Bioelectrochemistry 106 (2015) 88–96

Contents lists available at ScienceDirect

Bioelectrochemistry

journal homepage: www.elsevier.com/locate/bioelechem

All ecosystems potentially host electrogenic bacteria

Nicolas Chabert, Oulfat Amin Ali, Wafa Achouak *

[a] CEA, DSV, IBEB, Lab of Microbial Ecology of the Rhizosphere & Extreme Environment (LEMiRE), 13108 Saint Paul-Lez-Durance, France
[b] CNRS, BVME UMR 7265, ECCOREV FR 3098, 13108 Saint Paul-Lez-Durance, France
[c] Aix Marseille Université, 13284 Marseille Cedex 07, France

ARTICLE INFO

Article history:
Received 29 January 2015
Received in revised form 9 July 2015
Accepted 9 July 2015
Available online 26 July 2015

Keywords:
Biofilm composition
Exocellular electron transfer
Microbial fuel cells
Inocula source
Molecular tools
Imagery

ABSTRACT

Instead of requiring metal catalysts, MFCs utilize bacteria that oxidize organic matter and either transfer electrons to the anode or take electrons from the cathode. These devices are thus based on a wide microbial diversity that can convert a large array of organic matter components into sustainable and renewable energy. A wide variety of explored environments were found to host electrogenic bacteria, including extreme environments. In the present review, we describe how different ecosystems host electrogenic bacteria, as well as the physicochemical, electrochemical and biological parameters that control the currents from MFCs. We also report how using new molecular techniques allowed characterization of electrochemical biofilms and identification of potentially new electrogenic species. Finally we discuss these findings in the context of future research directions.

Contents

1. Introduction

In recent years, industrialization and the global economic system have led to the overexploitation of fossil fuels, especially oil and gas. Indeed, shortage of these latter products has resulted in a global energy crisis warning [1–3]. Alternative green energy has attracted great attention for new means of electricity production, including by

* Corresponding author at: CEA, DSV, IBEB, Lab of Microbial Ecology of the Rhizosphere & Extreme Environment (LEMiRE), 13108 Saint Paul-Lez-Durance, France.
E-mail address: wafa.achouak@cea.fr (W. Achouak).

http://dx.doi.org/10.1016/j.bioelechem.2015.07.004
1567-5394/

microorganisms [4,5]. One promising yet challenging emerging technology uses microbial fuel cells (MFCs), in which microorganisms generate electricity by exchanging electrons with electrodes while oxidizing organic or inorganic matter [6,7]. This principle makes use of the fact that bacterial exocellular electron transfer plays an important role in anaerobic microbial communities that degrade organic matter and those that use insoluble electron acceptors (such as iron- and manganese-oxide) for growth [8,9].

The ability of microorganisms to produce electricity was demonstrated at least one hundred years ago by immersing a platinum electrode in a suspension of *Escherichia coli* and *Saccharomyces* [10]. However, a greater interest in this phenomenon only arrived several decades later, when anaerobic bacteria such as *Clostridium butyricum* were used to enhance current density and power output [11]. During this same period, the first fuel cell was conceived with two chambers (one anodic and one cathodic) separated by an ion exchange membrane [12]. Since then, the design of MFCs has evolved, and electrical current output now reaches 2.87 kW m^{-3} [13]. While most prokaryotes can potentially generate electricity [1,14,15], only a few bacteria have been highlighted to form electrochemically active biofilm (EAB) to date. EAB is a generic term used to designate biofilm that are able to transfer electrons towards a final electron acceptor (such as electrodes in a MFC system), thus acting as the catalyst for redox reactions. Different pathways are currently known to be involved in this electron transfer. These species will be detailed later in this review.

Although biofilm construction is rapid and highly durable, EAB diversity can be highly variable depending on culture conditions (which can favor certain bacterial populations), which can therefore modulate electricity production. For example, Logan and Regan [16] reported that power production could vary from <1 $mW \cdot m^{-2}$ to >1500 $mW \cdot m^{-2}$ on the basis of different MFC architectures that use oxygen as the final electron acceptor. This huge variability could be explained by the different existing MFC types that produce more or less current, since the design of the MFC system affects power generation [17].

Different environments have been explored in an attempt to understand the diversity of microorganisms involved in this exocellular electron transfer. Many different types of environments harbor EAB, including anaerobic sludge from treatment plants, anaerobic sediment, and even soil. Although it is supposed that bacteria may belong to the rare biosphere, they may dominate when electrode are in contact with sample [18,19]. Since one of the most promising applications of MFC could be the treatment of wastewater, many efforts have been targeted at wastewater treatment plants, paper mill effluents, etc. [20–24].

The focus of this review is on environments that host EAB, the principle communities of these ecosystems, and their electrogenic potential. Ecosystems that host EAB and the optimal conditions for growth and electron transfer will be described in detail.

2. Electrogenic microorganisms

Many diverse electroactive microorganisms have been studied to date in an effort to improve the energy production of MFCs. An inventory of enrichment cultures as well as pure strains known to be involved in MFCs was made until late 2008 [15,25].

Different EAB communities can interact as consortia and generate energy. This is generally what characterizes natural ecosystems such as wastewater, river, rice field soils or compost [22,26–31]. Identification of single electrochemically active species in these natural environments has been performed with type strains that correspond to predominant species in wild EA biofilms. The few strains of bacteria directly isolated from EAB have displayed a higher electrochemical performance than their type strains [25,27]. For example, *Ochrobactrum anthropi* YZ-1, a strain isolated from EAB originally obtained in a primary clarifier overflow from a wastewater treatment plant, produced 89 $mW \cdot m^{-2}$. This value is two-fold higher than its type strain [32]. The comparison of electroactivity between pure cultures and microbial consortia in wastewater reveals a greater power density with higher columbic efficiency for the consortia [15,33].

3. Do all ecosystems host electrogenic bacteria?

The presence of diverse EAB raises the question of which environments are the most electrogenic. Although most electrical current studies have focused on effluent from diverse wastewater treatment facilities, EAB appear to be widely distributed as suggested by studies of different environmental types (Table. 1) [34]. Many soil and aquatic environments have been tested over the years, complicating the ability to address an exhaustive list. Furthermore, none of these studied environments have been tested under the same MFC conditions. Instead, many studies have focused on optimization and progress towards improving MFCs, even if accurate comparisons of natural inoculums and their electrical performances are lacking. Therefore, even if these advances are highly useful to future MFC commercialization, a basic understanding of MFC biology and electrochemistry is still necessary.

3.1. Natural environments

Various aquatic natural environments have been investigated (Table. 1), and one river with phototrophic biofilm has been reported to have a current of 3.7 $A \cdot m^{-2}$ [35]. River offers further possibilities such as sediment that could perform from about 0.2 to 0.3 $A \cdot m^{-2}$ [36–38]. Environments that assure the transition between continental and marine environments can lead to power production. Mangrove sediment is naturally rich in organic matter due to tides and rich forest litter [39], resulting in a potential for energy output as high as 12 $A \cdot m^{-2}$ [34, 40]. Beach sediments that play the same role as mangroves produce a current density with values ranging from 0.8 to 8.9 $A \cdot m^{-2}$ [26,34]. Even tidal mud has a slight potential to produce current [41]. Sampling direct microbial biofilm from salt marsh [34,42] or marine sediment [26] provides current densities from approximately 4.45 to 85 and 2 $A \cdot m^{-2}$, respectively. Two types of MFC can be employed in marine and salt marsh sediment: a traditional MFC, in which sediment is sampled and serves as the inoculum for a reactor; and a benthic MFC (BMFC), in which two electrodes are placed in situ (the anode is placed under the sediment and the cathode is floating). The BMFC design could be applied to any type of environment, but it is more often used in marine environments, since they could be an energy source for different autonomous oceanographic or environmental sensors [43–45].

Microbial fuel cells have been extensively exploited in aquatic environments such as marine sediments or wastewater [27,31]. Terrestrial environments have been comparatively underexploited despite they hold a high diversity of microorganisms and a wide variety of organic and/or inorganic matters widespread [27,34,46].

Soils that support plant growth (Table. 1) are naturally rich in nutrients (e.g. carbohydrates, amino acids, aliphatic acids, enzymes, vitamins…) and should correspond to an electrogenic environment [46]. Indeed, plants produce organic acids such as acetate that are known to induce a high power density and enrich EAB [47,48]. For example, one previous study demonstrated the potential of rice paddy fields to produce a current density reaching approximately 0.1 $A \cdot m^{-2}$ [49]. Soils with plants are not the only terrestrial ecosystem that can host EAB; rich soils such as compost can also offer a promising host environment for electricity production. Compared to ordinary soil, compost is more enriched in organic matter; this could increase bacterial activity and thus the potential to produce electricity using a MFC system. Composts can display current production up to four times greater than natural soil [29]. This confirms that composition and richness of organic matter greatly affect MFC potential. Garden compost is another good source of organic matter and EAB for MFC, as it displays a current production (1.5 $A \cdot m^{-2}$) on the same order as industrial composts (1.1 $A \cdot m^{-2}$) [29,50]. Anaerobic soils produce a good electrical current density

Table 1
Electrochemical activity of various environments.

