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Oxygen In The Mix: Is Oxic Microbial Electrosynthesis A Potential Alternative For Biomass Production?

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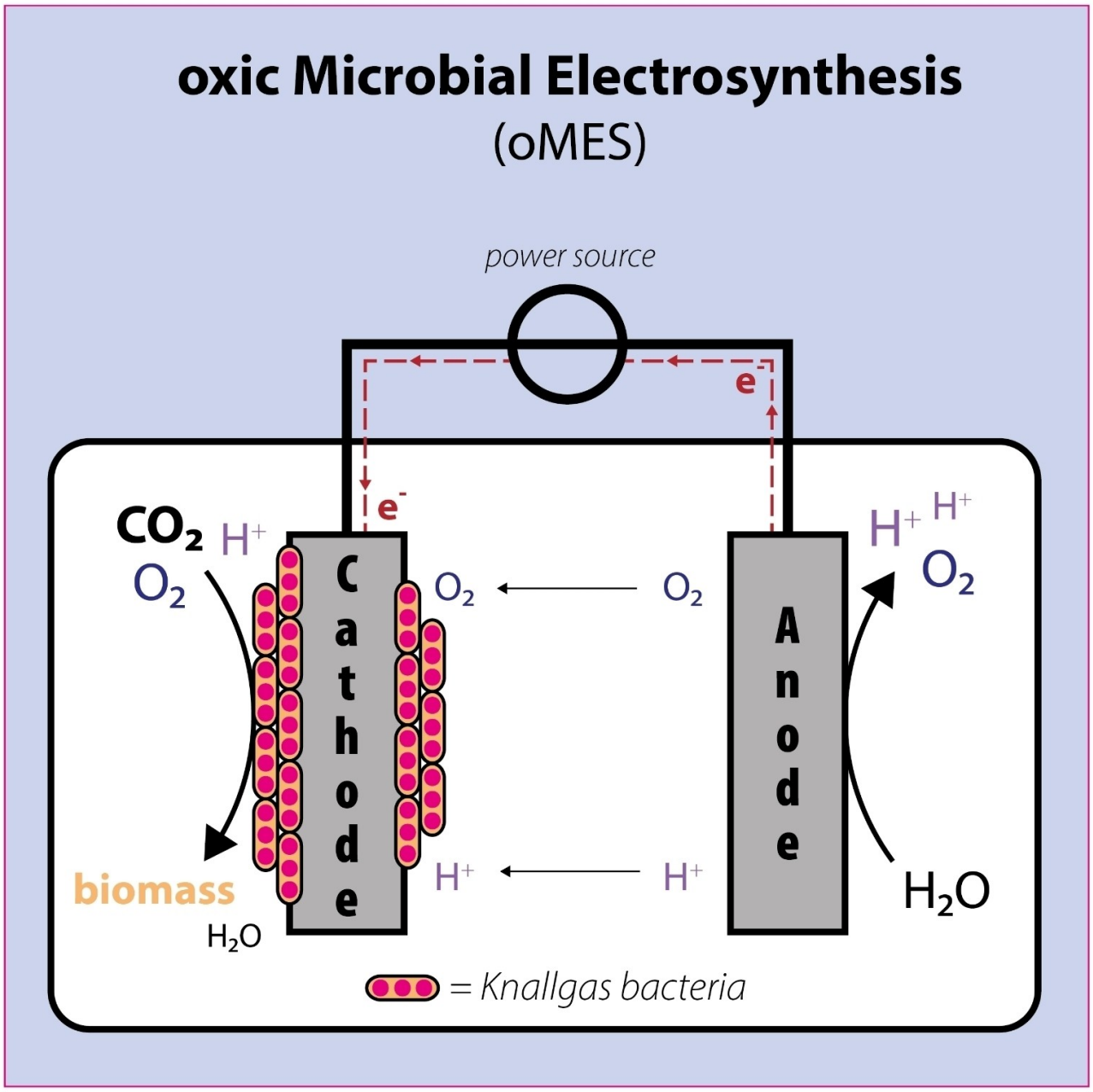

Oxic microbial electrosynthesis (oMES) allows the utilization of renewable electricity and industrial gas streams containing CO_2 and O_2 for biomass production by cultivating aerobic, autotrophic, hydrogen-oxidizing bacteria, commonly known as Knallgas bacteria. oMES is likely not a direct competitor to conventional anoxic microbial electrosynthesis as harnessing aerobic hydrogen-oxidizing bacteria depends on energetically inefficient assimilatory CO_2 reduction pathways. However, it might be a complementary approach to classical biomass production from the perspective of limited land use and the availability of cheap renewable energy. The best characterized Knallgas bacterium is *Cupriavidus necator*. Extensively studied as lithoautotrophic production host, *C. necator* already offers a broad arsenal of genetic tools. In contrast, mechanistical knowledge about the recently discovered *Kyrpidia spormannii* is limited, but this species shows remarkable growth when cultivated as cathodic biofilm in bioelectrochemical systems. In addition, first experiments indicate a low energy demand for biomass production, which is in the order of magnitude of gas fermentation with *C. necator* or heterotrophic and methanotrophic technologies. Still, many aspects of the electrochemical cultivation of *K. spormannii* need to be better understood and rigorously improved to be a competitive technology in the making, including electron transfer and microbial kinetics, cultivation conditions, mass and energy balances, and reactor design.

1. Introduction

The conventional way in which humanity has produced goods and commodities for centuries has led to some of the greatest challenges of our time, including climate change caused by anthropogenic greenhouse gas emissions or biodiversity loss due to extensive land use.[1] This comprises crops cultivation for human and livestock sustenance, as well as its use for direct incineration applications such as district heating or for the production of other energy carriers like biogas or biofuels.[2] Hence, microbial CO_2 conversion technologies harnessing lithoautotrophic organisms have repeatedly become the focus of scientific attention. Utilizing inorganic electron donors as energy source for the reduction of CO_2 might offer an alternative to the conventional production of biomass, other energy carriers, or even more defined organic building blocks, while circumventing the surface limitation of light-dependent phototrophic CO_2-fixation.[3] When lithoautotrophic microbial cultivation is based on gaseous feedstocks, for instance, H_2 as electron and energy donor and CO_2 as carbon source, the process is regarded as gas fermentation.[4] This approach has been followed for decades and has attracted vital industrial interest in producing biomass or energy carriers (e.g. methane or ethanol).[3,5,6] However, under physiological conditions, hydrogen exhibits a low solubility in aqueous systems, which results in productivity limitations.[7] Gas mass transfer can be improved by a higher operating pressure or thorough mixing, leading to a homogeneous distribution of small hydrogen bubbles in the reactor.[8] Still, both approaches require a considerable energy input. Furthermore, the potential for hydrogen accumulation in the gas phase poses the risk of forming explosive gas mixtures.[9] Hence, an in-situ hydrogen production in the bacterial growth medium is an opportunity to overcome these problems.[10] This can be achieved in a bioelectrochemical system (BES), interfacing microorganisms with electrochemistry.[11] Here, hydrogen is electrochemically produced at an integrated cathode and directly supplied to the microorganisms, allowing a lithoautotrophic CO_2 fixation while omitting process limitations caused by low hydrogen solubility. In such a biotechnological process – also referred to as microbial electrosynthesis (MES) – acetogens and hydrogenotrophic methanogens have predominantly been cultivated as pure cultures thus far.[12,13] Both physiological groups employ highly energy-efficient catabolic pathways to convert CO_2 either into acetic acid (Wood-Ljungdahl pathway in acetogens) or methane (hydrogenotrophic methanogenesis pathway in hydrogenotrophic methanogens).[14] These catabolic CO_2 reduction pathways are characterized by a higher metabolic flux and product selectivity (only a small fraction of the reduced carbon ends up as biomass) compared to a purely assimilatory CO_2 fixation pathway like, for example, the Calvin cycle.[15–17] This leads to unrivaled product yields for the production of acetate or methane from H_2 and CO_2.[10,18] However, the limited product spectrum requires further conversion steps to produce more valuable and complex compounds like longer-chain fatty acids, alcohols, or biomass.[19,20] Moreover, since acetogens and methanogens are strict anaerobes, methane or acetate production via hydrogenotrophic methanogenesis or the Wood-Ljungdahl pathway are prone to oxygen.[14] This is particularly relevant since oxygen is produced at the anode during MES – as water oxidation is the usual counter-reaction in a cathodically operated BES.[21] Therefore, the use of ion exchange membranes for separating anodic and cathodic compartments becomes crucial for cultivating methanogens and acetogens in a BES in order to ensure anoxic conditions in the cathode compartment. However, using membranes increases ohmic losses and mass transfer limitations, contributing to undesired pH shifts in the separated compartments.[12,22–25] These drawbacks of membranes, coupled with the resulting complexity of

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the reactor setup, culminate in a high-maintenance system that is likely to incur significant costs in long-term operation.[26] In addition, most industrial CO_2-rich gas streams, which are supposed to be used in MES and other microbial CO_2 conversion technologies, will not be strictly anoxic.[27] Therefore, oxygen removal is mandatory for the utilization of these off-gases by acetogenic and methanogenic microorganisms. In the case of catalytic oxygen removal, the associated provision of electrons as reducing agents (e.g. H_2S, H_2, CH_4) or electricity could significantly impair the overall process efficiency.[28] As recently emphasized by Bañeras et al. in a review about MES models, this oxygen obstacle could be circumvented when aerobic autotrophic bacteria are employed for MES.[29] Here, the organisms rely on O_2 as terminal respiratory electron acceptor, CO_2 as carbon source, and electrons provided (either directly or indirectly) by a cathode as the sole energy source. This process was recently referred to as oxic microbial synthesis (oMES).[30] In previous publications, also by the authors, the term *aerobic microbial electrosynthesis* was used for describing the process.[31] However, when referring to the oxygen dependency of microbial metabolisms, the classical terminology aerobic/anaerobic is correct. In contrast, when growth conditions, e.g., in a natural environment, or process conditions are the focal point, the terms anoxic/oxic should be used. Since the authors refer to "microbial electrosynthesis" as a biotechnological process and not as a physiological trait of a microorganism in this manuscript, the term oxic microbial electrosynthesis is used.

In addition to the intrinsic benefit of oxygen tolerance when employing aerobic organisms for MES, a simplified reactor design (i.e. no membrane) and a more straightforward and cost-effective medium (e.g. absence of reducing agents for oxygen removal) are required.[31] An inherent disadvantage of oMES compared to traditional, anoxic MES must be mentioned: Approx. 50% of the electrons consumed by an aerobic, autotrophic microorganism cannot be expended for the reduction of CO_2. Instead, they are channeled in the electron transport chain for catabolic oxygen reduction, providing ATP for growth and cell maintenance, ultimately resulting in water as a carbon-free electron sink.[30] Furthermore, due to the dependence on assimilative CO_2 fixation pathways, such as the Calvin cycle, the share of electrons that become available for CO_2 fixation ends up almost exclusively in biomass and not in a specific organic product, such as for example acetic acid.[32,33] Therefore, it is crucial to emphasize that oMES is not intended to compete directly with conventional anoxic MES. Given the distinct substrate and product range, oMES cannot fulfill the same role. Instead, it should be viewed as an additional and complementary CO_2-converting technology. For instance, when oxic, unpurified carbon dioxide waste streams like off-gas or CO_2 sourcing from direct air capture technologies are employed as a feed stock, oMES may carve out its unique role within the diverse potpourri of potential technologies in a future sustainable economy. Especially in a future scenario, which may be distant, where the pivotal cost factors are not exclusively tied to electricity sourced from renewables but instead revolve around physical resources essential for reactor construction and cultivation media, the relevance of oMES could be notably pronounced. Another conceivable application scenario for oMES is oxygen scavenging from an oxic CO_2 stream with the benefit of a simultaneous biomass production. For example, an upstream oMES process could be coupled to an anoxic MES reactor as the main CO_2 reduction stage.

