Selective enrichment of biocatalysts for bioelectrochemical systems: A critical review

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Abstract

Microbial electrochemical technologies (MET), also known as bioelectrochemical systems (BES), use microorganisms as biocatalysts to recover valuable resources like bioelectricity, hydrogen, nutrients, metals, and industrial chemicals from wastes and wastewaters. MET are therefore expected to play a key role in waste management and reduction of the carbon footprint in the near future. However, considerable fundamental and technological challenges still need to be addressed before using METs in practice. Rapid start-up, as well as an efficient and stable performance, are the pre-requisites to achieve commercialization of METs. Although considerable advancements have been made in this field in the past two decades, no general conclusion has been drawn about how to start-up BES in the most efficient manner. This review aims to survey and critically analyze start-up strategies proposed in the literature to favor a fast and efficient establishment of electrochemically active microorganisms onto bioanodes or biocathodes and promote their activity over a long period of operation. Various aspects of BES start-up, including inoculum selection, elimination of competitive microorganisms, and selection of operational parameters for enrichment of electroactive biofilms are covered. In summary, inoculation with already enriched culture, imposing of an anode potential or using polarity reversal at the cathode are the potential methods for ensuring fast and efficient BES start-up. Electrode configuration and hydrodynamic conditions are also major aspects to be considered for biofilm formation and development.

Highlights

- This review critically analyzes start-up strategies of BES
- Inoculation with already enriched culture cause fast start-up
- Imposing anode potentials < -0.2 V vs. SHE reduces performance and delays start-up
- Using polarity reversal reduces biocathode start-up time
- Electrode configuration and hydrodynamic conditions affect BES start-up

Keywords: Bioanode, Biocathode, Microbial electrochemical technology, Electroactive microorganisms, Start-up

List of abbreviations

BES – bioelectrochemical systems

BESA – bromoethane sulphonic acid

CE – coulombic efficiency

COD – chemical oxygen demand

DO – dissolved oxygen

MEC – microbial electrolysis cell

MES – microbial electrosynthesis cell

MET – microbial electrochemical technology

MFC – microbial fuel cell

SHE – standard hydrogen electrode

VOC – volatile organic carbon

Units

Time – day (d), hour (h)

Voltage – Volt (V)

Power density – mW/m^2

Volumetric power – W/m^3

Current – mA

Current density – A/m^2

Temperature - °C

1. Introduction

Increasing population, accompanied by industrial and economic growth continuously increases environmental pollution. Due to the inevitable decline of natural resources and environmental concerns, there is a growing demand for sustainable production of fuels, energy and chemicals, as well as efficient recycling of nutrients and metals. Thus, the industrial process and business models are slowly shifting from a supply based linear economy to a recovery based circular economy, and new technological solutions are being developed to harvest energy and resources from waste materials and side streams. Bioelectrochemical systems (BES) employing microbial electrochemical technology (MET) is one rapidly developing approach, in which microbial biocatalysts are used for conversion of organic or inorganic waste to energy, nutrients, metals or biochemicals (for reviews, see [1,2]).

A BES typically consists of an anodic and a cathodic chamber for oxidation and reduction reactions, respectively, separated by an ion exchange membrane [3]. Electroactive microorganisms catalyze the oxidation of organic and inorganic electron donors at the anode, and/or the reduction of electron acceptors at the cathode. Electron transfer from microbial cells to the electrodes occurs through either a series of components in the extracellular matrix (direct electron transfer), or via electron shuttles dissolved in the bulk solution (mediated electron transfer) [4]. Simultaneously, ions travel through an ion exchange membrane to maintain electroneutrality.

Microbial fuel cells (MFCs), microbial electrolysis cells (MECs), and microbial electrosynthesis cells (MESs) are the major applications of MET. In MFCs, electricity is captured directly from organic compounds via anaerobic respiration when the electrons move from the anode to the cathode through an external circuit. In MECs, the electrons released by microorganisms at the anode in absence of oxygen combine with protons to produce hydrogen at the cathode. This reaction does not occur spontaneously and external energy (in addition to that generated by the microorganisms) needs to be added to drive the process. In a MES, microbes can electrochemically reduce inorganic carbon to fuels and commodities like volatile fatty acids and alcohols at the cathode with the addition of external energy [3].

Though there has been considerable progress in MET research, the product titer, in terms of either power or value-added products, and the start-up time need further improvement for commercialization [5]. Efficient microbial activity, with high oxidation/reduction rates, is essential to liberate electrons to the anode or accept electrons at the cathode [6]. Start-up time

as well as steady-state performance of MET strongly depend on the start-up procedure [7,8]. The rapid establishment and maturation of an electroactive biofilm and a fast restoration of the microbial activity in case of system failure would support industrial adoption of MET. Formation of an electroactive biofilm can be triggered by providing favorable conditions for the microorganisms including environmental conditions like temperature, pH, an appropriate growth medium with suitable carbon and nitrogen sources and an optimum reactor configuration.

A large number of research papers have been published addressing the different aspects of MET and several review papers have consolidated the research foci, such as electron transfer [4,9], electrode design [10,11], cathodic catalysts [12], microbiology of electroactive biofilms [13], and practical implementation [14,15]. However, to our knowledge a systematic review comparing and critically evaluating different BES start-up strategies has not been published previously. The aim of this review is to evaluate advantages and disadvantages of the various BES start-up approaches reported in the scientific literature, and suggesting a start-up strategy for developing a robust electroactive biofilm for a stable long-term performance.

2. Electroactive anodic biofilms

If favorable growth conditions are provided, the electroactive microorganisms form a biofilm on the anode electrode and grow by oxidizing organic or inorganic matter, thereby transferring part of the electrons to the electrode [4]. Enrichment of these electroactive microorganisms is discussed in the following sections with particular focus on experiences reported on BES start-up (Fig. 1).

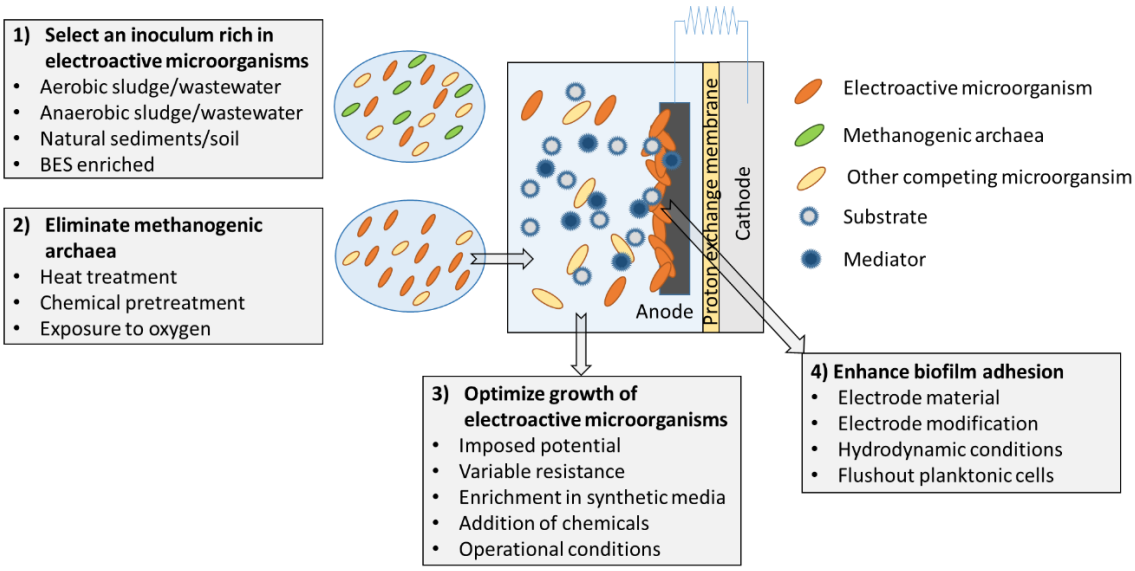

Figure 1: Steps to favor the development of an electroactive anodic biofilm in bioelectrochemical systems for efficient waste-to-current conversion. Similar strategies are also applied for cathodic biofilms depending on the application (described in Table 5).

