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Research review paper

Anodic electro-fermentation: Empowering anaerobic production processes via anodic respiration

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ABSTRACT

In nature as well as in industrial microbiology, all microorganisms need to achieve redox balance. Their redox state and energy conservation highly depend on the availability of a terminal electron acceptor, for example oxygen in aerobic production processes. Under anaerobic conditions in the absence of an electron acceptor, redox balance is achieved via the production of reduced carbon-compounds (fermentation). An alternative strategy to artificially stabilize microbial redox and energy state is the use of anodic electro-fermentation (AEF). This emerging biotechnology empowers respiration under anaerobic conditions using the anode of a bioelectrochemical system as an undepletable terminal electron acceptor. Electrochemical control of redox metabolism and energy conservation via AEF can steer the carbon metabolism towards a product of interest and avoid the need for continuous and cost-inefficient supply of oxygen as well as the production of mixed reduced by-products, as is the case in aerobic production and fermentation processes, respectively. The great challenge for AEF is to establish efficient extracellular electron transfer (EET) from the microbe to the anode and link it to central carbon metabolism to enhance the synthesis of a target product. This article reviews the advantages and challenges of AEF, EET mechanisms, microbial energy gain, and discusses the rational choice of substrate-product couple as well as the choice of microbial catalyst. Besides, it discusses the potential of the industrial model-organism *Bacillus subtilis* as a promising candidate for AEF, which has not been yet considered for such an application.

This prospective review contributes to a better understanding of how industrial microbiology can benefit from AEF and analyses key-factors required to successfully implement AEF processes. Overall, this work aims to advance the young research field especially by critically revisiting the fundamental aspects of AEF.

1. Introduction: Aerobic vs. anaerobic production processes

Biotechnology plays a key role in building a circular-based economy towards a safe, healthy and sustainable future. Especially, industrial microbiology paves the way for an ecofriendly and fossil fuel-independent production of chemicals, materials and energy-carriers. This technology features microorganisms as biocatalytical cell factories using biomass as a renewable feedstock for the synthesis of a broad spectrum of products in chemical-, health-, food- and feed-industries, among others (Lokko et al., 2018). Industrial microbiology is already well rooted in our economy. However, continuing research and development is crucial to increase the efficiency of this technology in order to promote competitiveness with fossil fuel-dependent manufacturing processes and to eventually supersede them. Also, extending the product spectrum of industrial biotechnology will support its deeper integration into our economy (Clomburg et al., 2017; Lee et al., 2019).

The success of a microbiological production process hinges on three factors: productivity (rate of production), titer (obtained product concentration) and yield (gained product per consumed substrate; Averesch

Abbreviations: AEF, anodic electro-fermentation; ATP, adenosine triphosphate; BES, bioelectrochemical system; CEF, cathodic electro-fermentation; EET, extracellular electron transfer; GRAS, generally recognized as safe; MET, microbial electrochemical technology; MES, microbial electrosynthesis; MFC, microbial fuel cell; NAD(P), nicotinamide adenine dinucleotide (phosphate); PPQ, pyrroloquinoline quinone; RuMP, ribulose monophosphate; SHE, standard hydrogen electrode..

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and Kromer, 2018). Anaerobic production processes are often preferable over aerobic processes due to lower operational costs, as well as advantages in yields and volumetric production rates (Weusthuis et al., 2011): the need for an adequate and thus continuous supply of oxygen in aerobic bioreactors is associated with high energy input resulting in up to 20% of all operating costs (Junker et al., 1998). The poor solubility of oxygen in aqueous cultivation media (27.6 mg/L at 30 °C) limits oxygen mass-transfer, and thereby the production rates (Wittmann et al., 2017). Oxygen transfer rates also restrict the upscale of bioreactors, resulting in higher capital costs compared to anaerobic systems (McMillan and Beckham, 2017). Additional costs arise due to cooling requirements to counter the heat generated by aerobic respiration and due to the addition of chemical antifoaming agents to prevent foam generation through aeration of the medium (Delvigne and Lecomte, 2009; Humbird et al., 2017). If the target product is not dependent on biomass formation (i.e. product formation coupled to growth), the production yield in aerobic processes can be lower than in anaerobic processes, due to substrate loss. For example, a fraction of the substrate can be completely oxidized to carbon dioxide, resulting in diversion of metabolic carbon flux away from the target product. Moreover, microorganisms gain more energy for reproduction via aerobic respiration compared to anaerobic fermentation, naturally driving the maximization of undesired biomass formation (Weusthuis et al., 2011). Nevertheless, oxygen is essential as the terminal electron acceptor for many industrial microorganisms to generate energy for cell growth and metabolic functions (oxygen-dependent pathways), as well as to balance their metabolic redox state.

However, the energy conservation of fermentation ($\Delta G^{\circ} \approx -218$ kJ per mol glucose) and anaerobic respiration, e.g. with nitrate as a final electron acceptor ($\Delta G^{\circ\prime} = -858$ kJ per mol glucose), is significantly lower in comparison to aerobic respiration ($\Delta G^{\circ\prime} = -2870$ kJ per mol glucose; Tran and Unden, 1998; Unden and Bongaerts, 1997). In fact, fermentation yields only two mol ATP per mol glucose via substrate-level phosphorylation, which results in low cell growth and therefore low catalytic biomass compared to aerobic processes (Fig. 1). Nevertheless, decreased carbon commitment to the formation of biomass can potentially lead to increased formation of desirable (by-)products. Under fermentative conditions, microorganisms regenerate their co-factors and stabilize their redox state through the synthesis of reduced products such as succinate, formate, acetate, lactate, and ethanol. While these products are considered valuable commodities and feedstocks for the chemical industry, a mixed production of these compounds in the same reaction broth leads to low yield and titer of one target product and complicates its downstream processing/purification (Förster and Gescher, 2014). While directing the metabolism towards one target product is desirable, the productivity of anaerobic processes can also be greatly increased by integrated in-situ product removal preventing product inhibition. In contrast, a well-established in-situ product extraction in aerobic processes might not enhance the productivity, as often the oxygen transfer rate remains the limiting factor (Weusthuis et al., 2011).

In conclusion, aerobic and anaerobic production processes, both have advantages and disadvantages, the verdict often depending on case-by-case. While oxygen utilization is linked with higher microbial energy conservation, less by-product synthesis during regeneration of co-factors and high theoretical product yields, anaerobic processes can achieve higher yields and productivities in-vivo if the product is a cell-growth independent metabolite and are associated with lower cost and energy requirements, making them generally the more desirable approach.

To advance higher oxygen independency in current aerobic production processes, different strategies have been developed. One approach is to separate growth and production through a two-stage process, allowing rapid accumulation of catalytic biomass in an aerobic phase, followed by high-yield product synthesis in a growth-arrested anaerobic phase (Lange et al., 2017). This concept is, however, limited to only a few facultative anaerobic organisms. Moreover, the transition phase from aerobic to anaerobic stage can lead to a long lag-phase and the rapid oxygen depletion at high cell densities after the aerobic phase often results in poorly adapted cells or even cell death, decreasing the productivity and yield in the second phase (Lange et al., 2017; Zhu et al., 2011). A second strategy to oxygen independency is the chemical addition of alternative terminal electron acceptors such as nitrate, sulfate, metals or organic matter. However, these electron acceptors are often toxic to many microorganisms, can only be provided in limited amounts and deplete fast, which reduces the productivity (Nealson et al., 2002; Nishimura et al., 2007; Tebo and Obraztsova, 1998). A third option is to utilize synthetic biology to engineer the cellular metabolism towards higher oxygen independency. One great example was provided by (Meadows et al., 2016) by rewiring the central metabolism of *Saccharomyces cerevisiae* for enhanced production of isoprenoids from glucose. Metabolic engineering allowed for the generation of acetyl coenzyme A (acetyl-CoA) with reduced energy requirement and CO_2-production, as well as optimized redox balance. This resulted in 25% higher yields and a 75% lower oxygen requirement compared to the wild-type (Meadows et al., 2016).