Accordingly, in this review, we compile knowledge on the cathodic cultivation of aerobic autotrophic microorganisms,

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Benjamin Korth has been researching electroactive microorganisms since 2013 in Falk Harnisch's lab, with a particular focus on their thermodynamics and kinetics. In 2017, he received the Helmholtz PhD award in the research area "Energy" for his doctoral thesis. Following a research stay in the lab of Sebastià Puig Broch, his research also covers the fundamentals and application of bioelectrochemical denitrification. Since 2020, he has led the Team "Thermodynamics of Electroactive Microorganisms" in the Department Microbial Biotechnology at the Helmholtz Centre for Environmental Research - UFZ.

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with a particular focus on the electrobiotechnological application of hydrogen-oxidizing Knallgas bacteria in oMES for the production of biomass using pure cultures. Especially, the well-characterized *Cupriavidus necator* and the recently discovered *Kyrpidia spormannii* are discussed as promising candidates for oMES. Subsequently, the biomass productivity and the energy demand of approaches cultivating *C. necator* and *K. spormannii* are compared to other microbial technologies for biomass production to estimate their economic viability. Finally, the focus of future research efforts is discussed in order to close research gaps of oMES and to understand how *K. spormannii* achieves its low energy demand for biomass production, making it a candidate calling for investigation for its future industrial applicability.

2. An Overview of the Cathodic Cultivation of Aerobic Autotrophic Microorganisms

Table 1 compiles – to the best of the authors' knowledge – most aerobic organisms cultivated in pure culture as cathodic biofilm in a BES. Furthermore, the table encompasses studies that enriched and characterized cathodic, oxygen-reducing, mixed-species-biofilms. Notably, the listed species exclusively fall within the physiological groups of either lithoautotrophic metal and/or sulfur oxidizers (e.g. *Mariprofundus ferrooxydans* PV-1, *Acidithiobacillus ferrooxidans*, *Acidithiobacillus thiooxidans* – see also Figure 1A, B and D) or autotrophic hydrogen-oxidizing bacteria (e.g. *C. necator* and *K. spormannii* – see Figure 1C, E–I), commonly known as Knallgas bacteria. The primary focus of most studies is not, as one might anticipate, on the development of a biotechnological process for CO_2 reduction into biomass or other organic products using cathodically supplied reduction equivalents. Instead, many of these studies targeted the enhancement of electrocatalytic properties of the cathode through the colonization by electroactive microorganisms. Here, the goal was to achieve a more effective cathodic oxygen reduction, ultimately leading to improved performance (i.e. power generation) of microbial fuel cells.[37,42–44] In these studies, no additional CO_2 was provided, and only the CO_2 present in the air was used by the microorganisms as carbon source. Other motivations for the research on cultivating lithoautotrophic microorganisms at cathodes were screening for heavy metals in tap water and developing an alternative cultivation method for the characterization of metal oxidizers – of which some can otherwise only be cultivated with considerable effort as a laboratory pure culture.[35,45] An indicator for the early stage of research on oMES and aerobic cathodic biofilm research in general are the reactors employed in the respective studies. Limited in electrode surface and reactor volume, the majority of the used BES, with a few exceptions, lack the capability for genuine upscaling. In addition, the achieved current densities have thus far been orders of magnitude too low for any substantial economic viability.[12] In this context, it is not only noticeable that there are generally few pure aerobic cultures that have been cultivated autotrophically with the aid of cathodes but also that the process parameters are not uniform and performance parameters (e.g. coulombic efficiency (*CE*), current and biomass production) stated in the respective studies are sometimes not comprehensive (often due to the different motivations behind the studies). A closer look at the listed current densities, *CE*, and cultivation conditions clearly illustrates the difficulties of comparing these studies and raises the question of whether one of the values can be used as a potential performance parameter to determine the most suitable organisms for a future oMES process. Comparing current densities (i.e. current normalized to cathode area) – a performance parameter frequently used in other microbial electrochemical technologies – is challenging in the case of cathodic cultivation of aerobic autotrophic organisms and should be treated with extreme caution, not only due to the use of different reactor configurations (e.g. electrode materials and hydrodynamic conditions) and varying cultivation conditions but also, in particular, due to the different ways in which oxygen is introduced to the reactors and, above all, at what concentration. Especially if a biofilm does not fully cover the electrode, abiotic oxygen reduction at the cathode can occur quite readily, depending on the chosen potential and cathode material, resulting in high current densities unrelated to microbial activity.[30,31,39] This might also account for the considerable variability in current densities, spanning several orders of magnitude between different studies when the same organism was employed as biocatalyst. As demonstrated in Table 1, *CE* of oMES processes were rarely determined, making it challenging to ascertain the percentage of cathodic electrons dedicated to CO_2 reduction versus those utilized for abiotic and biotic oxygen reduction. Consequently, future studies on oMES must prioritize the establishment of comprehensive electron and mass balances to allow a clear differentiation between abiotic and biotic processes. Achieving this requires quantification of biomass and/or other metabolic products, a practice observed in only a few studies listed in Table 1. To this end, the quantification of planktonic and biofilm growth is essential. Focusing on current density, it becomes apparent that the organisms that are mainly suspected of direct electron uptake from a cathode – e.g. most of the metal and sulfur oxidizers such as *Mariprofundus*, *Ancylobater*, or *Acidithiobacillus* – tend to form single-layer biofilms with a surface coverage of well below 100% (see Figure 1A and B). Although, these studies are certainly interesting from an ecophysiological point of view (e.g. how a potential direct electron uptake and energy metabolism are mechanistically coupled),[46] they are not further discussed in this review. In contrast, Knallgas bacteria like *K. spormannii* or *C. necator*, which are likely dependent on a hydrogen-mediated electron transfer and show a much more pronounced biofilm growth (sometimes accompanied by planktonic growth at high hydrogen production rates), are discussed in more detail below (see Figure 1C, E, F, and I).

It should be noted that it is still debated if some electroactive microorganisms receive electrons from the cathode directly or if the electron transfer is accomplished by cathodically produced hydrogen (or other small, reduced molecules)

Table 1. Cathodic cultivations of aerobic autotrophic organisms and the respective process parameters. (–) – no information given. (EET) – extracellular electron transfer. (m) – maximum current densities recorded during chronoamperometric measurement; (a) – average current densities of chronoamperometric measurements; (cv) – current densities derived from cyclic voltammetry; (cp) – fixed current density during chronopotentiometric operation. (Ref.) – reference.

Microorganism	Electrode material (projected area [cm^2])	Proposed EET mechanism	Cathode potential [V vs. SHE]	Current density [$\mu A\,cm^{-2}$]	Coulombic efficiency [%]	Gas phase composition	Reactor type ($Volume_{Cathode}$)	Duration [d]	pH	Temperature [°C]	Biofilm properties	Motivation	Ref.
Mariprofundus ferrooxydans PV-1	Graphite (18)	Direct	−0.076	−55.0 (m)	at least 80% of measured current due to abiotic O_2 reduction	8–10% O_2	(80 mL)	ca. 30	7.2	–	Biofilm of up to 1-5×10^6 cells cm^{-2}	Developing alternative cultivation method to study metabolism of obligate Fe(II)oxidizers	[35]
Ancylobacter sp. TS-1	Polished graphite (3.4)	Direct	−0.3	−42.5 (m)	–	Air	(40 mL)	18	7.2	ca. 24	Biofilm of up to 2.5×10^6 cells cm^{-2}	Characterisation of electroactivity of arsenic oxidizer for potential future arsenic electro-bioaugmentation and bioremediation	[34]
Acidithiobacillus ferrooxidans	Graphite (11.85)	–	0.245	−25.7 (a)	–	Air	Screening reactor system in disposable six-well plates (9 mL per well)	10	2.3	30	–	Screening for new cathodic biocatalysts	[58]
	FTO (3.14)	Direct	0.4	−2.2 (m)	–	Air	Single-chamber reactor with working electrode on the bottom (150 mL)	1.25	1.8	30	Single-layer biofilm of up to 9×10^6 cells cm^{-2}	Examination of a hypothetical metabolic pathway for electrolithoautotrophic carbon assimilation in microbial ecosystems of the deep ocean	[59]
	Graphite felt (11.8)	Direct	0.24	−194 (m)	–	Air	Glass electrochemical cell (90 mL)	ca. 25	2.0	30	–	To study the electrocatalytic properties of the colonized cathode for O_2 reduction at low pH in a MFC	[37]
	Carbon cloth (3)	Fe^{3+}/Fe^{2+}-mediated	0	−3861 (m)	–	Air	GL45-Bottle type reactor (200 mL)	ca. 21	2.0	30	Multi-layer biofilm with up to 30 μm thickness	Optimization of *A. ferrooxidans-based* biocathode for O_2 reduction in a MFC	[43]
	Carbon felt (–)	Direct	0	−56 (m)	–	–	–	8	2.0	30	–	Effect of quorum sensing autoinducers on inert carbon electrode biofilm formation	[60]
Acidithiobacillus thiooxidans	Graphite (11.85)	–	−0.3	−0.024 (a)	–	Air	Screening reactor system in disposable six-well plates (9 mL per well)	10	ca. 4.5	30	–	Screening for new cathodic biocatalysts	[58]
	Graphite (0.25)	–	0.1	−65.0 (cv)	–	Air	Glass jacketed reactor (100 mL)	7	1.9	28–30	Single-layer biofilm	Characterization biofilm formation of *A. thiooxidans* on a graphite surface (in the presence of S^0 as additional energy source)	[61]
Cupriavidus metallidurans	Graphite (11.85)	–	−0.3	−0.133 (a)	–	Air	Screening reactor system in disposable six-well plates (9 mL per well)	5	ca. 6.9	30	–	Screening for new cathodic biocatalysts	[58]
Cupriavidus necator	Graphite (11.85)	–	−0.3	+0.108 (a)	–	Air	Screening reactor system in disposable six-well plates (9 mL per well)	12	ca. 6.9	30	–	Screening for new cathodic biocatalysts	[58]
	Platinum (2.3)	Hydrogen	−1.3	−1565.0 (m)	–	Water electrolysis with addition of 10–15 ml CO_2 min^{-1} (40 mbar)	Modified Schott flask (–)	4	6.6	30	Planktonic growth: 0.09 h^{-1}	Characterization of the electrical properties of an optimized minimal medium	[50]
	Platinum (1.0)	Hydrogen	–	−500 (cp)	–	Flow of CO_2 and synthetic air (each 10 ml min^{-1})	Double wall bioreactor (250 mL)	1	7.0	30	Single-layer biofilm with 2×10^7 cells cm^{-2} (surface coverage 35%)	Impact of different current densities on the chemolithoautotrophic, cathodic biofilm formation of *C. necator*	[38]
	Platinum (22.5)	Hydrogen	–	−670 (cp)	–	Real flue gas after desulfurization (25 ml min^{-1})	Bottle type reactor (110 mL)	12	6.5	30	Planktonic growth: up to a OD_{600} of 2	Proof-of-principle for the production of PHB from real flue gas as carbon source and cathodically produced hydrogen	[62]
Kyrpidia spormannii	Graphite felt (16)	Direct/ Indirect	−0.531	−50.0 (m)	–	0.5% O_2 in CO_2	Cylindrical glass reactor (2 L)	11	3.5	60	Single-layer biofilm	Isolation of thermophilic species that grow as cathodic biofilm using CO_2 as carbon source	[41]

