

Focus on Bioelectrochemistry

## Review

# Electro-Fermentation – Merging Electrochemistry with Fermentation in Industrial Applications

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**Electro-fermentation (EF) merges traditional industrial fermentation with electrochemistry. An imposed electrical field influences the fermentation environment and microbial metabolism in either a reductive or oxidative manner. The benefit of this approach is to produce target biochemicals with improved selectivity, increase carbon efficiency, limit the use of additives for redox balance or pH control, enhance microbial growth, or in some cases enhance product recovery. We discuss the principles of electrically driven fermentations and how EF can be used to steer both pure culture and microbiota-based fermentations. An overview is given on which advantages EF may bring to both existing and innovative industrial fermentation processes, and which doors might be opened in waste biomass utilization towards added-value biorefineries.**

## From Fermentation to Electro-Fermentation

Fermentations have played a fundamental role in food production, transformation, and conservation throughout human history. For centuries, foods have been fermented to better facilitate their storage, increase their stability, and modify their organoleptic and textural properties [1]. Over the past few decades efforts have been oriented towards the design of metabolic pathways in bacteria traditionally used for the production of fermented foods and food ingredients. Thanks to some recent advances in genome sequencing, comparative genomic analysis, and gene cloning, specific compounds with particular sensorial, textural, nutritional, and health attributes have been synthesized [1,2]. Beyond food transformation with indigenous **microbiota** (see [Glossary](#)), metabolic pathway engineering has been used to target industrial syntheses of metabolites in bioreactors using **pure microbial cultures** (PMCs) with selected strains [3].

Industrial fermentations have different production targets: whole microbial cells (e.g., probiotics); primary metabolites (e.g., citric acid, glutamic acid, vitamins, etc.); secondary metabolites with antimicrobial, growth-promoting, enzyme-inhibiting, and pharmacologically important properties; enzymes; recombinant products; biofloculants; and the biotransformation of complex food ingredients [4,5].

Fermentation technologies at the industrial level have several constraints ([Box 1](#)) that often limit their application, their environmental sustainability, and their economic feasibility.

## Trends

Traditional industrial fermentations show several limiting constraints.

Electro-fermentation drives metabolism with the direct integration of an electrochemical cell.

Electro-fermentation offers a hybrid metabolism in which electrons are exchanged with solid electrodes as alternative oxidizing/reducing equivalents.

Electro-fermentation can (i) stabilize/optimize fermentation metabolisms by controlling imbalances due to substrate purity, redox/pH conditions, byproduct accumulation, etc.; (ii) establish oxidative or reductive conditions to drive carbon chain breakdown or elongation; (iii) increase ATP synthesis and improve microbial biomass yield; and (iv) extract target products by selective membranes.

Catalytic activity of electro-active microorganisms, metabolic pathways, electrode materials, reactor technology, and product recovery: a multidisciplinary research approach is needed.

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### Box 1. Constraints of Traditional Fermentations

Fermentations mainly rely on relatively pure substrates, such as glucose, glycerol, cornstarch, sugar cane hydrolysate, vanillin, and other chemicals [65]. Upstream production of pure and sterile carbon sources is often the most important fraction of the cost [66]. Dedicated land-use and agroindustrial transformations also limit the overall sustainability in terms of a life-cycle assessment of the whole production chain, especially for bulk chemicals produced in large quantities [67]. For succinic acid, for example, corn-based dextrose production accounts for almost 50% of the overall equivalent greenhouse gas (GHG) emissions and cost in terms of the energy invested [67].

Culture media are, in most applications, highly specific and optimized for each microbial strain and metabolic pathway. Microbial metabolism is controlled by well-defined concentrations and types of nitrogen sources, vitamins, minerals, buffers, chelating factors, and antifoaming agents. All these ingredients contribute heavily to the overall cost of production.

Air or pure oxygen is sparged into culture media as an electron acceptor to balance the redox equivalents of the microbial metabolism. Di-nitrogen is used in anaerobic fermentations to limit the accumulation of gaseous metabolites ( $H_2$ ,  $NH_3$ , etc.) [68]. By contrast, when additional electron donors are needed,  $H_2$  is sparged, or other low-molecular soluble compounds (formate, acetate, etc.) are used to enhance reductive metabolism [69,70]. Micro-bubbling has typically limitations in its mass-transfer efficiency owing to the limited solubility of the gas phase. To enhance the solubilization of the gas phase, fermenters are pressurized, with increased reactor engineering costs [1]. PMC stability and reactor sterility is also an issue when large quantities of ambient air are sparged, despite air filtering and sterilizing systems [1]. Finally, gas sparging increases foam formation in fermentation broths, requiring foam monitoring devices and continuous dosage of antifoaming agents.

pH is normally controlled by adding buffers to the culture media or by adding acid (e.g., HCl) or alkaline solutions (e.g., NaOH). One issue is maintaining the pH without influencing the osmotic conditions and salinity of the solution as a result of the accumulation of ions (e.g.,  $Na^+$ ,  $Cl^-$  etc.) [71].

All soluble metabolites are difficult to isolate from culture media and other secondary compounds and impurities. In addition, high concentrations of products or co-products in the medium may inhibit both microbial growth and metabolism.

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Product selectivity and purity are achieved using specific and pure substrates (e.g., refined sugars) obtained through dedicated agro-industrial production chains. This and other upstream operations (screening, selection, and maintenance of suitable hyper-producer strains; development of a suitable medium; starter culture propagation in sterile conditions and sterilization of the fermenter for inoculation) account for 20–50% of the final costs [6]. Fermentations are often redox-imbalanced, which severely limits the product selectivity from a given substrate [7]. Traditional strategies to alter redox balances include gas sparging (air,  $O_2$ ,  $N_2$ ,  $H_2$ ), the addition of co-substrates, and pH control. Chemical control of the medium conditions is also an issue (acid/base, chelating agents, buffers, antifoam agents). Finally, downstream processing for product separation and refining might account for up to 80% of the total cost.

