Microbial electrochemical systems for sustainable biohydrogen production: Surveying the experiences from a start-up viewpoint

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Abstract

The start-up of microbial electrohydrogenesis cells (MECs) is a key-step to realize efficient biohydrogen generation and adequate, long-term operation. This review paper deals with the lessons and experiences reported on the most important aspects of H_2 producing MEC start-up. The comprehensive survey covers the assessment and discussion of the main influencing factors and methods (e.g. inocula selection, enrichment, acclimation, operating conditions and cell architecture) that assist the design of MECs. This work intends to be a helpful guide for the interested readers about the strategies employed to successfully establish microbial electrochemical cells for sustainable biohydrogen production.

Keywords: bioelectrochemical systems, microbial electrohydrogenesis cell, microbial electrolysis cell, microbial fuel cell, biohydrogen, start-up

1. Introduction

In the last decade, bioelectrochemical systems (BES) have become an intensively studied platform technology in various fields of biotechnological processes [1]. BES are driven by special, electrochemically-active microorganisms to achieve goals such as (i) waste treatment to serve environmental remediation [2], (ii) the production of chemicals [3] and (iii) renewable energy recovery [4]. In the last aspect, microbial fuels cells (MFC) and microbial electrohydrogenesis cells (MEC) were shown as feasible approaches [5,6]. MECs are considered to combine MFC technology with electrolysis [7]. In both MFCs and MECs, bacteria work under anaerobic conditions at an anode to oxidize various substrates ranging from simple compounds i.e. sugars, organic acids [8] to complex organic matter including wastewaters of distinct origin [9-11] as well as fermentation effluents [12]. As a results, either bioelectric potential (in MFC) or H_2 gas (in MEC) is obtained. It was lately argued based on life-cycle assessment that the conversion of organic feedstock to bioH_2 in MECs is a highly attractive way to go from an environmental protection standpoint [13,14], which suggests the potential contribution of this technology to sustainability.

In principles, MECs apply two electrodes (the anode and the cathode) under anaerobic circumstances [15]. The anode is the important place for exoelectrogenic strains that after colonizing its surface, form an anode-respiring biofilm. In essence, attributed to the metabolic activity of the biofilm, electrons and protons are released from

successful substrate conversion/degradation. The electrons are transferred to the anode (as final electron acceptor) by different possible mechanisms (discussed later) and pass subsequently to the cathode via an external circuit. At the cathode, which plays the role of an electron donor, the reduction of H^+ to molecular H_2 gas takes place. Unfortunately, this phenomena is non-spontaneous (thermodynamically not favored due to the positive Gibbs free-energy of the reaction) and therefore an external voltage, practically at least 0.2-0.25 V must be supplemented to make it happen (**Fig. 1**). The consecutive reactions (anodic substrate degradation and cathodic product (H_2) formation) can be either done in single- or two-chambered arrangement. In the latter case, the anode and cathode are spatially separated, in general by a membrane possessing ion-exchange capacity.

Basically, the achievable, steady-state performance of MECs depends strongly on the way it is started-up, which is known as a crucial step for H_2 producing biotechnological systems [**16**]. The start-up could have a great importance to maximize the H_2 production capacity of the MEC and should involve the establishment of efficient and robust biofilms [**17-19**]. To achieve adequate start-up, the source of inocula (containing the exoelectrogenic strains), consequent enrichment and acclimation methods to select bacteria with high bioelectrochmical activity seem to be of high concern since the characteristics of the anode-surface grown biofilm (i.e. its composition and state) determine the attractiveness of BES [**20-23**]. In addition to these biotic factors, the start-up process ought to deal with the operating conditions (such as anodic potential, temperature, substrate and its concentration) and the cell architecture so as to positively

influence the biofilm development and optimize the MEC from the point of view of H_2 production rate/yield and other (energetic) process indicators e.g. Coulombic-efficiency, cathodic H_2 recovery, current density, etc. However, even though the start-up is a key-element for longer-term MEC viability [24], to our knowledge, no comprehensive article has been specifically dedicated to overview and assess the lessons and experiences gained in this field. Since MECs can be viewed as MFC-derived technologies with significant modifications on the cathode side, the start-up methods could reflect quite a number of similarities, especially related to the bioanode development [20]. Hence, in this paper, it was aimed to review the most essential factors and design considerations related to MEC start-up and give an insight to the progress how the recent accomplishments have improved the methodological approaches and enriched the international knowledge in this field.

2. Effects of start-up variables on MEC performance

2.1. Inoculum for MEC start-up

INOCULA containing anode-respiring bacteria can be delivered from various environments [25,26] and dozens of strains were found to have sufficient capability for powering BES via biocurrent generation by (i) exocellular electron transfer relying either on membrane cytochromes, (ii) artificial/self-secreted mediators or (iii) electro-

conductive appendages [27-29]. To select the microbial species (with appropriate electrochemical activity) to be used in biological fuel cells, a fast screening method was lately reported by Szöllősi et al. [30].