Table 1. continued

Microorganism	Electrode material (projected area [cm^2])	Proposed EET mechanism	Cathode potential [V vs. SHE]	Current density [$\mu A\,cm^{-2}$]	Coulombic efficiency [%]	Gas phase composition	Reactor type ($Volume_{Cathode}$)	Duration [d]	pH	Temperature [°C]	Biofilm properties	Motivation	Ref.
	Graphite (20)	Direct/ Indirect	−0.531	−85.0 (m)	7% (biomass as product, based on ^{13}C data)	5% O_2 in CO_2 (1.5 bar)	Pressurizable flow cell (ca. 1 L, flow-rate 100 mL min^{-1})	10	3.5	60	Multi-layer biofilm with an average height of 80 μm	Isolation of thermophilic species that grow as cathodic biofilm using CO_2 as carbon source	[41]
	Graphite (20)	Direct/ Indirect	−0.5	−13.0 (a)	–	0.5% O_2 in CO_2 (1.5 bar)	Pressurizable flow cell (ca. 1 L, flow-rate 100 mL min^{-1})	20	3.5	60	Multi-layer biofilm of up to 150 μm	Lab-scale flow cell system to monitor the cathodic growth of *K. spormannii* biofilms	[39]
	Graphite (2.25)	Direct/ Indirect	−1.425	−3770 (m)	0.2% (PHB as product)	5% O_2, 20% CO_2, 75% N_2	H-cell type reactor (250 mL)	ca. 3	5.5	60	Single-layer biofilm, 1.5×10^7 cells cm^{-2} (microscopy) 2.5×10^8 cells cm^{-2} (qPCR)	Optimization of biofilm growth of *K. spormannii* on a cathode	[40]
	Graphite plate with deposited Pt (2.25)	Direct/ Indirect	−0.625	−630 (m)	1% (PHB as product)	2.5% O_2, 20% CO_2, 77.5% N_2	Cylindrical glass reactor (1 L)	ca. 3	6.5	60	Single-layer biofilm 1.9×10^{10} cells cm^{-2}	Effect of cathode properties on biofilm formation and PHA synthesis by *K. spormannii*	[63]
	Graphite (700)	Direct/ Indirect	–	−3 (cp)	–	0.5% O_2 in CO_2 (0.5 bar)	Rotating disc bioelectrochemical reactor (10 L)	30	3.5	60	Biofilms of up to 100 μm	Proof-of-principle for an oMES process in a novel rotating disc bioelectrochemical reactor	[33]
	Graphite (20)	Direct/ Indirect	−0.5	–	up to 49.5% on day 4	O_2 (up to 4%) was adjusted in CO_2 according to biovolume (1.5 bar)	Pressurizable flow cell (ca. 1 L, flow-rate of 100 mL min^{-1})	7	4.0	60	Multi-layer biofilm of up to 250 $\mu m^3\,\mu m^{-2}$	Study biomass formation and energy efficiency of cathodic growth of *K. spormannii*	[30]
Mixed species	Carbon cloth (6)	–	0.05	−77 (m)	–	Air	Modified Duran bottles (650 mL)	23	7.8	–	Multi-layer mixed species biofilm	Comparing different methods to form oxygen-reducing biocathodes for a MFC	[44]
	Glassy carbon rotating disc electrode (1.5)	–	0.4	−22 (m)	–	Air	Open glass beakers (100 mL)	8	7.8	28	–	Cultivation of oxygen-reducing microbial cathodes for monitoring of heavy metals in tap water	[45]
	Carbon cloth (2)	–	−0.16	−37 (m)	–	Air	Modified Schott vial (500 mL)	ca. 14	7.6	40	–	Enrichment of oxygen-reducing biocathodes for enhancing MFC performances	[43]
	Carbon cloth (2)		0.34	−67 (m)		Air	Modified Schott vial (500 mL)	ca. 14	7.6	40			
	Graphite coupons (65.2)	Direct	0.310	−4.2 (m)	–	Air	Sediment-based MFC in food storage container (–)	14	–	30	–	Culture-independent characterization of aerobic, autotrophic cathodic enrichment	[64]
	Graphite coupon (58.8)	Direct	0.470	−64.1 (m)	–	Air	Dual chambered H-cell type reactor (2 L total volume)	ca. 4	–	30	–		[65]
	Stainless-steel mesh (200)	Hydrogen	–	Up to −1000 (cp)		H_2/O_2 from integrated electrolysis + Manual flushing with CO_2	Bottle type reactor (1 L)	Several weeks	6–7	25	Planktonic growth but 12% of biomass as cathodic biofilm	Effect of current density on biomass growth and nitrogen assimilation of a mixed species community	[66]

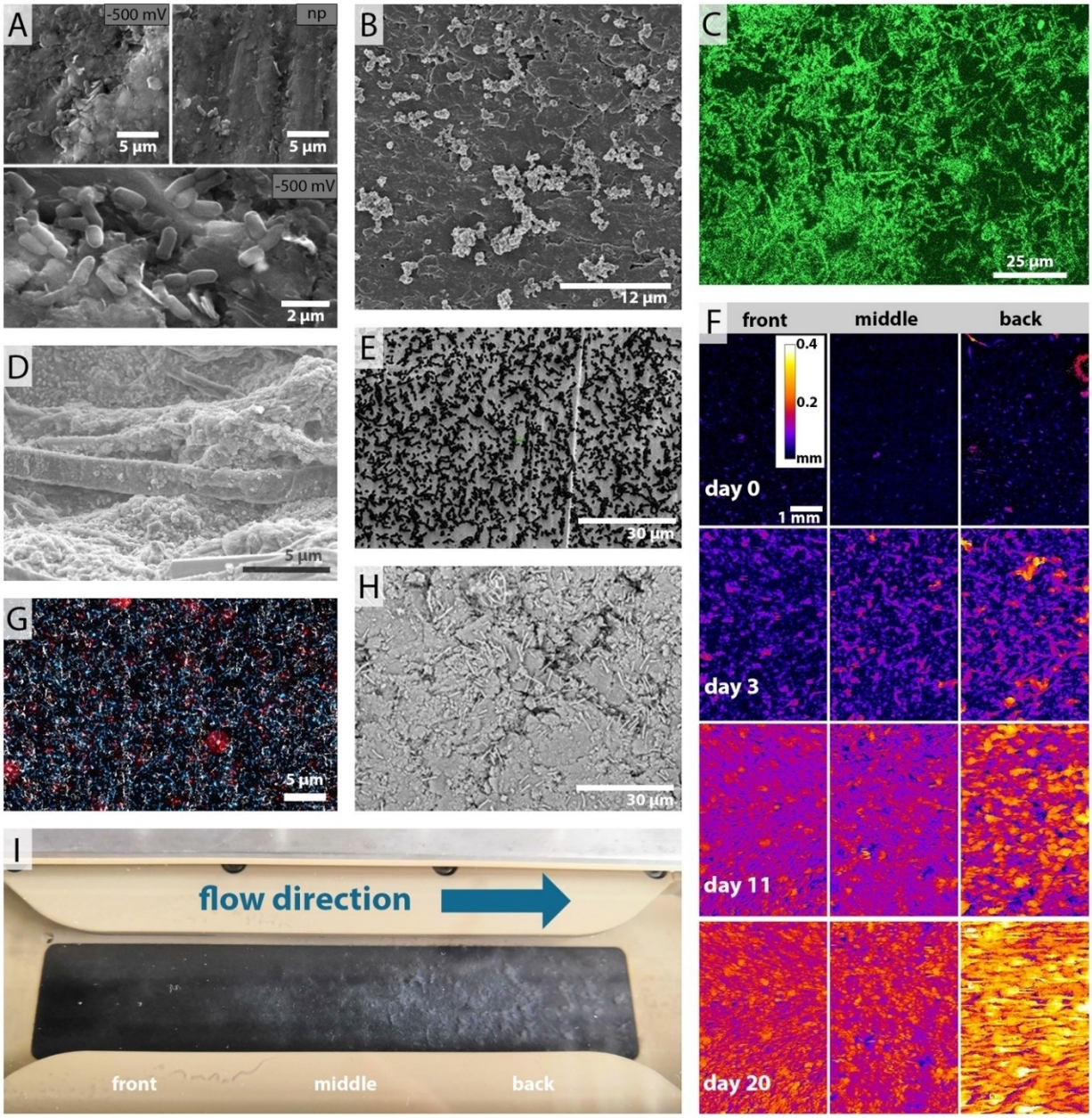

Figure 1. Compilation of various aerobic and cathodic pure culture biofilms recorded using different visualization techniques. Images were reused directly from the original sources without modifying. However, for the sake of uniformity and a clear overview, some of the images have been cropped and provided with a uniform scale bar. **A** – Scanning electron micrographs (SEM) of *Ancylobacter* sp. TS1 biofilms (after 72 h) on graphite electrodes poised to −500 mV vs. standard hydrogen electrode (SHE) and control electrode (not connected to a potentiostat, np). Reprinted from Anguita et al.[34] with permission from John Wiley and Sons; **B** – SEM of *Mariprofundus ferrooxydans* PV-1 attached to graphite electrodes. Reprinted from Summers et al.[35]; **C** – Fluorescence micrograph of a *Kyrpidia spormannii* EA-1 biofilm, cultivated in a pressurized flow cell (stained with RedoxSensor Green). Reprinted from Reiner[36] with permission from the author; **D** – SEM of a carbon felt fiber electrode 28 days after inoculation with *Acidithiobacillus ferroxidans* (0 V vs. SHE). Reprinted from Carbajosa et al.[37] with permission from Elsevier; **E** – SEM of a *Cupriavidus necator* biofilm at a current density of $-500\ \mu A\,cm^{-2}$ after 24 hours at a platinum electrode. Reprinted from Bause et al.[38] with permission from Springer Nature; **F** – Time series of height maps generated from optical coherence tomography data from three different positions of a cathode in a flow cell covered with *K. spormannii* EA-1 (a photograph of the cathode and the flow cell is shown in section I of this figure). Reprinted from Hackbarth et al.[39] with permission from Elsevier; **G** – Fluorescence micrograph of DAPI (blue) and Nile-red-stained *K. spormannii* EA-1 cells on a stainless-steel electrode. Reprinted from Pillot et al.[40] with permission from Elsevier; **H** – SEM of *K. spormannii* EA-1 cells on a graphite electrode after 9 days of incubation at −531 mV vs. SHE in a glass reactor. Reprinted from Reiner et al.[41] with permission from Oxford University Press; **I** – Photograph of a cathodic *K. spormannii* biofilm in a pressurized flow cell - visible to the bare eye. Reprinted from Reiner[36] with permission form the author.

acting as an electron shuttle. Although indications for both mechanisms were described in the literature, it becomes more and more evident that hydrogen-mediated electron transfer cannot be excluded in most studies (including the cultivation of acetogens and methanogens), even if the applied cathode potential is above the standard redox potential of the hydrogen

evolution reaction (HER, $E^{\circ\prime} = -0.420$ V). This can be explained by the fact that a high microbial affinity for hydrogen can lead to a low local H_2 partial pressure at the cathode surface – thereby shifting the redox potential of the hydrogen evolution reaction to more positive values. In summary, hydrogen-mediated electron transfer seems more widespread, and a direct electron uptake mechanism seems an exception in (o)MES instead.