By introducing electrodes, fermentation environments can be controlled and optimized to obtain products with higher purity, to favor microbial cell growth and density, or to achieve chain elongation [8]. This approach was until recently rarely reported in the literature and was only recently referred to as **electro-fermentation** (EF) by Rabaey and Rozendal in 2010 [9] and Rabaey and Ragauskas in 2014 [10]. Simply put, the key role for electrical current is as an alternative source of reducing or oxidizing power, potentially more sustainable than conventional substrates produced through crops (e.g., glucose) [11].

Over the past four decades a series of sporadic efforts have been carried out to apply electrical potentials to fermentative microbial populations to promote cell reproduction and metabolism for different microbial species (Figure 1). We review the principles of this approach, the results achieved so far, and the possibilities of applying EF to industrial fermentations and bio-based refineries.

### Principles of EF

To support their metabolism, all organisms need an electron donor and acceptor. An elegant alternative is to supply or subtract electrons to microbial cell metabolism via direct

electrochemical means [9,12]. Microorganisms able to use inorganic compounds as electron donors/acceptors are termed lithotrophic – either lithoheterotrophic or lithoautotrophic depending on whether they use organic compounds or CO<sub>2</sub>, respectively, as a carbon source [9]. These organisms were demonstrated to have the particular capacity of using solid conductors as electron donors or acceptors [13]. This effect can be achieved via direct **extracellular electron transfer** (EET) and transmembrane transport, using nanowires, cytochromes, NADH-ubiquinone oxidoreductase, hydrogenase, and other membrane-bound enzymes [14–16]. EET, as well as transmembrane transport to the cytoplasm, can also be mediated by specific molecules [12,15]. Soluble redox **mediators** (e.g., thionin, neutral red, methyl viologen, riboflavins, humic acids, etc.) can act as electron shuttles from the electrode surface to the microorganism [9], and also can diffuse through the periplasm to the cytoplasm as reducing/oxidizing agents for the NAD/NADH couple [15]. Through this mechanism, chemo-organoheterotrophic organisms (which normally find electron donors/acceptors in the form of organic molecules and do not use EET as a means of ATP synthesis) can also be electrochemically active [16]. Examples have been reported for acetogens including *Clostridium ljungdahlii*, *Moorella thermoacetica*, and *Sporomusa ovata* [17].

According to Thrash and Coates [12] and previous reports [18], the energy contained in the ATP a microbe can obtain from a given metabolic pathway is directly proportional to the potential energy difference,  $\Delta E^\circ$  (in volts), between the electron donor and the electron acceptor. The  $\Delta E^\circ$  can be calculated from the Gibbs free energy of the overall reaction, according to Thauer *et al.* [18]:

$$\Delta E^\circ = -\frac{\Delta G}{nF} \quad (1)$$

where  $\Delta G$  is the Gibbs free energy of the reaction (in J/mol),  $n$  is the number of electrons involved in the reaction (in mol), and  $F$  is Faraday's constant (96485.3 C/mol). The Gibbs free energy of a reaction represents the maximum amount of energy that the reaction can produce (exoergonic) or needs (endoergonic), and has been reported for several different reactions [18,19]. The sign of  $\Delta E^\circ$  determines whether electricity is produced or must be supplied to drive the reaction.

Anodic and cathodic currents can steer specific microbial metabolic reactions through different mechanisms (Figure 2). At the anode, the solid conductor accepts electrons derived from oxidative reactions and behaves as a favorable sink for reducing power [20]. Maximizing the efficiency of electron 'subtraction', through oxidation of intermediate metabolic electron acceptors, can foster NADH consumption and create proton gradients sufficient for ATP generation [15]. At the cathode, the electron supply from solid conductor to the cells forces the NADH pool to a more reduced state, induces the reduction of metabolites from their oxidized forms, and can also allow the production of additional ATP [15].

Fermentative routes can be electrically enhanced by influencing specific electron transport paths and by improving energy conservation mechanisms (i.e., ATP formation). H<sup>+</sup> (a key electron acceptor under anaerobic conditions) can be reduced to H<sub>2</sub> at the cathode, and H<sub>2</sub> can be oxidized to H<sup>+</sup> at the anode [18]. Similarly, other redox couples (both inorganic and organic molecules) can mediate electron transfer and serve as intermediate or final electron acceptors, depending on the free energy available from the overall reaction [21].

Fermentation pathways and electron transport routes can benefit from either electron supply (cathodic reaction) or subtraction (anodic reaction). This process depends on the degree of reduction of the product/metabolite couple and also on the stoichiometric production of NADH during substrate breakdown [16]. Determining the stoichiometric redox balance of a

## Glossary

**Counter electrode:** every electrochemical process requires a counter-reaction, which takes place at the counter electrode. It can be either a cathode or an anode.

**Extracellular electron transfer (EET):** the capacity of electrically-active microbes to exchange electrons with solid conductors.

**Electro-fermentation (EF):** microbial transformation of organic molecules enhanced by mediated electron transfer (MET). Electrodes can be substitute of chemical electron donors or acceptors, to stabilize/control/steer fermentation environment and conditions, in terms of redox balance, pH, thermodynamics, etc.

