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Microbial models for biocathodic electrochemical CO_2 transformation: a comprehensive review on pure cultures

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ABSTRACT

Electroactive microorganisms, either used as pure cultures or as mixed populations in complex communities, play a key role in microbial electrochemical technologies (MET) and are especially relevant in microbial electrosynthesis (MES). Although most MES research focuses on anaerobic organisms, the exploration of aerobic chemolithoautotrophs becomes relevant and may help mitigating the adverse effect of electrochemical evolution of oxygen. Critical avenues for future development involve increasing mechanistic characterizations of reactors and enhancing the understanding of thermodynamics and energy balance of electrode-cell electron transfer. In this review, we primarily concentrate on exploring and discussing the advancements and key findings in the field of microbial models for MES, and approximate to a model formulation. We highlight the potential benefits of using axenic cultures or mixed bacterial populations of known composition as a way to identify main knowledge gaps for further development of predictive modelling.

Keywords: electromicrobiology; modelling; biocathode; electron-transfer; *Clostridium*; *Cupriavidus*

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1. Introduction

Microbial electrosynthesis (MES), which involves the bioelectrochemically catalysed synthesis of chemical compounds using a bioelectrochemical cell, has centred most of the scientific attention over the last decade (Kerzenmacher, 2017; Rabaey and Rozendal, 2010). MES has obvious connections to the valorisation of CO_2 into valuable products (Dessì et al., 2021). These connections arise from the potential use of renewable energy (as the source of electric power for the operation of bioelectrochemical reactors), and a net carbon dioxide fixation during the production of chemicals and fuels (Nevin et al., 2010). *De novo* C-C bond formation is a high-energy-demanding metabolic process. The biological assimilation of carbon occurs through reductive C fixation pathways. The necessary energy (light or chemical) and reducing equivalents (water molecules or other reduced compounds) for the process to occur can be obtained in different ways and microbes have evolved interesting capacities which can be exploited industrially for such an activity (Schada Von Borzyskowski et al., 2020). The major and most studied C-fixation pathway (Rubisco-mediated photosynthesis) is probably not an interesting choice for the electrochemical fixation of CO_2. This is mainly due to the light dependency of the process, which may complicate reactor designs and scale-up. Nevertheless, photobioreactors have been used for such purposes, and more recently have been adapted satisfactorily for alternative activities such as minimizing oxygen inhibition over the algae *Chlorella* during growth (Song et al., 2019). In contrast, light-independent CO_2 assimilation processes have been widely used in MES. In this sense, bioelectrochemical reactor set-ups have made use of autotrophic light-independent bacteria, such as homoacetogens (Drake et al., 2008; Kracke et al., 2016; Philips, 2020). The amount of electricity (energy) that is needed for CO_2 fixation to take place is still considered a fundamental drawback for the scaling-up of microbial

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electrosynthesis technologies (Jourdin et al., 2020; Tremblay and Zhang, 2015). Nevertheless, this essential part of the process has not driven too much attention and only fundamental studies of energy conversion are available.

In addition to carbon fixation, electron transfer is a key function (biological) to be considered in MES processes. Microbial interaction with conductive inorganic materials, in either natural environments or engineered systems (bioelectrochemical systems, BES), allows electron transfer mechanisms between cells and solid substrates leading to exoelectrogenesis (power generation) or electrotrophy (electron capture) (Paquete et al., 2022). These electron transfer mechanisms can be either direct (electrons flowing between solid surfaces and the cells in close contact) or indirect (mediated by a soluble electron shuttle that diffuses in the medium). In complex systems, composed of more than one bacterial species, electrons are also supposed to move between species providing the basis for a complex network of communication (Costa et al., 2013; Paquete et al., 2022). In a recent review, Frühauf et al. (2020) listed 4 different MES types according to major electron use: (1) biofilm-based, typically relying on direct extracellular electron transfer, (2) based on soluble mediators transferring electrons to planktonic cells, (3) electro-fermentations aiming at improving fermentation processes by applying a potential, and (4) secondary microbial electrochemical technologies (MET) combining an electrochemical and a microbial conversion step, either in a single reactor or in 2 different ones.

Rate and yield-limiting steps, from microorganisms to reactor scales, have been investigated with relatively poor success in implementing reliable methods for scaling up (Brown et al., 2014). A recent review highlights the importance of the biological component in the overall efficiency of the technology and stresses the need for more intensive research into robust and adaptable biocatalysts, in parallel to the development of more efficient reactor configurations and electrode

materials for the MES technology to adapt to new challenges (Jourdin and Burdyny, 2021). New insights in the field will help understand and abate its limitations. In the present review, we focus on the living component of electrochemical systems for electrosynthesis purposes and highlight key aspects of modelling biological activities in MET systems that need further development. We provide a critical analysis of the potential applications of model pure cultures in both anaerobic and aerobic conditions with emphasis on *Clostridium ljungdahlii* and *Cupriavidus* sp. as model organisms. This approach serves as a step forward for testing genetically engineered strains, opening up the system possibilities beyond naturally produced compounds. This nuanced exploration is crucial for advancing the understanding and application of MET systems in future contexts.

2. The live component: facts and potentialities

Microorganisms, more likely bacteria, archaea and Eukarya, are essential components of MET. The live component constitutes the bone marrow of the bio-system making it at play in a series of changing conditions and settings within the limits of cell physiology, which in turn are the main constraints for further development of MET as a technology (Jafary et al., 2015; Logan et al., 2019). Strictly speaking, microbes boost electrochemical reactions by enhancing connectivity with biocompatible electrodes and opening up the lenses towards reactions that have a biological basis, including here all potential applications derived from genetic and metabolic improvement of microorganisms yet to be implemented. However, the microbes-power relationship in METs is limited to the ability of certain microorganisms to transform electrical energy into chemical energy and *vice versa*. Microorganisms use a plethora of mechanisms for electron exchange with organic or inorganic substrates. These substrates may be dissolved molecules, which can be

internalised through the cell membrane or may consist of solid substrates with which cells interact at the molecular level (Logan et al., 2019). All electron transport mechanisms have a common physiological goal of serving as electron donors or acceptors, fuelling metabolically relevant reactions for the cell. In cases where microorganisms exchange electrons with external components that cannot be imported, the biological energy balance is coupled to an external electron transfer mechanism (EET) (Kumar et al., 2016; Shi et al., 2016; Yang et al., 2012). In MES this is the first step in the electric-to-chemical transformation and involves proteins that extend outside the cell surface (Kracke et al., 2019). The capacity of some microorganisms to transfer electrons to or from a poised electrode is known as electroactivity (Lovley, 2012). True electroactive bacteria (e.g. *Geobacter sulfurreducens*, *Geobacter metallireducens*, *Shewanella oneidensis*, *Desulfovibrio* spp.) can perform ETT (Logan et al., 2019).

EET can occur after direct, mediated, and indirect transfer events (Conners et al., 2022; Horváth-Gönczi et al., 2023; Paquete et al., 2022; Sydow et al., 2014; Zheng et al., 2020). Direct electron transfer requires no soluble compounds for electrons to be transferred. Instead, intimate contact of the microorganism with the electrode is mandatory (Rabaey and Rozendal, 2010). *Geobacter* and *Shewanella* are model examples in which cytochrome c-type are likely participating in electron transfer (Rosenbaum et al., 2011). However, more limited knowledge exists about mechanisms employed by putatively electrotrophic bacteria, such as the acetogenic *Sporomusa ovata*, *Clostridium ljungdahlii*, *Moorella thermoacetica*, or methanogenic archaea, such as *Methanococcus maripaludis*, to highlight some examples (Lohner et al., 2014; Nevin et al., 2010, 2011). Mediated electron transfer requires shuttle molecules to dissolve freely in the medium. For instance, flavins and phenazines, naturally produced by *Shewanella oneidensis* or *Pseudomonas aeruginosa*, respectively, are active vehicles for electron transfer that may act in a