3. Potential Advantages of Biofilm and Planktonic Growth in Oxic Microbial Electrosynthesis

A general juxtaposition and discussion of the advantages of biofilm-based biotechnological processes is clearly beyond the scope of this review and has recently been discussed in detail elsewhere.[47] Nevertheless, a brief distinction between the two growth modes should be addressed in the context of oMES. Whether an organism grows during oMES as a cathodic biofilm or planktonically in the bulk phase of the BES depends on various aspects. Slow-growing organisms that exhibit a growth rate lower than the hydraulic retention time of a continuous system can only compete as biofilm against a wash-out.[48] Further, organisms depending on direct electron uptake (in the absence of an electron shuttle) need direct physical contact with the electrode and, therefore, are likely to grow as a biofilm.[49] However, biofilms can also form on the cathode in the case of hydrogen-mediated electron uptake.[38] It should be noted that in the case of hydrogen overproduction – i.e. when more hydrogen is produced than the cathodic biofilm oxidizes – the selection pressure for biofilm formation is eliminated, and planktonic growth is preferentially observed.[50] Yet, the major disadvantage here is that abiotic oxygen reduction at the uncovered cathode can significantly reduce the *CE* and can also lead to the formation of reactive oxygen species (ROS).[31,39,51] In addition, if the cathodically produced hydrogen is not entirely consumed by the microorganisms in the bulk phase, explosive hydrogen/oxygen mixtures may form in the gas phase.[9] Further, a biofilm can reduce the local H_2 partial pressure directly at the cathode to such an extent that hydrogen formation can be catalyzed even at cathodic potentials above the standard redox potential of HER.[52,53] Thus, the required energy input for an electrochemical hydrogen production can get reduced.

Cathodic biofilms can be regarded as counter-diffusional systems, as the electron donor, electron acceptor, and other nutrients enter the biofilm from different directions.[54] In oMES, oxygen, nitrogen source, and other micronutrients diffuse from the bulk phase, and hydrogen enters the biofilm from the cathode side. To ensure an optimal supply of substrates, such a counter-diffusional cathodic biofilm should not exceed a certain thickness (see Figure 2).[30] This maximum biofilm thickness can likely be regarded as organism-specific and cannot be generalized. In order to keep a biofilm-based oMES process as efficient as possible, the biofilm should, therefore, be sheared off regularly, allowing straightforward biomass harvest. This could be implemented, for example, by shearing using a type of moving blade, by the pulsed production of hydrogen bubbles, or via a short-term increase in shear forces by increasing the flow velocity over the biofilm.[30,31] Although the arguments presented above indicate a likely preference for biofilms over planktonic growth in conducting oMES, further investigations are necessary to definitively answer whether there is a superior growth mode. However, it may be succinctly summarized that the answer to this question culminates in a rate/yield trade-off. It is conceivable that oMES is more efficient with biofilm growth but faster with planktonic growth.

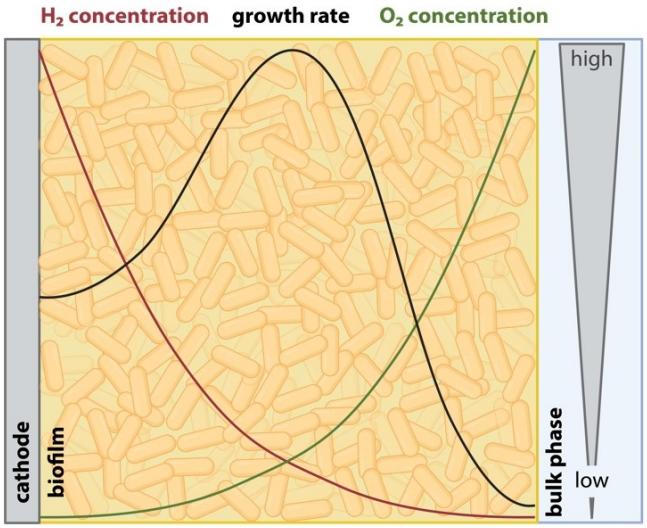

Figure 2. Knallgas bacteria cultivated as biofilms at cathodes represent a counter-diffusional system. Hydrogen is electrochemically produced at the cathode, and oxygen (and also other nutrients) diffuses from the bulk phase into the biofilm. The growth rate is maximized in biofilm layers with both hydrogen and oxygen availability. Adapted from Rominger et al.[30]

4. *Cupriavidus necator*

As early as 1965, the first oMES process using *Cupriavidus necator* as a biocatalyst was studied by Schlegel and Lafferty. The authors were able to show that the cathodic production of H_2, the anodic production of O_2, and the addition of CO_2 were sufficient for growth. This study cultivated hydrogen-oxidizing bacteria in a BES for the first time, and the authors observed rapid growth at high current densities, whereas growth at low current densities was limited by hydrogen availability.[55] Since this very early work, *C. necator* has proven to be one of the most promising production hosts for autotrophic industrial applications, particularly because of its rapid growth and genetic accessibility. Nevertheless, it took several decades before the conduction of oMES with *C. necator* as a production host came back into the focus of science. In order to understand the autotrophic metabolism of the organism in greater detail, it is crucial to understand the underlying hydrogen uptake mechanisms. *C. necator* has three distinct [NiFe]-hydro-

genases with different physiological roles. Firstly, the membrane-bound hydrogenase, which is coupled to the respiratory chain, belongs to the uptake [NiFe]-hydrogenases found in bacteria and archaea and is located at the inner membrane facing the periplasm. The second hydrogenase of *C. necator* is a heterodimeric soluble hydrogenase attached to a redox module, which interacts with other soluble cofactors. Lastly, the regulatory hydrogenase of *C. necator* is not directly involved in any energy conservation-linked processes but in transcriptional regulation. It consists of a heterodimeric core, which acts as H_2 sensor, and a histidine protein kinase, which transmits the signal via phosphorylation/dephosphorylation to a response regulator. Thereby, the expression of the membrane-bound hydrogenase and the soluble hydrogenase can be controlled.[56] Besides the characterization of the hydrogenases, the stoichiometry of its autotrophic growth is of major interest when using *C. necator* as a production strain. Yu and Lu investigated the stoichiometry of the different growth phases under autotrophic conditions with continuous gas supply. They found the early growth phase to be the most energy-efficient, resulting in energy costs of 263 kJ per mol CO_2 and the generation of 3 mol ATP per mol H_2 oxidation. However, they also showed that later growth phases were much less energy efficient.[57] In 2014, de Campos Rodrigues and Rosenbaum characterized different potential cathodic biocatalysts.

In their study, *C. necator* did not show any bioelectrochemical activity. Thus, the cathodic current did not change due to the organism. However, the growth of the organism itself was not investigated in the study. Possibly, the applied potential or the reactor setup did not allow for growth of *C. necator* in the system.[58] Three years later, Sydow et al. investigated an optimized media composition for oMES using *C. necator*. In their work, the authors developed a chloride-free medium with low buffer concentration. Using the optimized medium, no detectable hydrogen peroxide evolved, and a fast specific growth rate of 0.09 h^{-1} was achieved during oMES.[50] In the following years, different optimization strategies were pursued. In the study of Bause and colleagues, different cathodic currents ranging from 0 to $-2000\ \mu A\,cm^{-2}$ were tested, and a current density of $-500\ \mu A\,cm^{-2}$ was favorable for optimal cathodic biofilm growth (see Figure 1E). With lower current densities, hydrogen seemed to limit growth, whereas higher current densities led to a hydrogen saturation of the media and, thus, prevented biofilm formation.[38] Another approach to enhance the growth and productivity of *C. necator* is to modify the cathode for enhanced hydrogen production rates, surface area, and biocompatibility. To date, different cathode modifications have increased performances, such as using a cobalt-phosphorous alloy cathode, coating the cathode with nickel nanoparticles, or anchoring metal elements to porphyrinic triazine-based frameworks.[67–69]

Besides the optimization of the hydrogen evolution, a couple of studies focus on the formation of different products apart from biomass. Natively *C. necator* produces the hydrophobic storage polymer polyhydroxybutyrate (PHB), which can be used to produce bioplastics. The previously mentioned cathode optimization of Li and colleagues led to a PHB production of up to 9.94 $g\,m^{-2}\,h^{-1}$.[67] Recently, Langsdorf et al. demonstrated the cultivation of *C. necator* on cathodically produced hydrogen and flue gas from a coal-fired cogeneration plant as the sole carbon source. Over the total cultivation period of roughly 10 days a PHB yield of 333 $mg\,L^{-1}$ was reported.[62] Moreover, the authors estimated electricity costs of ca. 75 € per kg of PHB and claimed those costs as yet far too high for a possible commercialization. Besides PHB, biomass itself can also be considered a product. Chen and colleagues quantified the produced biomass of a genetically optimized *C. necator* strain, which expressed a superoxide dismutase for the decomposition of superoxide anion radical and produced $0.24 \pm 0.04\ gDW\,L^{-1}\,d^{-1}$ biomass.[70] Further, due to its easy genetic accessibility, research on non-native end products is increasing. As early as 2012, Li and colleagues introduced genes for isobutanol and 3-methyl-1-butanol production into *C. necator* and disrupted the PHB synthesis. Using formate as carbon source, a genetically engineered strain could produce 0.85 $g\,L^{-1}$ isobutanol and 0.57 $g\,L^{-1}$ 3-methyl-1-butanol within 140 h.[71] In the work of Torella and colleagues, the production of isopropanol was achieved via overexpressing a ketothiolase and an acetoacetyl-CoA transferase, and heterologously expressing an acetoacetate decarboxylase and an alcohol dehydrogenase from *Clostridium sp.* By doing so, a product yield of 0.22 $g\,L^{-1}$ in 120 h was achieved.[72] Another approach by Krieg et al., using *C. necator* as the production strain, heterologously expressed the mevalonate pathway from *Myxococcus xanthus*, leading to the production of up to $0.83\times10^{-4}\ g\,L^{-1}\,h^{-1}$ α-Humulene.[73] The production of lycopene using *C. necator* with a space-time yield of $0.17\times10^{-4}\ g\,L^{-1}\,h^{-1}$ was also demonstrated.[74] However, as seen from these examples, the space-time yields are meager, putting the application of *C. necator* in oMES for a significant production of chemicals out of reach.

5. *Kyrpidia spormannii*

Although a recently discovered organism, *Kyrpidia spormannii* EA-1 has already established a position of equal significance alongside *C. necator* as a crucial model organism for studying oMES. The strain was initially isolated in 2018 from a thermoacidophilic cathodic enrichment.[75] In contrast to *C. necator*, *K. spormannii* EA-1 currently lacks a developed genetic system, and its relatively recent discovery results in limited availability of bundled information in the scientific literature. Thus, this section aims to provide a comprehensive overview of the existing research on *K. spormannii* while addressing some potential research questions.