2.1 Source of inoculum

Both pure and mixed microbial cultures can be used to inoculate BES anodes. Pure cultures fit perfectly for studies focusing on fundamentals of MET, such as delineating electron transfer mechanisms [16] or metabolism of a specific electroactive microorganism [17]. In addition, for efficient removal of some particular compounds, such as phenols [18], VOCs [19] and pharmaceuticals [20], selection of microorganisms with a specialized metabolic capacity might be necessary. Mixed cultures are more suitable for most industrial and municipal applications, as they do not require sterilization, can be applied for treatment of complex substrates, and are less sensitive to changes in environmental conditions. Mixed cultures, in most cases, generate higher and more stable current densities than pure cultures [8].

The capability of microorganisms for donating electrons outside the cell, to the anode electrode, is a widespread mechanism in nature [21,22]. Typical anodic inocula are derived from anthropogenic environments, including aerobic sludge [23,24], anaerobic sludge [25,26], and digestates [27,28], or from natural environments such as soil [29,30] and sediments [22,31] (Table 1). Enriched electroactive microbial communities from previously operated anodes have also been used widely [32,33]. Wastewaters has also been reported as a source of potential exoelectrogenic microorganisms [34]. It was shown that, operating a MFC continuously with real wastewater at HRTs of 3 – 4 hours, addition of an external inoculum to the microorganisms present in the wastewater does not significantly affect current production [34].

The microbial diversity of the inoculum, and particularly the abundance of electroactive microorganisms, influences the start-up time of bioanodes [35]. Aerobic inocula may contain a low amount of electroactive microorganisms, but lack methanogenic communities that compete with current production, which are abundant in most anaerobic inocula [35,36]. Aerobic inocula have been shown to initiate electron transfer to MFC anodes within 5 days from start-up, against one day required by anaerobic microorganisms, whereas already enriched inocula start producing current in just a few hours [32]. Microbial consortia from natural biofilms, such as those forming on marine structures, rebuild an anodic electroactive biofilm faster than a planktonic community [30]. Enriched inocula, e.g. combining biofilms and effluent from previously operated BES, enable a fast start-up due to the presence of electroactive microorganisms, and possibly mediators [32,37,38]. For example, Liu et al. [39] observed a decrease in start-up time from 400 h to 48 h, as well as a two-times higher power density, by using an already enriched biofilm rather than domestic wastewater as inoculum. Combining inocula from different sources, e.g. anodic effluent or biofilm with aerobic or anaerobic sludge, can also be a good strategy to increase the diversity of the electroactive microbial communities [32,40].

Already established biofilms can also be used to restore BES performance upon failure. In fact, once a primary electroactive biofilm has been established, cells can migrate to colonize new surfaces with a short start-up time [39]. If not used immediately, electroactive communities should be stored at 4 °C, but too long storage times (more than one month) may negatively affect their biochemical and electroactive characteristics [41,42].

Bioaugmentation, which in the case of BES means seeding with pure cultures of known electroactive microorganisms, can be applied in certain cases to reinforce the mixed population or to revive the system after process disturbances [43]. Bioaugmentation of mixed populations with electroactive bacteria may result in stable and high electrogenic activity and substrate degradation in the long term, due to synergistic interactions between the native anodic populations and the added electrogenic microorganisms [44,45]. However, the impact of bioaugmentation on BES start-up has not been studied so far.

2.2 Inoculum pre-treatment

In MET, microorganisms such as methanogenic archaea, homoacetogens, and sulphate or nitrate reducers compete with the electroactive microorganisms by directing electrons from substrate oxidation to other purposes than current production, thus lowering the coulombic

efficiency [46]. Pretreatment of the microbial populations is one option for hindering the growth of undesired microorganisms. Methanogenesis is a major issue in MET and many studies have focused on its control [28,47,48], although methanogenic microorganisms can be useful for biofilm development by producing electron shuttles [49] or even perform direct interspecies electron transfer [50]. Since prolonged or repeated treatment of the microbial community is not cost-effective for full scale applications, an efficient inoculum pretreatment method must inhibit methanogens permanently, while avoiding damaging the electroactive microorganisms [26,51]. The most commonly applied inoculum pretreatment strategies are reported and discussed in details in the subsections 2.2.1 – 2.2.3.

Even if inoculum pretreatment is applied, continuous treatment of waste and side streams in unsterile conditions may introduce competing microorganisms to the anodes. In case of reappearance of methanogens during continuous MET operation, overcoming strategies such as regulation of the anode potential [52,53], prolonged exposure to oxygen [54], shortening the hydraulic retention time [55] or starvation [56] can be more feasible options than repeating the pretreatment. For example, methanogenic microorganisms present in anodic biofilms can detach when switching MFCs to open circuit mode [56].

2.2.1 Heat treatment

Heat treatment relies on the specific ability of bacteria of the phylum Firmicutes to produce heat-resistant endospores, which increase their survival compared to non-spore forming microorganisms when exposed to temperatures exceeding their tolerance limit [57]. Heat treatment is widely used in dark fermentative hydrogen production to eliminate non-spore-forming methanogenic archaea [58], and can be applied in MET for the same purpose [26,48]. The main drawback of heat treatment is that most of the known electroactive microorganisms, including Geobacter, are non-sporulating and therefore get eliminated as well [48].

2.2.2 Chemical pretreatment

Certain chemicals can specifically inhibit the growth of methanogens. These compounds include e.g. 2–iodopropane [48], lumazine, neomycin (NS), 2–bromoethanesulfonic acid (BESA) [51], chloroethanesulfonate, hypoxanthine [59], amoxicillin trihydrate, oxytetracycline tetrahydrochloride, thiamphenicol, chloramphenicol and chlortetracycline [60]. Among these, BESA is the most commonly used as it selectively inhibits methanogenic archaea at concentrations as low as 0.1 mM without damaging electroactive microorganisms [28]. Due to the high costs and possible toxic effects to the environment, continuous addition

of chemicals is not feasible in large-scale applications. Biologically synthesized chemicals, however, may be a sustainable alternative to inhibit methanogenesis. For example, 10 g/L of dried marine algae Chaetoceros, which produces hexadecatrienoic acid, a long-chain fatty acid toxic to methanogenic archaea, was used to pretreat anaerobic sludge obtaining 60% reduction in specific methanogenic activity and 77% increase in MFC power density [61] .

