mixed culture [44]. However, the bioelectrosynthesis starting from CO_2 to alcohols has a key disadvantage that CO_2 as an electron acceptor is the large electron requirement [12]. For instance, although the theoretical potentials for the reduction of

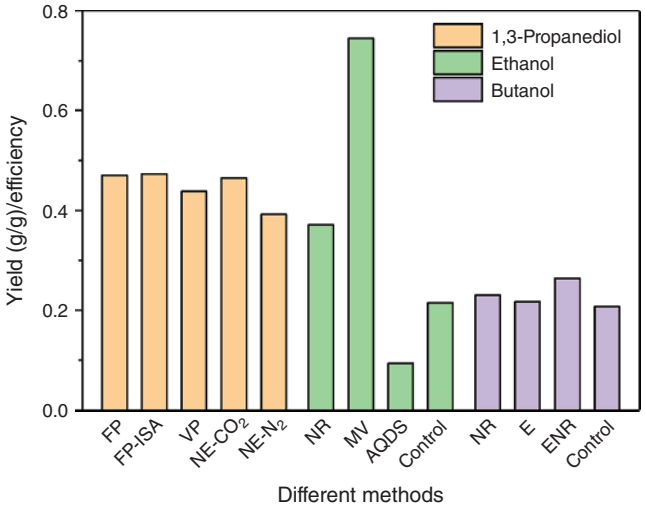

Figure 1.6 Yield (solvent g/substrate g) of 1,3-propanediol production, ethanol efficiency, and butanol yield in MEC [33, 83–85]. neutral red (NR); methyl viologen (MV); anthraquinone-2,6-disulfonate (AQDS); fixed electrode potential (FP); fixed-potential increased electrode surface area (FP-ISA); varying potential (VP); nonelectrochemical CO_2 spared (NE-CO_2); electrochemical N_2 spared (NE-N_2); electricity of cathodic potential (E); electricity reduced neutral red (ENR).

butyrate to butanol ($E_0' = -0.37$ vs. SHE) and the reduction of CO_2 to butanol ($E_0' = -0.30$ V vs. SHE) are similar, the reduction of butyrate to butanol requires only 4 electrons, whereas the reduction of CO_2 to butanol requires 24 electrons, which implies a sixfold higher current demand and an equivalently large power demand for this reaction. Besides, the conversion of CO_2 to butanol will also probably involve multiple synthesis steps, each with certain efficiency losses.

1.4.4 Short-chain Organic Acid Production

The production of formic acid, which is an important chemical used in pharmaceutical syntheses as well as in paper and pulp production, was achieved based on organic matter oxidation in the anode and CO_2 reduction in the cathode [46]. In the electrochemical processes driven by direct-current power supplies, the reduction of CO_2 to formic acid has been demonstrated by several studies on different metal cathodes, e.g. expensive Pt at a high Faraday efficiency (above 94%) [89]. To explore an environmentally friendly method, Zhao et al. utilized the electricity *in situ* generated from the degradation of the carbonaceous substances in the anodic chambers in a series-connected MFC stack, which also achieved the electrochemical reduction of CO_2 to formic acid at 4.27 mg/l/h [90]. The *Desulfovibrio* and *Sulfurospirillum* may contribute to H_2 and formate metabolism that may then support acetogenesis. *Desulfovibrio* in particular is well known for its ability to generate H_2 off of an electrode, and it has been shown to grow while converting formate into H_2 [91–93]. However, a CO_2 reductase in *Acetobacterium woodii* can catalyze the reversible and direct conversion of H_2 and CO_2

to formate, and it is thermodynamically favorable for sulfate reducing bacteria to generate formate from H_2 and CO_2 as long as sulfate is limiting and an acetogen is available to consume the formate [94, 95].