BES can apply both pure- and mixed cultures for inoculation. The use of pure isolates in bioelectrochemical applications could be important to conduct fundamental studies and to gain a better understanding about strain characteristics, behavior and functionalities (i.e. electron-transfer mechanism of the particular bacteria) [21]. Moreover, single-strains can be employed in the frame of bioaugmentation concept in order to reinforce mixed populations and obtain a better microbial equilibrium, which, in turn, leads to a higher capacity, exoelectrogenic biofilms and improved operational stability [20,31]. Systematic investigation and deeper comprehension on community ecology e.g. revealing the interactions in the fixed anodic-biofilms could be a valuable tool to enhance BES performance [22,32]. For instance, the syntrophy of anode-respiring bacteria with fermenting microorganisms seems to be advantageous [33] since the members of the latter class are able to efficiently decompose complex organic matter to simple compounds such as acetate, which represent easily biodegradable substrates for the former group.

Although pure culture BESs fit perfectly for principle studies, practical one should rely on mixed cultures. As concluded in the review by Liu et al. [27], these communities, in most cases, generate higher currents and provide better stability in comparison with single-strain systems. The further advantage of such communities could be the potential

versatility and flexibility that are required for real-case, non-sterile applications. These could be good reasons behind the fact that microbial consortia appear to be more feasible to inoculate BES. Nevertheless, depending on the source of mixed inoculum, the reactor start-up and concomitant operational behavior e.g. in terms of process lag-phase can be significantly different [**34**]. Hence, to increase the probability of appropriate start-up and fair performance in longer-terms, techniques can be proposed for mixed culture microbial electrochemical cells that may result in an enriched consortia with better properties.

These enrichment methods (being either electrochemical or chemical) make it possible targeting specific groups of efficient exoelectrogenic species such as *Geobacteraceae* [**35-37**]. This preliminary selection, controlled growth and acclimation of biocatalysts could have substantial practical advantage since besides the physiological state of the bacterial cells [**38,39**], the profile of the active microbial community developing on the anode during the start-up period is a factor that directly affects the MEC operation [**40,41**]. For instance, Boghani et al. [**42**] underlined that an optimized, electrochemical-strategy can be applicable to control biofilm enrichment, cut the start-up time demand and increase the capacity of the bioelectrochemical cell. Interestingly, Borjas et al. [**38**] demonstrated a 20-fold faster start-up period and a concurrent, 6-fold enhancement of COD removal during continuous MEC operation using chemostat-(pre)grown, ”plug and play” *Geobacter* culture instead of batch-grown cells. Thus, faster BES start-up seems to be possible employing pre-activated inoculum.

In mixed culture BES, however, the competition of various microbial groups for ecological niche and substrate [**43**] e.g. between suspended-form (bulky phase) and anodophilic (electrogen) biocatalysts [**9**] may occur and can be seen as a notable constraint. Although non-exoelectrogens are expected to fail after a certain period of time because of the gradual dominance of their anode-surface located, bioelectrochemically-active counterparts [**44**], preventive actions so as to restrict undesired microbiological phenomena are advisable. It is noteworthy that apart from classical substrate (e.g. acetate) degradation, H_2-scavenging reactions via interspecies hydrogen transfer e.g. by hydrogenotrophic methanogenesis could also take place in MECs [**45,46**]. Besides conventional and well-known methanogenesis, the H_2-recycling effect is also to avoid, which means that a part of H_2 evolved on the MEC cathode is utilized by bacteria on the anode i.e. to produce acetate via homoacetogenesis [**47,48**]. Besides, certain electrotroph microorganisms sticking to the cathode surface are able to capture the electrons transferred from the bioanode and directly convert them to methane via CO_2 reduction [**49-51**], referred as electromethanosynthesis [**52**]. According to Sun et al. [**53**], further internal factors that can deteriorate BES performance are (i) biofouling and membrane blockage (obstructing proton transport to the cathode chamber), (ii) excessive anodic biofilm growth (causing non-conductive (dead) layers and limited substrate diffusion rate) and (iii) cathode inactivation due to the deposition of salt aggregates (partly occupying the reactive sites and blocking the proton transport to the surface).

Typically, when the above-mentioned H_2-consuming bioreactions and/or the consumption of organic materials through non-bioelectrochemical pathways cannot be neglected, the MEC performance, characterized by energetic process indicators i.e. Coulombic efficiency, current density, cathodic hydrogen recovery and actual H_2 production rate undergo a decrease [**54**]. The Coulombic efficiency is a good tool to see what portion of the electrons liberated from oxidation of organic matter could be effectively captured by the anode and utilized in the bioelectrochemical reactions [**55**]. The amount of electrons reaching the anode (as terminal electron acceptor under anaerobic conditions) will influence the current (density), which is common measure to express the electrochemical activity of the biofilm [**56**] and determines the cathodic H_2 recovery as well as the H_2 production rate [**24,57**]. Apparently, to make MEC technology competitive with others existing in the field of renewable energy e.g. anaerobic digestion, as high efficiencies as possible should be attained for these parameters.