2.2.3 Exposure to oxygen

Known electroactive microorganisms include obligate anaerobes (e.g. Pelobacter and Desulfovibrio), aerotolerant anaerobes (e.g. Geobacter) and facultative anaerobes (e.g. Shewanella and Pseudomonas). Although electricity production by aerobic microorganisms has been reported [13,62], the anodic chamber is usually kept under anaerobic conditions to prevent oxygen exposure of the strict anaerobic microorganisms and loss of chemical energy by aerobic respiration. However, strict anaerobic conditions favor methanogenic activity. In a study, exposure of the anodic biofilm to 5 mg/L dissolved oxygen (DO) for 48 – 120 hours not only suppressed methanogens, but also enhanced the metabolic activity of the facultative and micro-aerobic electroactive microorganisms [54]. Oxygen exposure was also reported to increase current production in an aniline fed MFC, with an increase of the relative abundance of Proteobacteria (74%) in the anodic community compared to the inoculum (45%), as well as a decrease of Bacteriodetes from 24% to 8% [63]. A current production of 99.80 mA/cm^2 was obtained in a MFC upon aerobic start-up for 125 hours, 92% higher than a control MFC kept under anaerobic conditions from the beginning [64]. Quan et al. [65] reported a slightly higher power production in a MFC operated with an anodic community previously enriched in the presence of air (pumped at a rate of 1.5 L/min for about 550 hours) as compared to that enriched anaerobically, with 77% and 97% similarity of the anode-attached and planktonic microbial communities, respectively. Thus, despite the negative effects of aerobic metabolism in MFCs, intermittent air sparging could enable high coulombic efficiencies, as well as increase the COD removal efficiency, with a minimum effect on the microbial community [65].

Table 1: Summary of studies showing the effect of different inocula on start-up time and maximum power or current output, or coulombic efficiency (CE), obtained in MFCs. All the voltage values (V) reported refer to the applied anodic potentials *vs.* standard hydrogen electrode (SHE).

Source of inoculum	Enrichment strategy	Start-up time	Output	Reference
Domestic wastewater	0.4 V	17 d	686 mW/m^2	[39]
MFC biofilm	Secondary biofilm	1 d	1487 mW/m^2	
Domestic wastewater	-0.15 V	30 d	1057 mW/m^2	[66]
	0.15 V		886 mW/m^2	
	Fixed external resistance: 1000 Ω		924 mW/m^2	
Aerobic sludge	Fixed external resistance: 120 Ω	10 d	1942 mW/m^2	[38]
Anaerobic sludge		22 d	1.7 mW/m^2	
MFC biofilm		6 d	2946 mW/m^2	
Primary clarifier effluent	Fixed external resistance: 1000 Ω	60 d	600 mW/m^2	[67]
Anaerobic bog		9 d		
Salt lake sediment	0.44 V	1.8 d	8.5 A/m^2	[31]
Lagoon sediment		0.3 d	8.5 A/m^2	
Aerobic sludge	0.6 V	90 d	CE, 35 ± 4%	[68]
MFC effluent		30 h	CE, 30 ± 5%	
Aerobic sludge	Fixed external resistance: 120 Ω	12 d	150 mW/m^2	[35]
Anaerobic sludge		1 d	300 mW/m^2	
Aerobic sludge	- 0.2 V	5 d	Not available	[32]
Anaerobic sludge		4 d		
MFC effluent		8 d		
MFC effluent + anaerobic sludge		3 d		
MFC biofilm + MFC effluent		0 d		
MFC biofilm		0 d		
Natural biofilm	0.14 V	3 d	2.0 A/m^2	[30]
Marine sediments			0.4 A/m^2	
Beach sand			0.8 A/m^2	

2.3 Enrichment of electroactive microorganisms

In MFCs, the anode requires the presence of electroactive microorganisms able to transfer electrons from the cell to the solid anode electrode. Many studies have been dedicated to developing a strategy to efficiently enrich electroactive microorganisms during start-up and operation of MET, as discussed in the following subsections.

2.3.1 Electrochemical enrichment

2.3.1.1 Imposed anode potential

Electrode potential influences the composition of the microbial community, or at least the extracellular electron transfer pathways [69,70]. Since electroactive microorganisms gain energy for their growth by transferring electrons to the anode electrode, the energy gain can be increased by imposing the anode with a potential higher than the potential of the electron donor (Eq. 1) [70–74]:

$$\Delta G^{0\prime} = -nF(E^{0\prime}_{substrate} - E_{anode}) \quad \text{..... (1)}$$

where $\Delta G^{0\prime}$ (J/mol) denotes the change of Gibbs free energy at pH 7, n is the number of electrons involved in the reaction, F is the Faraday constant (9.64853 × 10^4 C/mol), and $E^{0\prime}_{substrate}$ (V) and E_{anode} (V) denote the standard biological potential of the substrate and the anode potential, respectively.

However, the metabolic energy gain is not directly linked to the applied anode potential as only the energy liberated via electron transfer outside the cell through the outer membrane proteins can be obtained by the microorganisms [73] (Eq. 2):

$$\Delta G^{0\prime}_{mic} = -nF(E^{0\prime}_{substrate} - E_{TED}) \quad \text{..... (2)}$$

where $\Delta G^{0\prime}_{mic}$ (J/mol) denotes the energy gain by the microbial cell and E_{TED} (V) denotes the standard biological potential of the terminal electron donor, which is the outer membrane protein for direct electron transfer and the mediator for mediated electron transfer.

The upper limit of the potential of the membrane proteins of several bacterial strains is around 0 V *vs.* standard hydrogen electrode (SHE), thus increasing the anodic potential beyond 0 V *vs*. SHE might not result in further energy gain for the microorganisms [73]. It has been suggested that imposing a negative anode potential selects electroactive microorganisms capable of respiring at low potentials [75,76]. Many microorganisms, including *Geobacter*, can

maximize their energy efficiency by adapting their electron transfer mechanism to the anode potential by using different outer membrane proteins [70,74,77,78].

In most cases, both direct and mediated electron transfer have a Nernst-Monod potential (anodic potential giving half of the maximum current density) close to –0.2 V *vs.* SHE [73,79]. There are exceptions, for example, pyocyanin, a known mediator produced and used for electron transfer by *Pseudomonas* sp., has a redox potential of −0.03 V *vs.* SHE [77]. Thus, it can be expected that *Pseudomonas* sp. will grow at higher anodic potentials [77]. In mediated electron transfer, the anode potential required and the current produced depend on the oxidation potential of the mediator. Electroactive microorganisms using mediators are incapable of producing high current densities due to diffusion limitations of the mediators [74,77]. More positive anodic potentials might thus be required to overcome the diffusion losses compared to the microorganisms performing direct electron transfer.

Despite the energetic advantage of more positive anode potentials, there is a lack of consensus whether a positive or negative anodic potential must be selected to support the growth of an electroactive anodic biofilm for maximizing power density (Table 2). A positive anode potential seems to be beneficial in the early stages of the anodic biofilm formation to increase its thickness, and thus its resistance to shear forces. Later, a low anode potential can be applied to increase the relative abundance of electroactive microorganisms. Although advisable during the start-up phase, the energy requirements for applying a potential for prolonged time periods may exceed the benefits obtained in power output, and thus such a technique can be used only for short time periods.