Environments	MFC architecture	Natural or enriched samples	Poised potential (V)	Carbon sources	Electrode materials	Performances ($A\cdot m^{-2}$)	References
Aquatic							
Anaerobic lake sediment	BMFC[a]	Natural	–	Acetate	Carbon fiber brush	0.125	[74]
Deep-ocean coldseep	BMFC	Natural	0.3	–	Graphite rod	0.085	[71]
Volcanic lake	BMFC	Natural	–	Acetate	Carbon paper	0.2	[70]
Anoxic marine marsh sediment	Single chamber MFC[b]	Enriched	0.5	Acetate	Graphite	0.2	[69]
Beach sand	Single chamber MFC	Natural	−0.1	Acetate	Graphite plate	0.6	[26]
Mangrove	Single chamber MFC	Enriched	−0.3	Acetate	Cylindrical graphite rod	10.27	[34]
Natural marine biofilm	Single chamber MFC	Natural	−0.1	Acetate	Graphite plate	2.6	[26]
Saline microbial mat	Single chamber MFC	Enriched	−0.3	Acetate	Cylindrical graphite rod	4.45	[34]
Salt marsh	Single chamber MFC	Natural	0.1	Acetate	Carbon felt	85	[42]
Acidic river	SMFC[c]	Natural	–	–	Graphite	3.5	[28]
Aquaculture pond sediment	SMFC	Natural	–	Cellulose	Graphite plate	0.02	[60]
Freshwater aquaculture sediment	SMFC	Natural	–	–	Graphite plate	0.03	[59]
Acid hydrometallurgical mining process waters	Two chamber MFC	Enriched	–	–	Graphite plate	0.433	[67]
River sediment	Two chamber MFC	Natural	–	Glucose	Carbon paper	3.5	[36]
Sea sediment	Two chamber MFC	Natural	–	Glucose	Carbon paper	2.8	[36]
Terrestrial							
Hydrocarbon contaminated soils	Air cathode MFC	Natural	0.25	Phenol	Carbon felt	0.18	[22]
Anaerobic soils	Single chamber MFC	Enriched	−0.3	Acetate	Cylindrical graphite rod	9.15	[34]
Garden compost leachate	Single chamber MFC	Natural	0.1	Acetate	Carbon felt	9.9	[27]
Iron rich soils	Single chamber MFC	Enriched	−0.3	Acetate	Cylindrical graphite rod	2.33	[34]
Living (rice) plant (rhizodeposit)	SMFC	Natural	–	Hogland's hydroponics solution	Graphite granules	0.12	[46]
Silt-rich soil	Two chamber MFC	Enriched	–	Lactate	Graphite felt	0.375	[75]

[a] Benthic microbial fuel cell.
[b] Microbial fuel cell.
[c] Sediment microbial fuel cell.

ranging from 8.6 to 9.2 $A\cdot m^{-2}$ [20]. Less typical soils also offer promise for MFC technologies. For example, iron-rich soil induces a current density around 2.3 $A\cdot m^{-2}$ [34].

3.2. Anthropogenic environments

Since effluents are organically rich and could potentially host EAB, many studies have been conducted to develop devices in which wastewater and organic wastes are converted into energy by living microorganisms (Table. 1). The Kraft pulp mill effluent can reach a current density of about 5.1 $A\cdot m^{-2}$ [51], while the highest current recorded using wastewater was ranging from 200 to 300 $A\cdot m^{-2}$ [52]. Since MFC technology has a great potential to decrease organic content and simultaneously produce energy, diverse industrial wastewater sources have been investigated including potato wastewater [53], municipal wastewater [54,55], cassava mill wastewater [56], and even domestic wastewater treatment plants [57]. Current between different wastewater effluents can range from 0.01 $A\cdot m^{-2}$ for brewery and bakery wastewater up to more than 0.1 $A\cdot m^{-2}$ for paper wastewater [58]. As in situ MFC offers a great possibility to enhance bioremediation; other organically rich environments have been investigated. The better example is aquaculture pond (Table 1), where although, the current production is relatively low (0.02–0.03 $A\cdot m^{-2}$), average chemical oxygen demand (COD) and total nitrogen (TN) removal efficiencies of 80.6 ± 0.3% and 83.0 ± 0.01% were obtained [59–61]. Nonetheless, groundwaters are also man-contaminated environments that had been tested for electricity efficiency and bioremediation using MFC. With hydrocarbon contamination, MFC produce up to 0.6 $A\cdot m^{-2}$ [62] while MFC use for nitrate removal could reach up to 1 $A\cdot m^{-2}$ [63].

As suggested by Dunaj et al. [64], MFC performance and EAB diversity depend strongly on soil composition and the availability of organic matter. MFCs have been shown as efficient technology combining electricity generation and treatment of recalcitrant compounds such as petroleum hydrocarbons [22,62,65]. For instance, a MFC inserted into a waterlogged soil contaminated with hydrocarbons (Table. 1) by simultaneous biodegradation of 27.6% hydrocarbon, 90% phenol removal and generation of 29.45 mW m^{-2} power densities [22].

By contrast, organic contaminated soils are limited to about 0.1 $A\cdot m^{-2}$ [22,66], although they perform extremely well in remediation with the MFC system.

3.3. Extreme environments

Some extreme environments can reduce the limitations of MFC, such as the pH gradient between the anode and cathode compartments (Table. 1). For example, acidic sediments display a current production reaching 3.5 $A\cdot m^{-2}$ that correlates with the organic content of the sediment and its bacterial diversity [28]. Other acidic environment have been found to perform electricity production such as acid hydrometallurgical mining process waters or even aerobic sludge with associated performance about 0.4 and 0.2 $A\cdot m^{-2}$ respectively [67,68]. Thermophilic bacteria showing a growth optimal temperature at 60 °C have also potential for electricity production with *Thermincola carboxydophila* that reach a performance from 0.209 to 0.254 $A \cdot m^{-2}$ [69]. Hydrothermal ecosystems can also be electroactive, including the Furnas Lake ecosystem, which displays a low current density around 0.2 $A \cdot m^{-2}$ [70]. Deep oceans are also a source of EAB. Indeed, Monterey Canyon, composed by cold seeps, had shown electrical performance of 0.1 $A \cdot m^{-2}$ using BMFC [71]. This performance is supported by sulfur and iron cycle with Deltaproteobacteria such as *Desulfuromonas*, *Desulfocapsa* and *Syntrophus*. Other deep-sea vents could potentially host EAB. Even if the natural environment is not directly tested using BMFC for example, the submarine volcano, lacated at Loihi Seamount, host the species *Mariprofondus ferrooxydans* belonging to Zetaproteobacteria [72] that is able to produce about 0.5 $A \cdot m^{-2}$ [73].

There is a broad diversity of potential electrogenic environments to exploit. Nevertheless, electrical current production depends on the characteristics of the sample, especially its organic matter content. The sample origin used for the MFC system is another important factor, since bacterial diversity is variable and can thus affect the production of energy. Indeed, highly conductive environments such as sea ecosystems are generally able to produce greater current density than other types of ecosystems.

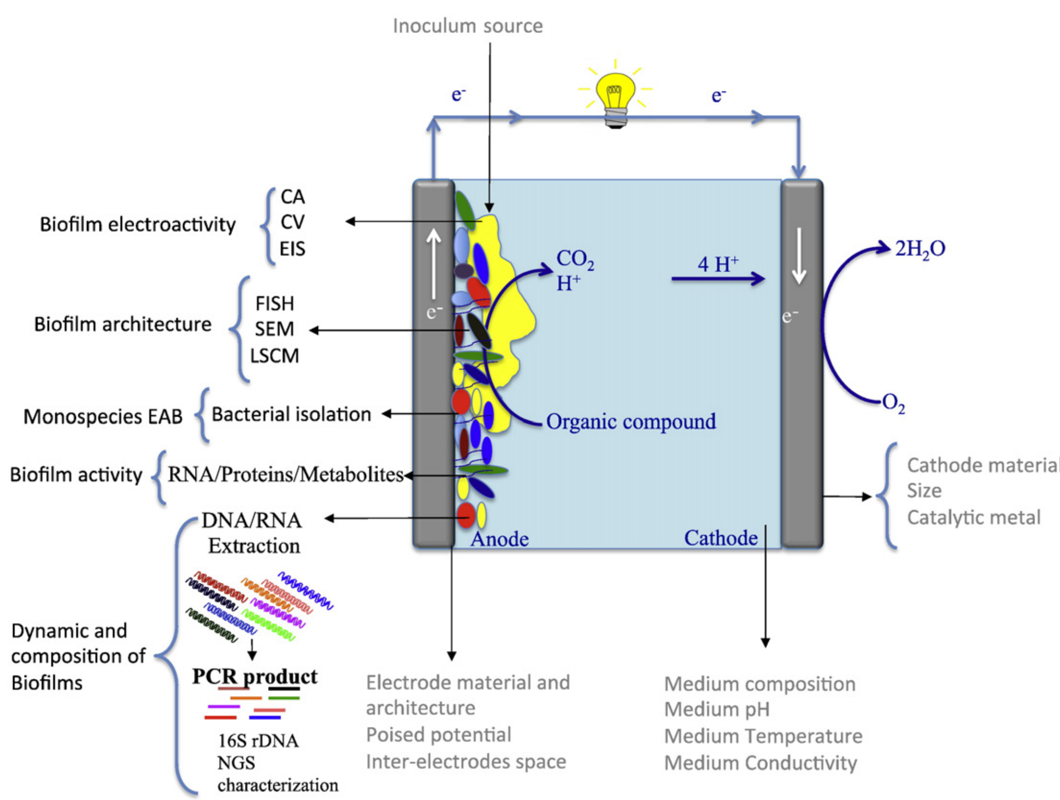
As shown above, EABs seem to be widespread and colonize a huge environmental diversity. Actually, these prokaryotes can be found almost everywhere, like in natural environments (e.g. river, sea, compost) as well as in extreme areas (e.g. acidic ecosytems, deep sea vents). However, more studies are required to highlight EAB diversity. Indeed, there is evidence that extremophile bacteria, such as *A. ferrooxidans*, *M. ferrooxidans* or *T. carboxtdophila* are involved in current production. Thus, extreme environments harboring similar microorganisms seem to be an interesting area to explore (e.g. desert soils, tundra, Arctic water).