As implied, a part of biocatalyzed side-reactions in MECs is encountered due to the presence of methanogens (**Fig. 2**). In addition to the fact that methane formation can lower the overall efficiency of MECs, it can also be responsible for reactor off-gas contamination, which makes the downstream more complicated. This problem is more considerable in single-chamber devices where the anode and cathode reactions are not spatially separated. Nonetheless, even in MECs constructed in two-chambered design, gases could diffuse over time through the membrane placed in-between the anode and cathode compartments [**58**]. If it occurs, the hydrogen gas recovered at the cathode will

contain impurities to be removed. To help the suppression of these unbeneficial organisms – in addition to the enrichment methods enlightened above – inoculum pretreatments i.e. by heat-shock, chemical inhibitors and pH adjustment were confirmed to eliminate/restrict methane-forming activity from mixed anaerobic communities [**59-61**].

However, there are occasions when competing microorganisms, despite the careful efforts, survive for longer-terms by alternative metabolisms e.g. fermentation and methanogenesis [**62**]. For instance, Escapa and co-scientists [**63**] have communicated residual methanogenic activity in MECs inoculated with heat-shock pretreated culture. In such cases, overcoming strategies i.e. by regulating anode potentials can have a positive contribution to control the intensity of CH_4 production and subsequently recover the system performance [**64**]. Furthermore, shortened MEC cycle time can also depress the methane formation activity [**46**]. Nonetheless, if considerable methanogenesis still exists i.e. due to the growth of archaea either on the reactor wall [**65**] or on the cathode, MEC re-start may be unavoidable.

Whether or not preliminary enrichment and/or seed pretreatment are carried out, MEC systems can be started-up by following two distinct approaches (**Table 1**): direct and indirect mode [**66**]. The former means one-step inoculation and adaption directly in the MEC, while the latter consists of a two-step, sequential procedure applying MFC as a first step to acclimate the biocatalysts and develop stable bioanodes. In this latter case, the steady, MFC-grown bioanodes can be transferred to the MEC device [**57,67**].

Interestingly, Liu et al. [15] found that the choice of MEC start-up mode can play an important role in preventing the growth of CH_4-forming archaea. In their investigation, the observable methane production during the start-up of single-chamber MECs, running preliminary in MFC mode was much lower compared to MECs begun to operate directly (without the MFC stage). Besides, Wang et al. [68] demonstrated that it is also possible to switch between MFC and MEC modes in the same reactor employing a time-relay method.

From another aspect, (sequencing) batch mode operation represents the simplest and most routine way for BES start-up, although some authors succeeded with start-up carried out in continuous mode. For instance, Escapa et al. [69] used domestic wastewater as inocula for continuous flow MEC and the start-up period was performed in continuous mode (6 days long start-up, 12 h of HRT) and an extra 29 days were ensured to further stabilize the biofilm after observing the stabilization of current. Following a similar strategy, Tartakovsky et al. [70] investigated the hydrogen production in membraneless MEC started-up in a continuous mode at 10 h hydraulic retention time.

2.2. The effect of operating variables on MEC start-up and its time demand

The time requirement of start-up period in microbiological fuel cells should be as short as possible [71]. Nevertheless, it can be dependent on (i) the traits of the inoculum

[71-73], (ii) the operating circumstances and (iii) the system architecture. Depending on the joint impact of these parameters, usual, system-specific start-up can last even for a couple of months [71]. Although the start-up of bioelectochemical systems seems to be laborious and time-consuming, some papers presented complete BES start-up only in several days [44,71]. Interestingly, Verea et al. [74] described a fast method for bioanode enrichment, which was done in 8 hours using 1 V voltage and facilitated MEC performance. In general, bioelectrochemical systems are considered to be started-up when performances (in particular steady-state voltages and current density profiles) are reproducible for a few (normally at least 3) consecutive (batch) cycles under the given operational conditions. Once such a state of the reactor is noted, it can be said that the anodic, exoelectrogenic biofilm is developed, mature enough [75] and accustomed to the reaction circumstances.

Since the significance of inoculum properties on start-up was already discussed in chapter 2.1., the following sections intend to present the role of (i) operating conditions and (ii) cell architecture on this critical phase of MEC operation.