2.3.1.2 External resistance

The anode potential can be indirectly controlled by selecting a proper external resistor, if the cathodic conditions are stable. Reducing the external resistance increases the anodic potential, thus leading typically to a higher biomass density of the biofilm [80–82], but often to a loose biofilm structure with a high proportion of extracellular polymeric substances [82,83]. This results in an increased number of void spaces, which is beneficial for proton and buffer transport but decreases the electrical conductivity of the biofilm [82,83].

Boghani et al. [7] and Premier et al. [84] used an algorithm to vary the external load depending on the power production of a MFC and were able to shorten the start-up time by half and to obtain a three times higher power density than a similar MFC equipped with a static load (Table 2). An automatic resistance control was also applied to MFCs treating swine wastewater,

resulting in a start-up time of only 15 days, compared to the 50 days required by a control MFC with a static resistance [85]. Such results suggest that controlling the anode potential by varying the external resistance is a suitable strategy to optimize MFC start-up.

Table 2: Summary of studies showing the effect of different electrochemical enrichment strategies on start-up time and maximum power or current output in MET. All the voltage values reported (V) refer to the applied anodic potentials *vs.* SHE.

Source of inoculum	Enrichment strategy	Start-up time	Output	Reference
Domestic wastewater	-0.15 V	30 d	1057 mW/m^2	[66]
	0.15 V		886 mW/m^2	
	External resistance: 1000 Ω		924 mW/m^2	
Aerobic sludge	Constant external resistance (500 or 1000 Ω)	40 d	133 mW/m^2	[7]
	Variable external resistance	22 d	222 mW/m^2	
Anaerobic sludge	Constant external resistance (200 Ω)	Not available	84 mW/m^2	[84]
	Variable external resistance		124 mW/m^2	
Activated sludge	-0.15 V	4 d	8.2 A/m^2	[77]
	-0.09 V	4 d	6.2 A/m^2	
	0.02 V	7 d	1.8 A/m^2	
	0.37 V	Start-up failed	0	
MFC effluent	-0.2 V	7 d	160 W/m^3	[6]
	0 V		199 W/m^3	
	0.2 V		160 W/m^3	
MFC effluent	External resistance: 10 Ω	6.3 d	14.6 mA	[83]
	50 Ω	4.3 d	11.2 mA	
	250 Ω	3.0 d	2.8 mA	
	1000 Ω	2.6 d	0.7 mA	
Shewanella oneidensis	0.2 V	3.75 d	1.8 mA	[86]
	0.4 V	2.08 d	1.7 mA	
	0.55 V	0.83 d	1.6 mA	
	0.7 V	0.2 d	1.6 mA	
	0.95 V	Failed start-up	Failed start-up	

2.3.2 Enrichment media

In BES inoculated with mixed cultures, the competition of various microorganisms for the substrate is a notable constraint. Preventive actions, such as pre-enrichment of electroactive microorganisms on non-fermentable substrates can favor the selective enrichment of electroactive microorganisms in the anodic biofilm. The structure of the anodic microbial community depends on the electron donor supplied [87]. Many substrates sustain the growth of electroactive microorganisms. A growth medium containing fermentable substrates, like glucose or sucrose, results in a diverse microbial community, including non-electroactive microorganisms [87]. In contrast, non-fermentable substrates such as acetate, in the absence of methanogens, select more for electroactive microorganisms than more complex substrates [31,88]. In fact, fermentable or other complex substrates might require syntrophic interactions between electroactive and non-electroactive microorganisms, whereas acetate can be directly converted to current by electroactive microorganisms [46].

In fed-batch systems, where the carbon source is added periodically, a substrate overload may occur during the start-up, favoring the growth of non-electroactive microorganisms [27]. Electroactive microorganisms require less energy than other groups of microorganisms, and hence can grow in substrate depleted conditions [27,89]. Therefore, BES start-up at low substrate loading rates will favor the development of electroactive microorganisms.

Unlike synthetic anolytes, real wastewaters typically have low conductivity and a complex and fluctuating composition, possibly containing compounds toxic to the microorganisms. Furthermore, the indigenous microorganisms contained in wastewaters can affect the electrogenic community in BES [34], and high COD concentrations may lead to reduced BES performance [90]. An adaptation period in which the wastewater is added gradually increasing the organic loading rate (OLR) might help to avoid inhibition of the microbial community [90,91]. Addition of simple substrates and electron acceptors at the anode can speed up the formation of a strong electrogenic community and thus increase power production in the long-term [91]. This is a very common practice for start-up of laboratory scale BES fed with real waste streams [92,93].

2.4 Operational conditions

The composition and activity of the electroactive biofilm strictly depends on the operating conditions of the BES. The following subsections deal with the operational parameters influencing the start-up.

2.4.1 Temperature

In BES operation, temperature is an important parameter as it shapes the microbial community composition and the metabolic pathways [40]. Due to kinetic constraints, low temperature increases the start-up time [51]. However, Michie et al. [94] observed that operation temperature does not affect the final power density attained. It was shown that electroactive microorganisms forming an anodic biofilm at 30 °C were capable to adapt to low temperatures (4-15 °C) without decreasing the power density [95]. In the same way, the performance of MFCs started up at 15 °C was not affected by a temperature increase to 25 °C [96,97]. On the contrary, Min et al. [98] observed both a decrease in start-up time and increased power production after increasing the operation temperature from 22 to 30 °C (Table 3). In general, biofilms grown at low temperature are less sensitive to temperature changes than biofilms grown at higher temperatures [99]. This is particularly important for treatment of wastewaters with seasonally varying temperatures, which should be preferably started-up when the temperature is lower, though it can result in a longer start-up time.

Thermophilic MET can be potentially applied for treatment of high temperature or pathogenic waste streams [100]. The fast microbial growth and reaction kinetics of thermophilic (>50 °C) electroactive microorganisms may, in theory, promote high power densities, but knowledge on thermophilic electroactive microbes is limited. Despite the kinetic and thermodynamic advantages, studies have reported lower power densities in thermophilic than that in mesophilic BES [40,101,102]. However, most studies on thermophilic BES focused on the fundamentals, using basic reactor configurations such as H-type MFCs, whereas most advanced reactor design and electrode materials have been widely tested under mesophilic conditions to maximize power production. The possible adaptation of mesophilic electroactive microorganisms to thermophilic conditions has not yet been reported in the literature, and thus requires more investigation.

Overall, the optimal temperature must always be determined for the specific MET application keeping in mind the electroactive microbial community as well as the economical feasibility. The temperature should be a trade-off between (i) start-up time, which increases with decreasing temperature, (ii) anodic electrocatalytic activity, which increases with increasing temperature and (iii) methanogenic growth, which increases with increasing temperature up to around 37 °C [94]. Thus, at the expense of a longer start-up phase, BESs acclimated and operated at low (< 30 °C) temperatures may be advantageous for the selective development of electroactive microbial communities and simultaneous depression of competing organisms.