cell-to-cell or a cell-to-electrode transfer (Light et al., 2018; Pham et al., 2008). Artificially added compounds, such as methyl viologen, may also serve as electron shuttles for electron transfer to occur. Lojou and coworkers reported that *Desulfovibrio vulgaris* (strain Hildenborough) significantly increased the catalysis of H_2 in the presence of methyl viologen at low redox potentials using a graphite electrode as the electron donor (Lojou et al., 2002). Indirect electron transfer refers to the transient synthesis of soluble molecules, such as H_2 and formate, that after capturing electrons can serve in biological reactions. Although METs take advantage of a unique feature of some microorganisms, the direct, indirect and mediated transfer mechanisms are suspected to occur simultaneously. In this sense, (bio)electrochemically evolved H_2 plays a major role in the process, and for sure is a determinant aspect in most processes involving METs (Perona-Vico et al., 2020; Puig et al., 2017). In addition, cathode interactions with free extracellular enzymes (hydrogenases and formate dehydrogenases) may also increase H_2 production thus fuelling MES reactions (Deutzmann, Jörg S. Sahin Merve, 2015; Lienemann et al., 2018; Tremblay et al., 2019). Operating bioelectrochemical systems with strongly colonized biocathodes with *Sporomusa sphaeroides*, *Sporomusa ovata* or *Methanococcus maripaludis*, as common examples, may cause extensive modifications of the cathode surface by chemical depositions of nickel and cobalt leading to cathode conditioning.

The energy gain that bacteria can derive from a poised electrode can be used for CO_2 transformation into organic compounds. These can range from basic CO_2 reduction to C1 compounds, such as methane (CH_4) (Batlle-Vilanova et al., 2015; Cheng et al., 2009; Marshall et al., 2012) and formate (Marshall et al., 2013; Rotaru et al., 2012; Yu et al., 2017), to the synthesis of Cn molecules, e.g. acetate and ethanol (Nevin et al., 2011; Jourdin et al., 2015; Batlle-Vilanova et al., 2016), butyrate, isobutanol, caproic acid, and 3-methyl-1 butanol, among

others (Batlle-Vilanova et al., 2017; Ganigué et al., 2015; Jourdin and Burdyny, 2021; Li et al., 2012; Prévoteau et al., 2020). The use of microorganisms as biocatalysts in BES takes advantage of the self-regeneration and the adaptability of cells to environmental conditions. However, microorganisms need part of the added substrate and energy for cell growth and division, which decreases the productivity of the desired product (Rabaey and Rozendal, 2010). A major limitation in BES processes is the rate at which microbial catalysts acquire electrons from the poised electrode for CO_2 reduction (Kracke et al., 2015). Consequently, optimization of microbial catalysts and metabolic processes of microbe-electrode interactions are among the largest challenges to drive microbial electrochemical technologies (METs) beyond fundamental studies.

3. Better alone? Co-culturing as opposed to pure cultures

Pure cultures or mixed microbial communities have been invariably used in combination with MES. Interestingly, MES technologies for the production of acetate by reduction of CO_2 as the only carbon source have concomitantly made use of pure cultures of *Clostridium ljungdahlii*, *Moorella thermoacetica* or *Sporomusa ovata* strains (Bajracharya et al., 2015; Nevin et al., 2011; Yu et al., 2017), or introduced operational conditions for a defined evolution of mixed microbial communities using different starting materials such as anaerobic sludge or wastewater (Arends et al., 2017; Patil et al., 2015; Rovira-Alsina et al., 2020; Van Eerten-Jansen et al., 2013). A recent publication by Hengsbach and coworkers critically reviews the use of pure cultures and naturally evolved populations in the two most prominent products obtained by MES so far, acetate and methane (Hengsbach et al., 2022). The authors identify some interesting points in their comparisons that may define a choice for one or another system, including stability and

robustness (*i.e.* resilience), product spectrum and production rates, and genetic potential (including editing possibilities). In general, mixed cultures result in higher productivity, but pure cultures are more prone to benefit from reactor modifications and process improvements. Co-culturing of bacteria in defined communities of known composition is a third option to be considered and has long been considered beneficial for many biotechnological applications (Diender et al., 2021). Contrarily to what may be thought, co-culturing requires tight control of operating conditions and a highly specific selection of microbes into play to meet the bio-based technology expectations, thus differentiating from mixed communities (Chen, 2012; Yao and Nokes, 2013). "Naturally" evolved (enriched) microbial communities, however, are frequently used in the field when axenic conditions can not be accomplished during operation. The three possibilities, enriched communities, defined co-cultures, and pure cultures have to be considered in MES developments according to the complementary properties they can offer (Figure 1).

Mixed cultures, usually of unknown composition at the moment of the reactor's startup, have been used thoroughly in biotechnological applications, and METs are not an exception to this common practice (Batlle-Vilanova et al., 2015; Dessì et al., 2021; Dolfing, 2014; Lovley, 2011; Stams and Plugge, 2009). However, synthetic biology and modelling sciences can not rely on such data due to the poor characterization of microbial players (e.g. community composition, species abundance, metabolic activity) despite the use of molecular methods. In those "complex" systems, syntrophic relationships among bacteria may easily appear. Pure electrochemical production of H_2 as an intermediate to fuel the C fixation metabolism has been usually considered in MET when complex systems are used (Arends et al., 2017; Marshall et al., 2012). However, this process cannot be dissociated from a biologically enhanced process (Batlle-Vilanova et al., 2014; Perona-Vico et al., 2020). In such systems, physiological parameters to

describe the biologically assisted process are difficult to be determined and incorporated into modelling attempts, thus requiring further investigation using pure isolated bacteria (Perona-Vico et al., 2020). Syntrophism among cell members is supposed to be frequent in naturally evolved METs, leading to a concomitant enrichment of producers and consumers. The use of synthetic consortia can partially override this problem by engineering a metabolic interdependence (Hengsbach et al., 2022). For instance, co-cultivation of strain IS4 (*Desulfopila corrodens* DSM 15630) together with the methanogen *Methanococcus maripaludis* or *Acetobacterium woodii* increased methane and acetate production rates, respectively (Deutzmann and Spormann, 2017). This synthetic co-culture allowed operation with less negative cathode potentials (-0.5 V *vs.* SHE) in comparison with those usually used for H_2 production (-0.8 to -1 V *vs.* SHE) resulting in an energy benefit for either acetate or methane production. More recently, the use of different methodologies for the conditioning of biocathodes (*i.e.* coating of active cells) before their use in MES together with modelling applications, has led to the emergence of a next-generation of electroactive cathodic biofilms. 3D-printed cathodes were proposed for electromethanogenesis, consisting of carbon aerogel coated with NiMo-alloy to facilitate H_2 evolution (Kracke et al., 2021). Such a method increased the methane production of a *Methanococcus maripaludis* pure culture to a remarkable volumetric rate of 2.2 LCH_4/Lcatholyte/day (99 % coulombic efficiency). Krige and coworkers created a synthetic biofilm that was 3D-printed (containing *Sporomusa ovata*) and showed a significant reduction in start-up time and higher acetate production rates about 10 times higher compared to conventional procedures H-type cells (Krige et al., 2021). Simpler methods to increase cells adherence to cathode materials, e.g. induction of stress conditions for enhanced biofilm formation (Perona-Vico et al., 2020) and the use of sprayable agarose-based hydrogels (Knoll et al., 2022), have

also been tested in electroactive bacteria resulting in significant increases of cells activity compared to conventional methods.

4. Translating microbe-electrode interactions into modelling approaches

Several models approaching BES functioning can be found in the literature. They expand from basic approximations (Matemadombo et al., 2017; Rousseau et al., 2014), to highly detailed models where mass transfer processes (Cabau-Peinado et al., 2021; Kazemi et al., 2015; Merkey and Chopp, 2014; Pinto et al., 2011b; Torres et al., 2008) and thermodynamics (Almeida Benalcázar et al., 2020; Harnisch and Holtmann, 2019; Korth et al., 2015, 2016) are included. In this report, we will focus on those related to the conversion of CO_2 (cathode reaction) using water splitting through the oxygen evolution reaction (OER) (anode reaction). Due to the lack of specific data for BES, information from other processes (*i.e.* MFC) is useful and frequently used in modelling since similar mechanisms in electron transfer are supposed (Hassan et al., 2019; Rosenbaum et al., 2011). Energy-demanding cell processes (production, growth, and structure maintenance) are ultimately fuelled by electrons, therefore electron transfer is a key process in BES. We have shown previously that different mechanisms coexist for electron transfer to occur, and consequently, different models apply.