Metabolic engineering is also regularly used to optimize anaerobic

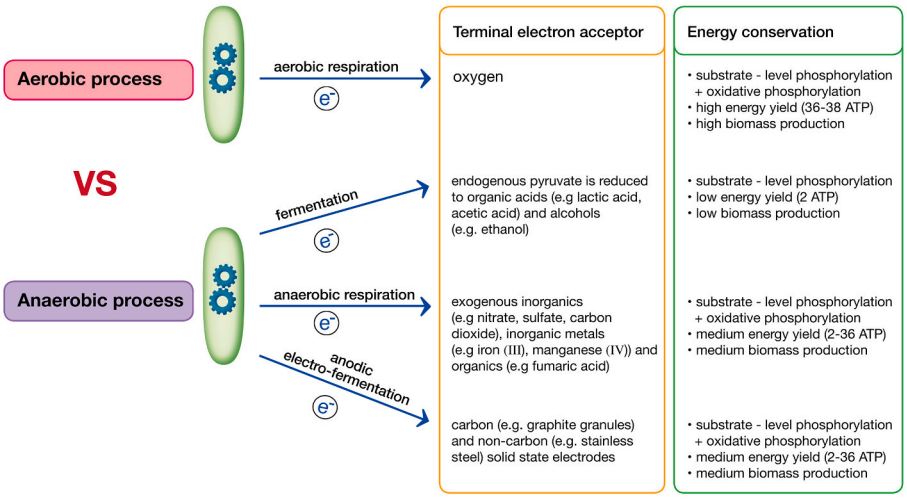

Fig. 1. Schematic comparison of aerobic and anaerobic microbial production processes using different terminal electron acceptors resulting in different energy and biomass yields. It is assumed that biomass and energy generation during anodic electro-fermentation is comparable to nitrate-respiration, which is explained in further detail in section 5.3.

production processes. Common strategies include the elimination of all major non-essential pathways that consume the target product and/or compete with its formation (e.g. those synthesizing by-products, which would decrease the product yield) by deleting or inactivating the corresponding genes (Lee et al., 2008; Liao et al., 2016; Wendisch et al., 2006). However, manipulation of the metabolism often results in reduced energy conservation and an imbalance of the redox state imposing a burden on the cells. For an efficient redirection of the carbon flux towards the desired product, an in-depth systems-level understanding of cellular functions, in particular, metabolism and its regulation is required. Additionally, suitable and well-established genetic tools must be readily available (Nielsen and Keasling, 2016; Yu et al., 2019).

Another promising and novel approach to optimize the efficiencies of aerobic and anaerobic industrial microbiology is the use of microbial electrochemical technologies (METs). Especially, anodic electro-fermentation (AEF) can combine the benefits of aerobic and anaerobic processes and eliminate their disadvantages by empowering anaerobic respiration of a solid-state electrode in bioelectrochemical systems (BES; Kracke et al., 2015; Moscoviz et al., 2016; Schievano et al., 2016). This technology is so far the only known approach to supply microorganisms with a non-depletable electron acceptor, which is provided in-situ by the anodic terminal of the BES (Figs. 1, 2). AEF has the potential to turn an aerobic process anoxic, replacing the commonly essential electron acceptor oxygen with an anode. Thereby, high yields may be sustained or even improved, by avoiding substrate loss in form of unwanted biomass formation as less energy is available for cell growth and/or by minimizing complete oxidation of substrate into CO_2 (Lai et al., 2016; Vassilev et al., 2018). Established anaerobic production processes can also benefit from AEF, since it can help to stabilize cellular energy and redox state. This is because co-factors can be potentially regenerated by transferring surplus electrons to the anode instead of producing undesired reduced by-products (Emde and Schink, 1990; Kracke et al., 2015; Sturm-Richter et al., 2015).

This article reviews the status of the novel and emerging technology AEF and its potential to electrify industrial biotechnology. Despite AEF being still in early development, this review highlights how this technology can be better understood by learning from the further advanced microbial fuel cell (MFC) and microbial electrolysis cell (MEC) research, as MFCs/MECs are related to AEF sharing strong similarities in terms of materials, configuration and operation. Furthermore, a rational choice of microbial candidates for AEF is discussed. Especially, the potential benefits of cultivating the industrial model-organism *Bacillus subtilis* in an anodic bioreactor are demonstrated in a theoretical exercise through metabolic modelling.

2. From microbial electrochemical technology to anodic electro-fermentation

Much of the current understanding of electroactive microorganisms and their interactions with solid terminal electron acceptors/donors as well as how these phenomena can be implemented stems from knowledge gained through research on METs, primarily throughout the last three decades of fundamental studies and applications of MFCs and MECs (Kadier et al., 2016; Santoro et al., 2017). In MFCs, the anaerobic respiration of sugars and volatile fatty acids with extracellular electron transfer to a solid anodic terminal (see Fig. 2) is exploited to produce electricity by forming an electron flow circuit with a complementary cathodic terminal where a reduction reaction occurs (typically O_2 reduction to H_2O; Santoro et al., 2017). While in MECs the microbial oxidation of organic matter is also catalyzed at the anode, externally supplied voltage via a power supply is required to allow hydrogen evolution at the cathode as MECs aim at the production of gaseous energy carrier (Kadier et al., 2016). AEF includes also hydrogen evolution at the cathode but with the target to enable anode-catalyzed bioproduction of industrially relevant chemicals. The quest to maximize electricity production and hydrogen gas production in MFCs and MECs, respectively, has led to a vast research portfolio which can directly benefit AEF, including: (*i*) electrochemical materials with adequate biocompatibility and stability under process conditions, including resistance to bio/chemical fouling and corrosion (Freguia et al., 2017), (*ii*) reactor configurations that maximize mass transfer while minimizing the activation/ohmic losses inherent to all electrochemical systems (Rozendal et al., 2008) and (*iii*) strategies for up-scaling and commercialization in consideration that electricity is an extremely low-value commodity per mol e^- (Mateo et al., 2018). A practical example of how MET knowledge can directly benefit AEF was recently demonstrated by (Rosa et al., 2019), whereby a standard laboratory fermenter was readily converted to electro-fermentation by the embedding of MET materials.

The broad research community of METs has used diverse terms to describe the process of utilizing an anode as an electron acceptor to promote the anaerobic synthesis of a target product. For example, the fermentation is outlined as ‘a production process in a poised-potential amperometric culture system’ (Emde et al., 1989), ‘driven procedure by a bioelectrochemical system’ (Lai et al., 2016), or ‘electrode-driven, electrode-assisted or MFC-assisted process’ (Forster et al., 2017; Zheng et al., 2021). To standardize the term definition this review article recommends using AEF, which is a term that clearly encompasses the applied technology, its purpose, and avoids any confusion with related techniques such as MFC, microbial electrosynthesis (MES) or cathodic electro-fermentation (CEF), the latter two already solidly established as cathodic-driven electron sources for the biosynthesis of value-added products from carbon dioxide and organic substrates, respectively. AEF, CEF and MES are often commonly reviewed in the same category because of sharing the same aim, the production of valuable chemicals (Gong et al., 2020; Lin et al., 2021). However, from fundamental point of view CEF and MES are indeed closely related with each other using both the cathode to promote bioproduction, but in this aspect AEF is more related to MEFs and MEC as the microbial process is driven by the anodic term of the BES.