In 2020, Reiner et al. characterized an autotrophic, cathodic biofilm community enriched for 13 months from hydrothermal water and sediment samples collected at Sao Miguel Island, Portugal.[41] In a subsequent isolation campaign, several distinct species were isolated from this initial thermoacidophilic, cathodic enrichment (pH 3.5, 60 °C, low nutrient minimal medium, −350 mV vs. standard hydrogen electrode (SHE)). Among the isolated strains, a facultatively autotrophic, aerobic hydrogen-oxidizing bacterium (strain EA-1) was found to be a

novel species belonging to the genus *Kyrpidia*.[41,75] This so far rather uncharacterized genus within the family of the Alicyclobacillaceae was established in 2011 by Klenk et al. with the reclassification of *Bacillus tusciae* as *Kyrpidia tusciae* comb. nov.[76] Members of the genus *Kyrpidia* are described as 0.7–1.2 μm×4–8 μm endospore-forming, long straight rods, and Gram-stain-positive. They exhibit chemolithoautotrophic or chemoorganoheterotrophic growth between 42 °C and 67 °C, with an optimum at 55 °C. Anaerobic growth was not observed so far.[75] Amplicon- and metagenome-based microbial community analyses revealed the occurrence of organisms affiliated to the genus *Kyrpidia* in a variety of high-temperature natural environments, including geothermal soils,[77] volcanic crater lakes,[78] subsurface coal-fire ground layers, and gas vents.[79,80] Additionally, members of the genus *Kyrpidia* have been identified in high carbon-flux settings like biogas reactors operated at 54 °C,[81] thermophilic enrichments from compost,[82] sugarcane bagasse,[83] or at the anode of an acetate-fed thermophilic microbial fuel cell.[84] To date, only four different pure culture strains belonging to two distinct species within the genus *Kyrpidia* have been isolated: *Kyrpidia tusciae*,[85] *Kyrpidia spormannii* EA-1^T,[75] *Kyrpidia spormannii* FAVT5 and *Kyrpidia spormannii* COOX1.[77] Noteworthy, complete genome sequences are available for all strains.[76,77,86]

In the isolation study, the electrocatalytic activity of *K. spormannii* EA-1 was demonstrated for the first time, achieving cathodic current densities of up to $-50\ \mu A\,cm^{-2}$ over 9 days in a simple membrane-less, cylindrical BES (see Figure 3A). However, *K. tusciae*, subjected to the same conditions (60 °C, pH 3.5, 0.5 % O_2 in CO_2, −531 mV vs. SHE), did not exhibit a distinct current density profile compared to the abiotic control reactors maintaining an average current of $-15\ \mu A\,cm^{-2}$.[41] The electrochemical performance of *K. spormannii* strains FAVT5 and COOX1 in a BES remains unexplored. Comparative transcriptomic analyses of differently grown *K. spormannii* EA-1 cells revealed a distinct gene expression profile of cells harvested from a poised cathode (−531 mV vs. SHE, 5 % O_2 in CO_2) compared to lithoautotrophically (H_2/CO_2/O_2) and heterotrophically (50 mM pyruvate) grown cells. The transcriptomic data showed a fully expressed Calvin cycle during cathodic and lithoautothrophic growth under the control of the regulator CbbR. The operon includes the RuBisCo that was found to be one of the most expressed genes during cathodic growth. Moreover, this cluster contains genes encoding for a (presumably anabolic) Nuo-complex, that might be involved in an (indirect) NADPH generation via a proton motive force-driven reversed electron flow, oxidizing the menaquinone (MK-7) pool of *K. spormannii* EA-1. The transcriptomic data showed no evidence for a *c*-type cytochrome-based direct electron uptake replenishing the reduced menaquinone pool with electrons directly received from the cathode. Furthermore, no evidence could be provided for a flavoprotein-based extracellular electron transfer (EET), such as described for the Gram-stain-positive bacterium *Listeria monocytogenes*.[87] Therefore, a direct EET according to currently known mechanisms was excluded. Finally, since no complete synthetic pathways for the excretion of flavins or phenazines as endogenous electron shuttles were found in the genome, the possibility of indirect electron uptake via hydrogenases or formate dehydrogenases was investigated. While no change in the expression of an already weakly expressed formate dehydrogenase could be detected, both subunits of a group 2a [NiFe]-hydrogenase showed strong positive regulation under lithoautotrophic and electroautotrophic growth conditions (p-value < 0.01; $\log_2 FC > 3.5$).[41,88] Summarized, based on the transcriptomic data from Reiner et al.,[41] a hydrogenase-based EET cannot be excluded at this chosen process conditions for strain EA-1. Furthermore, both hydrogenase subunits could be assigned to the 40 most highly expressed genes under electroautotrophic conditions. In addition to being the sole hydrogenase identified within the EA-1 genome, an analysis of the respective protein sequence data further revealed that this very distinct group 2a [NiFe]-

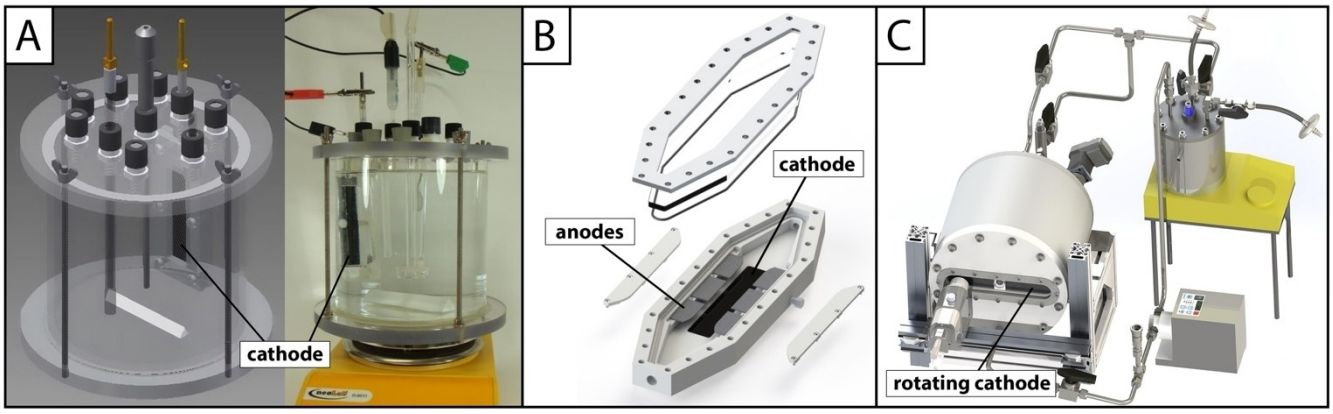

Figure 3. Selection of different reactor systems used for the cathodic cultivation of *Kyrpidia spormannii* EA-1. **A** – Cylindrical 2 L glass reactor as it was employed in the enrichment of the initial thermophilic, cathodic community from which EA-1 was isolated[41] with permission from the author. Moreover, the first pure culture experiments with EA-1 were conducted in the same reactor. A similar reactor type but loaded with multiple electrode setups was employed by Pillot and colleagues for their work on EA-1.[40] Reprinted from Golitsch[91] with permission from the author. **B** – Exploded-view drawing of a flow cell that allowed for the monitoring of the cathodic biofilm growth by means of optical coherence tomography.[39] Reprinted from Reiner[36] with permission from the author. **C** – 10 L rotating disc bioelectrochemical reactor (RDBER) that contains a set of rotating graphite discs as working electrodes. An optical window in the front allows biofilm quantification on the foremost cathode disc. Reprinted from Hackbarth et al.[31] with permission from Elsevier.

hydrogenase exhibits a notably low degree of sequence similarity when compared to other previously characterized group 2a [NiFe]-hydrogenases.[41,77,88] Islam et al. proposed – based on different whole-cell studies – that high-affinity lineages of group 2a [NiFe]-hydrogenases might play a role in atmospheric hydrogen oxidation to sub-atmospheric levels (< 530 ppb).[88] Furthermore, Grinter and colleagues recently conducted an enzyme assay, revealing that a purified group 2a [NiFe]-hydrogenase from *Mycobacterium smegmatis* appeared insensitive to inhibition by O_2 and exhibited the capacity to oxidize hydrogen at levels below atmospheric concentrations.[89] Interestingly, the scientific literature also designates representatives of the *Kyrpidia* genus as possible high-affinity trace gas oxidizers.[88,90]

In that context, the work of Hogendoorn and colleagues is particularly noteworthy.[77] Here, the kinetics of the hydrogen consumption by *Kyrpidia spormannii* strain FAVT5 were ascertained in whole-cell assays. Thereby, a high affinity for H_2 (K_s of 327 ± 24 nM) was determined for FAVT5 – the highest so far for any strain belonging to the phylum of Firmicutes. Maintaining a low H_2 partial pressure through microbial H_2 consumption thermodynamically promotes the HER at the cathode during (o)MES even at cathodic potentials far above the standard redox potential for H_2 evolution, a point Jo Philips discussed in detail recently.[52] Furthermore, using a high-affinity hydrogen oxidizer in an oMES process may enable an efficient operation at higher cathodic potentials, thereby reducing the production of harmful ROS at the cathode. However, it must be emphasized that no studies on the hydrogen affinities of *K. tusciae* and the electroactive isolate *K. spormannii* EA-1 are available yet. Accordingly, no conclusion can be drawn as to what extent the distinct *K. spormannii* EA-1 group 2a [NiFe]-hydrogenase is responsible for the electrocatalytic properties of EA-1. Especially because, under autotrophic conditions, FAVT5 expresses an additional group 2a [NiFe]-hydrogenase alongside the distinct *K. spormannii* hydrogenase. This additional hydrogenase is absent in the isolate EA-1, with a homolog of it being the sole hydrogenase in the genome of *K. tusciae*. It remains unclear which of these two hydrogenases is responsible for the high hydrogen affinity of FAV5, or whether there is an additive effect of both expressed hydrogenases. Additionally, there is no literature on whether the absence of the *K. spormannii*-specific hydrogenase genes in *K. tusciae* is responsible for its lack of electrocatalytic properties in a BES.[41,77] Regarding cathodic biofilm morphology of EA-1, scanning electron micrographs of cathodes from Reiner et al. exhibited a non-confluent single-layer of *K. spormannii* EA-1 cells on a poised cathode (−531 mV vs. SHE; see Figure 1H), whereas an OCP control cathode was only covered by individual scattered cells.[41] By cultivating *K. spormannii* EA-1 in similar cylindrical single-chamber glass reactors, Pillot et al. detected comparable cell numbers on solid graphite cathodes by means of fluorescence microscopy (see Figure 1G).[40,63] Likewise, the cathodic biofilm did not exceed beyond a single layer of cells. So far, it is unclear whether the cathodic cell numbers are an artifact caused by the phase transition during the fixation/staining of cells or whether the cathode coverage in the glass reactors is as meager as estimated from the different micrographs. In one of their studies, Pillot and colleagues optimized process parameters (cathodic potential, pH, and oxygen concentration) with regard to cell numbers obtained by qPCR and fluorescence microscopy after 3 days of cultivation in a BES.[40] A cathodic potential of −625 mV vs. SHE resulted in the highest cell numbers of around 10.5 $\log_{10}$cells cm^{-2} among the investigated potential window (ranging from −325 to −1425 mV vs. SHE). Cell counts decreased toward more negative cathodic potentials by up to three orders of magnitude from the maximum cell count. At the same time, the measured current densities increased steadily with lower potentials, which led to a significant deterioration of the *CE* (with regard to PHB and not biomass as a final product) in the lower potential range. The increase of current densities was discussed to be most likely caused by abiotic processes, like HER and oxygen reduction reactions. The formation of hydrogen bubbles leading to a biofilm disruption at low potentials could also explain the decrease of the measured cell numbers in the low potential experiments. Furthermore, they found that an oxygen concentration of 2.5 % in the gas feed resulted in the highest cathodic cell numbers after 3 days among the O_2 concentrations tested (0.5–20 vol%). Although the medium's pH between 3.5 and 7.5 had no significant effect on the cathodic cell numbers, Pillot and colleagues found that the *CE* (with regard to PHB) was highest at pH 6.5, with values around 3 %.[40] Those low *CE* could be explained on the one hand by the fact that most of the electrons consumed might have been spent on abiotic oxygen reduction since the cathode was not fully covered with a confluent biofilm. On the other hand, the external stimuli triggering PHB production in EA-1 are not fully understood yet, and it is not clear to what extent the fixed carbon is stored in PHB during cathodic growth under the chosen process conditions. For this reason, future calculations of *CE* should also include biomass quantification until the exact mechanisms of the PHB production machinery of EA-1 are thoroughly understood, leading to a reproducible fraction of PHB in the biomass.