2.4.2 pH

The development of electroactive biofilms is widely affected by the pH, which regulates the metabolic pathways of electroactive microorganisms [1,103,104]. Although bioelectricity production has been obtained at pH < 3 [105,106] and at pH > 12 [107], a neutral or weakly alkaline pH is usually required for the establishment of an electroactive biofilm from mixed cultures, particularly for complex organic substrates, as hydrolysis can only proceed at neutral pH [108]. It is particularly true if the inoculum is collected from neutral pH environments [103]. However, a neutral pH is also favorable for the growth of methanogenic archaea. Ren et al. [109] suggested that adjusting the pH to 9 in the start-up phase may improve the overall performance of MFCs in the long term. Specific MET applications, such as oxalate removal from aluminium refinery wastewater (pH 10), require start-up at high pH to adapt the microbial community to alkaline conditions [93,107].

2.5 BES design

The anodic electroactive biofilm develops from an initial attachment of planktonic microorganisms to the electrode [110]. The electrode material and the hydrodynamic forces control biofilm adhesion, detachment, thickness and composition, as well as the driving force behind substrate and metabolite diffusion inside and outside the biofilm, affecting the biofilm conductivity and electron transfer rate [111]. Several review papers have discussed the importance of electrode materials [112] and their modifications [113] to improve the overall BES performance.

2.5.1 Hydrodynamic forces

BES operated in batch and without recirculation may favor the growth of planktonic microorganisms performing mediated electron transfer, whereas BES operated in continuous mode or with recirculation favor the attachment of electroactive microorganisms to the anode [27]. Borole et al. [27] operated MFCs at an intermittent flow rate, successfully enriching an electroactive biofilm at the expense of the planktonic microorganisms. Mechanical mixing also increases the hydrodynamic forces in the anodic chamber, reducing the mass transfer resistance, increasing diffusion and preventing localized pH gradients in the biofilm [54]. Recirculation of the anolyte at different rates [111] or continuous bubbling with N_2 [5] can also be used to increase the hydrodynamic forces in the anodic chamber. However, too high hydrodynamic forces cause biofilm detachment, especially in the early stage of biofilm formation. To counteract this problem, Li et al. [110] used a vertical reactor configuration with

an anode placed at the bottom of the reactor, enabling gravity settling of planktonic bacteria on the anode, achieving a current density of 1.86 A/m^2 in 1.64 days as compared to 0.5 A/m^2 obtained in a horizontal MFC in 1.87 days (Table 3).

In summary, BES start-up in batch, with recirculation but avoiding severe hydrodynamic conditions, may favor the initial attachment of electroactive microorganisms to the electrodes. Once the biofilm is established, the operation can be switched to continuous mode to flush out the planktonic cells, and the recirculation ratio can be increased to promote mass transfer between the anolyte and the microorganisms. Using granular or foam electrodes rather than flat carbon electrodes increases the surface available for microbial adhesion, and protects biofilms from the shear forces generated by the anolyte flow in case of a high recirculation rate In addition, reactor and electrode configuration can significantly change the hydrodynamic forces, and comparing different reactor configurations for their start-up time and electroactive biofilm build-up requires more attention.

Table 3: Different operational and design parameters affecting the start-up time and power or current density in MET.

Source of inoculum	Operating strategy		Start-up time	Maximum power or current density	Reference
Domestic wastewater	Temperature (°C)	15	9 d	70 mW/m^2	[98]
		22	2.5 d	58 mW/m^2	
		30	1.25 d	2 mW/m^2	
	Secondary biofilm		1 d	1487 mW/m^2	
MFC effluent	Vertical anode		1.64 d	0.86 A/m^2	[110]
	Horizontal anode		1.87 d	0.5 A/m^2	
Anaerobic sludge	Temperature (°C)	10	60 d	1.2 W/m^3	[114]
		20	30 d	1.2 W/m^3	
		35	7 d	1.2 W/m^3	
Primary clarifier effluent	Temperature (°C)	15	40 d	Not available	[99]
		22	12 d		
		27	6.5 d		
		35	3.5 d		
Primary clarifier effluent	pH	3	Not available	0 A/m^2	[103]
		5		0 A/m^2	
		6		0.15 A/m^2	
		7		0.8 A/m^2	
		8		0.75 A/m^2	
		9		0.7 A/m^2	
		11		0 A/m^2	

2.5.2 Electrode surface modification

Anode material and its surface structure affect bacterial attachment and the electrical connections between bacteria and the electrode surface [115]. The anode surface can be modified to facilitate bacterial adhesion and electron transfer. Modification methods include (i) physical or chemical surface treatment, (ii) addition of highly conductive or electroactive coatings, and (iii) use of metal–graphite composite electrodes (for a review see [115]). Initial attachment of planktonic microorganisms to the electrode can be enhanced by treating conductive surfaces with specific chemicals [116]. Since the majority of the known electroactive microorganisms are negatively charged, the addition of positively charged metal oxides to the electrode can increase their adhesion [117]. Similarly, ammonia treatment favors microbial adhesion by generating positively charged functional groups on the electrode surfaces [116]. Guo et al. [118] modified the surface of glassy carbon anodes with $-OH$, $-CH_3$, $-SO_3^-$ and $-N^+(CH_3)_3$ functional groups, reporting the highest current density and the shortest start-up time for the anode doped with $-N^+(CH_3)_3$. Addition of ferric oxides onto carbon electrodes reduces the acclimation time because several electroactive microorganisms can use Fe^{3+} as electron acceptor [119].

Thermal treatment of carbon based electrode materials increases the surface area available for microbial adhesion [120]. Acid treatment not only increases the electrode surface area, but also facilitates protonation of the functional groups, increasing the net positive charge of the electrode [120]. Electrochemical oxidation of carbon based electrodes generates new functional groups, such as carboxyl groups, which facilitate the formation of peptide bonds between the electrode surface and microorganisms, acting as highways for electron transfer [121]. Summarizing, adhesion of microorganisms to solid electrodes can be improved by increasing the surface area and roughness as well as modifying the surface charge towards more positive values. However, to selectively target adhesion of electroactive microorganisms, electrode modification should be combined with the repression of competitors.

2.5.3 Membranes

In BES, the anode and the cathode compartments are usually separated by a membrane, though membraneless BES have also been reported [36]. While membraneless BES can be characterized by (i) less complex design, (ii) reduced capital cost as well as (iii) decreased internal resistance, their efficiencies are often reduced due to the occurrence of side-reactions [122]. In membraneless BES, oxygen transfer from the cathode to the anode can affect

anaerobic biomass growth and activity of electroactive microorganisms, thus increasing start-up time and decreasing power production [122]. It has been reported that the type of membrane used can affect the abundance and phylogenetic distribution of electroactive bacteria [122]. Sotres et al. [49] observed that the eubacterial community in a MFC is not affected by membrane materials, while the archaeal counterpart is highly dependent on the type of membrane used. A selective enrichment of *Methanosarcina* occurred in the MFC equipped with cation exchange membranes whereas both *Methanosarcina* and *Methanosaeta* were abundant in the MFC equipped with an anion exchange membrane [49]. Kook et al. [123] reported that the membrane type does not affect start-up time, although anion exchange membranes lead to 2–5 times higher power production than cation and proton exchange membranes.. Transfer of other cations than protons through cation exchange membranes can hamper proton migration. As a result, a pH gradient develops across the membrane, affecting biofilm formation and thus increasing start-up time [122].