3. Microorganism-anode interaction

3.1. How do microorganisms use the anode as the terminal electron acceptor?

The performance of AEF is driven by the microbial capability of using the anode as the final electron acceptor. A broad range of microorganisms discovered in recent years have demonstrated the ability to transfer electrons to solid-state electrodes (Logan et al., 2019; Sydow et al., 2014; Yee et al., 2020), but only a few have been sufficiently studied to understand their mechanism(s) of extracellular electron transfer (EET). In particular, the two dissimilatory metal-reducing bacteria *Geobacter sulfurreducens* (Bond and Lovley, 2003; Tabares et al., 2020) and *Shewanella oneidensis* (Bretschger et al., 2007; Fredrickson et al., 2008) have been studied in-depth as current-producing model organisms to clarify the mechanisms that allow microbes to transfer electrons beyond their membranes to anodes in MFCs. When the bacteria grow as biofilms on the electrode, direct cell-anode contact allows direct EET. The electrons are shuttled from the cell interior through the inner and outer membrane to the extracellular terminal electron acceptor (i.e. anode) via primary dehydrogenases, quinones, a chain of cytochrome complexes and/or terminal reductases (Fig. 2A; Kracke et al., 2015; Kumar et al., 2017; Shi et al., 2016). In particular, outer membrane cytochromes in Gram-negative bacteria have been identified as key players in this phenomenon (Mehta et al., 2005; Shi et al., 2007). Besides direct microbial-electrode connection, electrons are also shuttled between microorganisms via direct cell-cell contact and conductive extracellular polymeric substances in the biofilm (direct interspecies electron transfer, DIET) supporting thick biofilm growth of several cell-layers on the anode (Fig. 2A; Lovley, 2017; Shi et al., 2009; Stams and Plugge, 2009). The development of a thick biofilm as the catalyst with the outer layer still

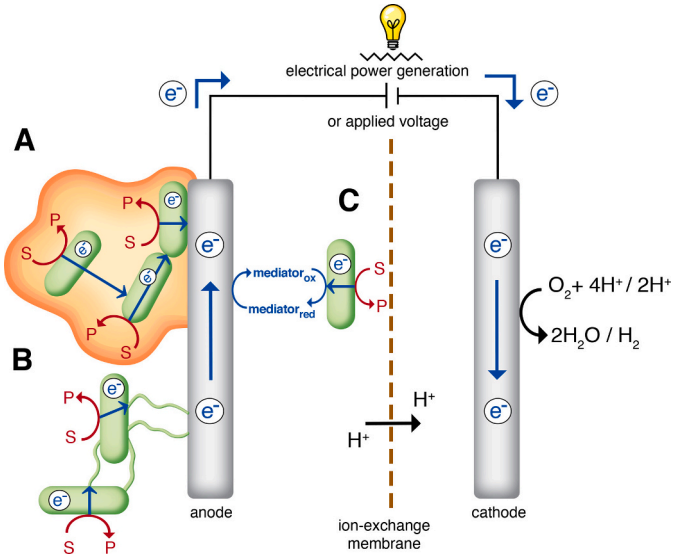

Fig. 2. Simplified schematic representation of extracellular electron transfer (EET) mechanisms during anodic electro-fermentation (AEF). A: Direct EET via physical contact of a cell with an anode and direct interspecies electron transfer (DIET) via cell-cell contact and extracellular polymeric substances in a biofilm. B: Direct EET via nanowires between cells among each other and the anode. C: Indirect EET via soluble extracellular mediators, which are continuously recycled at the anode. S = substrate; P = product; ox = oxidized; red = reduced.

being able to use the anode as the terminal electron acceptor via DIET is critical in MFC and AEF for efficient production of electricity and chemicals, respectively. In addition, metal-respiring bacteria have developed a strategy to increase the distance efficiency of direct EET (>10 μm) by growing electrically conductive appendages, termed nanowires (Fig. 2B; Sure et al., 2016). The structure of nanowires is a matter of intense debate: for *G. sulfurreducens*, it has been suggested that the framework consists either of a series of hexaheme cytochrome OmcS filaments (Wang et al., 2019) or an assembly of type- IV pilins (Lovley and Walker, 2019), while for *S. oneidensis* the dominant consensus is that the apparent nanowires are in fact similar to outer membrane vesicles, which can be seen as extensions of the outer membrane and periplasm comprising multiheme cytochromes (Subramanian et al., 2018).

An additional strategy to extend the range of EET is the use of extracellular redox-active metabolites, which act as electron carriers (soluble mediators) and are reduced by the microorganisms and re-oxidized by the anode in a continuous cycle enabling indirect EET (Fig. 2C; Martinez and Alvarez, 2018). *S. oneidensis* and *Pseudomonas aeruginosa* are among the best-studied natural mediator producers being able to excrete flavins (Marsili et al., 2008) and phenazines (Boon et al., 2008) into the medium, respectively, which facilitate indirect electron transfer to the anode. Non-electroactive microorganisms lacking the ability to synthesize mediators on their own can be supplemented with artificial mediators such as thionines (Sakai and Yagishita, 2007), potassium ferricyanide (Lai et al., 2016), methylene blue (Sturm-Richter et al., 2015) and 2-hydroxy-1,4-naphthoquinone (HNQ; Kim et al., 2017) to empower indirect EET. For a comprehensive review on mediators used in BESs the reader is referred to: (Martinez and Alvarez, 2018).

The underlying mechanisms for EET have been mainly studied for Gram-negative microorganisms, leaving the fundamentals of Gram-positive microorganisms' EET relatively unclear. It was generally assumed that the thick, non-conductive cell wall of Gram-positive bacteria would limit their capacity to use the anode as the terminal electron acceptor (Pankratova et al., 2019). Nonetheless, many Gram-positives including *Enterococcus faecalis* (Pankratova et al., 2018) and *B. subtilis* (Nimje et al., 2009) are indeed capable of interacting with an anode and producing an adequate current in MFCs. It is hypothesized that this is due to the diffusion of natural mediators through the peptidoglycan layer, serving as an electron shuttle. In addition, cell wall-associated proteins may also play a role in facilitating EET of Gram-positive bacteria (Pankratova et al., 2019). For a detailed mechanism of indirect and direct EET on a biomolecular level, the reader is referred to following comprehensive review articles: (Kracke et al., 2015; Kumar et al., 2017; Shi et al., 2016).

However, most researchers have studied EET in MFCs in terms of understanding the underlying mechanism to enhance the power output, while in AEF the goal is to efficiently produce value-added chemicals, power generation being peripheral. Therefore, the relevant EET pathways may differ significantly between AEF and MFCs, especially when using microorganisms other than *G. sulfurreducens* and *S. oneidensis* (Hernandez and Newman, 2001; Kracke et al., 2015).

3.2. How do microorganisms gain energy via anode respiration?

Energy generation plays a central role in bacterial growth and can significantly influence product synthesis. In the process of oxidative phosphorylation, electrons are transferred via an electron transport chain from a low-potential electron donor to an electron acceptor with more positive redox potential. The thermodynamic energy difference between electron donor and acceptor is used to create a proton gradient across the membrane in order to drive ATP synthesis via chemiosmosis (Anraku, 1988). Therefore, under aerobic conditions, the microorganisms always prefer O_2 as the terminal electron acceptor, as it has usually the largest potential difference to the electron donor. However, the high energy yield results in high biomass production, which might decrease the desired product yield if the product is not biomass-based. Due to the inherent mass-transfer limitations of oxic conditions, it is critical to supply the microorganisms with a permanently available terminal electron acceptor to keep the microorganisms catalytically-active and to ensure the efficient synthesis of the target product. In that context, the anode of an electrochemical cell is so far the only known undepletable electron acceptor in a bioreactor. Anodic respiration may also allow a better control of microbial energy conservation compared to aerobic respiration by avoiding surplus energy generation in microbes via controlled anode potential and/or via the implementation of mediators with specific redox potential. The supply of microorganisms with membrane-permeable mediators favors the diffusion of the mediators into the periplasm or beyond the inner membrane, allowing the possible interaction with different sites of the electron transport chain, which can result in different ATP yields depending on the chain element that transfers the electrons to the mediator (Martinez and Alvarez, 2018). For example, in a previous theoretical study, it was calculated that *E. coli* can yield up to 2.7 mol ATP by transferring 2 mol electrons from the cytochrome *bo* to a terminal acceptor given that 8 mol protons would be transported across the membrane using the suggested electron transport chain. Conversely, drawing the electrons directly from the cellular NADH pool via NADH dehydrogenase would result in no net ATP gain for *E. coli* via oxidative phosphorylation (Kracke et al., 2015).

A general prerequisite to enable interaction between the mediator and a member of the electron transport chain is that the redox potential of the mediator is high enough to thermodynamically drive the electron transfer to the mediator and to potentially promote proton transport across the membrane for ATP generation via chemiosmosis. For example, a recent AEF study using *Pseudomonas putida* demonstrated that only a mediator with a redox potential above 0.207 V vs. standard hydrogen electrode (SHE) significantly catalyzed electron transfer to the anode (Lai et al., 2016). In fact, the process productivity improved with the increasing redox potential of the mediator so that ferricyanide with a redox potential of 0.416 V vs SHE showed the best performance. Essential in such an application is also to apply a voltage, which is positive enough to re-oxidize the mediator at the anode to ensure constant availability of the mediator for the microorganisms. Mediated AEF

significantly enhanced the energy generation in *P. putida* as the intracellular adenylate energy charge was increased compared to cells lacking a terminal electron acceptor. Although no cell growth was observed, the cells could survive and were catalytically active under anaerobic conditions while cells in a control experiment lacking a terminal electron acceptor died (Lai et al., 2016).