The production of acetate (or acetyl-CoA) is central to the bioelectrosynthesis of chemicals beyond H_2 and methane. Acetate can be an important end product as well as a platform for further chemical syntheses [15]. Many acetogenic microorganisms and enriched microbial communities have been tested for the ability to produce acetate with electrons supplied at a cathode [15]. Generally, mixed cultures performed better than pure cultures (Tables 1.3 and 1.4). Nevin et al. demonstrated that an acetogenic microorganism *S. ovata* growing on graphite cathode surfaces could use electrons for the reduction of carbon dioxide to acetate and small amounts of 2-oxobutyrate concomitantly with electrons delivered directly from biocathode [13, 16]. Electron recovery in these products exceeded 85%, which is consistent with the reaction $2CO_2 + 2H_2O \rightarrow CH_3COOH + 2O_2$. It was subsequently found that several other acetogenic bacteria including two other *Sporomusa* species, *C. ljungdahlii*, *C. aceticum*, and *M. thermoacetica*, could consume current with the production of acetate, 2-oxobutyrate, and formate, which expanded the known range of microorganisms capable of bioelectrosynthesis [16]. Jourdin et al. have developed very stable and long-lasting electroacetogenic microbial community. The acetate production per total surface area of the cathode was very high (25.2 g/m^2/d) and was accomplished with an electron recovery in acetate of 100% [99, 100]. On the other hand, a handful of studies have focused on the development of prospective electrode materials for biocathode processes and the production of acetate in MES. For example, Lovley et al. modified electrode materials for the improvement of MES of acetate from CO_2 by pure cultures of *S. ovata*, which got a sevenfold higher production rate (c. 0.02 mM/cm^2/d) and current density (0.0475 mA/cm^2) on chitosan-modified carbon cloth over those of a nonmodified carbon cloth [17, 18]. It was reported that NanoWeb-reticulated vitreous carbon (RVC), a new electrode material for biocathode, can effectively enhance current consumption and MES rates of acetate from carbon dioxide with mixed cultures [99]. A very novel approach of supplying electrons for electroacetogenesis was done with *S. ovata* incubated with a photocathode of nanotubes of Si–TiO_2, which produced acetate at 0.24 g/l/d and reached a titer of 1.2 g/l in the system applying light simultaneously to a photoanode (also of Si–TiO_2) and the photocathode [96]. This is the highest rate and titer reported for electroacetogenesis with a pure culture, and interestingly, they were obtained with light as the sole energy source [15]. Moreover, Gong et al. investigated the integration of biological processes on bioelectrodes for sulfide-driven bioelectrosynthesis in BES, which resulted in an acetate production rate of 49.9 mmol/d m^2, with a CE of over 90% by using a strain of *Desulfuromonas* sp. as biocatalyst on the anode and *S. ovata* on the cathode [108]. Herein, abiotically oxidation of sulfide yielding two electrons on the anode and further biotic steps from the oxidation product, elemental sulfur, to sulfate by *Desulfobulbus propionicus* generating six additional electrons (eight electrons generated from the combined abiotic and biotic steps in total) were used to reduce carbon dioxide to acetate on a graphite cathode (Figure 1.7).

Table 1.3 Electroacetogenesis with pure cultures (maximum reported values).

Microorganisms	Cathode	$E°$ (mV vs. SHE)	J (A/m^2)	g_{acet}/m^2/d	g_{acet}/l/d	g_{acet}/l (d)	Electron recovery in acetate (%)	References
Sporomusa ovata[a)]	Graphite stick	−400	−0.695[e)] −0.207[f)]	4.64[e)] 1.38[f)]	0.01^{EV} 0.045^{STY}	0.0625 (6)	85	[13]
Sporomusa ovata[b)]	Chitosan-coated carbon cloth[d)]	−400	−0.475[e)]	2.75 ± 0.67[e)]	0.065 ± 0.16	0.59 (9)	82 ± 12	[18]
Sporomusa ovata[b)]	Ni-coated nanowire graphite stick	−400	−2.18[e)] −0.65[f)]	11.35[e)] 3.38[f)]	0.068	0.54 (8)	82 ± 14	[17]
Sporomusa ovata[c)]	Si–TiO_2 nanowire photocathode	−595 to −571	−3.5[e)] −0.12[f)]	ND	0.24	1.2 (5) 6.0 (ND)	86 ± 9	[96]
Sporomusa sphaeroides[a)]	Graphite stick	400	−0.057[e)] −0.017[f)]	0.208[e)] 0.062[f)]	0.000 35^{EV} 0.0027^{STY}	0.0028 (8)	39	[16]
Sporomusa silvacetica[a)]	Graphite stick	−400	−0.02[e)] −0.006[f)]	0.15[e)] 0.045[f)]	0.0002^{EV} 0.0025^{STY}	0.002 (10)	84	[16]
Clostridium aceticum[a)]	Graphite stick	−400	−0.08[e)] −0.024[f)]	0.02[e)] 0.006[f)]	0.0002^{EV} 0.0042^{STY}	0.0027 (13)	27	[16]
Clostridium ljungdahlii[a)]	Graphite stick	−400	−0.104[e)] −0.031[f)]	0.47[e)] 0.14[f)]	0.0009^{EV} 0.0054^{STY}	0.0065 (7)	72.2 ± 0.2	[16]
Clostridium ljungdahlii[b)]	Graphite felt + stainless steel	−695	−1.0[e)]	7.51–19.2[e)]	0.056–0.144	0.56 (11)	39–89	[97]