3 Electroactive cathodic biofilms

Although less studied than bioanodes, biological cathodes have recently attained increasing attention. Biocathodes are based on an uptake of electrons from the electrode to the microorganisms. In contrast to anodic microorganisms, which are mostly heterotrophs, the cathodic electroactive microorganisms are often autotrophic and usually require a long time to colonize the electrode. The electron transfer mechanisms of electroactive cathodic microorganisms are still not clear. A variety of terminal electron acceptors can be used by the electroactive cathodic microorganisms, such as protons, oxygen, nitrate, sulfate, iron, manganese, arsenate, fumarate, or carbon dioxide, depending on the MET application. Oxygen reducing cathodes have been widely studied, therefore, this review concentrates only on the enrichment of anaerobic electroactive microbial communities at the cathode performing i) nitrate or sulphate reduction and ii) electrically assisted synthesis of chemicals, hydrogen or methane. Similar to the anode, several strategies have been applied to improve the formation of an electroactive biofilm on the cathode electrode (Table 4).

Table 4: Comparison of operational conditions for enrichment of anaerobic biocathodes with the aim of increasing the output (H_2 or acetate depending on the application) and reducing start-up time. All the voltages reported (V) refer to the applied cathodic potentials *vs*. SHE.

Source of inoculum	Substrate	Start-up/Operation		Start-up time (d)	Output/Purpose		Reference
River mud	Bicarbonate	-0.6 V poised cathodic potential		No start-up	Electrosynthesis of acetate (mg/L at end of operation)	0	[124]
		0.4 V poised anode and polarity reversal to -0.6 V		4		158	
Anaerobic sludge		-0.6 V poised cathodic potential		14		162	
		0.4 V poised anode and polarity reversal to -0.6 V		3		70	
MFC biofilm	Acetate	Poised cathodic potential (V)	−0.5	30	H_2 production (the higher the current density, the more H_2 produced)	0.1 A/m^2	[71]
			−0.7	30		0.4 A/m^2	
			−0.8	30		0.6 A/m^2	
	Bicarbonate		−0.5	No enrichment		0.3 A/m^2	
			−0.7	60		0.8 A/m^2	
			−0.8	60		1.0 A/m^2	
Geobacter sulfurreducens	Acetate	Polarity reversal of bioanode with poised cathodic potential (V)	−0.6	Not available	H_2 production (mmol H_2/d)	0.1	[125]
			−0.7			0.3	
			−0.8			0.6	
Anaerobic sludge	Glucose	Poised whole cell potential (V)	0.0	Not available	H_2 production (mmol H_2/d)	0.70	[126]
			0.1			0.90	
			0.4			1.11	
			0.5			1.20	
			0.6			4.56	
			0.7			5.70	
			1.0			10.2	
Biofilm and effluent from a BES	Acetate	Poised cathodic potential (V)	-0.7	30	H_2 production (m^3 H_2/ m^3/d)	2.4	[127]
	$NaHCO_3$			60		2.7	
Moorella thermoautotrophica	Bicarbonate	Carbon cloth cathode	25 °C	Not available	Acetate production (mmol/m^2·d)	0.38	[128]
			37 °C			1.08	
			55 °C			1.46	
		Bacteria immobilized on carbon nanoparticles	55 °C			58.19	

3.1 Inoculum

Electroactive microorganisms from various natural environments can be acclimated on anaerobic biocathodes. The most common inoculum sources are anaerobic sludge [129,130], seawater or sediment [131,132], soil [83] and biofilm or effluent from a cathode of an operating BES [71,127]. The inoculum choice depends on the purpose of the biocathode. Pure cultures of metal reducing bacteria have been used for reduction of oxides of e.g. manganese, iron, and uranium [133,134]. Similar to anodic inocula, mixed cultures offer advantages over pure cultures such as the possibility to operate reactors without sterilization, and resistance to operational perturbations. However, when using mixed cultures, the cathodic product titer is often low due to competitive metabolic pathways, especially methanogenesis [135].

For methane synthesis in the cathodic chamber, a hydrogenophilic methanogenic culture was previously enriched from anaerobic sludge with hydrogen in the headspace [136]. Mixed anaerobic cultures [126,137] and planktonic microorganisms previously enriched on a biocathode [137] have been used for hydrogen production in MECs. Electroactive cathodic biofilm from an already existing BES [138], mixed culture of homoacetogenic bacteria [26,51] or a mixed culture of sulfate reducing microorganisms enriched in palm oil mill effluent [139] have been used for microbial electrosynthesis. Using sediment inoculum in MES, Mateos et al. [124] observed that a specialized biofilm grew on the electrode, resulting in high acetate and current generation. However, when using anaerobic sludge as inoculum, a non-specialized biofilm was developed and a lower acetic acid production was detected due to the influence of undesirable secondary metabolic pathways [124]. Similar to bioanodes, inoculation of biocathodes with an already acclimated microbial community may reduce the start-up time and increase MET performance, although how the different conditions affect the start-up of a biocathode is not widely discussed in the literature.

3.2 Imposed cathode potential

Cathodic microorganisms grow from the energy gain of the electron transfer from the cathode to the cell [126]. Hence, a more negative cathode potential results in faster start-up. In theory, a low cathode potential results in H_2 production, which is required for certain MET applications such as microbial electrosynthesis of VFAs from CO_2 [140], but can prevent biofilm attachment to the electrode due to the shear force generated by the produced H_2 bubbles. For H_2 producing biocathodes, a cathodic potential lower than the theoretical potential for H_2

production (-0.41 V *vs.* SHE) caused low biomass yield, due to an increased abiotic H_2 evolution reaction [141].

Jeremiasse et al. [71] examined the effect of controlled cathode potential (-0.5, -0.6, -0.7, and -0.8 V *vs.* SHE) for biohydrogen production and observed that a more negative cathode potential did not reduce the start-up time. In fact, despite the thermodynamic advantage, more negative cathodic potentials do not necessarily translate into faster start-up or improved performance [6]. The reason could be the increased abiotic hydrogen evolution with more negative cathodic potentials, which affects biofilm adhesion, as well as metabolic limitations of microbial species in relation to maximum energy gain (as discussed in section 2.3.1.1). A nitrate reducing cathode was enriched in electrochemical reactors with electrons supplied by graphite electrodes poised at -0.05 V and 0.2 V, and current production (-5 mA/m^2) was observed only when imposing -0.05 V [142]. In conclusion, slightly negative cathodic potentials, but higher than -0.5 V, enhance the start-up of biocathodes.

3.3 Polarity reversal

Polarity reversal (Table 4) consists of developing an electroactive microbial community at the anode and then reversing the polarity of the electrodes to let the community function as cathodic electron acceptor. Electrical inversion of bioanodes to H_2-evolving biocathodes was first demonstrated by Rozendal et al. [137], who enriched bioanodes on hydrogen to obtain hydrogen-evolving biocathodes based on the reversibility of hydrogenases. The same method was later applied in other studies for hydrogen production [125,139,143,144] and for wastewater treatment [145,146]. However, Jafary et al. [139] showed that the enrichment of anodic microorganisms in the presence of acetate may result in methane generation at the cathode upon polarity reversal, which reduces the product titer when the cathode is used for acetate synthesis.