If one considers energy conservation via direct EET for example for *G. sulfurreducens* and *S. oneidensis* growing as a biofilm on the anode, the terminal segment of the electron transport chain within the periplasm and across the outer membrane is unlikely to contribute directly to energy generation inside the cell. The terminal electron transport sites do not support the proton translocation across the inner membrane and therefore cannot drive the proton-motive force for chemiosmotic ATP production (Korth and Harnisch, 2019; Okamoto et al., 2017). Therefore, applying a high potential (close to water electrolysis) at the anode with the aim of thermodynamically increasing the energy difference between substrate and terminal electron acceptor (i.e. anode), will not necessarily increase the intracellular energy yield. From a thermodynamical point of view, the anode redox potential does not have an impact on microbial energy conservation, but it has been proposed that the anode potential can kinetically enhance the microbial energy harvest. A recent modelling study of electroactive microorganisms suggested that a potential of 0.2 V vs. SHE maximizes the direct EET kinetics by avoiding the accumulation of intracellular NADH thereby enhancing microbial energy harvesting, while an anode potential above 0.2 V vs. SHE does not significantly further improve the intracellular energy gain (Korth and Harnisch, 2019).

Similar to EET mechanism studies, research into microbial energy conservation via EET is lacking for Gram-positive bacteria. Considering the structure of the latter, the thick, non-conductive cell wall might function as a barrier potentially in a similar way as the outer membrane of Gram-negative bacteria. Therefore, only the sites of the electron transport chain in the plasma membrane are likely to contribute to the proton-motive force across the membrane for ATP generation via chemiosmosis, however, these assumptions require further in-depth investigation.

4. Potential benefits for industrial microbiology

4.1. Turning an aerobic process anaerobic via AEF

AEF provides a unique opportunity in applied microbiology to eliminate the dependency of aerobic strains on oxygen by replacing oxygen with an anode. Making aerobic processes anaerobic can potentially provide various benefits such as energy and cost savings as well as enhanced synthesis of a target product (Fig. 3A, C; Humbird et al., 2017; Lai and Krömer, 2019; McMillan and Beckham, 2017; Weusthuis et al., 2011). For example, the obligate aerobe *P. putida* was turned into an anaerobic producer using the anode as the sole electron acceptor mediated via ferricyanide (Lai et al., 2016). No microbial growth was observed, but AEF enabled the obligate aerobic cells to survive and to remain catalytically active under anoxic conditions producing 2–ketogluconate from glucose with a high yield of over 90% [mol/mol]. This investigation demonstrates the prospects of AEF to minimize the substrate consumption for anabolic processes and thereby achieve high product yields when an aerobic microbe is transformed into an anaerobic producer. However, the productivity achieved by *P. putida* was too low for real applications. Therefore, to enhance productivity, glucose dehydrogenase and gluconate dehydrogenase were overexpressed in the strain, which resulted in an increase of the production rate by 644% and current output by 327%, compared to the wild-type strain (Yu et al., 2018). Besides strain engineering, process engineering is also required for further improvement of AEF by *P. putida* as calculations suggested that mass transfer of mediator towards the anode was limiting, which was foremost attributable to a small electrode surface area (Lai and Krömer, 2019).

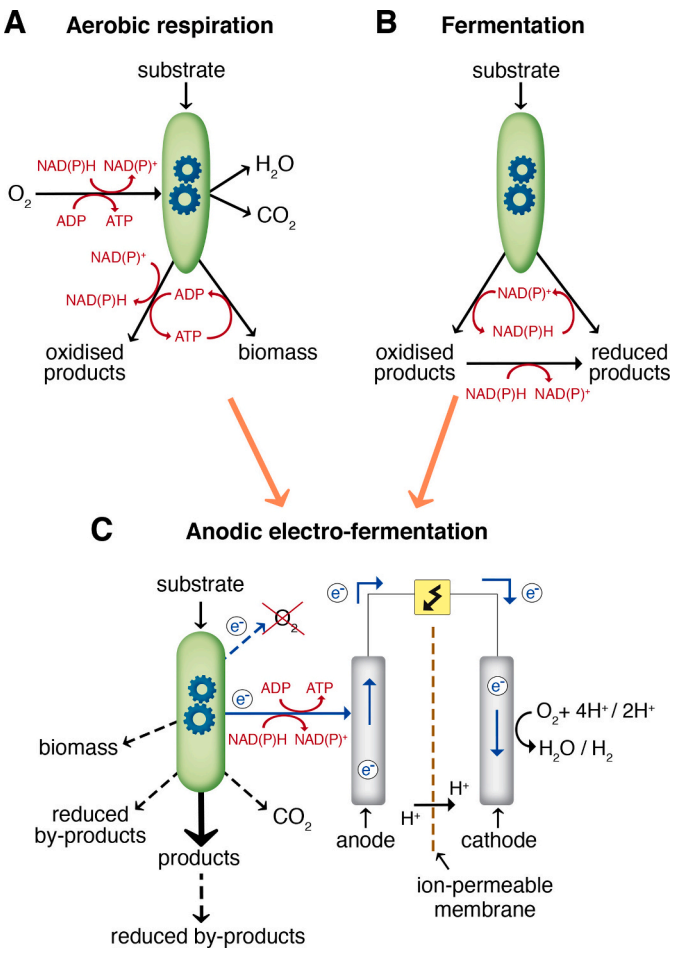

Fig. 3. Simplified flow schematic of substrate conversion into products via aerobic respiration (A), fermentation (B), and anodic electro-fermentation (C). During aerobic respiration, microorganisms use oxygen as the terminal electron acceptor to regenerate $NAD(P)^+$ and to generate ATP. The overflow of generated energy can be used to transform a part of the substrate into biomass, while a part of the substrate might be completely oxidized to CO_2, which will result in decreased product yields if the product is not biomass-based (A). During fermentation energy generation is limited and co-factors are regenerated by the synthesis of reduced by-products leading to decreased product yields (B). Anodic electro-fermentation has the potential to eliminate the disadvantages of aerobic respiration and fermentation by regenerating co-factors and stabilizing the energy state electrochemically via the anode of a bioelectrochemical system. Therefore, anodic electro-fermentation can steer the carbon flow from the substrate towards the target product and potentially minimize undesired oversynthesis of biomass and by-products (illustrated by dashed arrows; C).

Further AEF research showed an approach to empower the anoxic character of the industrial amino acid producer *Corynebacterium glutamicum* (Vassilev et al., 2018). The efficient reproduction of the organism relies on adequate oxygen supply. Although the bacterium is capable of fermentation, cell growth is restricted or even cell death occurs in the absence of oxygen likely due to finite energy gain (Michel et al., 2015; Vassilev et al., 2018). *C. glutamicum* can also use nitrate as terminal electron acceptor in the absence of oxygen, but its growth is limited due to the accumulation of toxic nitrite (Takeno et al., 2007). Therefore, an anode is an attractive alternative electron acceptor to promote its anaerobic production metabolism. Indeed, AEF enabled anaerobic growth and enhanced glucose consumption and biosynthesis of products such as L-lysine, indicating that AEF supported the energy and redox stabilization of anoxic *C. glutamicum* (Vassilev et al., 2018). These outcomes highlight the potential of AEF to develop an advanced amino acid production process without the need to be dependent on oxygen supply.

However, the few attempts to turn an aerobic process into anaerobic via AEF are mostly proof-of-concept studies. More understanding of the novel technology is needed, and further well-established production hosts should be validated for potential AEF applications to accelerate their development towards industrial applications.