Although, numerous environments haven't been explored yet, nonetheless, the diversity of ecosystems investigated to date suggests the important distribution of EABs in probably all kind of ecosystems.

4. Biological and electrochemical characteristics of EAB

Electrical current studies have essentially focused on biofilms associated with the anode, since one of the primary hindrances to current production is the cathode, where a slow oxygen reduction rate occurs. Here, a cathodic biofilm could increase the kinetics. The fact that the diversity of microorganisms involved in current production depends on available carbon sources and the materials employed [76] complicates the comparison of electricity output between different systems. Furthermore, it remains useful to include this characterization in comparative studies as microbial ecology is sensitive to a wide panel of parameters (Scheme 1) such as pH [77–79], temperature [77,80–83], substrate [78,63,84,85] or in the case of MFC, the nature of electrode material and MFC architecture or design [78,83,86–88] and poised potential [17,89–92]. However, a study using similar conditions could permit the comparison of different ecosystems and their associated communities. Miceli et al. [34] reported that the microbial community differs according to the inoculum used, thus indicating that EAB differ depending on various sample location parameters.

One interesting approach to optimize current output would be to mature EAB through successive enrichments of the dominant EAB community, resulting in a higher current output [93]. Finally, it appears that whichever community is involved in power generation depends on a wide range of factors, particularly sampling location [34,93].



Scheme 1. A schematic of microbial fuel cell. In blue characters are indicated the main reaction occurring within MFC and producing electricity. Microbial electricity generation in these systems is dependent on microbial inoculum source, the biofilm community composition, their spatial organization within the biofilm, the nature of electron donors, the structure and composition of electrodes materials, the imposed potential…In black characters are indicated electrochemical and microbiological analyses that can be performed. CA, chronoamperometry; CV, cyclic voltammetry; EIS, electrochemical impedance spectroscopy; FISH, fluorescent in situ hybridization; SEM, scanning electro microscopy; LSCM, laser scanning confocal microscopy; NGS, new generation sequencing. In gray characters are indicated parameters that may be modulated to improve electrical performance.

4.1. Structure and function of electrogenic communities of EABs

4.1.1. Molecular and imagery tools

Presuming that bacteria within electroactive biofilms play direct or indirect functional roles in the current generation, their identification is required, as well as that of the inoculum to determine the ability of electrodes to select certain bacterial populations from the reservoir. Using new molecular techniques, scientists are discovering new electrogenic bacteria. The structure of EABs may be determined by using fingerprinting techniques like denaturing gradient gel electrophoresis fingerprinting (DGGE) [36,68,94,95], terminal restriction fragment length polymorphism (T-RFLP) [96] or single strand conformation polymorphism (SSCP) [42]. Fingerprinting is a useful technique that can allow to quickly obtain an overview of the diversity within and between samples, but does not allow identification of rare biosphere and non-dominant organisms. For this technique, identification must be performed by sequencing after DGGE analysis and band-cutting. In the case of DGGE technique, identification must be performed by sequencing after DGGE analysis and band-cutting. The ribosomal RNA sequencing by high throughput metagenomic approach using new generation sequencing (NGS) technics is a powerful new tool that allows microbiologists to improve the accuracy of biofilm diversity identification [97–101] and to construct bacterial phylogenies (Scheme 1). The functional diversity might be performed by targeting a specific functional group such as denitrifying bacteria [102]. The 16S rRNA phylogenetic information might be used to set up growth conditions for bacterial isolation from EABs by adapting growth conditions of the close related known species. Furthermore, using phylogenetic information to construct highly specific ribosomal RNA probes as a means of identifying and tracking specific microorganisms within electroactive biofilms by fluorescence in situ hybridization (FISH) to visualize targeted bacteria distribution.

Characterization of biofilm architecture may be imaged by different microscopic technics (Scheme 1), such as laser scanning confocal microscopy (LSCM) mainly used for complex EABs by adding a green fluorescent nucleic acid stain to stain Gram-positive and Gram-negative bacteria, or scanning electron microscopy (SEM), transmission electron microscopy (TEM) or atomic force microscopy (AFM) (Scheme 1). LSCM coupled to FISH technic allows to visualize the distribution of targeted populations by using specific probes in a three dimensional structure [103–106]. Raman microscopy could also be used as a noninvasive approach for in situ observation [107]. Scanning electron microscopy with energy dispersive X-ray spectroscopy (*SEM/EDX*) is nice surface analysis technique providing information about chemical elements present into the biofilm structure and on its surface. This approach requires, nonetheless a prerequisite sample treatment [108–111]. Comparatively, AFM, doesn't demand any preliminary preparation of the biofilm to be observed. This advanced method shows adhesion force of cells with the surface they colonize. Moreover, its high resolution enabling observation at micrometric scales [112] could be exploited for microbial fuel cell, for providing interesting information on electron transfer mode. TEM, a well known microscopic technic, is mainly used to characterize electrode surface material in MFC [113–116] or bacterial structure such as pilli [117].

Other technics are used to characterize biofilms at physiological level as shown in Scheme 1, by profiling gene expression by transcriptomic approach [117,118] or identifying proteins by proteomics or by identifying all produced metabolites by the EAB by metabolomics analysis.

Using these technics to study bacterial physiology of electrochemically active biofilms made by single species or a mixture of species has exciting potential in the comprehension of MFC functioning and in the improvement of current production. These technics should improve our understanding of bacterial dynamics within MFCs and show that under an imposed potential certain communities are mostly enriched while others disappear and certain functions might be more expressed.

4.1.2. Electrochemical parameters

Biofilm formation and power output are directly affected by electrochemical parameters. First, the type of circuit used can alter the microbial community structure. In an open circuit, bacterial diversity is primarily influenced by the anode materials [30], whereas in a closed-circuit configuration, EAB growth is induced by power generation [17]. In one experiment, microbial anodes formed from compost leachate on carbon cloth electrodes were kept in an open circuit, and then the anodes were polarized at −0.2 V/SCE. By delaying the polarization, current production reached 9.4 $A \cdot m^{-2}$ after only 3–9 days of polarization, whereas the anodes that were polarized from the beginning attained 6–8 $A \cdot m^{-2}$ within 36 days [31]. The authors attributed these differences to the biofilm architecture, in which a thinner and more heterogeneous biofilm delayed polarization as compared to the thick and homogeneous biofilm resulting in full polarization.

There is no systematic rule to determine whether the power output will be better under an open- or closed-circuit operation, based on the inoculum source and the electrode material. Furthermore, in a closed-circuit, external resistance affects both the power output and biofilm growth. Thus, power output increases as external resistance decreases, due to a decrease in the internal resistance within the biofilm [119]. However, a very low external resistance promotes better biofilm growth and thus reduces electron transfer with the anode. The resistance within a MFC system is also linked to the space between the electrodes. A large space between the anode and the cathode yields a large resistance within the system; conversely, decreasing this space lowers the resistance and increases the power output [82]. The anode potential also affects power output. Biofilms grow rapidly at low anode potentials, with a large abundance of EAB such as *Geobacter sulfureducens*; in contrast, biofilms are more diverse and produce less electricity at higher potentials [52]. Electron transfer through the extracellular matrix towards the anode is dependent on the electron concentration on the anode, which may also directly affect EAB physiology.

4.2. Electroactive biofilm composition

Aquatic environments host a wide microbial diversity and are thus a potential reservoir for EAB. *Geobacter* sp. and *Shewanella* sp. are generally found, although species from Proteobacteria, Bacteroidetes, Chloroflexi and Cyanobacteria are also present [70,120]. Proteobacteria members typically dominate within biofilms. Among this group, *Geobacter* sp. is the most dominant and frequently identified EAB. In some cases where *Geobacter* sp. is absent from biofilms it can be replaced by other iron- and sulfate-reducing bacteria [111]. In raw paper mill effluent, *Geobacter* is not found, whereas *Desulfuromonas* is the predominant species [122]. Two acidophilic species from acidic sediments, *Acidithiobacillus ferrooxidans* and *Acidiphilum* spp., have been reported to predominate within biofilms on the anode [28]. Wastewaters have been extensively studied, and there is strong evidence that bacteria involved as sulfate-reducing bacteria in the sulfur cycle can play an important role in wastewater treatment using MFC technology [54,57,123,124].

Marine biofilm diversity has also been investigated as a source of EAB, since the conductive property of marine environments suggests the potential for current output. Sediment MFCs are essentially exploited in this case. Sampling biofilms directly from seawater yields a large diversity of species mainly dominated by Bacteroidetes, *Halomonas* and *Marinobacterium*; this diversity offers a higher power production than can be obtained from sediments sampled nearby, which are essentially composed of *Mesoflavibacter* [26]. In salt marshes, the composition of microbial communities associated with the anode is dominated by *Marinobacter* and *Desulfuromonas* [42]. These authors noted that the bioanode performance was decreased at the highest salinity (60 $g \cdot L^{-1}$), as well as the proportion of *Desulfuromonas* spp. relative to *Marinobacter* spp., suggesting that electron transfer to the anode is mainly performed by *Desulfuromonas*. Although the same inoculum is used, the physico-chemical parameters may favor different bacterial populations and/or alter their activity. Deltaproteobacteria dominate in high-salt content mangrove sediments, with a current output of 4.3 $A \cdot m^{-2}$. By contrast, a greater diversity of EAB that includes Gammaproteobacteria, Alphaproteobacteria and Clostridia is observed in low-salt content mangrove sediments, producing 10.77 $A \cdot m^{-2}$. These findings suggest the occurrence of interactions like syntrophy, which could favor current production [34].