Mateos et al. [124] observed that, regardless of the characteristics of the inoculum, biocathodes for microbial electrosynthesis struggled to form a biofilm by imposing reductive potentials (-0.6 V *vs.* SHE) directly to the biocathode, whereas a four times faster start-up was obtained applying an oxidative potential (0.4 V *vs*. SHE) and reversing it to a reductive potential. It was probably due to the fact that most of the bacteria in the inoculum used by Mateos et al. [124] oxidize organic compounds and cannot modify their metabolic pathways to be viable at reductive potentials. A methanogenic biocathode was established by inverting periodically the

anode and cathode polarity using a stack of rotatable conductive disks half submerged in wastewater and half exposed to headspace gas [147].

During periodic polarity reversal, switching the direction of the applied voltage between the anode and the cathode has been demonstrated as an effective method for pH control at the MEC cathode, enhancing hydrogen production as well [148]. In summary, polarity reversal could decrease the start-up time required for biocathodes, despite being a time-consuming multi-step procedure. The effectiveness of polarity reversal for biocathode enrichment is largely affected by the microorganisms present, electrode potential applied, electrode donors and the targeted products (for review see [149]). Thus, if polarity reversal is used to enrich the cathodic microbial community, it should be noted that high-current generating bioanodes do not necessarily translate into high-current consuming biocathodes [76].

3.4 Carbon source

Autotrophic microorganisms require more energy for growth than heterotrophic ones, resulting in a slow metabolism [71], and thus slow biocathode start-up. Jeremiasse et al. [71] and Croese et al. [127] investigated the effects of using either acetate or bicarbonate as carbon source on MEC biocathode start-up, and reported that acetate resulted in a higher cathodic biomass yield and two times faster start-up than bicarbonate. Hartline and Call [76] observed that formate-enriched cultures consume almost 20 times more current (-100 ± 26 A/m^3) than those established with acetate (-5.2 ± 2.9 A/m^3) in methanogenic biocathodes.

Using acetate as the carbon source in the biocathode chamber results in higher hydrogen production rates and decreases the start-up times in MECs [71]. However, an autotrophic carbon source and carbon-limited conditions during start-up can be used to outcompete methanogenic microorganisms [137].

3.5 Additives on the electrodes

Metals such as ferrous iron favor the initial contact between electroactive microorganisms and the electrode surface [86]. Graphene oxide was used to increase the conductivity of a carbon felt biocathode favoring the establishment of an acetate producing biofilm [138]. Many attempts have been made to immobilize microorganisms directly to electrode surfaces. Yu et al. [128] proposed, for a MES, a cathode consisting of bacteria immobilized on a carbon cloth modified with carbon nano-particles and Teflon emulsion, increasing acetate and formate production by 14 and 8 times, respectively, as compared to natural biofilms. Various matrices such as polyvinyl alcohol [150], latex [151], and hydrogel [152] have been used to entrap

microorganisms on the electrodes. However, these immobilization matrices are electrical insulators, which might completely inhibit microbial extracellular electron transfer. Despite possible advantages on the product titer, modification of cathodes does not appear a cost-effective approach for full-scale biocathodes.

4 Start-up strategies for bioanodes and biocathodes

The inoculum type and applied potential are the most studied factors related to bioanode start-up. The results obtained are not comparable as such among the different studies, as they depend on many variables, including reactor configuration, electrode and membrane materials, conductivity and pH of the anolyte and catholyte, substrate, and temperature.

In order to enable comparison between the different studies, a statistical analysis was performed for bioanodes (Fig. 2) by collecting data from studies that used different inocula (two or more) or different anode potentials (two or more) for BES start-up. The BES performance (P), in terms of power density, current density, voltage or Coulombic efficiency, and start-up time (L) of the investigated inoculum (or applied potential) were normalized to the maximum BES performance (P_{max}) or maximum start-up time (L_{max}) obtained in the same study. Fig. 2 shows that P/P_{max} close to one indicates that the inoculum (or the applied anode potential) investigated resulted in a higher power density than the other inocula (or applied anode potential) in the same study. On the contrary, a low L/L_{max} indicates that an inoculum (or applied anode potential) resulted in a faster start-up than the other inocula in the same study. The obtained P/P_{max} and L/L_{max} values have a wide variation due to different experimental set-ups and operational conditions.

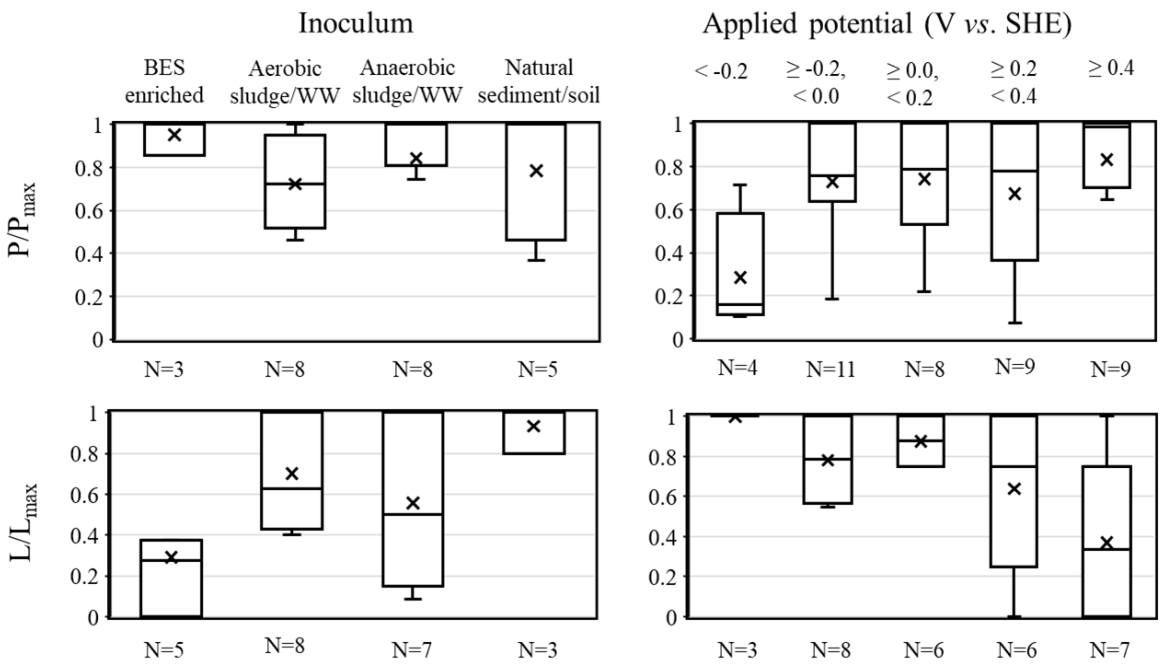

Figure 2: Statistical analysis on the effect of inoculum and applied potential on BES performance and start-up time. The P/Pmax and L/L_{max} denote, respectively, the BES performance (in terms of power density, current density, voltage or Coulombic efficiency) and the start-up time detected with the investigated inoculum/applied potential compared to the maximum BES performance or start-up time obtained in the same study. The cross and the horizontal line represent, respectively, the average and the median of the P/P_{max} or L/L_{max} distribution. The box plot represents the range of results included in the second and the third quartile (50% of the distribution), and the whiskers represent the first and the fourth quartile. N refers to the number of references taken into account for the statistical analysis (for more details, see File S1 in the supplementary material).