2.2.1. Anode potential

Among the MEC operating variables, *ANODE POTENTIAL* is definitely a significant one and its adjustment can be precisely done using a potentiostat against a reference electrode (e.g. Ag/AgCl, SCE – standard calomel electrode, etc.). As concluded

by Venkata Mohan and Lenin Babu [76], regardless of the transfer mechanism, the electrons released by the exoelectrogenic microoganisms have to move from higher negative potentials towards lower negative potentials. Thus, higher anode potentials will expectedly help the flow of electrons from the bacterial biofilm to the final terminal electron acceptor (anode). The relationship of the Gibbs free-energy with the potential difference between the electron (i) donor and (ii) acceptor suggests that higher energy can be gained by the cells via setting higher anode (electron acceptor) potentials. In other words, the potentials both of the anode and the terminal respiratory proteins will influence together the bacterial growth conditions and the amount of energy available for cell maintenance [77,78]. As summarized by Wagner et al. [77], literature studies demonstrate a general tendency of enhanced BES performance along with more positive anode potentials. For example, Wang et al. [73] demonstrated that positive posed potential on the anode (+200 mV vs. Ag/AgCl) was able to increase the activity of the electrochemical biofilm and thus, reduce the start-up time. The results showed that such strategy required 40% less time (35 days) in comparison with the control reactor (59 days) to get the system ready, without having significant differences in post start-up reactor performances. Similarly, Cho and Ellington [82] demonstrated the benefit of well-regulated anode potential conditions (+500 mV vs. Ag/AgCl electrode), which resulted in the drastic (over 90%) reduction of biofilm growth lag-phase. In another research, Aelterman et al. [83] drew supporting conclusions, as the outcomes indicated that an optimal anode potential can drive biofilm growth and activity, being accompanied by enhanced current generation and sustainable operation. Similarly, Commault et al. [84]

highlighted that well-regulated anode potentials are useful to select efficient, *Geobacter*-dominated biofilms and decrease the start-up time. However, it is noteworthy that even though many research works found better BES performance at higher anodic potentials, some others reported the preference of lower values [**77**]. Therefore, best anode potentials – defined as those resulting in high current densities and shorter start-up times – must be determined as a part of case-specific optimization due to factors (e.g. the composition of the microbial communities) that can vary from system to system) in order to help the development of proper anodophilic population and improve its e^- discharge capability [**56**].

In addition to the already described role that anode potential can have in BES, the value of fixed anodic (biofilm cultivation) potentials can metabolically stimulate the bacteria in a way that it may induce a switch in the electron transfer mechanism taking place between cells and the anode [**79-81**]. Furthermore, some researchers noticed a correlation between bacterial swimming speeds (using strains of *Shewanella*) and anode potential values [**85**] and it turned out also that carefully selected anodic potentials might have a beneficial effect in depressing methane formation activity [**64**], as implied above.

In summary, starting-up MEC systems with properly chosen anode potentials seems to be advantageous in promoting the colonization of anode by desired exoelectrogenic strains and in advancing robust bioanode formation [**86**]. Interestingly, Nam et al. [**65**] reported that strategies potentiostatically controlling the anode potential can be superior over simple “added voltage” operation using a DC power supply since it

could result in a higher cathodic hydrogen production and shortened MEC cycle time. Nevertheless, the application of external (DC) power sources to provide sufficient voltage is still a widespread alternative for start-up MECs, and its value was proven to affect biohydrogen recovery in MEC using recalcitrant substrate e.g. liquid fraction of municipal solid waste in a recent study by Zhen et al. [**122**]. In another example, Heidrich et al. [**87**] described (>2 months) anode-biofilm acclimation method, during which the externally supplemented voltage was step-wise increased until decent H_2 gas production could be observed. This start-up was proven successful and a stable biofilm could be obtained demonstrating a reliable performance in a long (1 year) interval [**88**]. In a paper by Jeremiasse et al. [**89**], MEC start-up took 3 weeks at 0.5 V applied cell voltage. Rozendal et al. [**90**] operated the MEC in start-up mode for 100 days, with a posed potential of 1 V. In a work by Wang et al. [**91**], 0.6 V was used to establish the bioanode. Last but not least, in some articles [**76,92**], the adaption of bacteria and bioanode establishment was achieved without supplementing any external (DC or potentiostatic) power.

2.2.2. Temperature effect on MEC start-up

The *ACCLIMATION TEMPERATURE* could have a defining role in the dynamic selection and subsequent enrichment of anodophilic bacteria [**93**]. Consequently, the operational temperature of bioelectrochemical systems should be chosen in accordance with the properties of the inocula in order to maintain sufficient performances in longer

terms. For example, as analyzed by Heidrich et al. [**88**], a likely cause for MEC failures can be the lack of sufficient adaption of mesophilic communities to lower temperatures. Additionally, operational temperature strongly regulates anode colonization [**94**]. In essence, the start-up time in bioelectrochemical cells was found to be in reverse relationship with system temperatures, while anodic biomass growth rate and accumulation showed a directly proportional correlation with elevated temperatures i.e. in the range of 10-35 ^{o}C [**94**]. For instance, MEC start-up time at 35 ^{o}C could be shortened by 90 % compared to 15 ^{o}C conditions [**95**]. Although it is commonly observed that MEC start-up increases with lower temperatures [**94-96**], findings about its effect on the final electrochemical activity of the bioanode are somewhat contradictory. For instance, in studies by Michie et al. [**94**] and Ahn and Logan [**121**] lower temperatures did not significantly affect the achievable, longer-term steady-state properties, meaning that of BESs acclimated at psychrophilic temperatures produced comparable voltages with mesophilic systems. In the case of the former study referred [**94**], a roughly 1 year process monitoring revealed that powers in MFCs operated at 10, 20 and 35 ^{o}C were all around 0.23-0.24 mW. On the other hand, Patil et al. [**95**] came to the conclusion that bioelectrocatalytic capacity of biofilms adapted at higher temperatures resulted in better, steady-state current densities. Noteworthy, even though greater biomass yield is generally reported with elevated temperatures, it can unfortunately be coupled with the improved proliferation of methanogenic archaea [**94**].