Terrestrial ecosystems produce current when electrochemical systems are applied. During MFC operation, different communities are enriched depending on what type of soil is used. Generally, Proteobacteria dominate on the anode biofilm, particularly the Deltaproteobacteria, represented by *Geobacter* spp. [27,54,55,64]. There is evidence from hydrocarbon-polluted soil that some Betaproteobacteria are involved in both current output and hydrocarbon remediation [66].

When *Geobacter* spp. are absent or in low abundance, Clostridia appear to dominate on the anode biofilm [34,64,125]. Since a large part of Clostridia enrichment appears to be related to *Clostridium* [125], the nitrogen cycle (and N_2-fixing bacteria) could play a key role in anaerobic current production. Therefore, it is possible that N_2-fixing bacteria could operate alone by oxidizing the substrate through N_2-fixation; alternatively, they could act in syntrophy by fermenting the substrates.

Plant roots, such as those in rice paddy fields, host an important diversity of bacteria involved in electrical performance. Deltaproteobacteria and Clostridia are reported to dominate when agricultural soil is used in MFCs and fed with glucose [64]. When electrodes are introduced in rice paddy fields, a wide diversity of Deltaproteobacteria (*Geobacter* spp., *Myxococcus*, *Deferrisoma*, and *Desulfobulbus*) and Alphaproteobacteria (*Rhizobiales*) dominate with the presence of Archaea from the Crenarchaeota group [30,126]. Here again, there is strong evidence that iron-, nitrogen- and sulfate-dependent bacteria play a key role in the anode-associated community. Acidobacteria such as *Geothrix* can also be enriched [49]. When rice paddy field soil is supplemented with cellulose, the community is dominated by rod-shaped cells affiliated with Rhizobiales and possessing filamentous appendages [126].

Aquatic environments host a wide microbial diversity and are thus a potential reservoir for EAB. *Geobacter* sp. and *Shewanella* sp. are generally found, although species from Proteobacteria, Bacteroidetes, Chloroflexi and Cyanobacteria are also present [70,120]. Proteobacteria members typically dominate within biofilms. Among this group, *Geobacter* sp. is the most dominant and frequently identified EAB. In some cases where *Geobacter* sp. is absent from biofilms it can be replaced by other iron- and sulfate-reducing bacteria [121]. In raw paper mill effluent, *Geobacter* is not found, whereas *Desulfuromonas* is the predominant species [122]. Two acidophilic species from acidic sediments, *A. ferrooxidans* and *Acidiphilum* spp., have been reported to predominate within biofilms on the anode [28]. Wastewaters have been extensively studied, and there is strong evidence that bacteria involved as sulfate-reducing bacteria in the sulfur cycle can play an important role in wastewater treatment using MFC technology [121,127,128].

Marine biofilm diversity has also been investigated as a source of EAB, since the conductive property of marine environments suggests the potential for current output. Sediment MFCs are essentially exploited in this case. Sampling biofilms directly from seawater yields a large diversity of species mainly dominated by Bacteroidetes, *Halomonas* and *Marinobacterium*; this diversity offers a higher power production than can be obtained from sediments sampled nearby, which are essentially composed of *Mesoflavibacter* [26]. In salt marshes, the composition of microbial communities associated with the anode is dominated by *Marinobacter* and *Desulfuromonas* [42]. These authors noted that the bioanode performance was decreased at the highest salinity (60 $g \cdot L^{-1}$), as well as the proportion of *Desulfuromonas* spp. relative to *Marinobacter* spp., suggesting that electron transfer to the anode is mainly performed by *Desulfuromonas*. Although the same inoculum is

used, the physico-chemical parameters may favor different bacterial populations and/or alter their activity. Deltaproteobacteria dominate in high-salt content mangrove sediments, with a current output of 4.3 $A \cdot m^{-2}$. By contrast, a greater diversity of EAB that includes Gammaproteobacteria, Alphaproteobacteria and Clostridia is observed in low-salt content mangrove sediments, producing 10.77 $A \cdot m^{-2}$. These findings suggest the occurrence of interactions like syntrophy, which could favor current production [34].

Aerobic bacteria that catalyze the reduction of oxygen at the cathodes can form a biocathode. The inoculum can be derived from different sources including bacteria present in the anode inoculum within a membrane-less single chamber MFC, from the air, and even from water. Mono- or multispecies biofilms can also be constructed at the cathode.

Biofilms at the cathode are less studied than those formed at the anode, even though the same trend is observed. Indeed, communities associated with the electrode will evolve depending on electrochemical sets such as circuit type [17] and electrode material [35] or environmental sets [129]. A limited number of bacterial populations may be specifically enriched on the cathode [130], although the opposite trend has also been reported [131]. This difference can be explained by the inoculum source, the substrate, and even the cathode material. The main concern with these studies is their lack of replicates and reproducibility. Biocathodes are mainly colonized by aerobic bacteria, which catalyze oxygen reduction. High-throughput sequencing techniques have demonstrated that Proteobacteria (including the three classes Alpha-, Beta- and Gammaproteobacteria) and Bacteroidetes dominate on cathodic biofilms [17,35,128–131]. Other studies have reported the enrichment of *Rhodobacter* and *Hydrogenophaga* [17], and the dominance of *Desulfobulbus*, *Comamonas* and *Desulfovibrio* [129]. Interestingly, the latter study reported that *Desulfobulbus* and *Desulfovibrio* were also enriched within the anodic biofilm, suggesting that sulfate-reducing bacteria can play a role in both the anode and cathode compartments. Bacteria such as *Nitrospira*, *Nitrobacter* or *Nitrosomonas* could also be found in denitrifying biocathode [132,133].

5. Extracellular electron transfer pathways

Monospecific EA-biofilms have been extensively investigated to decipher their electron transfer mechanisms. The thorough examination of electron transfer over the years has led to numerous discoveries of how microorganisms exchange electrons with extracellular insoluble electron donors and acceptors [134]. Briefly, electrons can be exchanged between microorganisms and electrode surfaces via three different pathways: (i) direct electron transfer (DET), (ii) mediated electron transfer (MET), and (iii) indirect electron transfer (IET).

In DET, electrons can be transferred directly to the electrode surface by cell membrane-bound c-type cytochromes composed of multiheme proteins [15,134,135]. DET can also occur through electrically conductive pili or "nanowires" [135,136]. These two pathways have been reported in pure cultures of *Geobacter sulfurreducens* and *Shewanella oneidensis* [15,136,137].

In MET, redox mediators are involved in electron transfer. They can be naturally secreted by microorganisms, or they can be artificial molecules added to the medium [135]. Mediators are essential for microorganisms that cannot transfer electrons, as they enable electron shuttle from cell membrane to electrode even at larger distance [15,135,138]. For example, *Shewanella sulfurreducens* can use flavin as a natural mediator for its electron transport [15].

The third mechanism referred by Sydow et al. [[]], is the IET. In this mechanism a wide range of microbial electron donors and acceptors (e.g. hydrogen, formic acids) are electrochemically synthetized and utilized by microorganisms. Moreover, electroactive (metabolic) substances can be secreted by microorganisms and transfer electrons between microbes and electrodes [15].

All three mechanisms detailed here have been examined in pure culture. Currently, genetic tools offer the best approach to improve the characterization of these pathways and to optimize EAB performance. Numerous model organisms can be used for this (such as *G. sulfurreducens*) due to the availability of their complete genomic sequences and their efficiency in electrochemical systems. Genetic tools have been similarly applied to study other microorganisms, including *S. oneidensis* and *G. metallireducens* [139]. The continuing investigation of genetic systems will offer many excellent opportunities to improve electrical current densities [15].

6. Future prospects

Although, great strides have been made in recent years in characterization of EAB and the isolation of new electrogenic bacteria, there are many undiscovered electrogenic bacteria. An accurate study of microbial diversity within EAB formed in different environments, coupled with improvements in culture conditions and analytical technology suggest that many more discoveries will be forthcoming. Moreover, using inocula from extreme environments will lead to discover new electrogenic bacteria and probably new modes of electron transfer to electrodes.

While it is clear that model organisms are well-suited to study detailed electrophysiological mechanisms, studies could be enhanced by the gradual addition of other bacterial species. Such an effort will improve our understanding of how the connection network occurs within electrochemically active multispecies biofilms. In order to improve our understanding of their role in natural systems, researchers must: focus on developing innovative strategies to isolate novel microorganisms from natural EA-biofilms; and make these microorganisms available for detailed physiological studies.

The contribution of electrical conductivity from the biofilm matrix must be investigated in order to improve the electron transfer rates between microorganisms and electrodes. In addition, the composition of exopolymeric components should be determined and their genetic determinants must be identified in order to obtain mutants that can decipher these roles. These factors are important, because the composition and spatial organization of each bacterial species within the biofilm, as well as their signaling exchanges, may be crucial for the expression of genes required in producing cytochromes, nanowires, mediators, and even the conductive matrix. Moreover, process-engineering approaches should be adopted for the design of MFC architecture and parameters, in order to improve the electron transfer rate while minimizing mass transfer effects.