**Ion (anion/cation) exchange membrane:** type of membrane that is selectively permeable to either anions or cations.

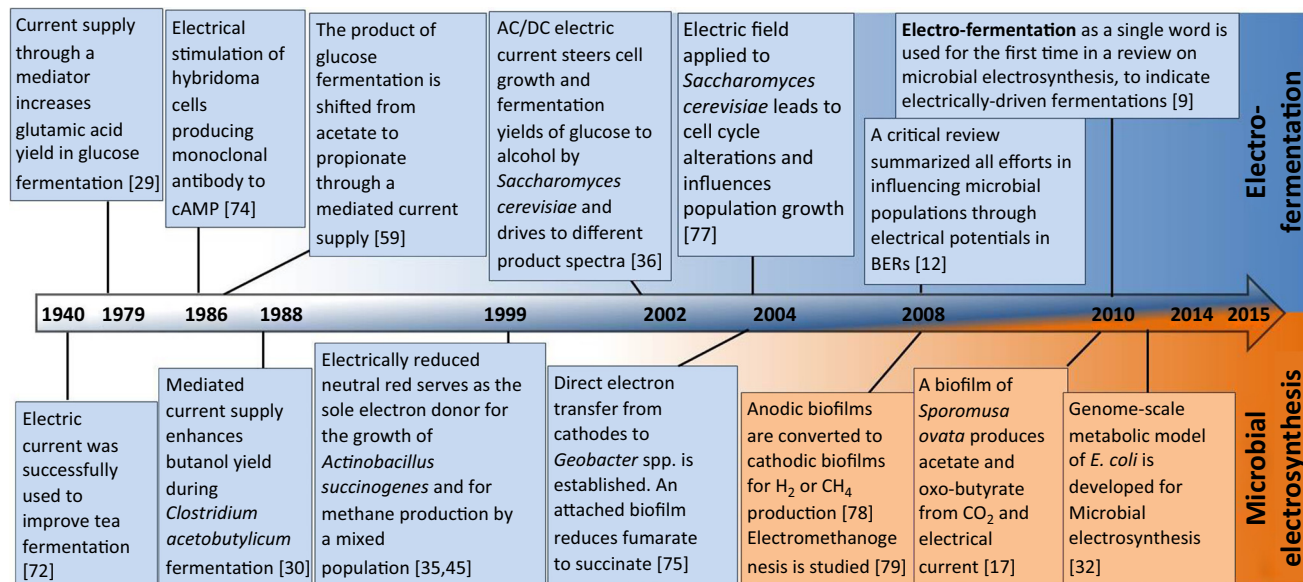
**Mediator:** also called an electron shuttle; are usually aromatic compounds or metal complexes with delocalized electrons, which can transfer electrons between an electrode and a redox-active substance.

**Microbial electrosynthesis:** cathodic microbial reduction of CO<sub>2</sub> to chain elongated organic compounds.

**Microbiota:** mixed/unknown microbial cultures. They can be co-cultures of more than one selected species or natural consortia of microbial populations that are obtained from environmental sources and enriched by natural selection in given fermentation conditions.

**Pure microbial cultures (PMCs):** in industrial fermentations, microbial strains are selected or genetically modified to favor 'hyperproducers' and are maintained in axenic conditions to avoid contamination by other species.

**Suspended microbial population:** in fermentations, microbial cells are normally suspended in the liquid bulk, which is agitated to ensure homogeneous distribution of both cells and soluble organics.



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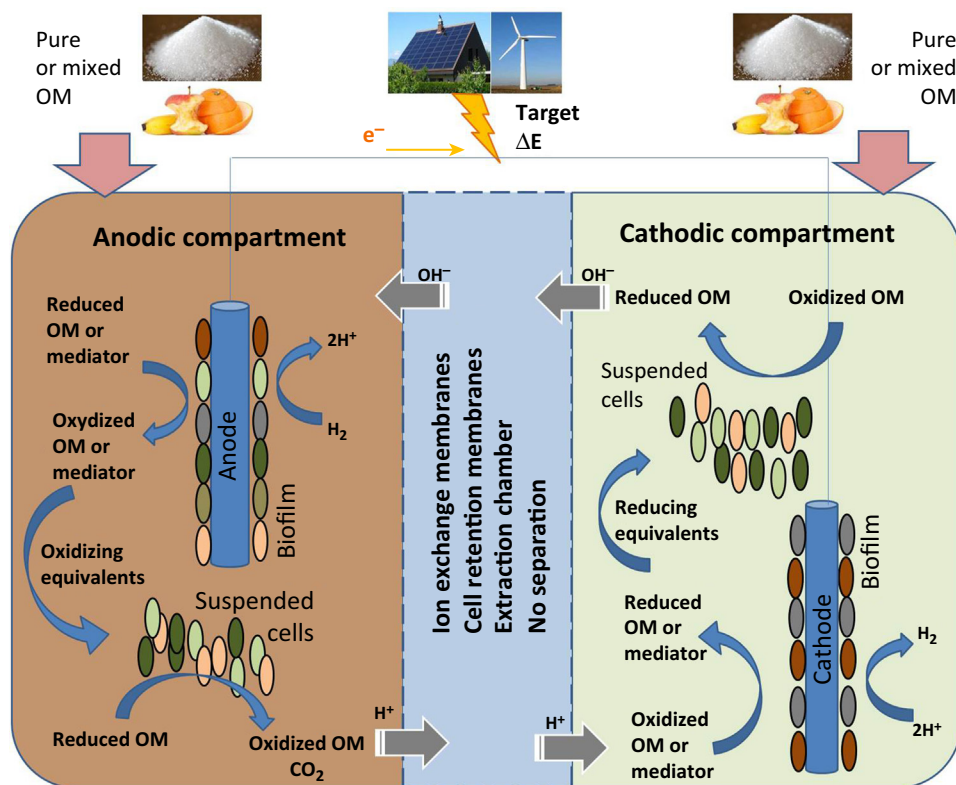
**Figure 1. Historical Overview of Efforts To Influence Fermentations by Electrical Current.** While the hybrid term 'electro-fermentation' has been used only sparingly in the literature, 'electro fermentation' has more often been used as two separate words; these works all concern electrochemically driven/influenced fermentations which originate from different approaches over the past 70 years. In 1940, Lominadze [72] reported evident positive effects of electric current applied to tea fermentation (process time reduced to half, improved brew and taste). Afterwards, in 1979, Hongo and Iwahara [29] showed that current supply through a mediator increased L-glutamic acid yield during glucose fermentation. In the following three decades, other studies have reported similar achievements with essentially only pure microbial culture (PMC; bacteria or yeasts) [30,35,36,59,73–77]. In 2008, Thrash and Coates [12], in a critical review, summarized all efforts to influence microbial populations behavior through the application of electrical potentials. Almost all these works were performed using PMC, or enriched MC, and started from pure substrates. Contemporarily (in 2008), the first approaches in microbial electrosynthesis were published. The pioneer efforts were driven by Rozendal *et al.* [78], in 2008, who for the first time converted anodic biofilms to cathodic biofilms for H<sub>2</sub> or methane production, by Cheng *et al.*, who introduced the concept of electromethanogenesis [79], and by Rabaey and Rozendal [9] who used for the first time the term 'electro-fermentation' to indicate electrical driving of fermentation pathways. Today, microbial electrosynthesis and electro-fermentation (EF) should be integrated, and new mediated electron transfer (MET) processes should be designed to simultaneously meet the differing requirements of process parameters typical of fermentation reactors and at the same time the electrochemical parameters. Abbreviation: BER, bio-electrochemical reactor.