Overall, the analysis of literature indicates that the optimal temperature must always be determined for the specific MEC application. This should however be a trade-off value that balances between (i) lag-phase time, (ii) anodic bioelectrochemical activity and (iii) methanogenic growth. Thus, at the expense of longer start-up phase, MECs acclimated and operated in the lower i.e. psychrophilic temperature range may be advantageous for the selective development of exoelectrogenically-active communities and simultaneous depression of competing organisms. Such psychrophilic MEC systems were shown to work well and produced decent amount of H_2 gas [**96**], which is an attractive outcome since conventional H_2 production methods i.e. by dark fermentation normally fail under low operational temperatures.

2.2.3. Substrate and its concentration

It was lately underlined that *SUBSTRATE QUALITY* and *CONCENTRATION* [**8,55**] can play determining roles in microbial electrochemical cells. The substrates used in MEC [**97**], depending on their characteristics e.g. source and complexity, influence the anode-surface biofilm growth, the composition of the bacterial community and in the end, the efficiency indicators e.g. Coulombic efficiency. As reported by Sleutels et al. [**55**], lower substrate concentrations along with increased anodic potentials could be able to boost anodic biofilm activity, making them more competitive in longer-terms with non-electrochemically useful microbes i.e. methanogens.

During start-up period, acetate is a widely recognized compound to attain sufficient, anode-surface biofilm build-up. Nonetheless, in the course of practical, post-start-up MEC operation where recuperation of bioenergy from inexpensive/waste resources is among the primary objectives, the simple substrates are normally changed to complex, problematic organic matter e.g. the effluent of dark fermentative H_2 producing bioreactors [**98**]. The feasibility of dark fermentation effluent (containing soluble metabolic products i.e. volatile fatty acids) for MEC set-ups was communicated in papers by Lalaurette et al. [**99**] and lately by Rivera et al. [**12**], as well (**Fig. 1**). According to such examples, it would appear that MECs hold the promise to be auxiliary (post-treatment) processes for classical dark fermentation in order to harness extra bioenergy (in the form of hydrogen) and thus, such combined applications can attract more attention to the emerging hydrogen energy sector.

As for substrate concentration, Escapa et al. [**63**] suggested a gradually-increased substrate loading for start-up to maintain its sufficient level but avoid its overdose causing an inhibition. In such a way, efficient stabilization of microbial bioanode was observed after 18-20 days. Furthermore, Liu et al. [**71**] studied the effects of medium amendments using compound such as acetate, fumarate, glucose and Fe(III) on the start-up time of the wastewater-inoculated MFCs and summarized that the applied wastewater itself was appropriate for the acclimatization period without any added chemicals or amendments.

In addition to the start-up of anodic biofilm, various authors took into account the development and application of H_2-producing biological cathodes, as alternative solutions to regular (and costly) metal-based ones [**14**]. It was reported however that biocathode start-up is quite sophisticated compared to bioanodes [**100**]. To reduce its time demand, Jeremiasse et al. [**101**] investigated the effect of the substrate type and cathode potential on the start-up process in microbial electrolysis cell inoculated with aged MEC anodic cells. They found that acetate feeding instead of bicarbonate resulted in higher cathodic biomass yield and two times faster start-up, while the cathode potential had no significant influence.

2.2.4. The cell architecture: anode and cathode materials, external and internal resistances

The *ELECTRODES* are crucial components of bioelectrochemical systems. Conductive anode and cathode materials will not only affect the investment costs, but also the attachment of microorganisms to the surface. Hence, electrode properties, at least in part, determine the time needed for biofilm growth [**102,103**] and as a consequence, the duration of start-up phase. Thus, their careful selection is a key-criteria for reactor design and stable operation. Anodes must be of biocompatible materials i.e. the already proven carbon or composites made of stainless steel [**104**], while chemical cathodes frequently contain platinum, nickel, stainless steel, etc. [**105- 108**] in order that H_2