(Continued)

Table 1.3 (Continued)

Microorganisms	Cathode	$E°$ (mV vs. SHE)	J (A/m^2)	g_{acet}/m^2/d	g_{acet}/l/d	g_{acet}/l (d)	Electron recovery in acetate (%)	References
Moorella thermoacetica[a)]	Graphite stick	−400	−0.03[e)] −0.009[f)]	0.35[e)] 0.104[f)]	0.0006^{EV} 0.0045^{STY}	0.0047 (9)	85 ± 7	[16]
Acetobacterium woodii[a)]	Graphite stick	−400	Not detected	Not detected	Not detected	Not detected	—	[16]
Acetobacterium woodii[b)]	Stainless steel felt	−690	−1.5[e)] (applied)	12.8[e)]	0.046	0.127	81	[98]

ND, not determined or not reported.

a) Estimates based on continuous feed linear production and flow rates reported (EV is rate calculated per exit volume, STY is the space-time yield).

b) Estimates based on batch operation (catholyte volume).

c) Light as energy source.

d) Several other materials/treatments of carbon cloth tested (cyanuric chloride, 3-aminopropyltriethoxysilane, polyaniline, melamine, ammonia, Au, Pd, Ni, CNT-cotton, CNT-polyester). Highest rates achieved with chitosan.

e) Based on projected surface area.

f) Based on total surface area.

Source: May et al. 2010 [15]. Reproduced with permission of Elsevier.

Table 1.4 Electroacetogenesis with mixed cultures (maximum reported values).

Microbial source (enriched genera)	Cathode	$E°$ (mV vs. SHE)	J (A/m^2)	g_{acet}/m^2/d	g_{acet}/l/d[a)]	g_{acet}/l (d)	Electron recovery in acetate (%)	Refs.
Brewery wastewater (*Acetobacterium*, *Sulfurospirillum*, and Rhodobacteraceae)	Graphite granules	−590	ND	ND	0.24	1.7 (12)	67 (incl. H_2)	[31]
Brewery wastewater (*Acetobacterium*, *Sulfurospirillum*, *Desulfovibrio*, and Rhodobacteraceae)	Graphite granules	−590	ND	ND	1.04	10.5 (20)	69	[32]
Brewery wastewater (*Acetobacterium*, *Sulfurospirillum*, *Desulfovibrio*, and Rhodobacteraceae)	Graphite granules	−800	ND	ND	3.1	8.7 (6)	47.5	[76]
	Graphite rod[a)]	−765	−428[b)], −122[c)]	ND	ND	ND	ND	[76]
	Graphite rods[a)]	−800	−91[b)], −26[c)] −210[b)], −60[c)]	ND	ND	ND	ND	[76]
	Graphite rods (4 d, sealed)	−600	-3.23 ± 0.42[b)] -0.92 ± 0.12[c)]	11.1–38.0[b)] 3.2–10.8[c)]	0.06–0.22	0.1–0.34 (4)	40 ± 4	[76]
Pond sediment and WWTP sludge (ND)	Carbon plate	−850	-1.76 ± 0.01[b), c)]	5.85 ± 1.35[b), c)]	0.068 ± 0.016	3.9 (100)	44 ± 22	[99]
	NanoWeb 3D RVC	−850	-37 ± 3[b)] -2.91 ± 0.04[c)]	195 ± 30[b)] 15 ± 1.5[c)]	0.025 ± 0.004	1.2 (100)	70 ± 11	[99]
Pond sediment and WWTP sludge (*Acetoanaerobium*, *Hydrogenophaga*, and *Methanobrevibacter*)	3D RVC with carbon nanotubes	−850	-102 ± 1[b)] -3.75 ± 0.04[c)]	685 ± 30[b)] 25.2 ± 1.1[c)]	0.36	11.0 (66)	100 ± 4	[100]
Wastewater sludge (ND)	Carbon felt stainless steel	−895	10.0[b)]	40.0[b)]	0.078	0.63 (73)	40–50	[97]
Domestic WWTP sludge (ND)	Carbon felt	−853 −953	−8.8[b)] ND	16.3[b)] 19.2[b)]	0.33 0.39	0.33 (1) 0.39 (1)	24 18	[101]