fermentative pathway from a given substrate is essential to understand whether electron supply or subtraction can enhance the formation of a target product [7].

An exhaustive mechanistic model for the EF of glucose and glycerol, with calculated theoretical yields of different metabolites, was recently reported by Kracke and Krömer [15]. According to these authors, while an excess of redox cofactors (NADH and NADPH) induced by a cathode can drive specific reduction reactions, the consumption of reducing power by an electron sink (i. e., an anode) can improve microbial biomass yields. The presence of a preferential and favorable electron acceptor (the anode), which is normally not available in anaerobic catabolism, drives additional ATP generation and a possible increase in biomass yields [15]. This was recently demonstrated for *Pseudomonas putida*, an obligate aerobe, in the production of 2-ketoglucuronate from glucose. Here an anode was used as alternative electron sink, substituting for O<sub>2</sub>, in an anoxic fermenter [22].

### Traditional Industrial Fermentations: Pure Microbial Cultures Versus Enriched Microbiota

Because microorganisms are rarely present as single cultures in nature, it does not come as a surprise that traditional microbial processes, for example in food and beverage production, are based on microbiota, enriched by adequate procedures. However, with industrialization, the practice of microbiota-driven fermentations was gradually replaced with PMC fermentations,



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**Figure 2. Possible Anodic and Cathodic Mechanisms.** At the anode, microorganisms utilize the solid conductor as a favorable electron acceptor during the oxidation of organic molecules (OM). In traditional oxidative fermentations, the lack of favorable electron sinks inhibits catabolic reactions towards target products, leading to partial formation of metabolites with different oxidation states. If this reducing power is efficiently consumed by an electron sink (solid conductor), the fermentation could be driven more homogeneously to a particular product. OM can be reduced at the cathode, where microorganisms utilize the solid conductor as a favorable electron donor and a chemical species as an electron acceptor. The supply of additional reducing equivalents can enhance target reductive pathways in particular metabolic steps, such as chain elongation of carboxylates or alcohols.  $H_2/H^+$  and other mediators can be utilized as electron shuttles to enhance oxidative/reductive fermentations of **suspended microbial populations**. This strategy could also substantially modify the environmental conditions (redox balance, pH, etc.), thus stimulating cell growth and concentration in the bulk. Anodic microbiota-driven oxidations of secondary organic matter (e.g., waste, wastewater) can be the source of electrons to be transferred to a sterile cathodic compartment where pure microbial cultures (PMCs) can efficiently catalyze reductive bioconversions. Anodic and cathodic environments might either coexist or be separated with specific ionic exchange membranes, micro/ultrafiltration septa for microbial cell retention, or other porous media. The extraction of target metabolites can be integrated into the process. Charged anions (e.g., carboxylates) can be continuously drawn through anionic exchange membranes by electro-osmotic forces between the fermentation broth and separate chambers.

which better control the microbial environment, growth, and product formation, and allow better process prediction and control [23].

PMC processes target maximal product formation from a single substrate, usually a sugar. In rare cases, redox balance can be achieved via perfectly balanced fermentations in which all electron equivalents are recovered in a single product, facilitating downstream product separation and purification [24]. More often than not, PMC fermentations generate arrays of metabolites because, even in a single microbial species, several pathways leading to different end-products exist, whereby the cell typically achieves homeostasis [7,12]. Such processes would benefit from a further increase in selectivity for the target product and in redirecting carbon and electron flow away from biomass synthesis to product formation [25]. This approach typically results in

increased product titers, and near-maximal yields and productivities, thus leading to reduced separation costs and improved economics. In other cases (e.g., probiotics production), maximizing biomass yields could be the target, and induced oxidative conditions (at an anode) would improve ATP availability [26].

Co-cultures or microbiota may offer several advantages in the process and its economics compared to PMCs. One microorganism may produce growth factors, remove inhibitors, or generate environmental conditions that are beneficial to a second microorganism. Some cascade pathways may not be possible in a single organism, necessitating at least two distinct organisms for full product formation. The formation of new primary or secondary metabolites can be triggered in co-cultivation conditions, offering opportunities for producing novel substances [23]. As an example, Farid *et al.* optimized the inoculation by including a second strain in co-culture (*Aspergillus awamori*), achieving increased ethanol production from starch by *Saccharomyces cerevisiae* [27]. Microbiota can bring about multistep transformations that would be impossible for PMCs. Owing to their diversity, they are able to handle variations in substrate composition and have the capacity to adapt. They do not require expensive procedures to avoid contamination and maintain axenic conditions. Microbiota have higher capacity to use mixed/impure/unrefined substrates such as waste biomass [23]. Particularly when industrial biotechnology aims at the use of complex substrates, such as lignocellulosic materials or mixed organic waste (Box 2), for the production of chemicals or fuels, an array of microorganisms possess a wider range of enzymes and can attack a greater variety of compounds than a PMC [23].