A decisive turning point in the cultivation of *K. spormannii* EA-1 was the development of pressurizable, bioelectrochemical flow cells by Hackbarth et al. (see Figure 1I, 3B).[39] These custom-built flow cells allow for non-invasive quantification of cathodic EA-1 biofilms using optical coherence tomography (OCT) while increasing the bioavailability of the substrate gases through elevated pressure (1.5 bar). A high volumetric flow rate was applied during cultivation to avoid sedimentation and agglomeration of cells on the electrodes. The OCT data presented by Hackbarth et al. revealed cathodic biofilms with thicknesses of up to 400 μm at an average biovolume of 150 $\mu m^3 \mu m^{-2}$ on a plane graphite cathode (−500 mV vs. SHE) after the experimental course of 20 days (see Figure 1F). Yet, after seven days, a complete surface coverage of the cathode was achieved, leading presumably to a decrease of abiotic redox processes (e.g. oxygen reduction) at the electrode surface and thereby to a decreased current density of approx. 13 $\mu A\,cm^{-2}$. The obtained biofilms are among the thickest cathodic biofilms described in the literature (see Figure 1I).[92] However, it is entirely unclear why the EA-1 biofilms cultivated in the glass reactors are so different from those in the flow cells

and whether this is an effect of flow velocity, better substrate availability due to the pressurized system, or simply a consequence of the non-invasive biofilm quantification. Autotrophic cathodic growth of *K. spormannii* in the flow cells was not only quantified via OCT, revealing mean biofilm accumulation rates of 11 $\mu m^3 \mu m^{-2} d^{-1}$, but also validated by Reiner and colleagues using a ^{13}C labeling approach.[41] In both cases, *K. spormannii* was cultivated in a minimal medium, omitting organic carbon sources and supplements like yeast extract or vitamins. The above carbon isotope data was also used to calculate an average *CE* of 7% over the whole cultivation period of 10 days, considering biomass as product (SI 1). Since the current densities of the first days (initial biofilm growth phase until the complete coverage of the cathode) were also included in the calculation, this comparatively low *CE* can be explained by efficiency losses due to abiotic oxygen reduction at the uncovered cathode in the early cultivation stage.[36] In another study, Jung et al. used a similar flow cell setup for an adaptive laboratory evolution experiment to enhance the cathodic biofilm growth capabilities of EA-1.[93] The adapted population showed not only a more uniform biofilm morphology compared to the progenitor strain but also an up to 4-fold higher biofilm accumulation rate (60 $\mu m^3 \mu m^{-2} d^{-1}$) and a faster substratum coverage (full substratum coverage was accomplished already after 3 days). A subsequent genomic variant analysis revealed a genomically heterogeneous population. However, Jung et al. reported that two loud single nucleotide variations occurred each in over 99% of the population: The first mutation affects the DNA-binding H_2O_2-sensor protein PerR and is discussed to lead to a higher expression of genes that cope with oxidative stress. Secondly, over 99% of the population possessed a mutation affecting a subunit in a gene cluster that might be involved in the respiratory oxidation of reduced sulfur compounds. Moreover, Jung and colleagues conducted autotrophic, aerobic growth experiments using elemental sulfur (S^0) as electron and energy source, showing for the first time the capability of EA-1 to use elemental sulfur as a respiratory electron donor. Noteworthy, the adapted population seemingly oxidized S^0 on average faster compared to the wild type, although the results were not statistically significant. Finally, the manuscript hypothesized that reduced sulfur species could be used as additional electron shuttles between the cathode and the organisms.[93] Yet, the molecular principles behind this postulated additional electron uptake mechanism remain obscure and require a profound understanding of the sulfur oxidation mechanisms in EA-1.

Recently, Rominger et al. published a manuscript on the efficiency of oMES, quantifying the biomass production of *K. spormannii* in the above-mentioned flow cells at different cathodic potentials.[30] In this study, the authors calculated time-resolved *CE* for the conversion of CO_2 into biomass of up to 51% at a cathodic potential of −500 mV vs. SHE. Furthermore, the study demonstrates that cultivation at a cathodic potential of −375 mV vs. SHE (compared to −500 mV vs. SHE) caused differences in the structure of the biofilm but had almost no impact on the biofilm growth behavior. On the other hand, at a cathodic potential of 0 mV vs. SHE, *Kyrpidia* exhibited a comparatively weak and linear growth behavior. This is particularly noteworthy because, at this potential, cathodic hydrogen production can be essentially ruled out for thermodynamic reasons (The hydrogen evolution potential calculated with the Nernst equation at a pH of 3.5 and an assumed hydrogen partial pressure of 10^{-4} atm amounts to −99.15 mV). It remains unclear to what extent a possible hydrogen-independent EET is responsible for the observed biofilm increase. Whether this observation has to be considered as substantial autotrophic growth must be clarified in future ^{13}C experiments. In the same study, Rominger and colleagues additionally investigated the application of a highly negative potential for the generation of hydrogen bubbles as a method for controlled biofilm detachment. After a controlled biofilm detachment and harvest, 30% of the cathode surface were not covered by biofilm anymore. However, the biofilm regenerated back to complete cathode coverage and more than 70% of its initial volume within 10 days.[30] Another approach that could allow for controlled harvesting of cathodic biomass – but by increasing shear forces – was recently pursued by Hackbarth et al.[31] This study demonstrated the construction and operation of a 10 L membrane-less BES that employs rotating graphite discs as working electrodes (see Figure 3C). Here, a temporal increase in the rotational speed of the stacked electrode discs might lead to a detachment of the biofilm. Hackbarth and colleagues developed this so-called RDBER (*rotating disc bioelectrochemical reactor*) as an upscaling strategy for their small-scale, custom-made flow cells (see Figure 3B). Since the energy demands for pumping required for successful cultivation of *K. spormannii* in an upscaled flow cell process would presumably render the overall process quickly uneconomical, such an RDBER might be an interesting option to generate high flow velocities over the working electrode independent from hydraulic retention time. However, EA-1 could not be successfully cultivated in the RDBER with the full electrode configuration comprising 13 graphite discs with a cumulative working electrode area of 1 m^2, presumably due to ROS production. Finally, an adjustment of the initial planktonic cell-to-cathode surface ratio (by reducing the amount of mounted graphite discs), together with a chronopotentiometric control of the cathodic current density, led to a successful cultivation of *K. spormannii* on 700 cm^{-2} cathode surface. Conclusively, the authors stated that the impact of ROS on biofilm growth should not be underestimated in the development and optimization of oMES processes – especially during the startup phase when the cathode is not fully covered with a biofilm.

6. How Does oMES Compare to Other Technologies for Biomass Production?

Knallgas bacteria gained particular attraction by industrial biotechnology for producing single-cell protein or biomass competing with heterotrophic, phototrophic, and methanotrophic approaches, which are in different development stages ranging from lab-scale to established industrial application

(Table 2).[3] A comprehensive discussion on the general benefits and advantages of these technologies is not in the scope of this article but can be found elsewhere.[94,95] Instead, this review provides a selective overview to compare the biomass productivity and the energy efficiency of biomass production, considering only the consumption of carbon and electron sources but neglecting the energy consumption for, e.g. cultivation temperature, pumping, and purification. This assessment may not fully reflect reality as all parameters required for calculations and data normalization were not always provided (see S2 for calculations), and simplifications were made (e.g. ideal gas and constant conversion factor for wet and dry biomass). Still, it allows a comparison of fundamentally different processes (e.g. gas fermentation, water electrolysis, heterotrophy) providing reasonable indications.

Gas fermentation with *C. necator* is a promising technology as it exhibits a superior biomass productivity of up to 54.7 $gDW\,L^{-1}\,d^{-1}$ while its energy demand is low (1.0 $kWh\,kgDW^{-1}$). The calculation of the latter is simply based on the standard free enthalpy of reaction of hydrogen oxidation thereby not considering, e.g. energy efficiencies of hydrogen production.[96] In contrast, supplying H_2 and O_2 via water electrolysis leads to significantly lower biomass productivity and higher energy demand for the cultivation of *C. necator* (Table 2). Cultivating *K. spormannii* in a BES, leads to a moderate median energy demand of 36.8 $kWh\,kgDW^{-1}$, however, the energy demand during periods of maximum *CE* is remarkably low (11.9 $kWh\,kgDW^{-1}$), according to Rominger and colleagues.[30] Conceivably, superior hydrogen uptake capabilities (low affinity and high uptake rate) of *K. spormannii* lead to thermodynamically favorable conditions for hydrogen evolution, resulting in comparable low energy demands for biomass production during optimal process operation. However, further research is required to elucidate the reasons for this low energy demand. Moreover, the low biomass productivity of 0.04 $gDW\,L^{-1}\,d^{-1}$ emphasizes the low stage of development. Interestingly, the highest biomass production rates in oMES were not achieved with pure cultures, but by cultivating a mixed species community dominated by *Xanthobacter* and *Thiobacillus* species.[66] Here, the manual control of the dissolved oxygen concentration, in order to minimize cathodic oxygen reduction (and thereby limiting ROS evolution), is likely one of the main reasons for the increased biomass productivity. Another approach using electrochemistry for biomass production is a two-stage process consisting of the electrochemical reduction of CO_2 to formate, which is subsequently fed to microorganisms. By doing so, a biomass production rate of 0.16 $gDW\,L^{-1}\,d^{-1}$ and an energy demand of 675 $kWh\,kgDW^{-1}$ were achieved when *C. necator* was cultivated for producing PHB. A better performance was obtained by Molitor et al. conducting a two-stage process anaerobically cultivating *Clostridium ljungdahlii* with a H_2/CO_2 mixture to produce acetate.[19] Subsequently, *Saccharomyces cerevisiae* was fed with the produced acetate, reaching a biomass production rate of 2.7 ± 1.2 $gDW\,L^{-1}\,d^{-1}$ and an energy demand of 51 $kWh\,kgDW^{-1}$.