(Continued)

Table 1.4 (Continued)

Microbial source (enriched genera)	Cathode	$E°$ (mV vs. SHE)	J (A/m^2)	g_{acet}/m^2/d	g_{acet}/l/d[a)]	g_{acet}/l (d)	Electron recovery in acetate (%)	Refs.
Cow manure (ND)	Graphite granules	−600	ND	ND	0.029	ND	28.9 ± 6.1	[102]
Bog sediment (*Trichococcus* and *Clostridium*)	Carbon fiber rod	−400	−0.1 ± 0.02[b)] −0.03 ± 0.006[c)]	0.21 ± 0.03[b)] 0.063 ± 0.01[c)]	0.0037	0.02 ± 0.0025 (54)	35.2 ± 4.4	[103]
Domestic WWTP sludge (*Acetobacterium*, *Advenella*, *Arcobacter*, and *Wolinella*)	Carbon felt	−703 −903	3.1[b)] ND	1.58[b)] 9.75[b)]	0.02 0.14	0.11 (5) ND	53.6 89.5	[104]
Domestic WWTP sludge (*Acetobacterium* and *Acetoanaerobium*)	Carbon felt	−1100	−3.3[b)]	10.28[b)]	0.18	1.38 (15 d)	65	[105]
Effluents from MFC anodes algae consuming UASBs (*Acetobacterium*, *Azovibrio*, Bacteroidales, Desulfovibrionaceae, and *Methanobacterium*)	Carbon felt	−1260 ± 80	−5.0 (applied)[b)]	19 ± 1.7[b)]	0.06 ± 0.006	1.29 ± 0.15 (~18 d)	58 ± 5	[106]
Effluents from MFC anodes algae consuming UASBs (*Acetobacterium*, *Azovibrio*, Bacteroidales, Desulfovibrionaceae, and *Methanobacterium*)	Carbon felt stainless steel	−1140 ± 0.04	−5.0 (applied)[b)]	20.4[b)]	0.58	13.5	60.8	[107]

ND, not determined; UASB, upflow anaerobic sludge blanket; WWTP, waste water treatment plant.

a) Per catholyte volume

b) Per projected surface area

c) Per total surface area

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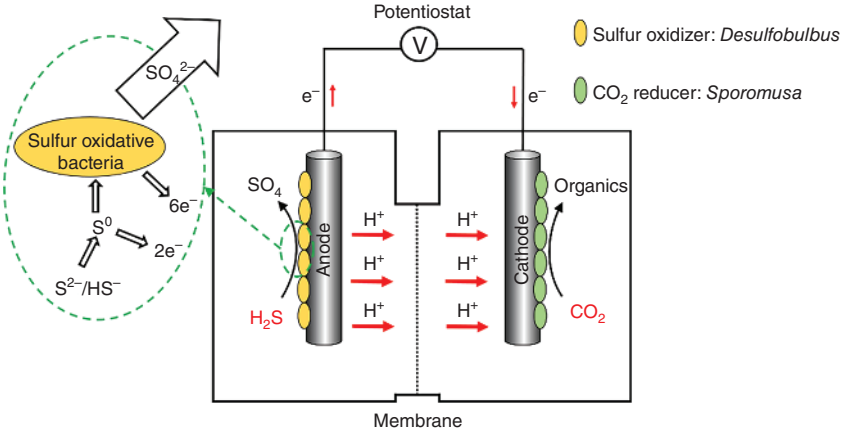

Figure 1.7 Sulfide-driven bioelectrosynthesis. Source: Gong et al. 2010 [108]. Reproduced with permission of Elsevier.

Interestingly, aimed to product ethanol by reducing acetate in MES using a cathode and a mixed microbial community, high yields of butyrate were found when the MV depleted owing to irreversible reduction at the cathode [33]. Van Eerten-Jansen et al. reached 6.8 mM of caproate and 3.0 mM of butyrate as main products in the cathode by biologically reducing acetate [34]. The work of Ganigue et al. proved for the first time the bioelectrochemical concomitant production of acetate and butyrate from CO_2 [36]. Besides, conversion of fumarate to succinate has been achieved using *A. succinogenes*, with electrically reduced neutral red as an electron donor and the same process was achieved without the addition of a mediator using *G. sulfurreducens* [12, 109]. Therefore, producing electrobiocommodities (E-BCs) directly via electrode-to-microbe electron transfer or indirectly with electrochemically generated electron donors such as H_2 or formate may be a possibly efficient and environmentally sustainable strategy.