However, microbiota are more difficult to control in terms of stability, side-product formation, and metabolic selectivity. The use of electrochemical means can be a successful approach in driving the metabolism of microbiota, controlling product formation, and boosting fermentation pathways, particularly when the substrate is not pure or sterile and the goal is to refine secondary streams and waste (Box 2).

### Electrochemically Driving PMC Fermentations Towards Target Chemicals

Many fermentations are redox-imbalanced, which implies that an external electron donor or acceptor needs to be provided. Commonly, oxygen is provided as the electron acceptor, but its distribution needs to be carefully managed to avoid anoxic conditions which would directly impact on the fermentation product spectrum. An electrode has been successfully applied to obtain the stoichiometric conversion of glycerol to ethanol using an engineered strain of *Shewanella oneidensis* [28]. The reverse, taking an electrode as the electron donor for L-glutamic acid formation, actually builds on the earliest work in microbial electrochemistry [29] (Figure 1). Similarly, Kim and Kim [30] demonstrated a shift towards more reduced products such as butanol and ethanol during fermentation of glucose with *Clostridium acetobutylicum*. Overall, the advantages of electrodes over supplying oxygen or hydrogen can be extensive provided that a good redox-link can be made between the electrode and the microorganisms: key examples are (i) enhanced process control because the electron flux is continuously steered; (ii) potentially lower competing biomass yields, particularly because an anode provides less thermodynamic gain relative to oxygen; and (iii) better distribution of electrons as provided *in situ*, in contrast for example to sparged hydrogen gas which needs to dissolve. Recently, Schmitz and coworkers [31] engineered a strain of *Pseudomonas putida* KT2440 to optimally make use of these advantages by inserting the ability to produce its own mediators, phenazines, thus potentially enabling it to produce biochemicals otherwise requiring oxygen sparging.

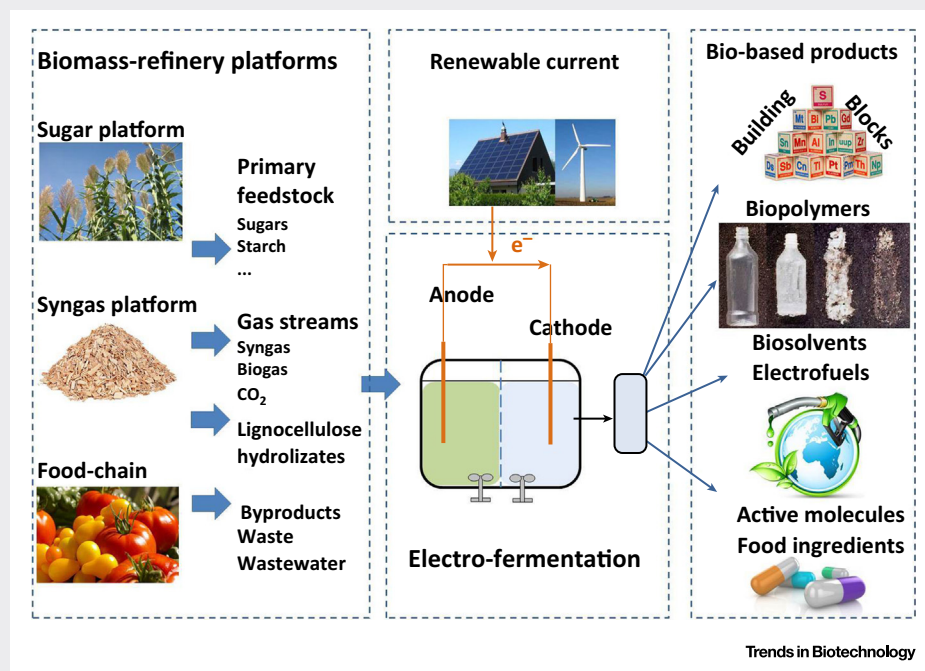
To better understand the potential benefit of electrical enhancement, Kracke and Krömer [15] created core networks of metabolic (anaerobic) carbon pathways in *Escherichia coli*, and elementary mode analysis revealed great potential to boost anaerobic glycerol and sugar fermentations, with yield improvements for 18 of 20 products of between 7% and 84%. Instead

### Box 2. Renewable Feedstock: Waste Organic Matter from Biomass Refinery Platforms

Fermentation technologies should, in the near future, embrace the use of waste/side materials as potential substrates to extract high-value compounds and complement the major platforms of industrial biomass utilization (see Figure 1 in Box 2).

First, the agro-food chain produces huge amounts of residual biomass in form of both solid biomass and wastewater byproducts [10]. In addition, a biomass-based industry is expected to develop and grow rapidly through the so-called sugar platform and the syngas platform [8]. In the sugar platform, enzymes convert biomass into five- and six-carbon sugars; in the syngas platform, thermochemical systems convert biomass into syngas (i.e., synthesis gas, such as CO, H<sub>2</sub>, and CO<sub>2</sub>). These intermediate feedstock chemicals are converted further by catalytic/chemical/microbial/enzymatic processes to bulk chemicals and fuels. Most of these processes require high substrate purity, and the industrial refinery chains generate huge amounts of residual organic matter as byproducts that will need to be further valorized to make the whole chain sustainable. EF could play a crucial role in this area.

Furthermore, the carboxylate platform was recently proposed [8,82] as a novel field of research and development, where an organic feedstock (especially waste/residual biomass) is first converted through hydrolysis and primary fermentations to soluble and bio-available intermediate organic matter, which can be then used as a substrate for secondary bio-conversions to produce target carboxylates or chemicals such as alcohols, solvents, short-chain fatty acids, aldehydes, poly-hydroxy-alkanoates (PHA), biopolymers, and methane. EF could help in optimizing the use of all of these organic materials, with increasing degrees of complexity and concentrations of impurities, to drive the synthesis of new products with higher degrees of purity.