A reference model for the simulation of biofilm behaviour was developed by *Marcus et al.* using a direct electrode-to-cell transfer mechanism (Marcus et al., 2007). The electrode working potential (which can be fixed by a potentiostat) was used as the model parameter for bioelectrochemical simulation since no overpotential was assumed during ion flow and electron transfer. The model assumed a steady-state potential and substrate concentration along the biofilm thickness, a Monod-Nernst equation was developed to simulate the uptake of the

substrate and a dynamic model for the biofilm. The model has been largely used including reactors with biofilms with different electrical conductivities and adapted to include ohmic losses due to external resistances (Kazemi et al., 2015; Teleken et al., 2017; Zhao et al., 2020). Moreover, other interesting modifications of Marcus' original model exist which considered, for instance, a biofilm simulation by a globalised approach based on oxidation and reduction of a (pseudo)intramolecular mediator (a proxy for NADH/NAD^+), or detailed simulations of the ohmic (Ohm law), activation (Butler–Volmer equation) and concentration overpotentials (Nernst equation) (Pinto et al., 2011a, 2010). Korth et al. (2015) also proposed a model based on intracellular mediators. However, in the latter case, internal (NADH) and superficial mediators (c-type cytochromes) were included for electron transfer.

External mediators, such as electron shuttles, have also been incorporated in electrode-biofilm simulation models (Picioreanu et al., 2007), although potential drops were not incorporated in this case. In the event of hydrogen being the mediator, it will be mostly taken from the bulk without the need for any direct cell-cathode interaction. Thus, electrochemical and biological processes could be simulated independently (although they would be related by mass transfer), combining pure water electrolysis models (Kubannek et al., 2020; Ni et al., 2006; Wang et al., 2015) and Monod based kinetics (Islam Mozumder et al., 2015). However, these basic models should have to include the fact that bacteria in bulk can increase the ohmic overpotential in the cathodic solution (Givirovskiy et al., 2019). Moreover, biomass growth has been also simulated by metabolic and thermodynamic approaches, including substrate inhibition (Cabau-Peinado et al., 2021; Tsipa et al., 2021).

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The combination of direct and indirect electron transfer events is a common situation in MES (Blasco-Gómez et al., 2019; Faraghiparapari and Zengler, 2017; Jourdin et al., 2015; Matemadombo et al., 2017). Indeed, bacteria can uptake electrons not only to reduce CO_2 but also to produce mediators. For hydrogen, this combined behaviour has been previously reported for acetogenic platforms and mixed cultures where hydrogen-producer microorganisms are involved (del Pilar Anzola Rojas et al., 2018; Perona-Vico et al., 2020). Previously, it has been established that biofilm could increase the ohmic resistance but when cells are also involved in hydrogen formation, the required potential for production is reduced, probably by decreasing the activation potential (Chandrasekhar et al., 2015). Nevertheless, modelling options that assume an overall behaviour by semi-empirical correlations can also be found in the literature (Lacroix et al., 2014; Rousseau et al., 2014). Pure data-driven models have been also developed to overcome the knowledge limitation on the involved mechanisms by mathematical algorithms fed with large/representative databases. In this sense, machine learning algorithms (like the combination of support vector regression, artificial neural networks, and Gaussian process regression) have been used to simulate the behaviour of bioelectrochemical systems with accuracies as high as 99% (Oyedeji et al., 2023; Zakir Hossain et al., 2023). Similarly, machine learning was also applied to predict the consortium composition depending on the media composition, leading to a kind of data-driven biosensor (Cai et al., 2019).

It should be noted that the mechanisms of electron uptake in microbes are still not known in detail. Different molecules have been proposed but most of the cellular compounds relevant to electron uptake remain unknown (Harnisch and Holtmann, 2019). From a modelling perspective, this is challenging because different processes will depend on the organism's fitness and on the conditions in BES, which can have a regulatory impact on metabolism activation. In addition,

mechanisms for biofilm formation in BES, and the composition of exopolymeric substances can also play a role in electron shuttling (Chen et al., 2017; Sydow et al., 2014; Zhuang et al., 2020).

4.1. Anaerobic approach: *Clostridium* platform

Clostridia are anaerobic or obligate anaerobes that are among a metabolically diverse group including cellulolytic, acetogenic, chain-elongating and solventogenic bacteria. They perform diverse metabolic functions, including the conversion of starch, protein and purines into organic acids (*e.g.* acetic, butyric, and caproic acids), alcohols, CO_2 and H_2 (Charubin et al., 2018). Because of their broad and flexible metabolic capabilities, *Clostridium* species are important to the commercial conversion of renewable resources into biofuels and commodity chemicals. For example, cellulolytic and solventogenic species (*C. thermocellum*, *C. saccharobutylicum*, *C. cellulolyticum* and *C. acetobutylicum*) are considered biomass-metabolizing bacteria with a high potential for sustainable biofuel production via consolidated bioprocessing (Lynd et al., 2005). For MET applications, *Clostridia* are an interesting microbial platform due to their metabolic potential covering the production of organics from CO_2 or synthesis gas (CO/H_2) via the Wood-Ljungdahl pathway. Oxygen penetration to the cathode (via anodic water electrolysis in METs) has to be excluded to avoid inhibition of anaerobes. Technical solutions should come for replacing OER as an anode reaction or at least minimising O_2 penetration to the cathode with oxygen scavengers (Abdollahi et al., 2022).

Specific microbial pure cultures of *Clostridium ljungdahlii* and *Clostridium aceticum* have been set up to perform MES for the synthesis of acetate and 2-oxobutyrate from CO_2. Different product amounts for both strains have been reported. While *C. ljungdahlii* was mainly producing acetate and, formate and 2-oxobutyrate remained at low concentrations, *C. aceticum* was able to

produce both acetate and 2-oxobutyrate. It was suggested that *Clostridium* strains interacted directly with electrodes since H_2 levels were below the 400 ppm required for acetogenesis (Nevin et al., 2011). Otherwise, Bajracharya and co-workers demonstrated higher acetate production rates (56 mg L^{-1} d^{-1}, maximum concentration 6.1 mg L^{-1}) in the presence of *C. ljungdahlii* (Bajracharya et al., 2015). Apart from *C. ljungdahlii* and *C. aceticum* other *Clostridia* have been studied in MES conditions even recent studies have demonstrated a major role of H_2 as a mediator for efficient CO_2 reduction (Boto et al., 2023; Im et al., 2022). Acetate and butyric acid cathodic production (0.03 and 0.01 g/L, respectively) were reported for *Clostridium scatologenes* ATCC 25775^T in a MET operated at -0.6V *vs.* SHE. Authors indicated that at such potential, no significant H_2 production could take place, and the high couloumbic efficiencies observed (~87%) could be forced by direct electron transfer events. Also, they demonstrated enhanced productions of acetate, butyrate and ethanol when utilizing more reducing potentials (-0.8, -1.5 and -1.2 V *vs.* SHE). In this case, both H_2-mediated and direct electron transfer could be occurring in the cathodic chamber and coulombic efficiencies decreased (Liu et al., 2018). Otherwise, *Clostridium kluyveri* was reported as not being able to directly interact with the electrode nor with several redox mediators (*e.g.* neutral red, methyl viologen, methylene blue) in MES (Koch et al., 2017). All together indicates that the exact mechanisms behind electrode-*Clostridium* interaction (direct electron transfer or H_2 mediated) is not yet completely understood and may be species-specific.

Additionally, BES-based approaches have been implemented to improve fermentative processes. Choi et al. demonstrated that *Clostridium pasteurianum* was able to take up electrons from a poised cathode without the use of a mediator. Using this system, additional energy was supplied from the cathode increasing the yield of conversion of glycerol to 1,3-propanediol or glucose to

butanol (Choi et al., 2014). Heterotrophic fermentation of fructose with *Clostridium autoethanogenum* under BES conditions (poised electrodes at -0.6 V *vs.* SHE) shift away from acetate towards lactate and 2,3-butanediol production with and without the presence of cobalt-complex redox mediators (Kracke et al., 2016).