1.4.5 Ammonia Production and Nitrogen Recovery

Recovery of nutrients as a more sustainable approach goes beyond removal and is of strong interest. Nitrogen recovery in a BES is mainly through ammonia recovery, namely ammonia production. Although photobioreactors containing algae can recover nitrogen via concentrating it in algal cells [110], recently, BESs are increasingly being considered to investigate as an alternative to NH_3 recovery from an ammonium-rich wastewater (e.g. urine). Ammonium ions are usually present in domestic wastewater with a much higher concentration than protons (assuming that wastewater has a neutral pH). It has been shown that cation NH_4^+ is transported against a concentration gradient through the cation exchange membrane from the anode into the cathode chamber driven by electromigration and diffusion, leading to an increase of the cathode pH, and the NH_4^+/NH_3 migration could account for about 90% of the ionic flux [111]. Villano et al. reported that ammonia moving resulted in ammonium accumulation in the cathode to

318 mg/l, almost 10 times the ammonium concentration in the anode in an MEC [112]. The concentrated ammonium ("ammonium" refers in a general sense to NH_3 and NH_4^+, whereas the chemical formulae are used to refer to its specific forms) is removed from the cathode chamber with increasing pH [96, 100]. One of the key factors in ammonia recovery is the high pH of catholyte, which can drive ammonium to ammonia gas [110]. NH_3 was stripped from the liquid–gas boundary via volatilization and subsequent absorption into an acid solution (Figure 1.8) [110, 113, 114]. The stripped ammonia can be absorbed in dilute sulfuric acid to form ammonium sulfate, which has the potential to be used in agriculture as a fertilizer, in the industry for synthesis of valuable nitrogen polymers, or for food production [115]. If the sulfuric acid solution as an absorption media is presaturated with ammonium sulfate, ammonia could also be collected as pure ammonium sulfate crystals, which is valuable as a laboratory chemical and fertilizer [116]. Furthermore, the MEC-forward osmosis system has also been used to achieve the recovery of both ammonia and water in leachate treatment [117].

The feasibility of ammonia recovery in a BES was intensively investigated through further understanding of ammonia moving mechanism. It was found that in the cathode chamber, ionic ammonium from urine was converted to volatile ammonia because of the high pH of the catholyte and the recovered ammonia via volatilization because of the aeration was subsequently adsorbed by an acid solution in an MFC [114]. Zhou et al. presented a possibility that

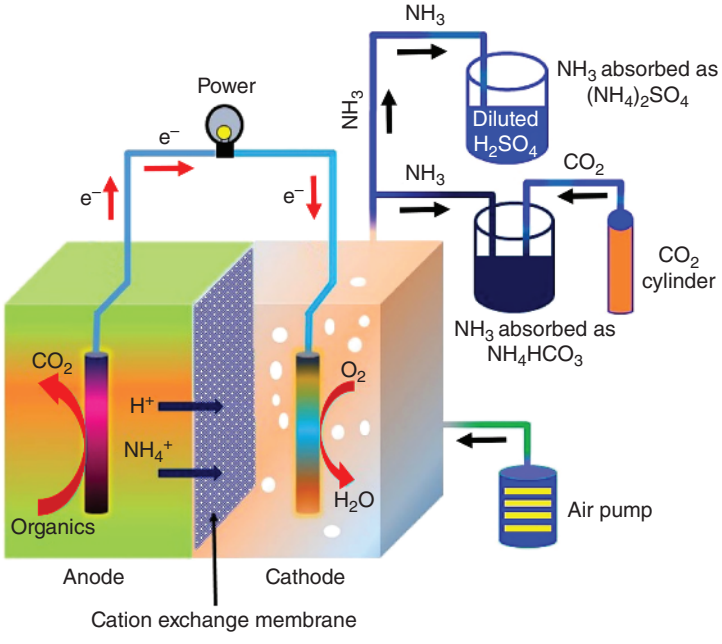

Figure 1.8 Ammonia recovery in BESs through ammonium transport and conversion to ammonia. The stripped ammonia can be collected as either ammonium bicarbonate or ammonium sulfate. Source: Iskander et al. 2010 [113]. Reproduced with permission of American Chemical Society.