**Figure 1. The Role of EF in Biorefineries.** Electrochemically induced microbial processes could broaden the possible range of fermentation substrates (including waste organic matter), optimizing traditional industrial fermentation processes, reducing chemical addition for process control (e.g., pH, redox regulation, antifoaming agents, etc.), steering continuous product separation through membranes, and widening the range of high-value compounds that can be produced. By improving metabolic pathways by electrochemical control, non-sterile microbiota-based fermentations could be more easily influenced to handle complex/impure organic substrates (such as waste-streams), where PMCs would fail, especially in commercial-scale applications. A significant step forward would be achieved when PMCs or microbiota, under electrode-induced reductive or oxidative conditions, could transform the wide variety of soluble organic compounds available as byproducts of food-chain, biomass refineries (side-products, waste, and wastewaters) and/or gaseous streams such as syngas (from biomass), fermentative gases (i.e., H<sub>2</sub> + CO<sub>2</sub>, biogas) and industrial flue emissions [80,81].

of the degree of reduction of the product, the electron transport mechanism determined the impact of electrical enhancement [15]. For particular networks, stoichiometric conversion of substrate to product at the cathode appeared to be possible in true catalytic mode. In line with this, Pandit and Mahadevan [32] showed that trade-offs can exist between improved growth

### Box 3. Microbe–Electrode Interaction and Possible EF Reactor Architecture

As extensively reviewed elsewhere [12,15], microbes can exchange electrons with solid conductors through (i) direct transfer (DT) and (ii) mediated transfer (MT). In DT, electrically-active populations typically form a biofilm in direct contact with the active sites of the solid through the transfer components of the cells (e.g., cytochromes or pili) [13]. In MT, soluble and redox-active molecules (e.g., H<sub>2</sub>, flavins, humic acids, neutral red, etc.) act as electron shuttles [15], and the microbial population can also be suspended in the bulk medium.

Carbon and graphene materials are widely used as electrodes in view of their high conductivity, good chemical stability, and relatively low cost [83]. By modifying the electrode properties, the electronic conductivity and electron transfer can be improved [84]. Depending on the porosity and surface area of the material and biocompatibility, biofilm adhesion can be enhanced or avoided [85,86]. The presence of surface functional groups such as oxygen and nitrogen can improve electrochemical reactions at electrode surface [87].

In EF applications, fermenters will need to be adapted to host electrodes and their structures might need to be revised according to electrochemical requirements [64]. Up to now, only laboratory-scale fermenters have been developed, and a challenge for scaling-up EF applications will be related to the materials of the electrodes and architecture of the fermenters.

Electrodes can be either fixed or mobile [64]. In the first case, the culture medium must remain in contact with the biofilm for a sufficient time; optimized designs would impose flow-through configurations in which the medium is agitated and the electrode surface area is maximized with respect to reactor volume [88]. Mobile electrodes are made of particulate solid conductors suspended in the bulk medium and colonized by biofilm; this technique can be used in fluidized bed reactors, where electron discharge to an external circuit takes place by stochastic contact between the working electrode and the conductive particles [89]. When the aim of EF is to stimulate suspended microbial populations, MT is preferred method of electron transfer, and a mediator should be chosen according to the microbial species [89].

In general, working and **counter electrodes** and their relative reactions can either take place in the same reactor section or be separated within different chambers by one or more separators (e.g., ion exchange membranes). If the counter-reaction products are compatible with the purity of the working reaction products, the single-chamber configuration is the ideal, and traditional fermenters with the simple addition of electrodes may act as EF systems. Where anodic and cathodic products or conditions can negatively affect the medium and the product purity, EF must take place in dedicated reactors with proper separators [89]. When *in situ* product extraction and concentration is the objective, multiple separators could be included [57].

rates and yields. In both cases, a sequential or dynamic strategy was proposed to optimize productivity by first maximizing growth rate, without electrical enhancement, and then improving product yield with electrical enhancement. Mathew *et al.* [33] reported a significant increase in both ethanol yield (twofold increase, reaching 14% v/v) and productivity (2–3-fold faster), by applying to a *Saccharomyces cerevisiae* culture a static potential of up to 15 V (without any resulting current). Similar results were shown for PMF using *Clostridia* spp. for butanol production or using *Brevibacterium* spp. for glutamate production [34].

The combination of fermentation and MET not only may improve yields and/or productivity but also can increase process kinetics, change and/or reduce the fermentation product spectrum [35–37], and drive thermodynamically unfavorable reactions [9]. These insights may lay the basis for a new platform of next-generation bioproduction strategies (Box 3).

### Electrochemically Driving Microbiota

Over 50 years of experience with anaerobic digestion (AD) have shown that beneficial interactions between various trophic levels are crucial in the biotransformation of complex and impure substrates like waste and residues [38,39]. Syntrophic interactions between groups of microorganisms in the anaerobic food web keep the concentrations of crucial intermediates below a threshold and increase the Gibbs free energy released by intermediate reactions [40]. In microbial electrochemical technologies, electrodes can serve as alternative means of drawing or supplying electrons to intermediate trophic levels [39]. Electrochemical approaches were demonstrated as innovative tools to manipulate microbiota and to link their structure, composition, and function to reactor performance, stability, and product formation [41].



Zhao *et al.* [42] recently demonstrated that inserting electrodes into an anaerobic digester led to the bioelectrochemical enrichment of *Geobacter* species in the suspended sludge and the anodic biofilm. To explain the increased methane production in the bioelectrochemical system and the fact that 50% of the produced methane resulted from an unknown pathway, they also hypothesized direct interspecies electron transfer between *Geobacter* and *Methanosaeta* species as an alternative to the long-held assumption that H<sub>2</sub> or formate are the primary interspecies electron carriers in conversion of organic matter to methane. With this strategy, EF would provide additional electron donors or acceptors to the cells [43,44]. This additional supply helps in overcoming the endothermic barrier of H<sub>2</sub> formation [44] or other metabolic redox limitations that can be crucial in determining the viability of an industrial biotechnology process [15].