Normally, a small number of C3-C6 compounds can be produced using MES and, BES-based production is mainly focused on C2 compounds, not being competitive since currently inexpensive C2 compounds are oversupplied by the chemical industry (Harnisch et al., 2015). The application of synthetic biology to MES may address that problem. For industrial and environmental biotechnology, genetically modified microbial organisms are used for many applications such as materials synthesis, chemicals, medicines, fuels, and remediation of waste products or toxins. Recent advances in synthetic biology have substantially improved our ability to program these microbes quickly and cheaply on a large scale with greater control (Johns et al., 2016). *Clostridia* have been demonstrated to be accessible for genetic modifications enabling metabolic engineering to improve CO_2 or synthesis gas valorization into more economically feasible products (Leang et al., 2013; Molitor et al., 2016; Ueki et al., 2014; Zhang et al., 2020). An engineered strain of *C. ljungdahlii* containing the butyrate production pathway of *C. acetobutylicum* has been reported as being highly efficient for the production and accumulation of butyrate (2.5-times higher) utilizing highly H_2 evolving electrodes (nickel-phosphide carbon felt electrode) (Wang et al., 2020). Consequently, greater opportunities for pure cultures (or defined co-cultures) in MES are possible if metabolic engineering methods to obtain microbial biocatalysts for non-natural reactions are optimized (Rosenbaum and Henrich, 2014).

4.2. Aerobic approach: *Cupriavidus* platform

Most work on CO_2 reduction in BES has been performed with anaerobic organisms, in particular with acetogens and to a similar extent with methanogens. In contrast, aerobic chemolithoautotrophs have received much less attention even though they exhibit fast growth. In particular, the best best-known representative *Cupriavidus necator* (formerly *Ralstonia eutropha*) has been listed among the promising microbial chassis to apply in MES for renewable energy storage and CO_2 conversion into carbon molecules that are inaccessible to abiotic electrochemistry (Abel et al., 2020). Knallgas bacteria, such as *Cupriavidus* spp., are chemolithoautotrophs. They can reduce CO_2 using H_2 as an energy source and O_2 as an electron acceptor and thus require a potentially explosive gas mixture to sustain growth and metabolite production. Product formation by *Cupriavidus* strains typically occurs in 2 steps: (1) biomass growth, and (2) product synthesis upon nutrient limitation (potentially with an additional induction step depending on the characteristics of the engineered organism).

While microbial attachment and biofilm growth on a Pt cathode could be demonstrated for *C. necator* (Bause et al., 2018), most BES studies with *Cupriavidus* strains make use of planktonic cells. This implies that they rely on indirect electron transfer via (dissolved) H_2. Investigations of CO_2 reduction by *C. necator* have typically been performed in membrane-less single-chamber set-ups, which supply both O_2 and H_2 via *in situ* electrolytic water splitting. These non-separated systems have the additional benefit that pH usually remains around neutral as desired for microbial growth. However, this may impede efficient O_2 evolution at low overpotentials and may lead to undesired side reactions that generate reactive O_2 species such as hydrogen peroxide, chlorine gas or hypochlorite, which are detrimental for microbial growth (Sydow et al., 2017; Torella et al., 2015). To overcome the drawback of inefficient O_2 generation at neutral pH, Torella et al. (2015) developed a cobalt phosphate-based water-splitting anode. At a lower

overpotential compared to that of noble metal electrodes, it supported the growth of wild-type *C. necator* H16 as well as production of 216 mg/L isopropanol by an engineered strain. Cell potential had to be optimized to favour biological growth over toxicity by reactive O_2 species. Liu et al. (2016) further improved the system and demonstrated the production of 700 mg/L PHB with a wild-type strain and 600 mg/L isopropanol, or 200 mg/L isobutanol + 3-methyl-1-butanol production by 2 different engineered strains. To avoid undesirable electrochemical side reactions, Sydow et al. designed a chloride-free electro-autotrophic medium that was further optimized to fulfil the requirements for both biotechnology and electrochemistry (Sydow et al., 2017). The optimized medium supported the growth of *C. necator* without lag phase in BES, but growth rates were lower than in autotrophic cultures probably due to H_2 limitations linked to the relatively low electrode surface area to liquid volume ratio. Under similar operational conditions, the same research group provided a proof-of-principle for 10 mg/L α-humulene production (Krieg et al., 2018) in MES mode by an engineered *C. necator* strain. In a more recent work, Wu and coworkers demonstrated de novo synthesis of 1.7 mg/L lycopene from CO_2 by another engineered *C. necator* strain (Wu et al., 2022). Interestingly, the terpene product protected the microbial cells against reactive oxygen species produced in the BES. Averesch *et al.* engineered C. necator to create cell factories capable of producing a broad range of polyhydroxyarylates, including for the first time novel biological aromatic polyesters (Averesch et al., 2023; Averesch and Kayser, 2020). In a single chamber BES test run under autotrophic conditions, nitrogen limitation and supply of the aromatic precursor D-phenyllactic acid resulted in the production of the copolymer poly(3-hydroxybutyrate-co-phenyllactate).

Optimal $H_2/CO_2/O_2$ gas mixtures for *C. necator* have been defined to be 64/16/20 (Krieg et al., 2018). Though the previously cited authors hint at H_2 or O_2 limitations to explain certain

experimental observations, no (or very little) information is provided on dissolved gas concentrations or headspace composition. However, these are important for several reasons. Too high dissolved O_2 levels, between 4 and 20% air saturation, are known to inhibit the *C. necator* hydrogenases enzymes and it is therefore recommended to keep them at 4% (Marc et al., 2017). On the other hand, both O_2 and H_2 are sparingly soluble and at the high growth rates of *C. necator*, fast limitations in dissolved gas availability can be expected, when gas demand exceeds supply, and high gas-liquid mass transfer efficiencies should be maintained in the cultivation process. Although dissolved gas levels can be estimated from the headspace composition via Henry's law, direct measurement of dissolved gases is preferred to assess actual substrate limitation or inhibition and dissolved H_2 and O_2 sensors are available to do so. Since the O_2/H_2 combination is potentially explosive, the O_2 level in the headspace should be kept below the lower explosion limit of 4% which is far below the optimal ratio mentioned above and may negatively impact growth and production rates. Careful consideration thus has to be given to optimal reactor and process design and control, to match gas supply with gas consumption throughout the various process steps while operating under safe conditions.

To avoid safety and solubility issues, there is however an increasing trend to couple pure electrochemical production of C1 and C2 intermediate products with subsequent biological transformation to multicarbon molecules (Table 1). Due to its metabolic versatility and flexibility, *C. necator* is a good candidate for such concepts. Li et al. assessed the synthesis of isobutanol and 3-methyl-1-butanol or intracellular accumulation of PHB from formate in a hybrid system with 2 spatially separated process steps (Li et al., 2012). To avoid the need for a concentration step or another pretreatment of the electrochemical output stream, Stöckl *et al.* (2020) aimed at synthesizing formate in a physiological buffer in terms of pH and salinity, and

used it for subsequent biological PHB production without intermediate purification (Stöckl et al., 2020). Similarly, an electrochemical biohybrid system consisting of a CO_2 electrolyser generating formate and a fermentor with *Cupriavidus* cells converting formate into PHB was tested (Lim et al., 2023). The catholyte was developed as a modified culture medium and was sent without purification to the fermentor, in which formate levels were controlled below 1 g/L to avoid toxic effects. A membrane filtration step ensured that a clarified catholyte was recycled to the electrolyzer and that cells were returned to the fermentor. The optimized system reached a PHB concentration of 0.6 g/L (at a PHB content of 80% on cell dry weight basis) after 120 hours. Al Rowaihi and co workers could demonstrate PHB production in a fully integrated one-pot electromicrobial setup with *C. necator* H16 (Rowaihi et al., 2018). In a cyclic approach, electrochemical formate production was performed in a comparatively high salinity buffer, and then microbially converted into PHB. Current results for direct microbial CO_2 conversion are mostly proofs-of-concept and electro-autotrophic performance is far from industrially relevant production rates and product titers. To assess what could potentially be gained from genetic engineering, Abel et al. (2020) modelled microbial O_2 respiration and carbon fixation strategies and concluded that the CO_2 fixation rate in aerobic MES is limited to < 6 μmol/cm^2/hr mainly because of O_2 mass transport. Overall, decoupling electrochemical and microbial processes in hybrid set-ups looks more promising in the short term, while their combination in a single multifunctional reactor still requires substantial compromising regarding optimal conditions of media, applied potential, etc. (Fruehauf et al., 2020) and the anticipated benefit of overall process intensification thus remains to be demonstrated. Through proper choice of intermediates, the secondary MET may improve the overall efficiency of commodity production because aerobic lithoautotrophs divert a high proportion of electrons to O_2 reduction to water and a large portion