ammonia inhibition of anode electroactivities could be mitigated concomitantly with ammonia recovery at a rate of $435.7 \pm 29.6\,g\,N/m^3/d$ from urine in a nitrogen-purged BES [118]. Still, effects of ammonia on the sustainable maintenance of BESs remain controversial. Some reports show that ammonia is oxidized with the anode, generally enriched with nitrifiers such as *Nitrosomonas europaea*, as an electron acceptor in BESs [119, 120] while others support adverse effects of ammonia on anode electroactivity [121]. These contradictory conclusions are believed to be dependent on the types of dominant bacterial species enriched on the anodes. Furthermore, recovery of ammonia in BES can greatly affect the energy performance (production and consumption) of the system. The theoretical analysis of energy consumption and production suggested that ammonia recovery in an MFC had significant energy advantage (with a positive energy balance) over conventional ammonia stripping [110]. However, it is reported that recovering ammonia in a MFC prefers an operation under high current generation, which will generate more electrons for driving the migration of ammonium ions but little power (or energy) production [113]. To simultaneously recover ammonia and generate energy, MECs may be a better option because hydrogen production in MECs usually occurs under high current condition. For instance, ammonia production from reject water at wastewater treatment plants could be modified to produce hydrogen simultaneously at a cathodic CE of 96% without affecting nitrogen recovery efficiency, even though the catholyte pH increased to above 12 because of current generation [122]. It was found that aeration in the cathode compartment is required to recover ammonia; otherwise, catholyte would accumulate a high concentration of ammonium [117], which increases the energy consumption of BESs. Therefore, development of an appropriate method for driving NH_3 out of the cathode without significant energy input will be essential for ammonia recovery in BES, from an environmental and economic perspective.

Nowadays, BESs are being explored for the removal of contaminants at the biocathode, such as the biological reduction of oxidized pollutants or the biological reduction of nitrate to nitrogen gas [8, 29, 123, 124]. Electrodes offer the possibility of supplying electrons for bioremediation in very specific locations and effectively colocalizing the electron donor and the appropriate organisms, offering the possibility of precolonizing the electrodes with the desired organisms. For example, it has been reported that *Geobacter* species are capable of using electrons derived from graphite electrodes for the reduction of a diversity of electron acceptors, including nitrate, fumarate, U(VI), and chlorinated solvents [16, 21, 125–127]. Among them, the current-driven microbial reduction of nitrate and nitrate (i.e. denitrification) for nitrogen recovery may be particularly attractive. The reduction of nitrate on cathodes with the existence of biofilms whose structure may include an outer layer occupied by nitrifying organisms and an inner layer with dominant nitrifying organisms has been observed along with simultaneous nitrification, suggesting the direct electron transfer (i.e. electrons move between the cell and the electrode via direct contact) in a biofilm to reduce nitrate [1, 128]. Besides, nitrate and nitrite as alternate electron acceptors can be reduced to drive electricity production from the oxidation of organics at the

anode, which produced comparable cell voltages because of its high solubility relative to oxygen [124, 129, 130]. In the study by Khunjar et al., nitrite was reduced electrochemically to ammonia in the first reactor, which was then used by ammonia-oxidizing bacteria *N. europaea* as a low cost, abundant, safe, and soluble redox mediator to facilitate the energy transfer into biomass [131].

Having the characteristics of effective and efficient nitrogen recovery makes BESs more advantageous over some existing technologies in meeting the stringent regulations of waste treatment. However, compared to nitrogen removal, nitrogen recovery in BESs may be more suitable for treating concentrated wastes including sludge, landfill leachate, animal wastes, and others containing a large amount of ammonia [110].

1.5 Key Factors for Improving MES Performance

Powering BES with electricity for bioelectrosynthesis from solar cells is a potent strategy for storing the sun energy into the chemical bonds of multicarbon compounds [8, 13]. However, the main obstacle for the development of MES as an economically viable technology is still the microbial reduction rate, stability, and long-term efficiency of CO_2 to multicarbon compounds in scalable BES reactors. Optimizing and scaling MES to practical applications relies on performance improvements while maintaining low costs. Enhancing microbial catalysts and electrochemical hardware and characterizing the electron transfer mechanisms from cathode to microbe make a lot of sense regarding efficiency, scalability, system lifetimes, and reliability for bioelectrosynthesis. The following sections will discuss the different options for electron transfer from the cathode toward microorganisms and the progress about electrochemical hardware.