formation is properly catalyzed. Alternatively, microbial cathodes can represent a solution [**109**]. The anode properties i.e. mass and charge transport speed take a direct effect on the MEC performance [**110**]. The modifications of electrode surface by heat- or chemical treatment have been recently used in the field of BESs to alter surface charge, hydrophobicity, etc. For instance, Guo et al. [**111**] investigated the formation and composition of anodic biofilms growing on $-N^{+}(CH_3)_3$, -OH, $-SO_3^{-}$ and $-CH_3$ groups modified anodes with different surface charges. It was found that the start-up time was the fastest in case of $N^{+}(CH_3)_3$ group (23 days), while the $-CH_3$ modification resulted in a longest one (37.2 days) and furthermore, the more (positively) charged and more hydrophilic surfaces could better promote the selective and efficient exoelectrogenic biofilm development. In order to perform the surface modification, Feng et al. [**112**] suggested a method by using quaternary ammonium compound directly added to the anodic electrolyte in wastewater utilizing BES. Assessing the behavior of BESs, the start-up time could be decreased by 29 % and 21 % using 0.01 M and 0.001 M quaternary ammonium concentration, respectively in comparison with the control system.

Besides the electrodes, the *EXTERNAL RESISTANCE* built in the electronic circuit of BES should be properly chosen, as well. In theory, the optimal value of external resistance should be close to the internal resistance of the bioelectrochemical system, which can improve the electrochemical performance e.g. in terms of current density [**102**]. In a study by Zhang et al. [**113**], the effect of static ohmic loadings on the biofilm formation and current production in MFC mode was sought. By using external resistance

values of 10 Ω, 50 Ω, 250 Ω and 1 kΩ, it could be deducted that the lowest external resistance resulted in shorter start-up time (2.2 days) and highest current production. However, regarding the maximum power density, the external resistance of 50 Ω (with a start-up time of 4.3 days) was found to be optimal because of the higher amount of active biomass formed.

The *INTERNAL RESISTANCE* of BES is dependent on factors such as electrode distances, solution (anolyte, catolyte) conductivity, electrode structures, etc. [**62,74]**. For example, the arrangement of the electrodes (anode, cathode) was shown to affect the internal resistance of the system [**67,70**] and smaller electrode spacing was found to increase H_2 production in MEC [**114**]. Therefore, constructional or in other words, architectural features of BES should be treated with care to minimize losses and enhance the performances [**115-117**]

Further considerations should be made regarding the *MEMBRANE* to be used in two-chambered arrangements, which represent the traditional design of bioelectrochmical systems. In such applications, the anodic and cathodic compartments are separated by various ion exchange membranes. In this regard, Rozendal et al. [**90**] compared cationic (CEM) and anionic exchange (AEM) membranes to be employed for such purposes. Using CEM, an issue with pH increase in the cathode side of the cell may be experienced due to migration of positively charged ions other than H^+ [**118**]. This phenomena depresses the system performance in a way that the higher pH gradients between anode and cathode cause greater potential losses. To overcome this problem, several different

strategies were already tested [**119**] e.g. the deployment of AEM. In that case, biocatalyzed H_2 production takes place from the reduction of water instead of via the recombination of protons with electrons [**90**]. Although membranes are recognized elements of classical BES, the construction of membrane-less systems may be suggested since the membrane itself acts as an ohmic resistance and thus, contributes to the overall internal resistance of the bioelectrochemical cell [**49**]. Moreover, the research of novel membrane separators can be taken as a way forward so as to improve the conductivity properties and facilitate more selective ion (proton) transport, which can expectedly lower internal resistances in the bioelectrochemical systems. From this point of view, recent findings demonstrated that membranes prepared with ionic liquids can be promising alternatives [**118,119**]. Besides, according to Tartakovsky et al. [**120**], a real-time strategy for adjusting external voltage can be suggested after proper start-up in order to minimize internal MEC resistance, reduce power supply demand and simultaneously achieve optimal hydrogen formation rate.

3. Concluding remarks

Factors taking part in MEC start-up are inoculum selection and enrichment, operating conditions and cell architecture (**Fig. 3**). The analysis based on a wide range of literature studies has the message that MECs started-up with pure cultures are feasible for fundamental studies, while practical MECs dealing with problematic feedstock treatment

and simultaneous energy (H_2) recovery rely typically on mixed communities. These, however, should be enriched and pretreated to attain a consortia with better electrochemical activity and to suppress the growth of competing, non-bioelectrochemical microorganisms. MECs – regardless of the type of inocula used – can be started-up in direct or indirect mode, where the latter means an MFC-based strategy for the development of sufficient anodic biofilms before their application for H_2 production in MEC. Successful MEC start-up has to consider proper reactor operation (in terms of anodic potential, temperature, substrate concentration, etc.) without which the full potential of the electrochemically-active bioanodes remains unexploited and operational failures may be experienced over time. To obtain as high process efficiencies as possible, cell design taking into account electrode materials, external- and internal resistances, membranes (where applicable) ought to be of primary concern to aid start-up and subsequent, steady-state operation.