4.2. Enhancing an anaerobic process via AEF

Anaerobic production processes via fermentation need to achieve redox balance. To reoxidize cellular co-factors, microorganisms are forced to produce a mixture of acids, alcohols and/or gasses, which decreases the yield of the target product and increases its separation costs (Förster and Gescher, 2014; Weusthuis et al., 2011). Missing an electron acceptor means that the electrons from the substrate need to be recovered in the products, which leads to products having primary the same or a higher degree of reduction than the substrate (Fig. 1, 3B). This makes the synthesis of oxidized products with high yield thermodynamically impossible. Thus, metabolic engineering strategies aiming at deleting synthesis pathways for reduced by-products to obtain higher yields of target products are unlikely to overcome the need of a cell to accomplish redox balance. 'Unbalanced' fermentation can be empowered via anaerobic respiration of an anode, which can support the reoxidation of co-factors and minimize the synthesis of undesired by-products.

4.2.1. Enhanced fermentation with pure cultures

For example, ferricyanide-mediated AEF induced a product shift in glycerol fermentation towards more oxidized products, i.e. from ethanol to acetate and lactate and from propionate to acetate by *E. coli* and by *Propionibacterium freudenreichii*, respectively (Emde and Schink, 1990; Emde et al., 1989). Further investigations in AEF with recombinant *Klebsiella pneumoniae* were done to enhance the anaerobic production of the important platform chemical 3-hydroxypropionic acid (Kim et al., 2017). In the two-step reaction, glycerol is first converted into 3-hydroxypropionaldehyde by glycerol dehydratase and then to 3-hydroxypropionic acid by aldehyde dehydrogenase. The first enzyme and coenzyme B_{12} (essential co-factor for the first reaction) are stable and efficiently synthesized only under anaerobic conditions. However, the lack of oxygen as a terminal electron acceptor impedes the regeneration of the required cofactor NAD^+ for the second step. In order to regenerate NAD^+, *K. pneumoniae* is forced to produce 1,3-propanediol, lactate and/or ethanol, which decreases the 3-hydroxypropionic acid yield. An alternative solution to regenerate NAD^+ is via anodic respiration. Indeed, AEF mediated via 2-hydroxy-1,4-naphthoquinone improved electrochemical NAD^+ regeneration, and in combination with overexpression of aldehyde dehydrogenase, the product spectrum was shifted from more reduced by-products to the more oxidized target product, 3-hydroxypropionic acid (Kim et al., 2017). In another key example, glycerol fermentation with *Enterobacter aerogenes* using thionine as a mediator in AEF did not result in a product shift but allowed the consumption of higher glycerol amounts, increased glycerol consumption rates and production rates of ethanol and hydrogen, which indicates that AEF supported *E. aerogenes* in balancing its redox state more efficiently (Sakai and Yagishita, 2007).

4.2.2. Enhanced fermentation with co-cultures

It is worth noting that not only pure culture but also co-culture fermentation processes can benefit from AEF. Especially, the combination of a producer and an electroactive strain can lead to synergetic benefits enhancing the production process. For example, the anode interaction with the producer strain can be enhanced by naturally-secreted mediators from the electroactive strain, or by embedding the producer strain in the conductive biofilm of the electroactive strain (Lovley, 2017; Stams and Plugge, 2009). Productivity can be enhanced by synchronization of the co-metabolism of two cultures as shown by *Clostridium cellobioparum* and *G. sulfurreducens* (Speers et al., 2014). *C. cellobioparum* converted the substrate glycerol into ethanol, while the produced by-products (i.e. acetate, formate, H_2) were consumed by *G. sulfurreducens* using the anode as the electron acceptor, which boosted glycerol consumption and ethanol production (Speers et al., 2014).

5. What are the ideal microbial candidates for AEF?

The success of an industrial microbiology process is determined strongly by the type of end product and the approach of how it is produced. Ideally, the end product has a high market price and great demand. The microbial catalyst should be able to utilize preferably a broad range of inexpensive substrates and it should be easy to cultivate under moderate conditions, keeping the production process simple. Further, the availability of metabolic engineering tools is advantageous to perform strain design-optimization for advanced production (Clomburg et al., 2017; Lokko et al., 2018). A special requirement for AEF is that the biocatalyst can efficiently transport surplus electrons across its cell membrane(s) to the anode in order to attain redox stabilization and energy conservation under anaerobic conditions.

5.1. Electroactive microorganisms vs. industrial microbial producers

Scanning for an optimal AEF candidate, *G. sulfurreducens* and *S. oneidensis* have been identified as excellent current-producers being proficient in using the anode as terminal electron acceptor (Shi et al., 2009). However, the capacity of their *wild type* strains to produce valuable and industrially-relevant chemicals is narrow, which limits their application in AEF. On the other hand, industrially established microorganisms, which serve as cell factories for the production of diverse commodity chemicals, have shown to date limited capabilities of anode-interaction. The product spectrum of highly electro-active bacteria may be broadened by means of metabolic engineering by inserting industrially relevant production pathways into the electro-active bacteria. Alternatively, the electro-activity of industrial microbial producers may be enhanced through metabolic engineering of their electron transport chain pathways or by supplying the microorganisms with an artificial mediator to enable EET to the anode (Kracke et al., 2018; Rosenbaum and Henrich, 2014; TerAvest and Ajo-Franklin, 2016). A prerequisite for these bioengineering approaches is that the host be genetically tractable, which is not a given in case of newly-discovered electroactive bacteria (Li et al., 2018). Furthermore, the current limited understanding and complexity of EET mechanisms make the engineering approach of EET pathways and the coupling of the electron flow with the core carbon metabolism of model organisms particularly challenging.

For example, *E. coli* was used as a model organism to demonstrate an engineering method to enhance microbial electroactivity by expressing the *c*-type cytochromes CymA, MtrA and STC from the Mtr EET pathway of *S. oneidensis* (Sturm-Richter et al., 2015). The expressed *c*-type cytochromes allowed electron transfer from the cytoplasm across the inner membrane into the periplasm and the added mediator methylene blue shuttled the electrons from the periplasm across the outer membrane to an anode. As a result, AEF of glycerol by the engineered *E. coli* induced a product shift towards a more oxidized product (i.e. from ethanol to acetate; Sturm-Richter et al., 2015). To eliminate the need for mediator addition, the complete Mtr pathway was expressed including the outer membrane-associated proteins MtrB and MtrC, which enable electron transfer from the periplasm to the anode (TerAvest et al., 2014). But in that approach, the microbe-electrode interaction was weaker compared to methylene blue mediated electron transport, presumably due to low expression of Mtr cytochromes limiting EET (Su et al., 2019).

The alternative strategy, engineering a production pathway into electrogenic bacteria was demonstrated with *S. oneidensis*. Acetoin synthesis genes from *B. subtilis* were expressed in the bacterial host, which enabled an unbalanced fermentation in the absence of oxygen, namely the conversion of lactate into acetoin by using the anode as the

sole electron acceptor (Bursac et al., 2017).

5.2. Summarized AEF approaches with different microorganisms

Table 1 summarizes the different microorganisms which have been assessed as potential candidates for AEF. These different studies show common features in substrate, mediator, electrode-material and applied potential usage. Glycerol was often the feedstock of choice, which is an economically-attractive and more sustainable substrate compared to sugar substrates such as glucose as it is highly available in waste streams of biodiesel industries (Speers et al., 2014). Additionally, the consideration that glycerol has a higher degree of reduction than glucose supports the rationale of using the anode as an electron sink to dispose of 'surplus' electrons. Other even more reduced feedstocks such as methanol, which can be produced electrochemically or found in waste streams of pulp factories and petroleum industry, might also have great potential in AEF (Zhang et al., 2018).

In most studies in Table 1, a mediator was essential to empower microbial-anode interaction. An artificial electron-carrier was not required when a natural electro-active bacterium was used as the producer strain (Bursac et al., 2017; Flynn et al., 2010), or when the producer strain was co-cultured with an electro-active strain (Awate et al., 2017; Speers et al., 2014). A further common feature in most of the studies includes the usage of carbon-based anodes with an average applied potential of 0.4 V vs. SHE. Carbon materials exist in various morphologies and structures and are usually highly biocompatible supporting colonization of biofilms and microbial-electrochemical interactions, which makes them attractive anode materials (Freguia et al., 2017; Li et al., 2017).