of carbon to biomass (Bajracharya et al., 2019). However, the range of microbial substrates that can currently be generated by electrochemistry is rather limited and should be improved. A recent review on *C. necator* cell factories for CO_2 conversion adds the need for further strain engineering to reduce the toxic effects of electrolysis byproducts such as H_2O_2, reactive O_2 and NO (Tang et al., 2023).

5. Today's challenges in BES modelling

The behaviour of a bioelectrochemical system can be represented fairly well through existing mathematical models. However, due to the complexity involved (e.g. electrochemistry and microbial activity), the fundamentals behind are not utterly defined and understood. For instance, simulation studies carried out in MES by Cabau-Peinado and co-workers (Cabau-Peinado et al., 2021) indicated that continuous CO_2 feeding was key for the formation of dense biofilms on the cathode and to achieving higher current densities thus pointing to the need to overcome substrate limitation events in biofilm-based electroactivity for process enhancement. In addition to biological effects, small changes (*e.g.* changes in the electrodes' position of millimetres) in the reactors' configuration can lead to dramatic differences between BES reactors that were supposed to run as duplicates (Givirovskiy et al., 2019). Thus, eventually, the reported parameters are particular solutions that cannot be directly transferred to other models without a re-evaluation.

For the *Cupriavidus* and *Clostridium* platforms reported above, modelled systems would include a single-chamber reactor (allowing a combination of O_2 and H_2 production at the anode and cathode, respectively) or a two-chambered system to maintain anaerobic conditions at the cathode and enhance CO_2 transformation. The energy balance is often not included in modelling

approaches, especially in laboratory-scale reactors. Often, published techno-economic assessments on electrochemical reactors do not include the heat generation/consumption inside reactors and are limited to accounting for sensible heat (input and output temperatures), and electric power consumption (Christodoulou et al., 2017; Jourdin et al., 2020; Marshall et al., 2013). Nevertheless, when scale-up is considered the final application of predictive modelling, a comprehensive energy estimation is essential and should be included. Concerning thermodynamics, Gibbs free energy has been successfully used to estimate the process stoichiometry (Almeida Benalcázar et al., 2020).

The total energy required for water electrolysis (the enthalpy) has two different terms: the electrical energy externally supplied (related to the Gibbs energy) and a reversible heat demand due to the reaction in association with the reaction entropy) (Mendoza-Hernandez et al., 2019). Additionally, the fact that overpotentials are present also means another source of (irreversible) heat (Ni et al., 2008; Ramousse et al., 2009). Therefore, the total heat flow can be obtained from the supplied electrical energy, the enthalpy changes of the products and the heat due to the overpotentials. In the presence of bacteria, the anabolic and catabolic heat must be also considered (Korth et al., 2016). The former can be estimated from the Gibbs free energy approach and the latter from the main reactions (products and reagents) that occur in the reactor. Korth and coworkers (Korth et al., 2016) used a microbial electrochemical Peltier plate to measure heat production (heat transfer from the biofilm to the electrode) or consumption (in the opposite case). This heat is due to the entropy changes related to the electron exchange between a biofilm and an electrode (specific for every specific process) and has been just measured experimentally using a bio-electro calorimeter. In principle, this contribution would not be negligible since its pure electrochemical equivalent can involve circa 20% of the total chemical

energy. Despite being experimentally challenging, considering heat flow in the electrode-cell interphase is mandatory to minimize a significant error source for energy estimations in BES modelling.

Despite numerous attempts to define a model that could reproduce the behaviour of a BES have been produced in the past, no one can be claimed as an overall solution. A thorough literature review on the subject points to three main limitations as responsible for the lack of reliable models (Table 2). First, there is a lack of knowledge about phenomenology, on how specific experimentation of individual research is translated into an understandable format to be translated into a model. Second, there is often a lack of reproducibility of the experimental setups, specifically at the fundamental level (*e.g.* small-scale laboratory reactors and inter-laboratory comparisons), probably indicating the importance of operational variables that are not considered. Third, there is a manifested lack of studies reporting energy balance information. Trying to enhance the development of an overall solution toward the implementation of reliable models in BES technology is mandatory and efforts are needed to implement solutions to the main limitations. In our view, these solutions will need to include exhaustive characterizations of mechanistic mechanisms (molecular level) in BES, an agreement in basic guidelines for BES set-up and monitoring practices to help inter-laboratory comparisons such as done in microbial fuel cells (Santoro et al., 2021), and increased knowledge in thermodynamics and energy measurements. Additionally, data-driven models are an option to develop tools that could reproduce the system behaviour as a function of the operational conditions, which would be useful not only for scaling-up and optimisation but also for control. However, the physical information that can be retrieved from them is often limited thus minimizing their real application into the real processes. Thus, data-driven models should be considered as a useful

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complement, but not as the only solution. Indeed, a combination of both (like physics-informed models) could be a better approach. In the following section, we develop a modelling strategy that balances essential detail for simulating key BES phenomena and the lack of reliable data.

6. Proposed modelling methodology

Pure electrochemistry plays an essential role in BES operation, therefore modelling equations should be a combination of electrochemical and biological parameters (like the aforementioned Monod-Nernst equation). Nonetheless, these models usually require specific experiments for a correct parametrisation. For this reason, empirical characterisation of the electrochemical behaviour is usually a suitable option (Sánchez et al., 2018; Ulleberg et al., 2018). This approach focuses on using the Faraday law to estimate the number of gases produced during the electrolysis (n_j), but includes an efficiency factor (ε) that accounts for the non-idealities as shown in equation (1) (Table 3). The efficiency factor could be obtained by equation (2) if the correlation parameters f_i and f_{ii} can be derived from experimental-based parameters (see equation 3). Finally, the cell voltage (V_{cell}) will be a function of the reversible voltage at the working pH ($V_{rev}(pH)$), the temperature, and the internal (ohmic), and activation overpotentials (η_{int} and η_{act}, respectively) (equation (4)). η_{int} would consider the electrical resistance of the internal elements: membrane, electrolytes, suspended biomass (if any), and biofilm. Among them, the most dominant is assumed to be the biofilm (closer to the electrodes) and the membrane (R_{bio} and R_m, respectively) and could be obtained by the addition of these two resistances (equation 5) and an empirical factor to include the role of pressure (f_p).

The electrical resistance of the membrane should be measured or provided by the supplier, but, for a proton exchange membrane, correlations exist in the literature (Ni et al., 2008). The resistance of the biofilm (f_p) can be estimated as a function of mass and biomass conductivities (Korth et al., 2015). Regarding the pressure role, it is purely empirical and could be obtained by equation (6), where d_i and d_{ii} are empirical parameters (Sánchez et al., 2018), and P is the working pressure (bar). In the case of isolated compartments, the anode and the cathode can work at different pressures. In such a case, the average value or the pressure for the working electrode compartment (biotic) could be used. Concerning η_{act}, its calculation has been proposed as an empirical correlation similar to the one shown in equation (7), where s, t_i, t_{ii}, and t_{iii} are fitted parameters (Sánchez et al., 2018).