1.5.1 Electron Transfer from the Cathode to the Cell

Before assessing how microorganisms deal with electrical current, it is important to understanding the electron transfer mechanisms involved in MES. It is commonly believed that EET, including direct and indirect EET, from electrodes to microorganisms is the key driver for the electrocatalysis conversion of carbon dioxide involved in the MES process [12, 30, 44, 54, 55]. Considering these differences in potentials, it is likely that different pathways exist for electron uptake in microorganisms (Figure 1.9). The first means of cathodic EET is through H_2. This gas can readily be produced at cathodes and can serve as a driver for microbial metabolism without an apparent negative effect on microbial integrity. This fact and the versatile range of products that can be formed when microbial metabolism is driven by H_2 make this approach a good first stepping stone toward electricity-driven bioproduction of chemicals such as methane. However, the shortcomings of H_2 as a driver of microbial metabolism are the low solubility and high overpotential at noncatalyzed electrodes. Therefore, it is recommended to circumvent H_2 for effective and

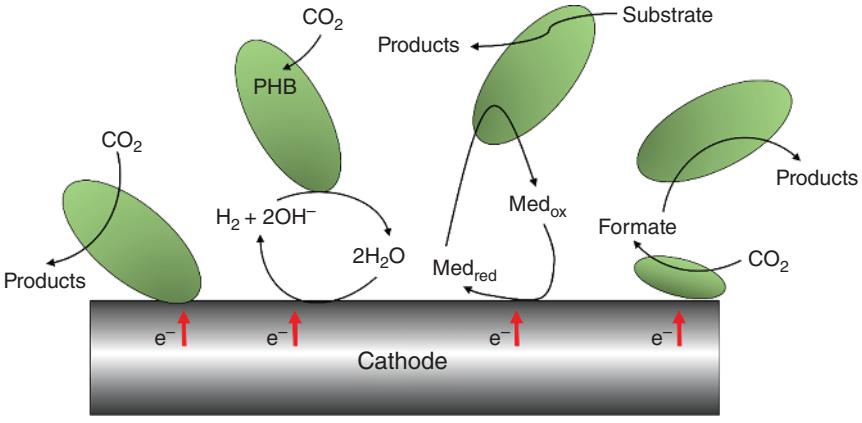

Figure 1.9 Mechanisms for electron transfer from electrodes to microorganisms [12]. The direct route of electron transfer (far left) seems the most attractive, but it is currently only speculative. The production of H_2 for subsequent microbial conversion (middle left) and the use of mediators (Medred and Medox for reduced and oxidized mediator, respectively; middle right) (for example, methyl viologen) are more established. Finally, the production of intermediate building blocks such as formate (far right) has been shown to be useful for single enzymes and needs to be demonstrated with whole microorganisms. PHB, poly-β-hydroxybutyrate. Source: Rabaey and Rozendal 2010 [12]. Reproduced with permission of Springer Nature.

safe cathodic bioproduction. The second method of cathodic EET is through electron shuttles, e.g. anthraquinone-2,6-disulfonate (AQDS), MV, and neutral red, which can provide an effective conduit for electrons toward a microorganism during cathodic EET. They can be dissolved at a higher concentration than H_2, decrease the overpotential at the electrode, and be reused many times, even in a large reactor, but their disadvantages of shuttles include their limited stability, possible toxic effects on the microorganisms, and their loss in flow-through systems. The third, perhaps the most attractive means of achieving EET from cathodes is through direct biocatalysis when high cathode potentials, limited or no H_2 production, and biofilm-based activity were recorded. Last, rather than achieving direct production based on electrical current, an intermediary microorganism or biocatalyst could be used to produce an initial building block, such as formate or acetate, from CO_2. Such building blocks are subsequently used by other microorganisms for the production of larger molecules.

1.5.2 Cathode Materials

Enhancement of bacterial attachment, biofilm development, electron transfer rate at the cathode surface (microorganism–electrode interaction), and chemical production rate for bioelectrosynthesis will require optimization of several key factors, particularly cathode materials [18]. The improved electrode material must be scalable, highly conductive, and cost effective.