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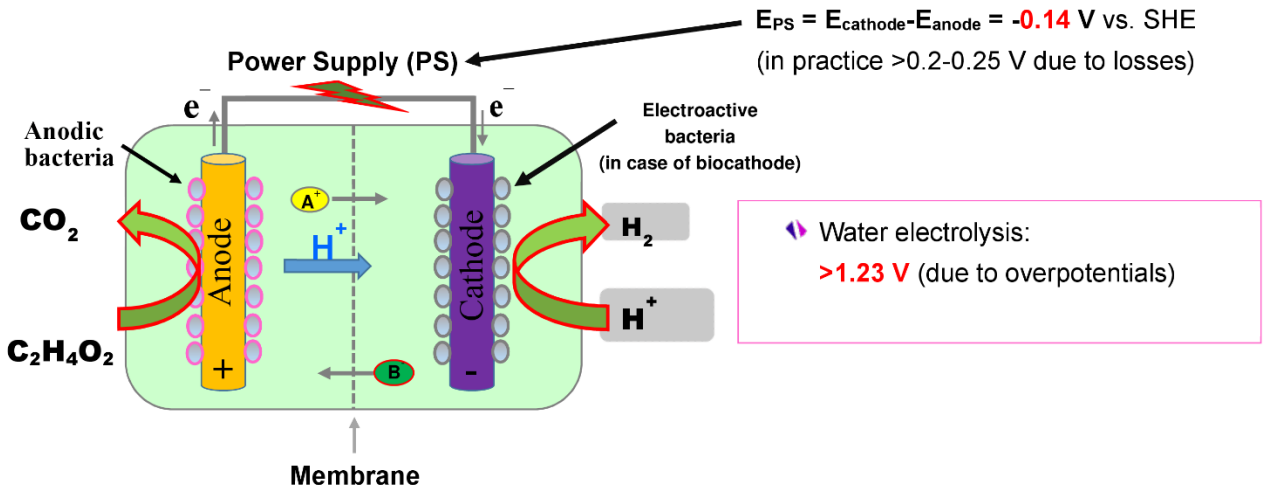
Table 1 – Constructional features of MECs for system start-up

Start-up mode	Cell design	Membrane	Inoculum	Substrate	Anode material	Cathode material	Anolyte	Catholyte	Reference
direct	two chamber	Nafion® 117 PEM	activated sludge	Na-acetate	graphite granules	graphite granules	anaerobic basal medium (pH: 7-7.5)	anaerobic basal medium (pH: 7-7.5)	[86]
direct	two chamber	Rhinohide®	indigenous wastewater microflora	municipal wastewater	carbon felt	stainless steel wool	N.A.	sterilized phosphate buffer (50 mM, pH: 7)	[88]
direct	two chamber	Neosepta® AEM	MEC effluent	Na-acetate	graphite felt	Ni foam	microbial nutrient medium	0.1 M KCl	[89]
direct	two chamber	Nafion® 117 PEM	activated sludge	Na-acetate	carbon cloth	carbon paper with Pt	nutrient solution (pH: 6.9)	phosphate buffer (50 mM, pH: 7)	[92]
direct	two chamber	Nafion® 117 PEM	sewage sludge	Na-acetate	carbon cloth	carbon paper with Pt	nutrient solution (pH: 7)	sterilized phosphate buffer (10 mM, pH: 7)	[91]

direct	two chambers operated in single chamber configuration	Fumasep® FAB AEM	bioelectrochemically -active culture	Na-acetate	graphite felt	Pt coated Ti mesh	nutrient solution (pH: 7)	only for gas collection purposes (no liquid catholyte)	[90]
direct	single chamber	-	anaerobic sludge	Na-acetate	carbon felt	gas diffusion Ni catalyst	nutrient solution		[44]
direct	single chamber	-	anaerobic sludge	(i) Na-acetate, (ii) synthetic wastewater	carbon felt	carbon paper with Ni	(i) acetate based nutrient solution, (ii) synthetic wastewater		[120]
direct	single chamber	-	enriched anaerobic sludge	glucose	graphite plate	graphite plate	designed synthetic wastewater		[76]
direct/indirect	single chamber	-	municipal wastewater	Na-acetate	carbon cloth	modified carbon cloth	mixture of phosphate buffer (50 mM, pH: 7.0) and nutrient solution		[15]
indirect	single chamber	-	MFC effluent	Na-acetate	graphite fiber brush	platinized carbon cloth	mixture of phosphate buffer (50, 200 mM, pH: 7.0) and nutrient solution		[57]
indirect	single chamber	-	wastewater	Na-acetate	graphite brush	carbon cloth with Pt layer	mixture of phosphate buffer (50, 200 mM, pH: 7.0) and nutrient solution		[65]

indirect	single chamber	-	wastewater	Na-acetate	graphtite felt	carbon cloth with Pt layer	mixture of phosphate buffer (50 mM, pH: 7.0) and nutrient solution		[67]
indirect	single chamber	-	heat-treated anerobic sludge	glycerol	graphtite felt	gas diffusion cathode with Pt	nutrient solution	gas-phase cathode	[63]
indirect	two chamber	CMI-7000 CEM	municipal wastewater	dark fermentation effluent	graphite cloth	carbon paper with Pt	synthetic and real dark fermentation effluent	phosphate buffer (50 mM, pH: 7)	[12]
indirect	single chamber	-	anaerobic sludge	Na-acetate	carbon cloth	Pt containing cathode	synthetic wastewater (pH: 9)		[74]
indirect	single chamber	-	H_2 fermentation effluent	Na-acetate in start-up, later dark fermentation effluent	graphtite felt	carbon cloth with Pt layer	mixture of phosphate buffer (50 mM, pH: 7.0) and nutrient solution		[98]