Beyond AD, more specific microbiota-driven fermentations can benefit from EET to/from electrodes. EF has shown the possibility to better control the metabolic pathways of microbiota and to optimize waste-derived substrates conversion to target compounds. Park and Zeikus [45] observed that an electrically reduced mediator (neutral red) served as the sole source of reducing power for the growth and metabolism of pure and mixed cultures of H<sub>2</sub>-consuming bacteria. The same mediator was subsequently oxidized by *Actinobacillus succinogenes* to produce succinate by fermentation of H<sub>2</sub> and fumarate from CO<sub>2</sub>. Greenfield Ethanol Inc. [46] patented a method for producing hydrogen from organic material, in which both dark fermentation and electro-assisted fermentation are applied in a single bioreactor using a microbiota. In addition to producing H<sub>2</sub> gas, the process and system are also applicable to the production of other chemicals, including acetone, butanol, ethanol, acetic acid, and butyric acid [38].

EF has also been tested for improving the rates and yields of glycerol conversion. Selembo *et al.* [47] were the first to employ polarized anodes and cathodes in single-chamber, batch operating glycerol fermentations, and they were able to increase the hydrogen yields produced by conventional glycerol fermentations. Later, Dennis *et al.* [48] studied the metabolites produced during continuous, bioelectrochemically altered glycerol fermentations. The electrical current did not significantly alter 1,3-propanediol (1,3-PDO) production (e.g., polymer synthesis, solvents, antifreeze); however, alcohols and medium chain fatty acids were formed. Zhou *et al.* [49,50] demonstrated that the conversion of glycerol to 1,3-PDO can be stimulated by imposing a cathodic potential (−0.9 V [49]) or cathodic current (from 1 A·m<sup>−2</sup> to 10 A·m<sup>−2</sup> [50]) to microbiota fermenting glycerol. Both strategies redirected glycerol metabolism from propionate fermentation to 1,3-PDO production. Finally, Xafenias *et al.* [51] studied glycerol-fermenting biocathodes, reporting the highest concentrations of 1,3-PDO in a glycerol EF study (42 g/L) at a cathode potential of −1.1 V (inoculum: anaerobic sludge from municipal wastewater treatment).

EF of added value products from organic wastes was also reported in the literature. Steinbusch *et al.* [52] reported on the biological reduction of acetate with hydrogen to ethanol, applying a cathode potential of −0.55 V. Four major products were formed: ethanol, H<sub>2</sub>, *n*-butyrate, and the non-reversible reduced methyl viologen. Ethanol production (1.82 mM) had a Coulombic efficiency of 49%. The same researchers produced medium-chain fatty acids by decreasing the cathode potential to −0.9 V, without adding an external mediator. Caproate, butyrate, and smaller fractions of caprylate were the main products formed from acetate with hydrogen produced *in situ* [53].

All these experiments have demonstrated the possibility of using EF as a mean of controlling the metabolism of microbiota or co-cultures. This achievement would open great perspectives in waste refineries (see Figure 1 in Box 2).

### EF Integrated with Separation/Extraction Technologies

Production and recovery in bio-processes are inexorably linked, particularly in the conversion of complex substrates such as organic waste [54]. An ideal production strategy has a single substrate that is converted through a defined pathway to a single product. Ideal recovery takes place in a stream in which the target product is present in a sufficiently large fraction relative to other compounds, and the product exhibits some unique physicochemical properties by which it can be separated. Organic waste-streams tend to be complex broths that can contain a range of gases, salts, small organics, and complex polymers. Converting such a broad range of substrates to a single product is nearly impossible, and bio-production from wastes therefore often targets specific elements of the stream. These substrates still participate in bioprocesses, however, and therefore some fraction of the organism or the community will likely be devoted to production outside of the target.

In fermentation, alcohols and volatile fatty acids (VFAs) are interesting target products because they can be differentiated by volatility and hydrophobicity. This possibility is attractive in physicochemical separations, but problematic in production and titer, because many hydrophobic compounds have strong toxicity to bacteria. Low-titer production can manifest in high capital and operational costs in concentration by dewatering and distillation, with an equivalent impact for added energy and chemicals. Following the concentration of the target product, removing trace organic compounds with similar physicochemical properties can contribute to the high costs of extraction. The recovery and purification of succinic acid was estimated at 60% of the total capital costs, in part due to co-products such as acetic and formic acids [55].

VFAs exist as charged anions in neutral broths, and short- to mid-chain VFAs, including acetic, butyric, lactic, and caproic acids, have been demonstrated to migrate across **an ion exchange membrane**, in this case an anion exchange membrane (AEM) in a process termed membrane electrolysis [56]. This process was demonstrated for the **microbial electrosynthesis** of acetic acid from CO<sub>2</sub> [57], the anaerobic elongation of C6-VFA to C8 [58], and the homolactic acid fermentation of glucose by *Lactobacillus delbrueckii* [59]. AEMs are permeable to a wide range of charged species, including other chemical precursors such as succinic acid [55]. An electrochemical extraction is able to extract directly from a fermentation broth, and this can prevent product inhibition [57]. The extraction efficiency strongly depends on the product concentration (a higher concentration implies a greater efficiency). This property represents a core challenge for electrochemical extraction because the product must be at a sufficiently high concentration to allow efficient extraction, although it must be below inhibition thresholds. Concentration inhibition for VFAs in anaerobic sludge was observed at an inhibition threshold of around 15 g/L (as chemical oxygen demand) for short-chain VFAs, with longer-chain VFAs tending to be more toxic [60]. Because of this toxicity, the active extraction of mid-chain VFAs such as caproic acid (C6) is recognized to be crucial for practical bioproduction [61–64], thus requiring a careful balance in the production and recovery of VFAs.