Our modelling strategy explicitly includes the role of the biofilm as a source of electrical resistance and here it is computed as a constant biofilm mass. The model applies when BES reaches a stable situation, with no further biofilm growth and considers its effects on the process efficiency. While the model could be adapted to include a variable biofilm mass, this would increase the number of experiments needed. Moreover, aiming for a system with a stable bioelectrode aligns with industrial production goals. It should be considered that given the dilution in the bulk, the electrical resistance of suspended biomass is often negligible in comparison to the membrane. The presented methodology simulates the main (bio)electrochemical processes involved in a BES with the minimum set of experiments. Although being empirical, the method would require additional calibration if significant changes apply. In any case, we consider developing a model for an individual cell, to be later used in a larger set, offers a suitable estimate of the expected behaviour of the whole system.

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6.1 Kinetics for biomass production and product yield

In this example, we have considered the production of lactate from molecular hydrogen, carbon dioxide and oxygen, occurring when bacteria face multiple nutrient limitations. The model microorganism used in the process has been *Cupriavidus* sp.. The use of a pure culture will simplify modelling since specific parameters could be derived from independent experiments. Biomass growth was simulated using a multi-substrate Monod equation, considering nutrients with a Haldane relation. Inhibition from oxygen and acids was also considered, with sudden oxygen inhibition based on a specific partial pressure and a gradual acid inhibition addressed through a tailored empirical function. The growth kinetics (m) can be expressed as in equation (8). C_j is defined as the bulk concentration of the compound "j" (g/L), K_j is the affinity constant of the microorganism for compound j (g/L). n_c is the number of compounds affecting growth, C_N is the concentration of the nutrients in the bulk (g/L), K_N is the affinity constant for the nutrients (g/L). The equation includes substrate (nutrients) inhibition, being ${K^i}_N$ is the inhibition constant for the nutrients (g/L). P_{O_2} is the oxygen partial pressure in the system (bar), $P_{O_2}^{limit}$ is the oxygen partial pressure that leads to growth inhibition (bar). Finally, In_{acid} is the inhibition term due to the presence of acids, and m_m is the maximium growth rate (h^{-1}).As previously mentioned, the inhibition due to acids was included by an empirical function using equation (9), where $a - d$ are fittable parameters obtained experimentally, and C_{Acids} is the concentration of acids in the bulk (g/L).

Regarding product yielding (q_l), a similar expression should be proposed. However, in this case, oxygen inhibition would be gradual, while hydrogen would lead to a strong and almost instant inhibition when a certain value is achieved. Moreover, it has been observed that production

suddenly starts when growth stops. So, for hydrogen inhibition and growth role, a logic expression should be used, while an empirical correlation was developed for oxygen inhibition. The kinetics can be seen in equation (10), where q_{m_l} is the maximum production rate for the product "l", In_m is the inhibition term due to the growth, In_{o_2} is the inhibition term due to the oxygen, P_{H_2} is the hydrogen partial pressure (bar), and $P_{H_2}^{limit}$ is the maximum hydrogen partial pressure before having inhibition (bar). As aforementioned, the growth inhibition was set to be a logic constrain as shown in equation (11). The empirical function for the oxygen inhibition is displayed in equation (12), where $a - b$ are fittable parameters obtained from the experiments, and C_{H_2} is the concentration of hydrogen dissolved in the bulk (g/L).

After establishing the kinetics, the subsequent modelling step would involve formulating the mass balances to simulate the products and reagents' evolution during the operation. Given the involvement of gas phases in the system (CO_2 supplied and H_2 and O_2 produced), it becomes necessary to develop a mass balance for each compound in each phase. The mass balance for the gas phase can be seen in equation (13), where f_g is the gas fraction in the cell, C_j^g is the cocentration in the gas phase of the compound "j" (g/L), t is the simulation time (s), Vol is the cell volume (L), Q_g is the volumetric flow of gas leaving the cell (L/s), and MT_j is the mass transfer between the liquid and gas phase for the compound "j". The mass balance for the liquid is shown in equation (14), where f_l is the liquid fraction in the cell, C_j^l is the cocnetration in the liquid phase (bulk) of the compound "j" (g/L), Q_l is the liquid volumetric flow (L/s), $C_j^{l,in}$ is the input concentration of the liquid for the compound "j" (g/L), $g_j$is the stoichiometric amount of the compound "j" consumed during the growth ($g_j/g_{Biomass}$), C_{bio} is the biomass concentration suspended in the liquid (g/L), $p_{j,l}$ is the stoichiometric amount of the compound "j" to yield the

product "l" (g_j/g_l), and n_p is the number of products. Balances are expressed as ordinary differential equations so, defining the initial conditions would be required to solve the two equations. The proposed mass balances imply the use of several assumptions, which are shown below.

First, equations (13) and (14) were developed per volumetric unit of the cell, assuming constant volume and liquid to gas phase ratio. Note that the biomass was assumed to behave like another compound dissolved in the liquid (despite it is in suspension). For this reason, constant gas and liquid volumetric fractions could be used, being related by: $f_l = 1 - f_g$. Furthermore, isobaric operation was also imposed and, so, the input and output volumetric flows in both phases must be the same. For the liquid flow, no additional consideration is needed since it can be easily defined. However, to ensure this situation in the gas phase, the output flow must be equal to the difference between the gas produced and the amount exchanged with the liquid (see equation (15), where $\rho_g$is the gas density in g/L). Second, balances were computed assuming a perfect mixture was used since it is the usual regime aimed during the experiments (and in the majority of the previous models). A continuous operation was included to provide a versatile solution that could be used for batch ($Q_l = 0$) and continuous systems. In addition, no pH balance was included despite the acid production since pH control is assumed for most reactors. Third, equations (13) and (14) have a sign definition for both, kinetics and mass transfer. As for the former, this implies that the stochiometry used must also have pre-defined sign criteria to make sense. For the amount of subtractive/nutrients consumed, g_j will be always positive, while it will be zero for the products and solvents. Regarding biomass, it should be -1. In contrast, $p_{j,l}$ will be negative for the subtrates, and 1 when $j = l$. It would be zero for biomass and solvents.

Focusing on the mass transfer, it was evaluated using the two-layer theory with emphasis on the liquid layer while adjusting the units to be volumetric per cell. The mass transfer expression is gathered in equation (16), where $k_l a$ is the liquid mass transfer coefficient multiplied by the volumetric exchange area (s^{-1}), H_j is the equilibrium constant between the liquid and gas phase for the compound "j", and P_j is the partial pressure of the compound "j". $k_l a$ can be determined

6.2 Thermodynamic framework and transport properties

The model depends on achieving equilibrium between the gas phase and different transport properties such as density. Gas-liquid interaction could be estimated using the Henry constant, and the Antoine equation would be applied for the vapour-liquid cases. In our proposal, ideal fluids are assumed and the solvent is treated as pure water and using the ideal gases equation used for the gas phase. However, since polar compounds and biomass are involved, more detailed thermodynamic models including equations of state and excess properties could be needed if the dilution factor is reduced. An alternative is to use empirical corrections to ideal models. The energy needs are estimated assuming isothermal conditions. Thus, this part focuses on computing the electricity required for the operation and the cooling/heating duty to keep the working temperature constant. For the former, since an expression is developed to compute the cell voltage for a certain current (equation (4)), it can be directly obtained using equation (17).

Regarding the cooling and heating needs, this estimation requires an evaluation of the different heat generation/consumption processes that are involved. For heat generation, the first aspect to consider is the Joule effect due to the overpotentials (P_{over}) as can be seen in equation (18). Additional terms can either imply heat generation or consumption. The Peltier effect (P_{pt}),

determined experimentally, depends on the electron flow between the microorganism and the electrode which can be exothermic or endothermic. In a BES, this value is positive, indicating heat consumption. If unavailable, the reversible heat for the equivalent pure electrochemical process could be used as a rough estimate. The second term, reaction heat (or the catabolism heat, P_{cat}), depends on the enthalpies of formation of products and reagents, contributing to heat generation as liquids form from gases. The last term, the anabolism heat (P_{an}), would ideally be determined experimentally or using average values from the literature (Doran, 2012). The reactor's cooling duty would be therefore obtained from equation (19), with a negative value indicating a heating duty.