In the search for an ideal AEF candidate, it can be concluded that AEF is too young of a research field with too many knowledge gaps to nominate an ideal microorganism for AEF. In fact, only a few bacteria have been evaluated to date as potential aspirants for AEF (Table 1). After reviewing the emerging research field of AEF, the authors believe that many more microorganisms could benefit from an undepletable electron acceptor to improve industrial microbiological processes. Therefore, in the following sub-chapter, the possible benefits of AEF are analyzed in a theoretical exercise for a well-known industrial microorganism, which has not been yet considered as an AEF candidate, namely *B. subtilis*.

5.3. Bacillus subtilis: A promising AEF candidate?

B. subtilis is probably the best-studied Gram-positive organism. Its natural capability to uptake extracellular DNA makes the bacterium easy to genetically engineer and its versatile metabolism allows for the production of various bulk and fine chemicals (Xiang et al., 2020). Especially due to its outstanding ability to secrete great quantities of proteins into the reactor broth, *B. subtilis* has become an essential workhorse in industrial microbiology for the production of degradative enzymes and other proteins (Hohmann et al., 2016). In addition, in the last decade, it was demonstrated that *B. subtilis* is electroactive being capable of producing current in MFCs (Nimje et al., 2009). Researchers suggested that anode interaction is facilitated by the natural production of redox-active mediators and a conductive biofilm (Nimje et al., 2009; Pankratova et al., 2019; Qin et al., 2019). Different pure strains of *B. subtilis*, as well as mixed cultures, were analyzed as current producers in MFCs in combination with treating municipal (Ismail and Jaeel, 2013) and swine wastewater (Jeon et al., 2016), and remediation of waste streams containing azo dyes (Kalleary et al., 2014), engine oil (Sabina et al., 2014) and phenols (Hassan et al., 2016; Mohan et al., 2020).

The properties of *B. subtilis* as a safe (GRAS status, Generally Recognized as Safe), established industrial producer and an electroactive species identify the organism as an intriguing candidate for AEF. To highlight its potential, a theoretical exercise was conducted using *B. subtilis* and acetoin as a feasible example for an organism-product couple to explore the benefits of AEF for anaerobic production of acetoin. Acetoin – CAS 513–86-0, also known as 3-hydroxybutanone or acetyl methyl carbinol with a chemical formula $C_4H_8O_2$ – is a bulk industrial chemical with a global market of ca. 12,000 tons (NNFCC, 2008). It is mainly used as an aroma compound (buttery flavor) in the food industry but also in the chemical industry as a building block for various products (e.g. alkyl pyrazines, diacetyl and acetylbutanediol; Xiao and Lu, 2014; Yang et al., 2017). Further, the fact that biosynthesis of acetoin from pyruvate is only a two-step pathway (Drejer et al., 2020), simplifies the organism-product couple study (Fig. 4A). However, efficient conversion of glucose into pyruvate during glycolysis requires a constant regeneration of NAD^+, which demands a continuously available electron acceptor, making the anode an interesting contender.

In this theoretical exercise, the metabolic models of *B. subtilis* were derived from previously established ones (Averesch and Rothschild, 2019) and modified for operation with different electron-acceptors and electro-donors (substrates). Specifically, in addition to oxygen as electron-acceptor, nitrate-respiration with full ammonification was assessed as an electron sink, where *B. subtilis* nitrite reductase does not result in a proton gradient (Nakano et al., 1998). An anode was implemented similarly by means of a "proxy" metabolite-couple allowing for the oxidation of ubiquinone without contribution to the proton gradient and generation of ATP. For fermentative metabolism, metabolic network modelling revealed that in addition to the reported major products lactate, acetate, and 2,3-butanediol (Ramos et al., 2000), an additional electron-sink had to be present to allow solutions to the network, either in form of succinate or formate as metabolic end-products. No pyruvate-formate lyase homologue has been found among the protein sequences deduced from the *B. subtilis* genomes, however, succinate was previously suggested as a potential product (Nakano et al., 1997), which was therefore assumed in the models. For the scenario where methanol was investigated as a potential substrate, a pyrroloquinoline quinone (PPQ)-dependent methanol dehydrogenase was implemented into *B. subtilis*, feeding the ribulose monophosphate (RuMP) pathway, as previously described (Averesch and Kracke, 2018). This simulated methanol conversion to acetoin is similar to what has been previously described for *Bacillus methanolicus* (Drejer et al., 2020).

Elementary flux modes were calculated in MATLAB® (MathWorks®), using the most recent implementation 'FluxModeCalculator' (van Klinken and Willems van Dijk, 2016), and evaluated as described before (Averesch and Kracke, 2018). Balances were established around boundary reactions, allowing carbon-yields [C-mol/C-mol] for all products to be determined. Accordingly, the ratio of electron-acceptor to substrate was determined as [mol/C-mol] for all flux modes. For the scenarios with oxygen as terminal electron acceptor, this corresponds to the net in-flux of O_2 per acetoin production in C-mol, nitrate respiration is analogous (net in-flux of NO_3 per acetoin production in C-mol). In the case of an anode as an electron acceptor where a proxy-compound was introduced in order to simulate an anode, one mol of this compound corresponds to one electron pair.

Sucrose, glucose, xylose, glycerol and methanol were investigated as carbon-sources under the different scenarios 'oxygen', 'nitrate', and 'anode' as electron acceptors, as well as for fermentative anaerobic conditions (Fig. 4B). The model indicated that with glycerol as substrate, no anaerobic metabolism was possible unless employing an anode as an electron acceptor. This is due to the uptake mechanism for glycerol, which relies in *B. subtilis* on a ubiquinone dependent glycerol-3-phosphate dehydrogenase (Averesch and Rothschild, 2019). Similarly, methanol uptake would require an alternative for oxidation of PQQ, in the case of a PQQ-dependent methanol dehydrogenase. This would not be necessary if a NAD-dependent methanol dehydrogenase was deployed.

The highest theoretical acetoin yield under no-growth conditions (no carbon flow into biomass) could be reached with oxygen or an anode as an electron acceptor, which was 67% for all tested substrates. When employing nitrate as an electron acceptor, the yield was reduced to 50%.

Table 1
Natural and engineered microorganisms cultivated in a bioelectrochemical system using the anode as the terminal electron acceptor to steer bioproduction, a technology referred to as anodic electro-fermentation (AEF).