Technological advances that facilitate whole-genome sequencing could be coupled with experimental evolution approaches to determine the amount and type of genetic changes that accumulate in evolving populations on electrodes over several generations; in this approach the biofilm of a known EAB could be used to inoculate the next MFC, and so on. Mutation rates can change over evolutionary time, leading to the preferential accumulation of genetic variants that are better adapted to the MFC environment. It is possible to identify genetic changes between ancestral and derived organisms on a whole-genome scale.

Acknowledgments

This work was supported by an IRTELIS Ph.D. program grant from CEA/DSV. We knowledge funding from the ANR BioElec, and DRT/DSV program from CEA.

References

[1] Z. Du, H. Li, T. Gu, A state of the art review on microbial fuel cells: a promising technology for wastewater treatment and bioenergy, Biotechnol. Adv. 25 (2007) 464–482.
[2] J.X. Leong, W.R.W. Daud, M. Ghasemi, K.B. Liew, M. Ismail, Ion exchange membranes as separators in microbial fuel cells for bioenergy conversion: a comprehensive review, Renew. Sustain. Energy Rev. 28 (2013) 575–587.
[3] A. ElMekawy, H.M. Hegab, K. Vanbroekhoven, D. Pant, Techno-productive potential of photosynthetic microbial fuel cells through different configurations, Renew. Sustain. Energy Rev. 39 (2014) 617–627.
[4] Y. Feng, H. Lee, X. Wang, Y. Liu, Electricity Generation in Microbial Fuel Cells at Different Temperature and Isolation of Electrogenic Bacteria, Power and Energy Engineering Conference, APPEEC, Asia-Pacific 1–5, 2009.
[5] G.G. Kumar, V.G. Sarathi, K.S. Nahm, Recent advances and challenges in the anode architecture and their modifications for the applications of microbial fuel cells, Biosens. Bioelectron. 43 (2013) 461–475.
[6] B.E. Logan, B. Hamelers, R. Rozendal, U. Schrorder, J. Keller, S. Freguia, P. Aelterman, W. Verstraete, K. Rabaey, Microbial fuel cells: methodology and technology, Environ. Sci. Technol. 40 (2006) 5181–5192.
[7] I. Vandecandelaere, O. Nercessian, M. Faimali, E. Segaert, A. Mollica, W. Achouak, P. De Vos, P. Vandamme, Bacterial diversity of the cultivable fraction of a marine electroactive biofilm, Bioelectrochemistry 78 (2010) 62–66.
[8] A.J. Stams, F.A. De Bok, C.M. Plugge, M.H. Van Eekert, J. Dolfing, G. Schraa, Exocellular electron transfer in anaerobic microbial communities, Environ. Microbiol. 8 (2006) 371–382.
[9] N. Pous, C. Koch, J. Colprim, S. Puig, F. Harnisch, Extracellular electron transfer of biocathodes: revealing the potentials for nitrate and nitrite reduction of denitrifying microbiomes dominated by *Thiobacillus* sp, Electrochem. Commun. 49 (2014) 93–97.
[10] M.C. Potter, Electrical effects accompanying the decomposition of organic compounds, Proceedings of the Royal Society of London, Series B, Containing Papers of a Biological Character 260–276 (1911).
[11] S. Suzuki, I. Karube, T. Matsunaga, S. Kuriyama, N. Suzuki, T. Shirogami, T. Takamura, Biochemical energy conversion using immobilized whole cells of *Clostridium butyricum*, Biochim. 62 (1980) 353–358.
[12] H. Bennetto, J. Stirling, K. Tanaka, C. Vega, Anodic reactions in microbial fuel-cells, Biotechnol. Bioeng. 25 (1983) 559–568.
[13] Y. Fan, S.K. Han, H. Liu, Improved performance of CEA microbial fuel cells with increased reactor size, Energy Environ. Sci. 5 (2012) 8273–8280.
[14] A. Cournet, M.-L. Délia, A. Bergel, C. Roques, M. Bergé, Electrochemical reduction of oxygen catalyzed by a wide range of bacteria including Gram-positive, Electrochem. Commun. 12 (2010) 505–508.
[15] A. Sydow, T. Krieg, F. Mayer, J. Schrader, D. Holtmann, Electroactive bacteria-molecular mechanisms and genetic tools, Appl. Microbiol. Biotechnol. 98 (2014) 8481–8495.
[16] B.E. Logan, J.M. Regan, Electricity-producing bacterial communities in microbial fuel cells, Trends Microbiol. 14 (2006) 512–518.
[17] J. Huang, Z. Wang, C. Zhu, J. Ma, X. Zhang, Z. Wu, Identification of microbial communities in open and closed circuit bioelectrochemical MBRs by high-throughput 454 pyrosequencing, PLoS One 9 (2014) e93842.
[18] N. Wang, N. Wang, Z. Chen, H.-B. Li, J.-Q. Su, F. Zhao, Y.-G. Zhu, Bacterial community composition at anodes of microbial fuel cells for paddy soils: the effects of soil properties, J. Soils Sediments 15 (2015) 926–936.
[19] B. Hou, J. Sun, Y. Hu, Effect of enrichment procedures on performance and microbial diversity of microbial fuel cell for Congo red decolorization and electricity generation, Appl. Microbiol. Biotechnol. 90 (2011) 1563–1572.
[20] C.I. Torres, On the importance of identifying, characterizing, and predicting fundamental phenomena towards microbial electrochemistry applications, Curr. Opin. Biotechnol. 27 (2014) 107–114.
[21] J. Desloover, S. Puig, B. Virdis, P. Clauwaert, P. Boeck, W. Verstraete, N. Boon, Biocathodic nitrous oxide removal in bioelectrochemical systems, Environ. Sci. Technol. 45 (2011) 10557–10566.
[22] D.Y. Huang, S.G. Zhou, Q. Chen, B. Zhao, Y. Yuan, L. Zhuang, Enhanced anaerobic degradation of organic pollutants in a soil microbial fuel cell, Chem. Eng. J. 172 (2011) 647–653.
[23] S. Gajaraj, Z. Hu, Integration of microbial fuel cell techniques into activated sludge wastewater treatment processes to improve nitrogen removal and reduce sludge production, Chemosphere 117 (2014) 151–157.
[24] J. Yu, Y. Park, B. Kim, T. Lee, Power densities and microbial communities of brewery wastewater-fed microbial fuel cells according to the initial substrates, Bioprocess Biosyst. Eng. 38 (2014) 85–92.
[25] O. Nercessian, S. Parrot, M.L. Delia, A. Bergel, W. Achouak, Harvesting electricity with *Geobacter bremensis* isolated from compost, PLoS One 7 (2012) e34216.
[26] B. Erable, M.-A. Roncato, W. Achouak, A. Bergel, Sampling natural biofilms: a new route to build efficient microbial anodes, Environ. Sci. Technol. 43 (2009) 3194–3199.
[27] B. Cercado, N. Byrne, M. Bertrand, D. Pocaznoi, M. Rimboud, W. Achouak, A. Bergel, Garden compost inoculum leads to microbial bioanodes with potential-independent characteristics, Bioresour. Technol. 134 (2013) 276–284.
[28] J. Garcia-Munoz, R. Amils, V.M. Fernandez, A.L. De Lacey, M. Malki, Electricity generation by microorganisms in the sediment-water interface of an extreme acidic microcosm, Int. Microbiol. 14 (2011) 73–81.
[29] S. Mocali, C. Galeffi, E. Perrin, A. Florio, M. Migliore, F. Canganella, G. Bianconi, E. Di Mattia, M.T. Dell'Abate, R. Fani, A. Benedetti, Alteration of bacterial communities and organic matter in microbial fuel cells (MFCs) supplied with soil and organic fertilizer, Appl. Microbiol. Biotechnol. 97 (2013) 1299–1315.
[30] A. Cabezas, B. Pommerenke, N. Boon, M.W. Friedrich, Geobacter, *Anaeromyxobacter* and *Anaerolineae* populations are enriched on anodes of root exudate-driven microbial fuel cells in rice field soil, Environ. Microbiol. Rep. 7 (2015) 489–497.
[31] C. Zhao, J. Wu, Y. Ding, V.B. Wang, Y. Zhang, S. Kjelleberg, J.S.C. Loo, B. Cao, Q. Zhang, Hybrid conducting biofilm with built-in bacteria for high-performance microbial fuel cells, Chemelectrochem. 2 (2015) 654–658.