7. Insights and Implications

The pivotal role of microorganisms and their ability to enhance electrochemical reactions by interacting with a biocompatible material (electrodes) and facilitating electron exchange with various substrates pose both valuable insights and implications for further development and optimization of microbial electrochemical technologies, including the biotransformation of CO_2 into organic compounds. The choice between pure cultures, mixed microbial communities, or defined co-cultures depends on specific goals, considering stability, productivity and genetic potential and required reactor modifications and operation. Selecting the appropriate option depends on specific goals, with pure cultures being favourable for process improvements, co-cultures requiring precise control of the counterparts to be maintained in the reactors, and naturally evolved communities are suitable when axenic conditions are unattainable. To maximize cell density, it is essential for the culture to adhere to the biocompatible electrode material. Achieving this can involve the induction of nutritional stress to enhance a natural

biofilm formation (Perona-Vico et al., 2020), or using any immovilization method such as sprayable agarose-based hydrogels (Knoll et al., 2022) or 3-D printing strategies. In theory, these approaches not only enchance biofilm formation but also leverage its beneficial features, such as surface protection and functionalization. Moreover, there is a growing exploration of spatially defined consortia, capitalizing on the ability to immobilize cells of different species in specific locations of the engineered biofilm. This facilitates the creation of reaction cascades involving multistep enzymatic pathways with whole-cell biocatalysts or a combination of cells and isolated immobilized enzymes (Mukhi and Vishwanathan, 2022; Philipp et al., 2023).

To integrate these cultures into biofilm reactor development, recent advancements in promising reactor designs have emerged. Two standout setups, the rotating disc bioelectrochemical reactor (RDBER) and the zero-gap flow cell, have garnered attention offering scalable and performance-improving solutions for microbial electrochemical technologies. The RDBER is a scalable setup for cultivating both cathodic and anodic biofilms, aligning well with the requirements of biotechnological pure culture cultivations (Hackbarth et al., 2023). These reactor concepts link back to the earlier discussion on selecting appropriate microbial cultures, emphasizing the importance of adherence to biocompatible electrode materials. The zero-gap flow cell, with its vapour-fed anode design, achieves low internal resistance (due to the minimal distance between working and counter electrodes) and minimizes pH drop between compartments (Baek et al., 2022). This innovative design holds the potential for efficient chemical production from electrical current compared to previous systems.

In advancing the modelling and optimization of microbial electrosynthesis beyond the current technology readiness levels (TRL) of 3-4, further investigation is imperative. Specifically,

addressing the challenge of the anode reaction (counter electrode) linked to the oxygen penetration into the cathode compartment is crucial to avoid toxicity and efficiency losses (Abdollahi et al., 2022). This aspect reinforces the significance of the discussed reactor designs. Simultaneously, the characterization of microbial players in complex systems adds another layer of importance. The integration of specific microbial cultures into biofilm reactor development aligns with this need, offering a strategic approach to address the challenges associated with anode reactions and oxygen penetration.

Microbial electrochemical models, including those accounting only for basic approximations, or more detailed ones (*i.e.* incorporating mass transfer processes, thermodynamics, and microbial community structure), have been developed. More recently, factors such as electron transfer mechanisms, biofilm behaviour, intracellular and external mediators, and the complex interactions between electrochemical and biological processes have been incorporated into modelling. However, some challenges remain to understand electron uptake and biofilm-mediated electron shuttling in BES reactors. Clostridia, including cellulolytic and acetogenic bacteria, hold promise for biofuel and chemical production through microbial electrosynthesis, with genetic editing offering additional optimization potential to the basic metabolisms. While most research focuses on anaerobic organisms, exploration of aerobic chemolithoautotrophs like *Cupriavidus necator* is needed for renewable energy storage and CO_2 conversion, especially if inherent risks of oxygen diffusion to the cathode chamber can not be prevented. To overcome the lack of understanding and variability in bioelectrochemical mechanisms, it is essential to conduct mechanistic characterizations, establish standard guidelines, and enhance knowledge of thermodynamics and energy measurements to ensure reliable modelling and implementation. This includes addressing containment measures, challenges in modelling electrode-cell electron

transfer, and the need for reproducible setups. In addition, comprehensive modelling would require a complete energy balance, which could be used to compute a precise estimation of the duty (thermal and electrical). To achieve this, dedicated experiments involving a Peltier plate should be conducted to account for the interactions between bacteria and the electrochemical system in terms of energy flow. These models will serve as the first step towards the digital transformation of microbial electrochemical systems, enabling process intensification and optimization through advanced computational techniques. This shift empowers researchers to explore these systems with unprecedented precision, enabling real-time monitoring, adaptive control, and predictive modelling. The synergy between biological insights and computational prowess holds the promise of unlocking novel avenues for discovery and optimization. The continuous refinement and application of these models will undoubtedly reshape the landscape of microbial electrochemical systems.

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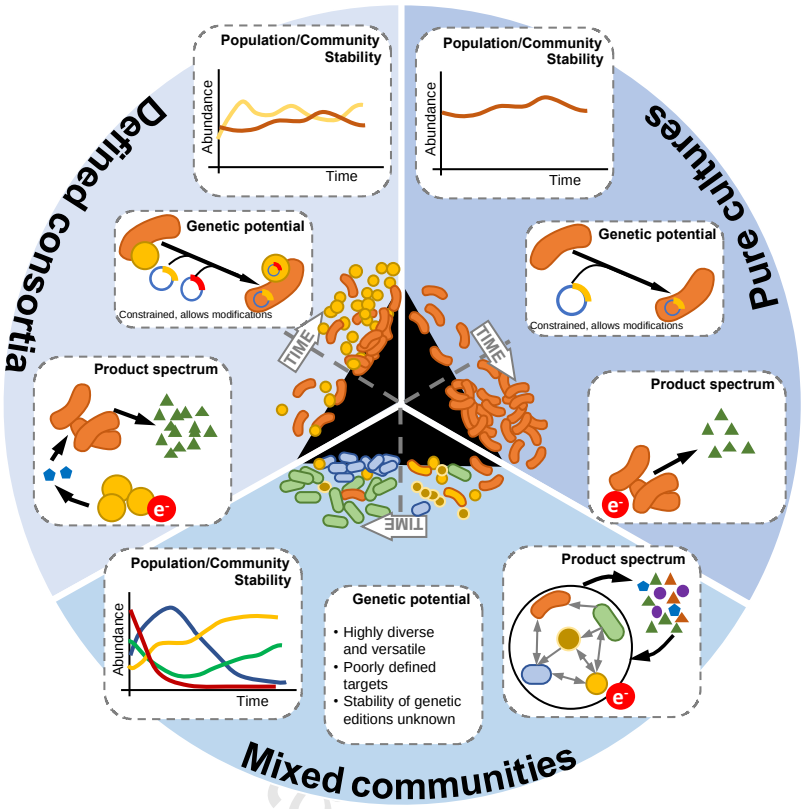

Figure 1. Key properties of using pure microbial cultures, defined consortia, or mixed microbial communities (non-controlled composition) in MES reactors. Changes in size, morphologies and colours of cells depicted in the centre indicate different species (or taxa). The cathode surface is indicated as the black area in the centre of the figure. Cells may remain attached (direct contact) to the cathode or suspended in solution. White "TIME" arrows indicate example progressions of both cathode biofilm and suspended cells on an idealized MES reactor. Inserted graphs show some key features of Population community dynamics (depicted as growth kinetics), Genetic potential (briefly indicating editing possibilities), and ideal electron-to-product conversion and product spectrum (indicated here as small coloured dots). **Pure cultures** offer a limited product spectrum and conversion efficiency, but higher population stability (providing optimized conditions are applied). Gene edition of specific properties is possible. **Defined consortia** in co-cultures allow compartmentalization of the reactor (optimized reactions can be set for biofilm and bulk, independently) and may maximize energy-to-product conversion, increasing yields without a compromise of product selectivity. Gene editing of the consortium members is possible. **Mixed bacterial populations** are less predictable during start-up and operation and eventually evolve into unwanted activities if conditions are not maintained. Product spectrum increases due to larger metabolic potential and inter-species relationships. Some products can be used as substrates for bacteria thus minimizing yields. Genetic edition in these conditions is challenging due to the poor stability of engineered cells.