Until now, most of biocompatible cathodes of MES relied mainly on carbonaceous materials such as graphite, but the most efficient cathode material to date for electron transfer is SS with current drawn as high as 30 A/m^2 with *G. sulfurreducens* as the microbial catalyst [132]. Conductive materials that will self-assemble in the cathodic biofilm are an alternative to improve electron transfer in BESs. A *Sh. oneidensis* biofilm assembled with embodied graphene oxide could uptake electrons 74 times more efficiently [55]. Compared to other carbonaceous cathodes, granular graphite is the high specific area for bacterial adhesion, which can produce acetate from a mixed community catalyst reaching 51.6 mM/d or 3.0 g/l/d [76].

On the other side, some cathode materials were treated or coated with other materials resulting in modifications of their surface for enhancing chemical production in MES. For example, Lovley et al. altered the surface chemistry of carbon cloth by the immobilization of positively charged molecules, which increased in both current density and acetate production rate, compared to untreated carbon cloth [17, 18]. Carbon nanotube (CNT)-based electrode materials have become extremely attractive for application in BESs because of large aspect ratios, high surface area, an exceptional electric conductivity along their length, and allowing for bacteria immobilization and proliferation [133]. It has been reported that CNT ink deposition on cotton and polyester fabrics also yielded biocathodes with up to three times higher current density (0.021 mA/cm^2) and acetate MES rates (0.010 $mM/d/cm^2$) than carbon cloth controls [18]. Jourdin et al. coated CNTs on reticulated vitreous carbon (NanoWeb-RVC) with high surface-to-volume ratio, which enhanced bacterial adhesion and effective mass transfer within the electrode–biofilm superstructure [99]. Moreover, electrophoretic deposition (EPD), one of the most efficient methods of generating thin films from colloidal suspensions, has been extensively used in the deposition of CNT to form highly porous electrodes for biocathodes of electrochemical applications. It has been used for producing biocathode electrode on a MES process, achieving a high acetic acid production rate of up to 685 $g/m^2/d$ from CO_2 [100].

A number of approaches that can improve microbe–electrode electron exchange have been identified in the studies of anode material studies for biosensors and MFCs [18]. For instance, a positive charge at the electrode surface, established with ammonia gas treatment, chitosan, cyanuric chloride, 3-aminopropyltriethoxysilane, melamine, or PANI, has the potential of leading to better electron transfer. Thin layers of metal catalysts, such as Au, Pd, or Ni, can reduce the activation energy threshold of electron transfer from electrodes to bacteria. Fabrics coated with CNTs offer an open, three-dimensional, conductive matrix for microbial growth. The increased knowledge about electron transfer between electrode and microorganisms and the characteristics of different electrode materials gained over the past few years and the several decades of more empirical use of electrical current and microorganisms are driving rapid development of bioelectrosynthesis in this area. Given that the mechanisms for electron transfer from electrodes to microbes are still poorly understood, the approaches to improve cathode design are likely to be largely empirical, but still potentially productive.

1.6 Summary

Bioelectrosynthesis is a promising strategy for the microbial conversion of carbon dioxide and other organic feedstocks to transportation fuels and other organic commodities. In addition to the electricity-driven bioproduction of hydrogen, methane, alcohols, short-chain organic acids, ammonia, etc., BESs can also be used for the bioelectrosynthesis of some other higher value compounds. For example, a kinetically enhanced process has been established to convert 6-bromo-2-tetralone to 6-bromo-2-tetralol, which is an intermediate in the synthesis of the potassium channel blocker MK-0499 (a chiral drug candidate) [134]. For such higher value compounds, considering that the resource cost (in energy and chemicals) is typically a small fraction of the production cost, it remains to be seen whether redox control or electron supply with a cathode is sufficiently attractive compared with the existing approaches. Even so, like BES, the systems designed for the bioelectrosynthesis of valuable products also need to address the challenges such as system scaling up, understanding of microbiological processes, demonstration of long-term operation and stability, capital investment and operational cost, and better assessment of economic and environmental benefits of using those systems (e.g. life cycle analysis).

Overall, bioelectrosynthesis has the potential to become a key process in future bioproduction. The several decades of more empirical use of electrical current and microorganisms are driving rapid development in this area of BES. As more and more fuels and chemicals can be produced from CO_2 or basic organics based on the principle of MES, a broad opportunity exists for the development of bioelectrosynthesis from a scientific phenomenon into a technical process.

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