[32] Y. Zuo, D. Xing, J.M. Regan, B.E. Logan, Isolation of the exoelectrogenic bacterium *Ochrobactrum anthropi* YZ-1 by using a U-tube microbial fuel cell, Appl. Environ. Microbiol. 74 (2008) 3130–3137.
[33] K.P. Nevin, H. Ritcher, S.F. Covalla, J.P. Johnson, T.L. Woodard, A.L. Orloff, H. Jia, M. Zhang, D.R. Lovley, Power output and columbic efficiencies from biofilms of *Geobacter sulfurreducens* comparable to mixed community microbial fuel cells, Environ. Microbiol. 10 (2008) 2505–2514.
[34] J.F. Miceli, P. Parameswaran, D.-W. Kang, R. Krajmalnik-Brown, C.I. Torres, Enrichment and analysis of anode-respiring bacteria from diverse anaerobic inocula, Environ. Sci. Technol. 46 (2012) 10349–10355.
[35] E. Lyautey, A. Cournet, S. Morin, S. Bouletreau, L. Etcheverry, J.Y. Charcosset, F. Delmas, A. Bergel, F. Garabetian, Electroactivity of phototrophic river biofilms and constitutive cultivable bacteria, Appl. Environ. Microbiol. 77 (2011) 5394–5401.
[36] J.R. Kim, N.J. Beecroft, J. Varcoe, R.M. Dinsdale, A.J. Guwy, R.C.T. Slade, A. Thumser, C. Avignone-Rossa, G.C. Premier, Spatiotemporal development of the bacterial community in a tubular longitudinal microbial fuel cell, Appl. Microbiol. Biotechnol. 90 (2011) 1179–1191.
[37] H. Lin, X. Wu, C. Miller, J. Zhu, Improved performance of microbial fuel cells enriched with natural microbial inocula and treated by electrical current, Biomass Bioenergy 54 (2013) 170–180.
[38] N. Touch, T. Hibino, Y. Nagatsu, K. Tachiuchi, Characteristics of electricity generation and biodegradation in tidal river sludge-used microbial fuel cells, Bioresour. Technol. 158 (2014) 225–230.
[39] E. Kristensen, S. Bouillon, T. Dittmar, C. Marchand, Organic carbon dynamics in mangrove ecosystems: a review, Aquat. Bot. 89 (2008) 201–219.
[40] P. Salvin, C. Roos, F. Robert, Tropical mangrove sediments as a natural inoculum for efficient electroactive biofilms, Bioresour. Technol. 120 (2012) 45–51.
[41] J. An, S.-J. Lee, H.Y. Ng, I.S. Chang, Determination of effects of turbulence flow in a cathode environment on electricity generation using a tidal mud-based cylindrical-type sediment microbial fuel cell, J. Environ. Manage. 91 (2010) 2478–2482.
[42] R. Rousseau, C. Santaella, W. Achouak, J.-J. Godon, A. Bonnafous, A. Bergel, M.-L. Délia, Correlation of the electrochemical kinetics of high-salinity-tolerant bioanodes with the structure and microbial composition of the biofilm, ChemElectroChem 1 (2014) 1966–1975.
[43] L.M. Tender, et al., The first demonstration of a microbial fuel cell as a viable power supply: powering a meteorological buoy, J. Power Sources 179 (2008) 571–575.
[44] Y.R.J. Thomas, M. Picot, A. Carer, O. Berder, O. Sentieys, F. Barrière, A single sediment-microbial fuel cell powering a wireless telecommunication system, J. Power Sources 241 (2013) 703–708.
[45] I. Lee, K. Gyouho, B. Suyoung, A. Wolfe, R. Bell, J. Seokhyeon, K. Yejoong, J. Kagan, M. Arias-Thode, B. Chadwick, D. Sylvester, D. Blaauw, L. Yoonmyung, System-on-mud: ultra-low power oceanic sensing platform powered by small-scale benthic microbial fuel cells, Ieee Transactions on Circuits and Systems I-Regular Papers 62 (2015) 1126–1135.
[46] L. De Schamphelaire, L. Van den Bossche, H.S. Dang, M. Höfte, N. Boon, K. Rabaey, W. Verstraete, Microbial fuel cells generating electricity from rhizodeposits of rice plants, Environ. Sc. & Technol. 42 (2008) 3053–3058.
[47] J.Y. Lee, N.T. Phung, I.S. Chang, B.H. Kim, H.C. Sung, Use of acetate for enrichment of electrochemically active microorganisms and their 16S rDNA analyses, FEMS Microbiol. Lett. 223 (2003) 185–191.
[48] A.P. Borole, C.Y. Hamilton, T. Vishnivetskaya, D. Leak, C. Andras, Improving power production in acetate-fed microbial fuel cells via enrichment of exoelectrogenic organisms in flow-through systems, Biochem. Eng. J. 48 (2009) 71–80.
[49] A. Kouzuma, T. Kasai, G. Nakagawa, A. Yamamuro, T. Abe, K. Watanabe, Comparative metagenomics of anode-associated microbiomes developed in rice paddy-field microbial fuel cells, PLoS One 8 (2013) e77443.
[50] B. Cercado-Quezada, M.-L. Delia, A. Bergel, Treatment of dairy wastes with a microbial anode formed from garden compost, J. Appl. Electrochem. 40 (2010) 225–232.
[51] S.F. Ketep, A. Bergel, M. Bertrand, W. Achouak, E. Fourest, Sampling location of the inoculum is crucial in designing anodes for microbial fuel cells, Biochem. Eng. J. 73 (2013) 12–16.
[52] S. Chen, G. He, Q. Liu, F. Harnisch, Y. Zhou, Y. Chen, M. Hanif, S. Wang, X. Peng, H. Hou, U. Schröder, Layered corrugated electrode macrostructures boost microbial bioelectrocatalysis, Energy. Environ. Sc. 5 (2012) 9769–9772.
[53] I. Durruty, P. Sebastian Bonanni, J. Froilan Gonzalez, J. Pablo Busalmen, Evaluation of potato-processing wastewater treatment in a microbial fuel cell, Bioresour. Technol. 105 (2012) 81–87.
[54] S. Ishii, S. Suzuki, T.M. Norden-Krichmar, A. Wu, Y. Yamanaka, K.H. Nealson, O. Bretschger, Identifying the microbial communities and operational conditions for optimized wastewater treatment in microbial fuel cells, Water Res. 47 (2013) 7120–7130.
[55] H.J. Mansoorian, A.H. Mahvi, A.J. Jafari, M.M. Amin, A. Rajabizadeh, N. Khanjani, Bioelectricity generation using two chamber microbial fuel cell treating wastewater from food processing, Enzyme Microb. Technol. 52 (2013) 352–357.
[56] P. Kaewkannetra, W. Chiwes, T.Y. Chiu, Treatment of cassava mill wastewater and production of electricity through microbial fuel cell technology, Fuel 90 (2011) 2746–2750.
[57] J. Yu, J. Seon, Y. Park, S. Cho, T. Lee, Electricity generation and microbial community in a submerged-exchangeable microbial fuel cell system for low-strength domestic wastewater treatment, Bioresour. Technol. 117 (2012) 172–179.
[58] S.B. Velasquez-Orta, I.M. Head, T.P. Curtis, K. Scott, Factors affecting current production in microbial fuel cells using different industrial wastewaters, Bioresour. Technol. 102 (2011) 5105–5112.
[59] T.K. Sajana, M.M. Ghangrekar, A. Mitra, Application of sediment microbial fuel cell for *in situ* reclamation of aquaculture pond water quality, Aquac. Eng. 57 (2013) 101–107.
[60] T.K. Sajana, M.M. Ghangrekar, A. Mitra, Effect of presence of cellulose in the freshwater sediment on the performance of sediment microbial fuel cell, Bioresour. Technol. 155 (2014) 84–90.
[61] T.K. Sajana, M.M. Ghangrekar, A. Mitra, Effect of operating parameters on the performance of sediment microbial fuel cell treating aquaculture water, Aquac. Eng. 61 (2014) 17–26.
[62] J.M. Morris, S. Jin, Enhanced biodegradation of hydrocarbon-contaminated sediments using microbial fuel cells, J. Hazard. Mater. 213–214 (2012) 474–477.
[63] H. Zhang, X. Chen, D. Braithwaite, Z. He, Phylogenetic and metagenomic analyses of substrate-dependent bacterial temporal dynamics in microbial fuel cells, PLoS One 9 (2014) e107460.
[64] S.J. Dunaj, J.J. Vallino, M.E. Hines, M. Gay, C. Kobyljanec, J.N. Rooney-Varga, Relationships between soil organic matter, nutrients, bacterial community structure, and the performance of microbial fuel cells, Environ. Sci. Technol. 46 (2012) 1914–1922.
[65] O. Adelaja, T. Keshavarz, G. Kyazze, The effect of salinity, redox mediators and temperature on anaerobic biodegradation of petroleum hydrocarbons in microbial fuel cells, J. Hazard. Mater. 283 (2015) 211–217.
[66] L. Lu, T. Huggins, S. Jin, Y. Zuo, Z.J. Ren, Microbial metabolism and community structure in response to bioelectrochemically enhanced remediation of petroleum hydrocarbon-contaminated soil, Environ. Sci. Technol. 48 (2014) 4021–4029.
[67] M.L.K. Sulonen, M.E. Kokko, A.-M. Lakaniemi, J.A. Puhakka, Electricity generation from tetrathionate in microbial fuel cells by acidophiles, J. Hazard. Mater. 284 (2015) 182–189.
[68] C. Gao, A. Wang, W.-M. Wu, Y. Yin, Y.-G. Zhao, Enrichment of anodic biofilm inoculated with anaerobic or aerobic sludge in single chambered air-cathode microbial fuel cells, Bioresour. Technol. 167 (2014) 124–132.
[69] B.J. Mathis, C.W. Marshall, C.E. Milliken, R.S. Makkar, S.E. Creager, H.D. May, Electricity generation by thermophilic microorganisms from marine sediment, Appl. Microbiol. Biotechnol. 78 (2007) 147–155.
[70] G. Martins, L. Peixoto, D.C. Ribeiro, P. Parpot, A.G. Brito, R. Nogueira, Towards implementation of a benthic microbial fuel cell in lake Furnas (Azores): phylogenetic affiliation and electrochemical activity of sediment bacteria, Bioelectrochem. 78 (2010) 67–71.
[71] C.E. Reimers, P. Girguis, H.A. Stecher, L.M. Tender, N. Ryckelynck, P. Whaling, Microbial fuel cell energy from an ocean cold seep, Geobiology 4 (2006) 123–136.
[72] D.D. Emerson, J.A. Emerson, T.G. Rentz, R.E. Lilburn, H. Davis, C. Aldrich, C.L. Chan, Moyer, a novel lineage of proteobacteria involved in formation of marine Fe-oxidizing microbial mat communities, PLoS One 2 (2007) e667.
[73] Z.M. Summers, J.A. Gralnick, D.R. Bond, Cultivation of an Obligate Fe(II)-Oxidizing Lithoautotrophic Bacterium Using Electrodes, mBio 4 (2013) e00420-12.
[74] U. Karra, G. Huang, R. Umaz, C. Tenaglier, L. Wang, B. Li, Stability characterization and modeling of robust distributed benthic microbial fuel cell (DBMFC) system, Bioresour. Technol. 144 (2013) 477–484.
[75] H. Futamata, O. Bretschger, A. Cheung, J. Kan, R. Owen, K.H. Nealson, Adaptation of soil microbes during establishment of microbial fuel cell consortium fed with lactate, J. Biosci. Bioeng. 115 (2013) 58–63.
[76] B.E. Logan, Essential data and techniques for conducting microbial fuel cell and other types of bioelectrochemical system experiments, ChemSusChem 5 (2012) 988–994.
[77] S.A. Patil, F. Harnisch, C. Koch, T. Huebschmann, I. Fetzer, A.A. Carmona-Martinez, S. Mueller, U. Schroeder, Electroactive mixed culture derived biofilms in microbial bioelectrochemical systems: the role of pH on biofilm formation, performance and composition, Bioresour. Technol. 102 (2011) 9683–9690.
[78] L. Zhang, C. Li, L. Ding, K. Xu, H. Ren, Influences of initial pH on performance and anodic microbes of fed-batch microbial fuel cells, J. Chem. Technol. Biotechnol. 86 (2011) 1226–1232.
[79] S. Puig, M. Serra, M. Coma, M. Cabre, M.D. Balaguer, J. Colprim, Effect of pH on nutrient dynamics and electricity production using microbial fuel cells, Bioresour. Technol. 101 (2010) 9594–9599.
[80] B. Erable, R. Lacroix, L. Etcheverry, D. Féron, M.-L. Délia, A. Bergel, Marine floating microbial fuel cell involving aerobic biofilm on stainless steel cathodes, Bioresour. Technol. 142 (2013) 510–516.
[81] I.S. Michie, J.R. Kim, R.M. Dinsdale, A.J. Guwy, G.C. Premier, Operational temperature regulates anodic biofilm growth and the development of electrogenic activity, Appl. Microbiol. Biotechnol. 92 (2011) 419–430.
[82] Y. Liu, V. Climent, A. Berna, J. Miguel Feliu, Effect of temperature on the catalytic ability of electrochemically active biofilm as anode catalyst in microbial fuel cells, Electroanalysis 23 (2011) 387–394.
[83] H. Liu, S. Cheng, B.E. Logan, Power generation in fed-batch microbial fuel cells as a function of ionic strength, temperature, and reactor configuration, Environ. Sci. Technol. 39 (2005) 5488–5493.
[84] K.-J. Chae, M.-J. Choi, J.-W. Lee, K.-Y. Kim, I.S. Kim, Effect of different substrates on the performance, bacterial diversity, and bacterial viability in microbial fuel cells, Bioresour. Technol. 100 (2009) 3518–3525.
[85] D. Pant, G. Van Bogaert, L. Diels, K. Vanbroekhoven, A review of the substrates used in microbial fuel cells (MFCs) for sustainable energy production, Bioresour. Technol. 101 (2010) 1533–1543.
[86] S.P. Jung, M.-H. Yoon, S.-M. Lee, S.-E. Oh, H. Kang, J.-K. Yang, Power generation and anode bacterial community compositions of sediment fuel cells differing in anode materials and carbon sources, Int. J. Electrochem. Sci. 9 (2014) 315–326.
[87] C. Li, L. Zhang, L. Ding, H. Ren, H. Cui, Effect of conductive polymers coated anode on the performance of microbial fuel cells (MFCs) and its biodiversity analysis, Biosens. Bioelectron. 26 (2011) 4169–4176.
[88] Y. Liu, F. Harnisch, K. Fricke, U. Schroeder, V. Climent, J. Miguel Feliu, The study of electrochemically active microbial biofilms on different carbon-based anode materials in microbial fuel cells, Biosens. Bioelectron. 25 (2010) 2167–2171.