Microbial organism	Substrate	Production				Electrochemical properties			Highlights	Reference
		Product	Volumetric rates [mg/(L·h)]	Titers [g/L]	Yields [$mol_{product}/mol_{substrate}$]	Electrode	Artificially added mediator	Applied potential [V vs. SHE]		
Escherichia coli	Glycerol	Acetate, ethanol, lactate, hydrogen	2.3, 1.3, 1.3, 0.02	0.12, 0.07, 0.07, 0.001	0.41, 0.29, 0.15, 0.08	Platinum net	Potassium ferricyanide	0.51	AEF induced a product spectrum shift and increased product yields	Emde et al. (1989)
Propionibacterium freudenreichii	Glycerol & propionate (GP), or lactate & propionate (LP), or only propionate (P)	Acetate	17.8 (GP), 19.4 (LP), 21.9 (P)	0.38 (GP), 0.42 (LP), 0.47 (P)	0.56 (GP), 0.60 (LP), 0.68 (P)	Platinum net	Potassium ferricyanide	0.43	Enhanced bacterial growth and substrate consumption	Emde and Schink (1990)
Enterobacter aerogenes	Glycerol	Ethanol, hydrogen	81.9, 2.9	3.93, 0.14	0.92, 0.74	Carbon cloth	Thionine	0.40	Increased glycerol consumption	Sakai and Yagishita (2007)
Engineered *Shewanella oneidensis*	Glycerol	Ethanol, acetate	~26, ~6	1.28 ± 0.02, 0.29 ± 0.08	0.85, 0.15	Carbon fiber	No	0.44	Transformation of a plasmid with glycerol utilization and ethanol production modules	Flynn et al. (2010)
Clostridium cellobioparum + *Geobacter sulfurreducens*	Glycerol	Ethanol, 1,3-propanediol	~48, ~61	8.7 ± 0.83, 11.03 ± 0.76	0.40, 0.31	Graphite rod	No	0.45	*Geobacter sulfurreducens* consumed by-products from glycerol fermentation by *Clostridium cellobioparum* and supported H_2 evolution at the cathode	Speers et al. (2014)
Engineered *Escherichia coli*	Glycerol	Ethanol, acetate	6.06 ± 0.8, 4.47 ± 0.15	0.69 ± 0.09, 0.51 ± 0.02	0.53 ± 0.07, 0.30 ± 0.01	Graphite felt	Methylene blue	0.20	Heterologous expression of CymA, MtrA and STC from *Shewanella oneidensis* for enhanced EET	Sturm-Richter et al. (2015)
Pseudomonas putida	Glucose	2–Keto-gluconate, acetate	4.00 ± 0.09, 0.19 ± 0.01	1.25 ± 0.03, 0.06 ± 0.00	0.90 ± 0.02, 0.14 ± 0.01	Carbon cloth	Potassium ferricyanide	0.70	Enabled anaerobic cell maintenance and catalysis of an obligate aerobic strain	Lai et al. (2016)
Engineered *Pseudomonas putida*	Citrate	4-Hydroxybenzoic acid	0.09	0.04	0.01	Graphite rod	Potassium ferricyanide	0.70	AEF in a controlled stirred-tank bioreactor	Hintermayer et al. (2016)
Engineered *Shewanella oneidensis*	Lactate	Acetoin	3.35	0.24	0.39	Not reported	No	0.00	Acetoin synthesis genes from *Bacillus subtilis* were induced and AEF allowed unbalanced fermentation	Bursac et al. (2017)
Cellulomonas uda + *Geobacter sulfurreducens*	Cellobiose	Ethanol	56 ± 3	1.23 ± 0.07	2.04 ± 0.12	Graphite rayon felt	No	0.44	AEF allowed removal of byproducts by *Geobacter* and enhancement of ethanol production by *Cellulomonas*	Awate et al. (2017)
Engineered *Escherichia coli*	Glucose	Acetoin	9.56 ± 0.22	0.86 ± 0.02	0.79 ± 0.02	Graphite felt	Methylene blue	0.20	High yield acetoin production via expression of genes for EET from *Shewanella oneidensis* and for acetoin synthesis from *Bacillus subtilis*	Forster et al. (2017)
Engineered *Klebsiella pneumoniae*	Glycerol	3-Hydroxypropionic acid, 1,3-propanediol (and other fermentation products)	60.63 ± 6.25, 22.50 ± 8.44	1.94 ± 0.20, 0.72 ± 0.27	0.18 ± 0.02, 0.08 ± 0.03	Carbon cloth	2-hydroxy-1,4-naphthoquinone	0.70	AEF decreased the $NADH/NAD^+$ ratio in the cell and shifted product spectrum from 1,3-propanediol towards 3-hydroxypropionic acid production	Kim et al. (2017)
Corynebacterium glutamicum	Glucose	Lactate, succinate, acetate, lysine	4.02 ± 0.19, 0.67 ± 0.19, 0.11 ± 0.01, 0.20 ± 0.002	5.15 ± 0.30, 1.42 ± 0.31, 0.13 ± 0.01, 0.42 ± 0.002	1.14 ± 0.04, 0.20 ± 0.05, 0.03 ± 0.003, 0.06 ± 0.003,	Carbon cloth	Potassium ferricyanide	0.70	Enabled anaerobic growth and enhanced anaerobic production	Vassilev et al. (2018)
Actinobacillus succinogenes	Glycerol	Succinate, acetate, formate	390 ± 1.30, 18.75 ± 12.56, 41.90 ± 1.79	23.92 ± 0.08, 1.15 ± 0.77, 2.57 ± 0.11	0.68, 0.07, 0.19	Graphite	Neutral red	Operated as MFC	Enabled anaerobic growth on glycerol and increased titers and yields of succinate. Transmembrane transport of neutral red was improved by atmospheric and room temperature plasma mutagenesis.	Zheng et al. (2021)

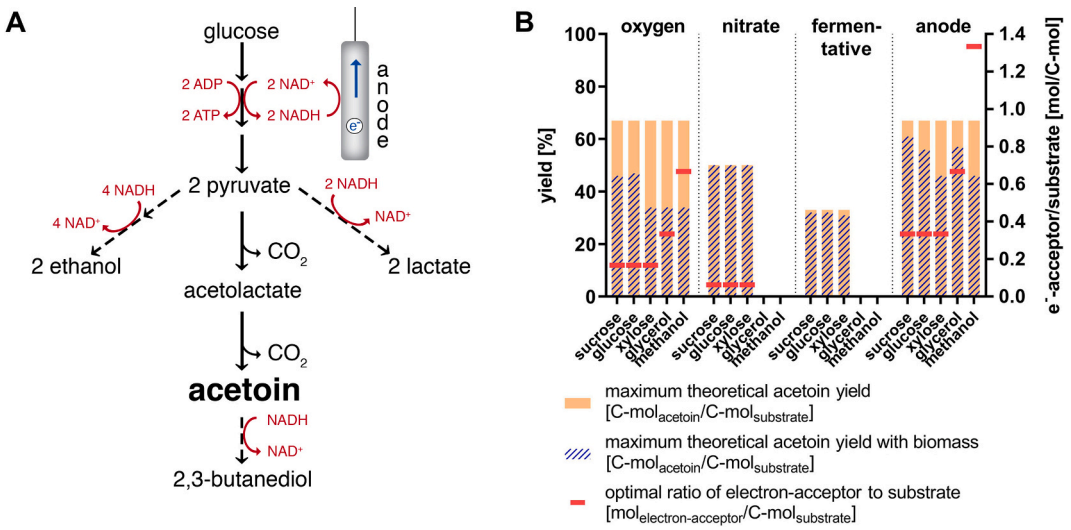

Fig. 4. Simplified metabolic pathway for the production of acetoin (solid black lines). AEF empowers to use the anode as a terminal electron acceptor thereby potentially regenerating NAD^+, which can minimize the synthesis of by-products (dashed lines; A). Summarized results of the theoretical exercise with *B. subtilis* testing different substrates (sucrose, glucose, xylose, glycerol and methanol) under fermentative conditions and using different electron acceptors (oxygen, nitrate and an anode). The bar chart shows the maximum theoretical acetoin yields with and without biomass formation (i.e. growth) and the optimal ratios of electron-acceptor to substrate (B).

Under fermentative conditions, only 33% maximum theoretical acetoin yield could be reached for sucrose, glucose or xylose as substrate. Interestingly, the case with the highest yields that also allowed the formation of biomass (i.e. growth) were obtained with an anode as an electron acceptor, highlighting the potential of AEF to improve acetoin production with *B. subtilis*. With nitrate, the maximum yield with biomass formation remained at 50%, while oxygen was even below that (34–47%), but higher than fermentation, for which yields were slightly below 33%. Glycerol and methanol are seen as sustainable and promising next-generation substrates in industrial biotechnology but those alcohols are more reduced than the investigated sugar substrates. Therefore, higher ratios of electron-acceptor to substrate are required for the alcohols, respectively 2- and 9-fold higher for glycerol and methanol, than for the sugars. Since the anode is in fact an undepletable electron acceptor, AEF would efficiently support the utilization of such reduced substrates.

This short theoretical exercise underpins the perspective of this review article. AEF has the potential to improve the synthesis of a target product by eliminating the need of providing the microorganisms continuously with a consumable electron acceptor by instead supplying an undepletable electron acceptor, an anode.

6. Outlook on anodic electro-fermentation

As discussed above, AEF offers numerous advantages over conventional industrial fermentation processes. The aim of AEF should not only be to match the rates/yields to current production processes, but to transform the way in which commercial fermentations are undertaken due to e.g. its unique capacity to provide an undepletable electron acceptor. For that to occur, however, significant progress remains to be made with regards to the up-scaling of AEF reactors to meet industrial-scale demands (Krieg et al., 2014). This review highlights that such advancements are within reach because AEF can directly benefit from decades of research into METs and fermentation systems, particularly materials and reactor architecture/configurations.