Enriched inocula from previous BES produce, on average, slightly higher power outputs than other inocula. This is likely due to the higher proportion of electroactive microorganisms in the enriched inocula, although the differences are not statistically significant due to the high variability in operational conditions (Fig. 2). As expected, enriched inocula often result in faster start-up, whereas natural sediment or soils, which mostly have low initial percentage of electroactive microorganisms, result in the slowest start-up in most cases (Fig. 2). Combining an enriched inoculum to anaerobic sludge (with addition of BESA to inhibit methanogens) may allow fast start-up of scaled up reactors, ensuring a broad microbial community composition, including electroactive microorganisms, able to resist harsh conditions and fluctuating composition typical of real wastewaters.

Applied potentials below -0.2 V *vs*. SHE must be avoided, as they have resulted, in average, in a low P/P_{max} (Fig. 2), likely due to the low number of electroactive microbial species able to grow at such low potentials. Furthermore, as shown by the L/L_{max} of 1, an applied potential of –0.2 V *vs*. SHE resulted in a slower start-up than higher potential in all the studies (N=3) included in the statistical analysis. No relevant differences on power production have been individuated applying potentials above –0.2 V *vs*. SHE, likely due to the energy gain limitation of cell membrane proteins (see section 2.3.1.1). The results suggest that potentials higher than – 0.2 V *vs*. SHE speed up anodic biofilm formation resulting in shorter start-up times, although no statistical significant differences can be seen on the final power obtained.

Favoring the growth of anodic biofilms, rather than planktonic cells, is crucial to obtain high and steady power production. Reactor configurations with good mixing capabilities should therefore be preferred in order to ensure a regular washout of planktonic cells, as well as decreasing diffusion resistance. However, severe mixing conditions in early stage may hinder biofilm attachment on the electrode. Electrodes with a rough surface and high active surface, as well as chemical modification of electrodes, promote adhesion of microorganisms by reducing the shear forces, but may also favor competing microbial communities. Furthermore, a cost/benefit analysis is required if expensive electrode materials are used. Due to cost constraints, extensive addition of chemicals (e.g. buffers or conductive solutions) to the anolyte is not recommended. Bioanodes can be started at ambient temperature, resulting in more versatile biofilms, if a fast start-up is not required.

Biocathode-based MET are in their infancy, and further investigations are required on biocathode start-up before drawing general conclusions, as the research articles currently available in the literature are not enough for a statistical analysis. However, similar to the bioanodes, inoculation of biocathodes with an already acclimated electroactive microbial community may reduce start-up time and increase performance in terms of cathodic product titer. Anaerobic sludge is also a potential inoculum for cathode applications such as electrosynthesis, hydrogen production, methanogenesis, and sulfate and nitrate reduction. Starting-up as a bioanode and then reversal of polarity to biocathode is often an effective choice for biocathode enrichment, irrespective of the MET application. Using a heterotrophic rather than an autotrophic substrate during start-up will result in shorter start-up times. As with the anode, a proper mixing will enhance microbial attachment to the cathode electrode. Further studies are required to determine the optimum temperature and pH for the different cathode applications. For example, in electrosynthesis reactions, the pH determines the product

spectrum [153]. Based on the extensive and critical literature survey, strategies that result in a fast and efficient development of bioanodes and biocathodes are summarized in Table 5.

Table 5: Proposed strategies for an efficient and fast start-up of bioanodes and biocathodes. The four steps refer to Fig. 1.

Step	Anode			Cathode		
	Strategy	Advantage	Disadvantage	Strategy	Advantage	Disadvantage
1) Select an inoculum rich in electroactive microorganisms	Enriched in earlier bioelectrochemical reactor +anaerobic sludge or wastewater	Wide microbial community, fast start-up	Competitive microorganisms (e.g. methanogens)	Enriched in earlier bioelectrochemical reactor or batch bottles	Fast start-up, presence of specialized anaerobic microorganisms for electrosynthesis, hydrogen or methane production, or sulfate or nitrate reduction	Low diversity and thus, low versatility of the microbial community
2) Eliminate methanogenic archaea	BESA treatment	Selective inhibitor of methanogens	Does not ensure long-term effect in case of unsterile conditions	BESA treatment (for biocathode applications other than methanogenesis)	Improves product titer; Selective inhibitor of methanogens	Does not ensure long-term effect in case of unsterile conditions
3) Optimize growth of electroactive microorganisms	Imposed potential ($\geq$ -0.2 V *vs.* SHE)	Fast start-up; Required only in the initial stage	Energy requirements	Polarity reversal	Fast start-up	Multi-step start-up procedure; possible enrichment of methanogens if not previously inhibited
4) Enhance biofilm adhesion	High shear stress	Flush-out planktonic cells; Improved diffusion and mixing	Biofilm detachment in early stage; High energy requirements	High shear stress	Flush-out planktonic cells; Improved diffusion and mixing	Biofilm detachment in early stage; High energy requirements

5 Conclusions and future outlook

This review thoroughly analyzes BES start-up as a major constraint for MET commercialization. The following start-up strategies for bioanodes and biocathodes are proposed:

- Mixed cultures are preferred over pure cultures as a source of inoculum for full-scale operation of MET. Addition of several inocula of different origin and properties widens the electroactive microbial community, if combined with inhibition of competitors. In case of reactor failure, the performance can be recovered by replacing part of the electrode or electrolyte with an enrichment culture. A secondary electrode from a well performing reactor is another good strategy to start-up or to revive BES.
- Chemical inhibition of methanogens may be necessary for efficient start-up of bioanodes and biocathodes for applications other than methane production. However, use of chemicals may not be sustainable in large-scale operation and other options to inhibit methanogens must be developed.
- A negatively poised anodic potential and use of non-fermentable substrates such as acetate for the start-up of MET anodes often selects for electroactive microorganisms. However, applying potentials lower than –0.2 V *vs.* SHE for the bioanode is not advisable due to the low number of microorganisms growing at low potential. Polarity reversal is a promising strategy for a fast start-up of MET biocathodes.
- Operation with improved hydrodynamic conditions of the electrolyte flushes out planktonic microorganisms and mitigates pH gradients and diffusion limitations.

The scalability of BES is a key parameter for their practical implementation. Reduction of start-up time, and the development of strong, resilient electrogenic biofilms, are major requisites for commercialization of BES. More research efforts are required on the following topics:

- Increasing the technological level of biocathodes, as done for bioanodes in the last years, to speed up start-up and achieve more consistent biocathode performance.
- Application of bioaugmentation to revive system performance after process disturbances.
- Strategies to avoid contamination with non-electroactive bacteria when treating real wastewaters, specially during BES start-up, and understanding the role of methanogens and other competing microbes on biofilm formation.

- Impact of different electrode materials and their modification on start-up time.
- Application of the strategies summarized in Table 5 on BES fed with real wastewater to solve application based issues.
- Optimize hydrodynamic conditions for a fast biofilm formation in full-scale BES.

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