[89] Y. Fu, Q. Xu, X. Zai, Y. Liu, Z. Lu, Low electrical potential anode modified with Fe/ferric oxide and its application in marine benthic microbial fuel cell with higher voltage and power output, Appl. Surf. Sci. 289 (2014) 472–477.

[90] A. Larrosa-Guerrero, K. Scott, K.P. Katuri, C. Godinez, I.M. Head, T. Curtis, Open circuit versus closed circuit enrichment of anodic biofilms in MFC: effect on performance and anodic communities, Appl. Microbiol. Biotechnol. 87 (2010) 1699–1713.

[91] D. Pocaznoi, B. Erable, L. Etcheverry, M.L. Delia, A. Bergel, Forming microbial anodes under delayed polarisation modifies the electron transfer network and decreases the polarisation time required, Bioresour. Technol. 144 (2012) 334–341.

[92] C.I. Torres, R. Krajmalnik-Brown, P. Parameswaran, A.K. Marcus, G. Wanger, Y.A. Gorby, B.E. Rittmann, Selecting anode-respiring bacteria based on anode potential: phylogenetic, electrochemical, and microscopic characterization, Environ. Sci. Technol. 43 (2009) 9519–9524.

[93] S.F. Ketep, A. Bergel, M. Bertrand, W. Achouak, E. Fourest, Lowering the applied potential during successive scratching/re-inoculation improves the performance of microbial anodes for microbial fuel cells, Bioresour. Technol. 127 (2013) 448–455.

[94] J. Cai, P. Zheng, Y. Xing, M. Qaisar, Effect of electricity on microbial community of microbial fuel cell simultaneously treating sulfide and nitrate, J. Power Sources 281 (2015) 27–33.

[95] C.W. Lin, C.H. Wu, W.T. Huang, S.L. Tsai, Evaluation of different cell-immobilization strategies for simultaneous distillery wastewater treatment and electricity generation in microbial fuel cells, Fuel 144 (2015) 1–8.

[96] O. Lefebvre, T.T.H. Nguyen, A. Al-Mamun, I.S. Chang, H.Y. Ng, T-RFLP reveals high beta-proteobacteria diversity in microbial fuel cells enriched with domestic wastewater, J. Appl. Microbiol. 109 (2010) 839–850.

[97] T.K. Lee, T.V. Doan, K. Yoo, S. Choi, C. Kim, J. Park, Discovery of commonly existing anode biofilm microbes in two different wastewater treatment MFCs using FLX titanium pyrosequencing, Appl. Microbiol. Biotechnol. 87 (2010) 2335–2343.

[98] D. Sanchez-Herrera, D. Pacheco-Catalan, R. Valdez-Ojeda, B. Canto-Canche, X. Dominguez-Benetton, J. Dominguez-Maldonado, L. Alzate-Gaviria, Characterization of anode and anolyte community growth and the impact of impedance in a microbial fuel cell, BMC Biotechnol. 14 (2014).

[99] A. Yamamuro, A. Kouzuma, T. Abe, K. Watanabe, Metagenomic analyses reveal the involvement of syntrophic consortia in methanol/electricity conversion in microbial fuel cells, PLoS One 9 (2014) e98425.

[100] T.J. Park, W. Ding, S. Cheng, M.S. Brar, A.P.Y. Ma, H.M. Tun, F.C. Leung, Microbial community in microbial fuel cell (MFC) medium and effluent enriched with purple photosynthetic bacterium (*Rhodopseudomonas* sp.), AMB Express 4 (2014) 22.

[101] N. Shebab, D. Li, G.L. Amy, B.E. Logan, P.E. Saikaly, Characterization of bacterial and archaeal communities in air-cathode microbial fuel cells, open circuit and sealed-off reactors, Appl. Microbiol. Biotechnol. 97 (2013) 9885–9895.

[102] A. Vilar-Sanz, S. Puig, A. Garcia-Lledo, R. Trias, M.D. Balaguer, J. Colprim, L. Baneras, Denitrifying bacterial communities affect current production and nitrous oxide accumulation in a microbial fuel cell, PLoS One 8 (2013) e63460.

[103] H. Richter, M. Lanthier, K.P. Nevin, D.R. Lovley, Lack of electricity production by *Pelobacter carbinolicus* indicates that the capacity for Fe(III) oxide reduction does not necessarily confer electron transfer ability to fuel cell anodes, Appl. Environ. Microbiol. 73 (2007) 5347–5353.

[104] X. Tang, Z. Du, H. Li, Anodic electron shuttle mechanism based on 1-hydroxy-4-aminoanthraquinone in microbial fuel cells, Electrochem. Commun. 12 (2010) 1140–1143.

[105] K.C. Wrighton, J.C. Trash, R.A. Melnyk, J.P. Bigi, K.G. Byrne-Bailey, J.P. Remis, D. Schichnes, M. Auer, C.J. Chang, J.D. Coates, Evidence for direct electron transfer by a Gram-positive bacterium isolated from a microbial fuel cell, Appl. Environ. Microbiol. 77 (2011) 7633–7639.