Currently, AEF research (Table 1) is performed in custom-made bioelectrochemical reactors with relatively small volumes (mainly <500 mL), the scalability of which has not been considered. Given, however, that the main objective of AEF is the recovery of valuable fermentation products, the latter must be prioritized in AEF for any up-scaling and practical implementation. Accordingly, any AEF up-scaling efforts should be based on state-of-the-art fermenters specifically modified for electro-fermentation. First attempts to integrate electrochemistry into 0.75–2.4 L commercial fermenters by the addition of solid electrodes within the fermenting vessel have been reported by (Rosa et al., 2017), (Krieg et al., 2018), and (Rosa et al., 2019). For example, the AEF process by *C. glutamicum* was scaled-up from a 0.35 L custom-made bioelectrochemical reactor to a 2.4 L electrochemically modified fermenter resulting in comparable performance on both scales (Krieg et al., 2018). If the electrochemical components are optimally positioned into the fermenter the mixing is not negatively affected and the mass transfer performance is comparable with conventional bioreactors or even improved (Krieg et al., 2018; Rosa et al., 2019).

Thus, the first results on integrating electrochemistry into fermenters are promising. However, further reactor optimization requires detailed fundamental knowledge about microbial EET mechanisms as microorganisms-anode interaction determines the efficiency of the AEF process. Understanding EET mechanisms will assist in choosing the ideal electrode material, structure and configuration (Xie et al., 2015). While flat electrode materials are often used in laboratory experiments, 3D-electrode materials enable higher surface area for biofilm growth, which supports maximizing microbial cell retention and electron transfer to the anode (Yu et al., 2017). In addition to the electrode surface area, the placement of the electrodes and the membrane needs to be considered to minimize overpotentials and other losses (Clauwaert et al., 2008). Carbon-based electrodes are commonly used anodes in BES (Table 1) due to their great biocompatibility, good conductivity and low cost (Li et al., 2017). For example, packed bed reactors with, e.g. graphite granules, are scalable and showed promising results in MFCs, while the mass transfer in the reactor could be further enhanced when the bed was fluidized (Borsje et al., 2019; Quejigo et al., 2019). High electrode surface and efficient mass transfer are especially important, if utilizing soluble mediators for electron transfer (Lai and Krömer, 2019). In regard to mediated electron transfer, the supply of artificial mediators is only economically feasible in AEF when the mediator can be efficiently recycled (Arinda et al., 2019). However, microorganisms capable of natural production and secretion of mediators into the broth are preferred as it maintains the AEF process sustainable and simple (Schmitz et al., 2015). Fig. 5 summarizes which aspects are crucial when designing a reactor for AEF. Besides, the design of a AEF process can be

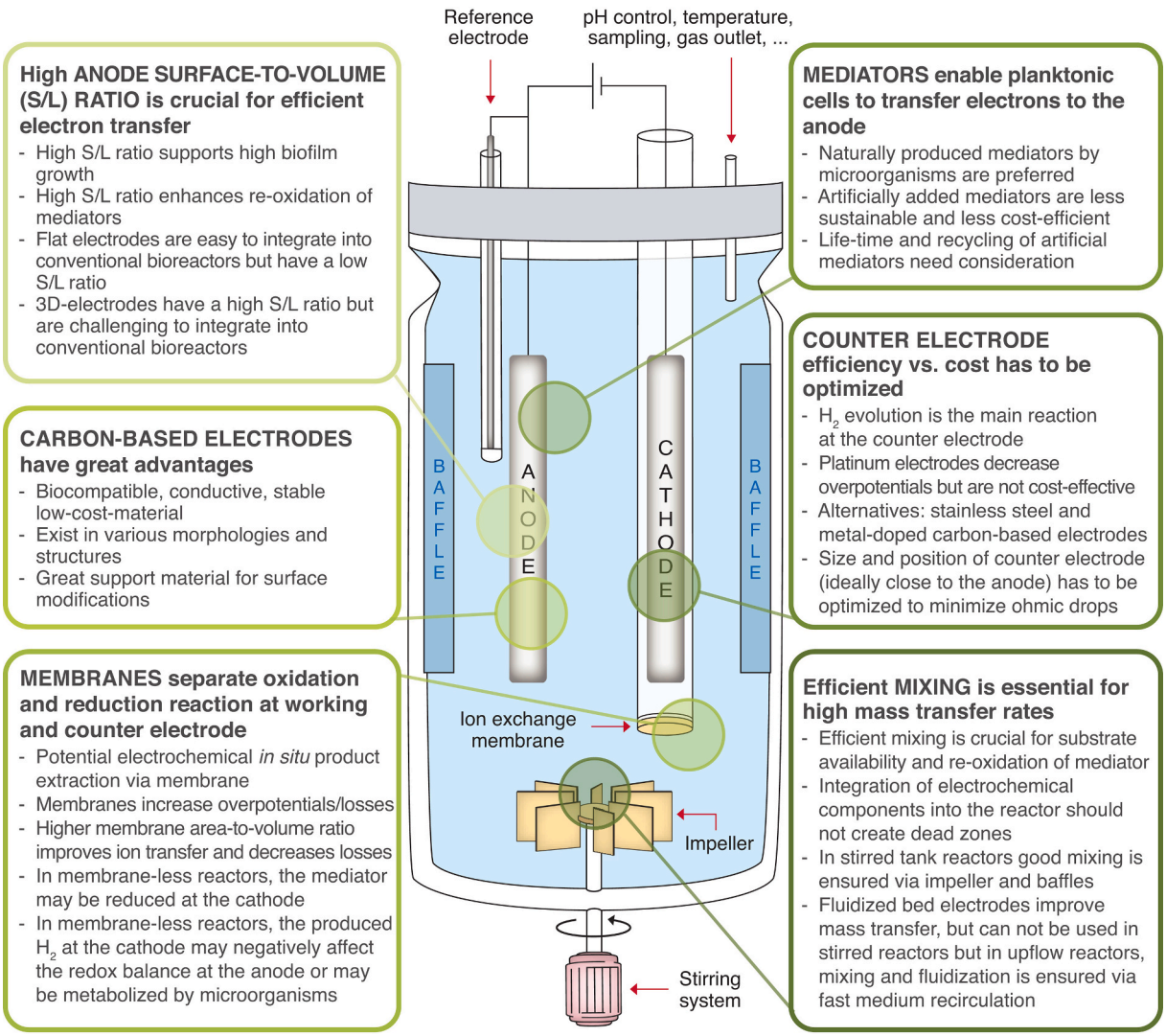

Fig. 5. Aspects needed to be considered when designing a bioreactor for AEF. The illustration shows a stirred tank reactor with integrated electrochemical components for AEF inspired by (Krieg et al., 2018). The discussed reactor design aspects are referred to (Roy et al., 2016) and (Krieg et al., 2014).

assisted by mathematical models improving biological, reactor configuration and operational parameters (Gadkari et al., 2018). Finally, the optimized process design should be followed by a techno-economic assessment.

7. Conclusions

Biotic anodes are mainly associated with MFCs for power generation, while microbial production of chemicals in BESs is mainly undertaken with biotic cathodes, especially the conversion of CO_2 into organic acids and alcohols via MES using the cathode as an electron donor. However, depending on the substrate-product combination and the microbial catalyst in a bioproduction process, the right choice of electrode can fall on an anode, which can be used by the microorganisms as an inexhaustible electron sink to discharge "surplus" electrons, stabilizing microbial redox and energy states. AEF can be seen as a tool to enhance the bioproduction of chemicals by combining advantages of aerobic respiration and fermentation via respiration of an anode under anaerobic conditions. Furthermore, AEF supports the use of substrates with a high degree of reduction (e.g. glycerol and methanol) and the synthesis of more oxidized products than the substrate.

However, AEF is a very young technology and besides technical challenges, such as scaling up AEF reactors, only a few microorganisms have been analyzed as candidates for AEF. More research is needed to better understand the mechanism(s) of microbial electron transfer to the anode and how the electron transport chain can be efficiently coupled to the central metabolism to direct carbon flow towards a target product. A key challenge in this emerging research area is that the electron transport mechanisms vary greatly in different microorganisms, which makes it difficult to identify a universal model organism. Therefore, this perspective review article does not only summarize the current stage of AEF research but also aims to encourage further studies on different potential microbial candidates such as *B. subtilis* for AEF, and to advance this emergent field, with the ultimate goal of bringing this technology one step closer to real-world applications.

Declaration of Competing Interest

None.

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