Bañeras et al. Table 1.

Table 1. Main limitations, challenges and feasible solutions for the development of an overall model for BES systems.

Limitation(s)	Research Challenge(s)	Proposed solution(s)
Uncertainty in the use of previously calibrated models due to the complexity of the real phenomenology behind them	Exhaustive characterization of metabolic mechanism(s) involved and development of plausible models for a generalized solution	Comprehensive modelling (molecular scale/CFD) to include the identified mechanism(s) in parallel to a deep statistic design of experiments for complete characterization.
Low reproducibility due to unpredicted changes in the reactor configuration/design	Strengthen the replicability of reactor set-up and operation of main operational variables imposed (i.e. temperature, working voltage, gas composition, the activity of the living component)	Developing "good practices" guidelines for the design, building, and running of BESs at different scales, focusing on identified critical issues (e.g. changes in the position of electrodes, liquid-to-gas ratio, selectivity of membranes)
Poor information on energy balance for evaluation of the operational costs and scaling	Electrochemistry + Biology imply processes of different nature, requiring a deep understanding of thermodynamics. Lack of information about the energy interaction between biofilms and the electrodes.	Specific energy consumption measurement (evaluation of energy flow) is mandatory in Research oriented to high TRL >5. The information could be used in models to evaluate consumption on larger scales.

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Table 2: Summary of MET concepts evaluated for growth and product formation by *Cupriavidus*. PHB: polyhydroxybutyrate, an intracellular product formed by wild type strains; all other products require engineered strains.

***Cupriavidus* platform**	**Tested Products**	**Benefits**	**Challenges**
Single chamber BES	Isopropanol Isobutanol 3-methyl-1-butanol PHB* Polyhydroxyarylates α-humulene Lycopene	*In situ* production of both O_2 and H_2 Neutral pH	Higher Explosion risk Poor solubility of gases Inefficient O_2 production Production of reactive oxygen species Need for chloride-free electro-autotrophic medium
Secondary MET (so far, only formate tested as intermediate)	Isobutanol 3-methyl-1-butanol PHB*	No explosion risk Water miscible substrate	Formate toxicity Impact of impurities formed in the electrochemical step Diluted product stream from the electrochemical step Need to match electrochemistry and biotechnology (medium) requirements

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Table 3. Detailed equations and strategies proposed to fulfil the compromise between the degree of details required and the available data in the literature. The model was developed in the frame of the BioRECO$_2$VER project.

	Description / Process	Equation
Electrochemistry		
(1)	Gases produced during electrolysis	$n_j = \varepsilon \cdot \frac{i}{F \cdot n_e} \cdot MW_j [=] g/s$
(2)	Efficiency factor	$\varepsilon = f_{ii} \cdot \frac{J^2}{f_i + J^2}$
(3)	Temperature dependence of correlation parameters (*f*)	$f_x = f_x^1 + f_x^2 \cdot T; x \in [i, ii]$
(4)	Cell Voltage	$V_{cell} = V_{rev}(pH) + \eta_{int} + \eta_{act} [=] V$
(5)	Internal (Ohmic) overpotential	$\eta_{int} = i \cdot (R_m + R_{bio} + f_p) [=] V$
(6)	Pressure effects on Internal overpotential	$f_p = d_i + d_{ii} \cdot P [=] \Omega$
(7)	Activation overpotential	$\eta_{act} = s \cdot \log\left(j \cdot \left(t_i + \frac{t_{ii}}{T} + \frac{t_{iii}}{T^2}\right) + 1\right) \cdot i [=] V$
Kinetic expressions for biomass production and product yielding		
(8)	Growth kinetics	$m = \begin{cases} if\ P_{O_2} < P_{O_2}^{limit} \rightarrow m_m \cdot In_{acid} \cdot \frac{C_N}{C_N + K_N + \frac{C_N^2}{K^i{}_N}} \cdot \prod_{j=1}^{n_c} \frac{C_j}{K_j + C_j} [=] s^{-1} \\ if\ P_{O_2} \geq P_{O_2}^{limit} \rightarrow 0 \end{cases}$
(9)	Growth inhibition due to acid accumulation	$In_{acid} = a + \frac{b}{1 + \left(\frac{C_{Acids}}{c}\right)^d}$
(10)	Product yielding	$q_l = \begin{cases} if\ P_{H_2} < P_{H_2}^{limit} \rightarrow q_{m_l} \cdot In_m \cdot In_{o_2} \cdot In_{acid} \cdot \prod_{j=1}^{n_c} \frac{C_j}{K_j + C_j} [=] g_l/g_{biomass} s \\ if\ P_{H_2} \geq P_{H_2}^{limit} \rightarrow 0 \end{cases}$

(11) Growth inhibition constrains

$$In_m = \begin{cases} if\ m > 0 \rightarrow 0 \\ if\ m = 0 \rightarrow 1 \end{cases}$$

(12) Growth inhibition due to oxygen production

$$In_{o_2} = \frac{1}{1 + e^{a+b \cdot C_{H_2}}}$$

Mass balances

(13) Gas phase

$$f_g \cdot \frac{dC_j^g}{dt} = \frac{n_j}{Vol} - \frac{Q_g}{Vol} \cdot C_j^g - MT_j$$

(14) Liquid phase

$$f_l \cdot \frac{dC_j^l}{dt} = \frac{Q_l\left(C_j^{l,in} - C_j^l\right)}{Vol} + MT_j - g_j \cdot m \cdot C_{bio} + \sum_{l=1}^{n_p} p_{j,l} \cdot q_l \cdot C_{bio}$$

(15) Gas produced and exchanged with the liquid phase

$$Q_g = \frac{1}{\rho_g}\left(\sum_{j=1}^{n_c} n_j - \sum_{j=1}^{n_c} MT_j\right)$$

Mass transfer and stoichiometry

(16) Mass transfer

$$MT_j = f_l \cdot k_l a \cdot \left(H_j \cdot P_j - C_j^l\right)$$

Energy requirement

(17) Power

$$Power = V_{cell} \cdot i[=]W$$

(18) Heat generation

$$P_{over} = (\eta_{int} + \eta_{act}) \cdot i[=]W$$

(19) Cooling

$$Cooling = P_{over} + P_{cat} + P_{an} - P_{pt}[=]W$$

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Declaration of interests

☒The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests:

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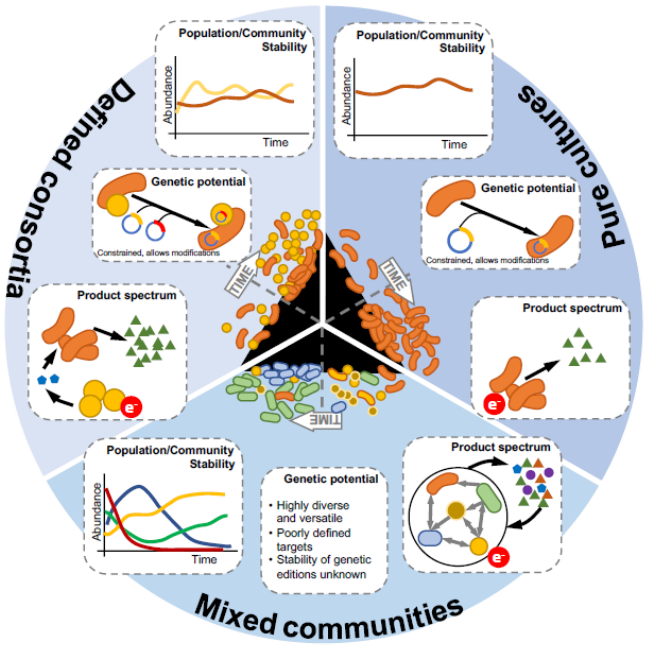
Graphical abstract



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Highlights

- The biological compartment plays a key role in Microbial Electrosynthesis Systems.
- Aerobic and anaerobic model organisms need to be considered separately
- Modelling needs to improve the thermodynamic and electrode-to-cell aspects.
- Pros and Cons of axenic cultures and mixed communities in MES are highlighted.

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