Fig. 1 – Principles of H_2 production in microbial electrolysis cell



Biological fermentation

$C_6H_{12}O_6 + 2\ H_2O \rightarrow 4\ H_2 + 2\ CO_2 + 2\ C_2H_4O_2$ (acetic acid)

MEC process

Anode: $C_2H_4O_2 + 2H_2O \rightarrow 2CO_2 + 8H^+ + 8e^-$ E_{anode} = -0.279 V vs. SHE

Cathode: $8H^+ + 8e^- \rightarrow 4H_2$ $E_{cathode}$ = -0.414 V vs. SHE

Fig. 2 – Possible methane-forming side-reactions in single-chamber MEC

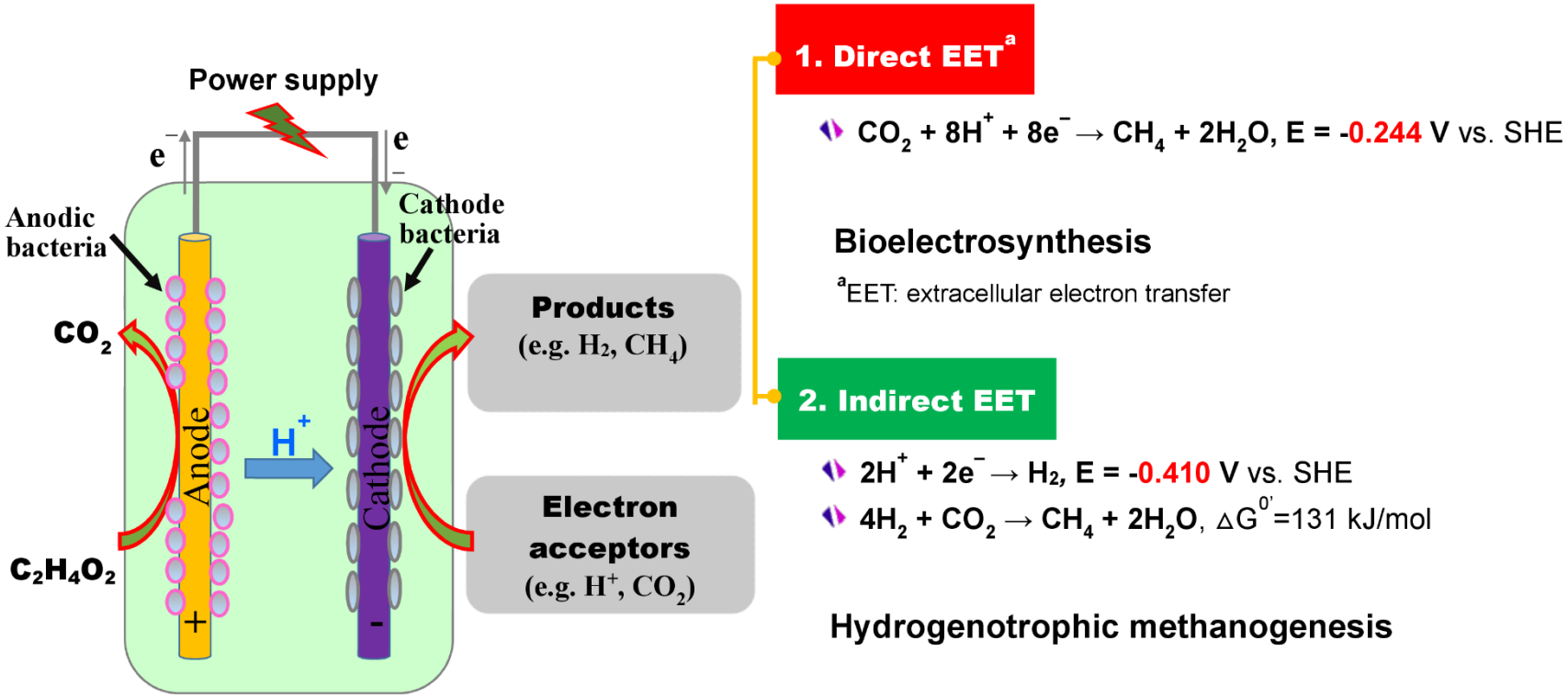


Fig. 3 – Aspects to consider for MEC start-up