[106] A.E. Inglesby, K. Yunus, A.C. Fisher, *In situ* fluorescence and electrochemical monitoring of a photosynthetic microbial fuel cell, Phys. Chem. Chem. Phys. 15 (2013) 6903–6911.

[107] B. Virdis, F. Harnisch, D.J. Bastone, K. Rabaey, B.C. Donose, Non-invasive characterization of electrochemically active microbial biofilms using confocal Raman microscopy, Energy Environ. Sci. 5 (2012) 7017–7024.

[108] O. Lefebvre, Z. Tang, M.P.H. Fung, D.H.C. Chua, I.S. Chang, H.Y. Ng, Electrical performance of low cost cathodes prepared by plasma sputtering deposition in microbial fuel cells, Biosens. Bioelectron. 31 (2012) 164–169.

[109] J. Kramer, S. Soukiazian, S. Mahoney, J. Hicks-Garner, Microbial fuel cell biofilm characterization with thermogravimetric analysis on bare and polyethyleneimine surface modified carbon foam anodes, J. Power Sources 210 (2012) 122–128.

[110] L. Ren, J.C. Tokash, J.M. Regan, B.E. Logan, Current generation in microbial electrolysis cells with addition of amorphous ferric hydroxide, Tween 80, or DNA, Int. J. Hydrog. Energy 37 (2012) 16943–16950.

[111] R. Liu, Y. Zhao, S. Lu, Q. Huang, Electricity generation from lactate using microbial fuel cell and the distribution characteristics of anode microbial community, Acta Microbiol. Sin. 52 (2012) 744–752.

[112] N.S. Malvankar, M.T. Tuominen, D.R. Lovley, Lack of cytochrome involvement in long-range electron transport through conductive biofilms and nanowires of *Geobacter sulfurreducens*, Energy Environ. Sci. 5 (2012) 8651–8659.

[113] X.B. Gong, S.J. You, X.H. Wang, Y.G.R.N. Zhang, N.Q. Ren, Silver–tungsten carbide nanohybrid for efficient electrocatalysis of oxygen reduction reaction in microbial fuel cell, J. Power Sources 225 (2013) 330–337.

[114] Y. Wang, B. Li, L. Zheng, D. Cui, X. Xiang, W. Li, Polyaniline/mesoporous tungsten trioxide composite as anode electrocatalyst for high-performance microbial fuel cells, Biosens. Bioelectron. 41 (2013) 582–588.

[115] Y. Wang, B. Li, D. Cui, X. Xiang, W. Li, Nano-molybdenum carbide/carbon nanotubes composite as bifunctional anode catalyst for high-performance *Escherichia coli*-based microbial fuel cell, Biosens. Bioelectron. 51 (2014) 349–355.

[116] Z. Yan, M. Wang, Y. Lu, R. Liu, J. Zhao, Ethylene glycol stabilized $NaBH_4$ reduction for preparation carbon-supported Pt–Co alloy nanoparticles used as oxygen reduction electrocatalysts for microbial fuel cells, J. Solid State Electrochem. 18 (2013) 1087–1097.

[117] W. Zhi, Z. Ge, Z. He, H. Zhang, Methods for understanding microbial community structures and functions in microbial fuel cells: a review, Bioresour. Technol. 171 (2014) 461–468.

[118] D.E. Holmes, S.K. Chaudhuri, K.P. Nevin, T. Mehta, B.A. Methé, A. Liu, J.E. Ward, T.L. Woodard, J. Webster, D.R. Lovley, Microarray and genetic analysis of electron transfer to electrodes in *Geobacter sulfurreducens*, Environ. Microbiol. 8 (2006) 1805–1815.

[119] L. Zhang, X. Zhu, J. Li, Q. Liao, D. Ye, Biofilm formation and electricity generation of a microbial fuel cell started up under different external resistances, J. Power Sources 196 (2011) 6029–6035.

[120] N.J. Sacco, E.L.M. Figuerola, G. Pataccini, M. Celina Bonetto, L. Erijman, E. Corton, Performance of planar and cylindrical carbon electrodes at sedimentary microbial fuel cells, Bioresour. Technol. 126 (2012) 328–335.

[121] K.P. Katuri, K. Scott, On the dynamic response of the anode in microbial fuel cells, Enzyme Microb. Technol. 48 (2011) 351–358.

[122] S.F. Ketep, A. Bergel, M. Bertrand, M. Barakat, W. Achouak, E. Fourest, Forming microbial anodes with acetate addition decreases their capability to treat raw paper mill effluent, Bioresour. Technol. 164 (2014) 285–291.

[123] P. Cristiani, A. Franzetti, I. Gandolfi, E. Guerrini, G. Bestetti, Bacterial DGGE fingerprints of biofilms on electrodes of membraneless microbial fuel cells, Int. Biodeterior. Biodegrad. 84 (2013) 211–219.

[124] J.H. Ahn, W.S. Jeong, M.Y. Choi, B.Y. Kim, J. Song, H.Y. Weon, Phylogenetic diversity of dominant bacterial and archaeal communities in plant-microbial fuel cells using rice plants, J. Microbiol. Biotechnol. 24 (2014) 1707–1718.

[125] P.Y. Wong, K.Y. Cheng, A.H. Kaksonen, D.C. Sutton, M.P. Ginige, Enrichment of anodophilic nitrogen fixing bacteria in a bioelectrochemical system, Water Res. 64 (2014) 73–81.

[126] S. Ishii, T. Shimoyama, Y. Hotta, K. Watanabe, Characterization of a filamentous biofilm community established in a cellulose-fed microbial fuel cell, BMC Microbiol. 8 (2008) 6.

[127] L. De Schamphelaire, P. Boeckx, W. Verstraete, Evaluation of biocathodes in freshwater brackish sediment microbial fuel cells, Appl. Microbiol. Biotechnol. 87 (2010) 1675–1687.

[128] Z. Wang, Y. Zheng, Y. Xiao, S. Wu, Y. Wu, Z. Yang, F. Zhao, Analysis of oxygen reduction and microbial community of air-diffusion biocathode in microbial fuel cells, Bioresour. Technol. 144 (2013) 74–79.

[129] Z. Wang, T. Lee, B. Lim, C. Choi, J. Park, Microbial community structures differentiated in a single-chamber air-cathode microbial fuel cell fueled with rice straw hydrolysate, Biotechnol. Biofuels. 7 (2014).

[130] G.W. Chen, S.J. Choi, T.H. Lee, G.Y. Lee, J.H. Cha, C.W. Kim, Application of biocathode in microbial fuel cells: cell performance microbial community, Appl. Microbiol. Biotechnol. 79 (2008) 379–388.

[131] K. Rabaey, S.T. Read, P. Clauwaert, S. Freguia, P.L. Bond, L.L. Blackall, J. Keller, Cathodic oxygen reduction catalyzed by bacteria in microbial fuel cells, ISME J. 2 (2008) 519–527.

[132] Y. Du, Y. Feng, Y. Dong, Y. Feng, Y. Qu, J. Liu, X. Zhou, N. Ren, Coupling interaction of cathodic reduction and microbial metabolism in aerobic biocathode of microbial fuel cell, RSC Adv. 4 (2014) 34350–34355.

[133] R.R. Sayess, P.E. Saikaly, M. El-Fadel, D. Li, L. Semerjian, Reactor performance in terms of COD and nitrogen removal and bacterial community structure of a three-stage rotating bioelectrochemical contactor, Water Res. 47 (2013) 881–894.

[134] J.P. Busalmen, A. Esteve-Núñez, A. Berná, J.M. Feliu, C-type cytochromes wire electricity-producing bacteria to electrodes, Angew. Chem. Int. Ed. 47 (2008) 4874–4877.

[135] K. Hasan, S.A. Patil, D. Leech, C. Hagerhall, L. Gorton, Electrochemical communication between microbial cells and electrodes via osmium redox systems, Biochem. Soc. Trans. 40 (2012) 1330–1335.

[136] Y.A. Gorny, S. Yanina, J.S. McLean, K.M. Rosso, D. Moyles, A. Dohnalkova, T.J. Beveridge, I.S. Chang, B.H. Kim, K.S. Kim, D.E. Culley, S.B. Reed, M.F. Romine, D.A. Saffarini, E.A. Hill, L. Shi, D.A. Elia, D.W. Kennedy, G. Pinchuk, K. Watanabe, S. Ishii, B. Logan, K.H. Nealson, J.K. Fredrickson, Electrically conductive bacterial nanowires produced by *Shewanella oneidensis* strain MR-1 and other microorganisms, Proc. Natl. Acad. Sci. U. S. A. 103 (2006) 11358–11363.

[137] H. Richter, K. McCarthy, K.P. Nevin, J.P. Nevin, V.M. Rotello, D.R. Lovley, Electricity generation by *Geobacter sulfurreducens* attached to gold electrodes, Langmuir 24 (2008) 4376–4379.

[138] P. Kavanagh, D. Leech, Mediated electron transfer in glucose oxidising enzyme electrodes for application to biofuel cells: recent progress and perspectives, PCCP. 15 (2013) 4859–4869.

[139] P.L. Tremblay, T. Zhang, S.A. Dar, C. Leang, D.R. Lovley, The Rnf complex of *Clostridium ljungdahlii* is a proton-translocating ferredoxin: NAD+ oxidoreductase essential for autotrophic growth, MBio. 4 (2012) e00406–e00412.