Heterotrophic and methanotrophic processes achieve high biomass productivities of 28 $gDW\,L^{-1}\,d^{-1}$ and 48 $gDW\,L^{-1}\,d^{-1}$, respectively, with low to moderate energy demands. Therefore, these processes outcompete other approaches and are well-established in the market (Qourn™) or recently revived (Uniprotein®).

Table 2. Selective comparison of heterotrophic, phototrophic, methanotrophic, and autotrophic approaches for biomass production regarding biomass production rate ($r_{Biomass}$) and energy demand for biomass production ($P_{Biomass}$).

Microorganism	Process type	$r_{Biomass}$ [$gDW\,L^{-1}\,d^{-1}$]	$P_{Biomass}$ [$kWh\,kgDW^{-1}$]	Reference
Kyrpidia spormannii	Electrochemical cultivation	0.04[a]	11.9[b,c]	[30]
Cupriavidus necator (genetically modified)	Water electrolysis	0.24 ± 0.04[c]	53.8[a]	[70]
Mixed culture	Water electrolysis	0.47 ± 0.16[c]	37–45[c]	[66]
Nocardioides nitrophenolicus KGS-27	Water electrolysis	0.24 ± 0.05[c]	64.3[a]	[104]
	Gas fermentation	0.19 ± 0.03[c]	1053[a]	
Cupriavidus necator	Gas fermentation	54.7[c]	1.0[a]	[96]
Cupriavidus necator	Two-stage process (CO_2 to formate first)	0.16[c]	675[a]	[105]
Clostridium ljungdahli and *Saccharomyces cerevisiae*	Two-stage process (CO_2 to acetate first)	2.7 ± 1.2[a]	51[a]	[19]
Mixed culture	Cultivation of purple phototrophic bacteria	0.21[a]	24[a]	[106]
Scenedesmus obliquus	Cultivation of microalgae	0.7 ± 0.1[c]	1140[a]	[107]
Fusarium venenatum	Qourn™, heterotrophic fermentation	28[a]	8.7[a]	[108]
Methylococcus capsulatus	UniProtein®, methanotrophic fermentation	48[a]	31.0–42.5[c]	[94,109]

[a] See S1 for calculation. [b] Assuming the highest coulombic efficiency observed in the experiments. [c] See reference.

7. Outlook: Research Gaps and Engineering Potential of Oxic Microbial Electrosynthesis using Knallgas Bacteria

As indicated by several industrial projects, employing Knallgas bacteria for biomass production in a gas fermentation process is an economically promising concept. Electrochemical cultivation of Knallgas bacteria is regarded as an alternative to gas fermentation as it mitigates associated challenges, like the low solubility of H_2 and the danger of an explosive gas mixture in case of, e.g. unforeseen cultivation events. Nevertheless, the biomass production rates of electrochemical cultivations of Knallgas bacteria are still significantly lower than (1) during gas fermentation employing these organisms (Table 2)[96,97] and (2) compared to anoxic microbial electrosynthesis now achieving titers and rates that are well in the range of conventional gas fermentations.[10,98] The exact reasons for the low rates of oMES are to be revealed in future studies. At this point, the authors can only speculate: In addition to growth inhibition due to radical formation at both electrodes, researchers might have legitimate concerns while experimenting with explosive gas mixtures in BES. Hence, the exploration of the limits of oMES might proceed slowly and cautiously.[9,96]

Likewise, the electrochemical cultivation of the recently discovered *K. spormannii* EA-1 is still in its infancy and exhibits significant knowledge gaps. A detailed understanding of its physiology, including energy metabolism (e.g. energy dissipation during growth and maintenance requirements), comprehensive mass and energy balances, and electrochemical and microbial kinetics, is indispensable for tailored process enhancements, leading to optimized cultivation conditions, customized feeding regimes, and flexible process operation without significant efficiency losses. In addition, reactor design and electrode configuration require improvements to enhance biomass productivity while keeping a low energy demand for biomass production (Figure 4).

7.1. Deciphering Electron Transfer Mechanisms and Electrochemical Kinetics

One of the essential aspects for investigating and improving the biomass production of Knallgas bacteria is elucidating remaining uncertainties regarding electron transfer mechanisms (Figure 4). Whereas *C. necator* likely takes up cathodically produced hydrogen, the mechanism for *K. spormannii* EA-1 is not fully clarified yet.[38] Although most experimental observations suggest a hydrogen-mediated electron transfer, a direct electron uptake mechanism cannot be ruled out as an increasing biovolume is observed at potentials as low as 0 V (vs. SHE).[30] At this cathode potential, the local partial pressure of H_2 needs to be lower than 10 Pa to thermodynamically allow the HER (SI 3), which is in the range of the lowest H_2 thresholds experimentally determined for acetogens.[52] Moreover, this estimation does not consider the HER overpotential at the used graphite electrodes. The observed capability of EA-1 to use insoluble elemental sulfur also indicates the presence of direct electron uptake mechanisms.[93] If this observation would be confirmed, many follow-up questions and corresponding research arise: Which redox centers are responsible for the direct electron uptake from cathodes? Is the cathodic electron uptake the limiting step for the growth of *K. spormannii* EA-1? How are electrons conducted within the biofilm? Are the electron fluxes during direct electron uptake comparable to hydrogen evolution? Consequently, fundamental electrochemical methods (e.g. cyclic voltammetry, differential pulse voltammetry, double potential step chronoamperometry) ought to be applied for determining electron transfer constants (via the Laviron equation) and charge transport parameters (via the Cottrel equation) like it was conducted for anodic biofilms.[99,100]

Different research questions would arise in case of a fully confirmed hydrogen-mediated electron transfer, which mainly revolve around electrode materials and electrode/reactor geometry to mitigate, e.g. hydrogen losses and efficiency losses due to pH gradients (Section 7.3). As it was done for the

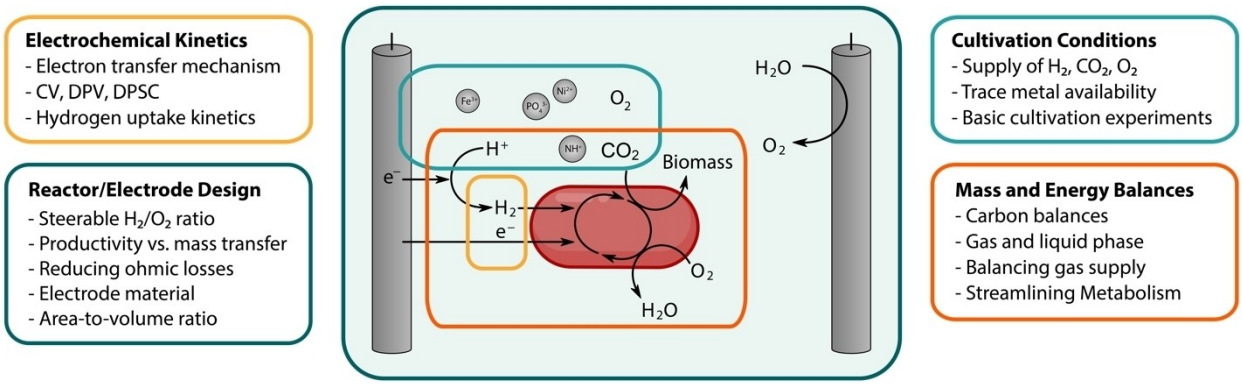

Figure 4. The research on the electrochemical cultivation of Knallgas bacteria, especially of *Kyrpidia spormannii* EA-1, for biomass production is in the beginning, and several knowledge gaps must be resolved to develop a viable process. These include improving cultivation conditions, unraveling electrochemical kinetics, establishing comprehensive mass and energy balances, and optimizing reactor and electrode design. CV: Cyclic voltammetry, DPV: Differential pulse voltammetry, DPSC: Double potential step chronoamperometry.

K. spormannii strain FAVT5, hydrogen uptake kinetics (i.e. affinity constant, maximum rate) need to be revealed as it represents a measure to minimize H_2 losses and to optimize O_2/CO_2 supply for balancing electrochemical and microbial kinetics.[77] A different scenario that cannot be ruled out yet is the secretion of surface-associated redox enzymes, which would require the application of enzyme electrochemical methods for determining kinetics.

7.2. Optimization of Cultivation Conditions

Considering that *K. spormannii* was only recently discovered, it is evident that its cultivation likely requires modification opportunities to increase its growth rate (Figure 3). Undoubtedly, there is a requirement for a general medium optimization based on fundamental microbial growth experiments for optimizing the availability of nutrients like nitrogen, sulfur, phosphorus, and trace metals like nickel and iron.[40,101] For instance, as [NiFe]-hydrogenases are essential for the activity of EA-1, an optimal supply of nickel and iron could foster the intracellular synthesis of these redox enzymes, resulting in higher growth rates. Interestingly, EA-1 exhibits notable growth with a minimal medium containing only a few nutrients (e.g. ammonium as nitrogen source) and trace metals but no vitamins, significantly simplifying its cultivation but also providing opportunities to accelerate growth.[30] Cultivation of EA-1 without an additional nitrogen source may also be an option, as *K. spormannii* FAVT5 was described to perform nitrogen fixation.[77] Although this would further decrease the environmental impact of the cultivation process, it likely leads to a slowdown of biomass growth.

In general, the optimal cathodic cultivation of aerobic autotrophic microorganisms requires a finely tuned and steerable dosage of of the electron donor (see Section 7.1), the electron acceptor O_2, and the carbon source CO_2. The supply of oxygen is critical not only for the growth itself but also for the overall energy efficiency. In the case of surplus oxygen, oxygen penetrates the biofilm and gets reduced at the cathode, resulting in a decreased *CE*. In addition, ROS can form, impeding biofilm growth.

On the other hand, if the dissolved oxygen concentration falls below a certain threshold, anoxic niches can form locally in the biofilm or in the bulk phase of a BES, which can lead to a hydrogenotrophic reduction of anaerobic electron acceptors.[102] These side reactions, which can either be unwanted electron sinks or result in the production of small, reduced intermediates that can subsequently act as electron donors for other non-hydrogenotrophic autotrophs, occur mainly when mixed species are employed for oMES or when working under non-sterile conditions like for example during the cultivation of aerobic autotrophs for ammonium recovery from wastewater.[97,103]

However, this interspecies network of possible physiological interactions may not be discussed in detail in this manuscript as it mainly focuses on oMES using pure cultures and as such reactions can be avoided by the axenic cultivation of a suitable biocatalyst lacking these metabolic traits in a medium designed without the addition of unnecessary electron acceptors.

Noteworthy, a gas mixture of 2:1 H_2:O_2 as generated by water electrolysis does not represent ideal growth conditions. While this stoichiometric ratio might be ideal for the catabolic water formation (i.e., Knallgas reaction), reduction equivalents required for CO_2 reduction (or anabolism in general) are not taken into account here. Therefore, the ideal H_2:O_2 ratio is shifted towards hydrogen.[57] If the different solubilities of the two gases and the resulting significantly lower bioavailability of hydrogen are also considered, the optimal theoretical ratio is even more shifted towards hydrogen. Moreover, elevated levels of dissolved oxygen have been shown to impede the activity of *C. necator* hydrogenases. Consequently, it is advised to maintain dissolved oxygen levels below 4 %.[29,110]

Although few experimental efforts were conducted on the optimal oxygen concentration for the cathodic cultivation of *K. spormannii*, the literature is inconclusive. Whereas Pillot et al. obtained the highest growth rate for 2.5 % O_2 in the gas phase (corresponding to 1.59 mg L^{-1} dissolved oxygen assuming ideal gases, SI 4), Rominger et al. identified 3 mg L^{-1} dissolved oxygen as the best condition in their model-assisted study.[30,40] Microsensor measurements of oxygen and hydrogen gradients in the biofilm could underpin mathematical models such as that of Rominger et al.[30] and help to determine the optimum substrate concentrations.[111] In addition, knowledge transfer into the electromicrobiology community of the exact mechanisms of ROS formation in a BES (precisely which ROS compounds are formed at which concentrations) and their effects on microbial growth is needed. For example, considerable knowledge can be obtained from the wastewater treatment community as electrogenerated H_2O_2 is commonly used in so-called advanced oxidative processes for the remediation of wastewaters. Moreover, as hydrogen peroxide is one of the 100 most used chemicals globally, intensive research has been carried out on the electrogeneration of hydrogen peroxide on carbon electrodes via a 2-electron oxygen reduction reaction (ORR).[112] Since this ORR is a complex reaction forming different ROS as intermediates, a detailed description thereof is beyond the scope of this manuscript. Interested readers are referred to more in-depth literature, which diligent scientists have already compiled.[112–116] However, much of this work on H_2O_2-electrogeneration has not been carried out under typical cultivation conditions (e.g. in microbial growth media). Furthermore, there is no clarity if the ORR at the cathode is the only source of ROS in oxic microbial electrosynthesis and what role anodically produced radicals play in one-chamber BES.[117]

7.3. Design of Reactor and Electrode Configuration

One of the major unresolved challenges in scaling up and designing appropriate (even lab-scale) BES for oMES is the discrepancy between the 2:1 H_2/O_2 ratio engendered by electrochemical water splitting and the optimal H_2/O_2 ratio required for cultivating Knallgas bacteria (see Section 7.2). Although the oxygen tolerance and ROS detoxification capa-

bilities of Knallgas bacteria can potentially be enhanced through adaptive laboratory evolution or targeted genetic engineering, the fundamental stoichiometric requirements of biomass production from H_2, O_2, and CO_2 in these bacteria are not easily altered by simple genetic modifications or laboratory adaptations. Therefore, the primary challenge is engineering a reactor that allows the control of the H_2/O_2 ratio independent of the electrochemical reactions without significantly complicating the simplicity of single-chamber BES. For instance, an additional feed of pure hydrogen, for example via an additional electrolyzer, to adjust the H_2/O_2 ratio may offer a solution. Furthermore, it is crucial to consider whether the cultivation process is biofilm-based or planktonic. In biofilm-based processes, the problem might be mitigated by strategically positioning the electrodes or employing a directional flow of the bulk phase. Conversely, in planktonic cultivation within a stirred tank reactor type BES, uniform mixing prevents the formation of local niches with varying oxygen concentrations. Finally, while a two-chamber BES using a cation ion exchange membrane would allow higher flexibility in process control, one of the core advantages of oMES, its simple reactor design, would be compromised.

The electrode area is another decisive parameter for high biomass yields because EA-1 forms biofilms at the cathode. 3D electrode geometries like felts, foams, reticulated vitreous carbon, or brushes are favored as they offer high electrode area-to-reactor volume ratios (Figure 4). However, this increase in electrode area can be accompanied by ohmic losses and mass transfer limitations affecting biomass productivity and, in turn, the overall energy efficiency of biomass formation.[118] For anodic processes, it was shown that pore sizes of around 1 mm minimize clogging and internal acidification, leading to a better performance of the microorganisms.[119,120] For CO_2-based MES, macropore sizes of 0.6 mm were demonstrated to be optimal for acetate production using a mixed culture.[121] For hydrogen-mediated electron transfer, increasing the cathodic surface is recommended to obtain lower current densities, which mitigates mass transfer limitations, bubble formation, and local pH gradients.[122] Another opportunity to cope with the limited mass transfer of gases is using gas diffusion electrodes, which have already been applied to cathodic biofilms.[123]

On the system level, process development should aim at minimal-gap configurations for decreasing the absolute electrolyte resistance between anode and cathode. However, using 3D electrodes is always accompanied by ohmic losses due to the varying distance between cathode segments and anode. Depending on the used material and its conductivity, this also leads to cathode potential heterogeneities and, thus, varying currents over the cathode area. Spatial proximity of both electrodes could lead to increased availability of anodically produced oxygen at the cathode, especially during early-growth phases with uncomplete biofilm coverage and, thus, an increased formation of ROS. On the other side, anodically produced oxygen can also serve as electron acceptor for Knallgas bacteria, decreasing the required supply of external oxygen and increasing the overall energy efficiency. Consequently, the reactor design must permit permanent biofilm growth monitoring in the best case to allow a tailored supply of oxygen and current. Several reactor types were already developed and applied for biofilm cultivation, partially even on an industrial scale, including fixed and fluidized bed reactors, rotating drum reactors, membrane reactors, and hollow fiber membrane reactors (the authors refer to[47,124] for overviews on biofilm reactors). However, the fact that the substratum (i.e., cathode) is actively polarized certainly shapes reactor design, which must balance electrode surface-to-reactor volume, mass transfer, and energy efficiency. For instance, rotating disc reactors offer high surface-to-volume ratios and aerosol-based reactors have an improved mass transfer while minimizing the demand for resources (e.g., water).[125,126] Certainly, parallelizing smaller reactors (numbering-up) is an alternative strategy to a pure scale-up approach for keeping ohmic losses passable.[127,128]

Besides the reactor geometry, the anode and cathode materials are further parameters for improving energy efficiency. Materials that exhibit high biocompatibility, avoid ROS formation, and have a high catalytic activity for HER or direct electron uptake are required for the cathode. A high catalytic activity for the oxygen evolution reaction is the most crucial property of the anode material. Secondly, when the anode compartment is not separated from the cathode compartment by a membrane, care must be taken to avoid the anodic oxidation of chloride ions into toxic chlorine compounds. Although an efficient anode reaction is indispensable for improving the global energy efficiency of biomass production using Knallgas bacteria, this is not in the focus of this manuscript, as it was extensively discussed elsewhere.[129–131] A few electrode modifications for improved electrochemical cultivation of Knallgas bacteria have already been tested. For instance, Liu et al. used a cobalt-phosphate catalyst at the anode for oxygen evolution and a cobalt-phosphorous alloy cathode for hydrogen production at the cathode. The cathode modification resulted in less ROS evolution, higher biocompatibility, and storage of more than 50% of the input energy into biomass.[67] Nickel nanoparticles are another cathode coating to improve biocompatibility and reduce ROS formation. In the work of Li and colleagues, these nanoparticles are embedded into carbon nanotubes to prevent leaching of Ni^{2+}, leading to excellent biocompatibility and stability.[68] Another biocompatible cathode material that showed excellent performance in oMES with *C. necator* was a porphyrinic triazine-based framework that anchored metal elements such as iron, cobalt, nickel, and copper, resulting in almost no ROS formation and superior growth of *C. necator*.[69] Besides improving electrode material for more efficient electrode reactions, surface modifications could also be used to optimize biofilm adhesion decreasing the start-up phase.[132]

7.4. Establishing Complete Mass and Energy Balances

Establishing complete mass and energy balances are opportunities to understand the mechanisms behind the electrochemical cultivation of Knallgas bacteria and potentially advance it to

higher economic feasibility by optimizing resource utilization (Figure 4). Quantifying the consumption of the substrate gases (H_2, O_2 and CO_2) might be challenging due to the occurrence of a liquid and a gas phase, but it is a prerequisite for complete mass balances.[133] Based on the established mass balances, the supply of O_2, CO_2, and H_2 (and or electrons) can be adjusted to minimize gas losses and ROS formation and to avoid short circuits between O_2 and the cathode, which is detrimental to *CE*. Analyzing the composition and the degree of reduction of the formed biomass by elemental analysis could be beneficial for providing valuable insights into microbial metabolism and for optimization of the cultivation (Section 7.2).[134] Metabolic side or storage products must also be identified and quantified to close the balances. So far, polyhydroxyalkanoates (PHA) are the only known storage compound produced by EA-1.[40] As already conducted for other Knallgas bacteria, PHA production genes can be knocked out to increase the protein fraction if the induced metabolic burden does not outbalance the increase in biomass formation.[135] Another opportunity to optimize cultivation and increase the energy fluxes towards biomass formation is modifying the CO_2 fixation pathway. EA-1 uses the Calvin cycle, which is an energetically inefficient pathway compared to other autotrophic metabolisms and could be replaced with, for instance, the reductive glycine pathway, which is proposed to increase the energetic efficiency of electromicrobial biomass production and was already inserted in *C. necator*.[136–138]

Certainly, due to the low *technology readiness level* of oMES – a technical and economic roadmap guiding towards future research and application of this technology, as provided by Jourdin and colleagues in a techno-economic assessment of anoxic microbial electrosynthesis, is lacking.[139] However, such an assessment requires almost complete energy and mass balances, which are currently hardly available in the literature on oMES. Therefore, future studies must provide conclusive data on mass/energy inputs and efficiencies to guide future research and to determine if – as well as to which extent – oMES can establish itself in the emerging niche of biotechnological CO_2 sequestration technologies. Reference should be made to Pous et al., which carefully discussed considerations for the energy bill of oMES processes.[66]

8. Summary

This literature review on the cathodic cultivation of aerobic, autotrophic microorganisms clearly reveals that oxic microbial electrosynthesis (oMES), in particular for biomass production based on Knallgas bacteria, is still in its infancy. Only a handful of aerobic, autotrophic microbial pure culture strains have been cultured in bioelectrochemical systems (BES), with most studies not even targeting CO_2 conversion. Among the studied biocatalysts for oMES, *Cupriavidus necator* and *Kyrpidia spormannii* are the most prominent, with *K. spormannii* demonstrating remarkable biofilm formation on cathodes of customized flow cells. However, whether this trait is inherent to *K. spormannii* or a result of the flow cell design requires further investigation and can only be determined if other Knallgas bacteria, such as *C. necator* or other *Kyrpidia* strains are cultivated in the same type of flow cells. A selective comparison of the energy demand for biomass production using EA-1 in the flow cells mentioned above with other approaches for biomass production, indicates the ability of oMES to rival leading contenders in biomass production. However, biomass production rates are still far from being economically viable, and several challenges remain to be addressed before oMES could be implemented. These challenges include understanding and optimizing the microbial catalysts, improving electrode materials and reactor designs to enhance electron transfer rates and growth, and addressing economic factors. Additionally, the scalability of oMES processes from laboratory experiments to industrial applications needs further exploration. Scaling up oMES while maintaining efficiency and cost-effectiveness requires innovative engineering solutions and integration with existing industrial processes. Overall, while oMES shows promise for sustainable biomass production using renewable electricity, further research, development, and collaboration between scientists, engineers, and industry stakeholders are needed to overcome existing challenges and realize its full potential.

Supporting Information Summary

The authors have cited additional references within the Supporting Information.[140–142]

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Conflict of Interests

The authors declare no conflict of interest.

Data Availability Statement

Data sharing is not applicable to this article as no new data were created or analyzed in this study.

Keywords: Biomass · oMES · *Kyrpidia* · Extracellular electron transfer · Hydrogen · Hydrogen-oxidizing bacteria

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