### Outlook: Potential and Future Role of EF

Both PMC- and microbiota-driven EF can optimize microbial processes and could have an important impact on emerging biomass refinery chains (see Figure 1 in Box 2). What needs to be understood in much more detail is the way that the electrical potential and current impact on the metabolism of the organism of interest and which microbial strains can benefit from EET. Another important gap to be closed is the lack of fermenter designs incorporating electrodes for efficiently steering the EF. In addition, it will be important to clarify whether a surface-based technology, in contact with complex culture broths, is feasible and stable in long-term operations. Moreover, a techno-economical evaluation should of course point out what the additional costs of the EF might be to achieve these gains in process efficiency (see Outstanding Questions).

EF is rapidly emerging at the intersection of MET and fermentation. The potential of EF is very wide, and its versatility in integrating bio-based production chains (food, food additives, biofuels, industrial green chemistry) through renewable current production chains (solar, wind, etc.), will attract the interest of the industrial world and be an important new frontier of the upcoming bio-based economy.

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### References

- Socol, C.R. *et al.*, eds (2013) *Fermentation Processes Engineering in the Food Industry*, CRC Press
- Couto, S.R. and Sanromán, M.A. (2006) Application of solid-state fermentation to food industry – a review. *J. Food Eng.* 76, 291–302
- Mariano, A.P. *et al.* (2015) Butanol production by fermentation: efficient bioreactors. In *Commercializing Biobased Products: Opportunities, Challenges, Benefits, and Risks* (Snyder, S.W., ed.), pp. 48–70, Royal Society of Chemistry
- Doran, P.M. (2013) *Bioprocess Engineering Principles*, Elsevier
- Peng, L. *et al.* (2014) Characterization and application of biofloculant prepared by *Rhodococcus erythropolis* using sludge and livestock wastewater as cheap culture media. *Appl. Microbiol. Biotechnol.* 98, 6847–6858
- Binod, P. and Pandey, A. (2009) Bioprocess technology and product development. In *Bioprocess and Bioproducts – Technology, trends and opportunities* (Biswas, S. *et al.*, eds), pp. 10–27, Asiatech Publishers
- Moat, A.G. *et al.* (2002) Fermentation pathways. Chapter 11 In *Microbial Physiology* (4th edn) (Moat, A.G. *et al.*, eds), John Wiley & Sons
- Agler, M.T. *et al.* (2011) Waste to bioproduct conversion with undefined mixed cultures: the carboxylate platform. *Trends Biotechnol.* 29, 70–78
- Rabaey, K. and Rozendal, R.a. (2010) Microbial electrosynthesis – revisiting the electrical route for microbial production. *Nat. Rev. Microbiol.* 8, 706–716
- Rabaey, K. and Ragauskas, A.J. (2014) Editorial overview: energy biotechnology. *Curr. Opin. Biotechnol.* 27, 5–6
- Rabaey, K. *et al.* (2011) Metabolic and practical considerations on microbial electrosynthesis. *Curr. Opin. Biotechnol.* 22, 371–377
- Thrash, J.C. and Coates, J.D. (2008) Review: direct and indirect electrical stimulation of microbial metabolism. *Environ. Sci. Technol.* 42, 3921–3931
- Lovley, D.R. (2011) Powering microbes with electricity: direct electron transfer from electrodes to microbes. *Environmental Microbiology Reports* 3, 27–35
- Reguera, G. *et al.* (2005) Extracellular electron transfer via microbial nanowires. *Nature* 435, 1098–1101
- Kracke, F. and Krömer, J.O. (2014) Identifying target processes for microbial electrosynthesis by elementary mode analysis. *BMC Bioinformatics* 15, 410–423
- Kracke, F. *et al.* (2015) Microbial electron transport and energy conservation – the foundation for optimizing bioelectrochemical systems. *Frontiers in Microbiology* <http://dx.doi.org/10.3389/fmicb.2015.00575>
- Nevin, K. *et al.* (2011) Electrosynthesis of organic compounds from carbon dioxide is catalyzed by a diversity of acetogenic microorganisms. *Appl. Environ. Microbiol.* 77, 2882–2886
- Thauer, R.K. *et al.* (1977) Energy conservation in chemotrophic anaerobic bacteria. *Bacteriol. Rev.* 41, 100–180
- Flickinger, M.C. and Drew, S.W., eds (1999) *Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis, and Biopurification*. John Wiley & Sons
- Sharma, M. *et al.* (2014) A critical revisit of the key parameters used to describe microbial electrochemical systems. *Electrochim. Acta* 140, 191–208
- Berrios-Rivera, S.J. *et al.* (2002) Metabolic engineering of *Escherichia coli*: increase of NADH availability by overexpressing an NAD<sup>+</sup>-dependent formate dehydrogenase. *Metab. Eng.* 4, 217–229
- Lai, B. *et al.* (2016) Anoxic metabolism and biochemical production in *Pseudomonas putida* F1 driven by a bioelectrochemical system. *Biotechnol. Biofuels* 9, 39
- Bader, J. *et al.* (2010) Relevance of microbial coculture fermentations in biotechnology. *J. Appl. Microbiol.* 109, 371–387
- Van Hecke, W. *et al.* (2013) Pervaporative recovery of ABE during continuous cultivation: enhancement of performance. *Bioresour. Technol.* 129, 421–429
- Calli, B. *et al.* (2008) Dark fermentative H<sub>2</sub> production from xylose and lactose – effects of on-line pH control. *Int. J. Hydrogen Energy* 33, 522–530
- Arioli, S. *et al.* (2013) Increasing the heme-dependent respiratory efficiency of *Lactococcus lactis* by inhibition of lactate dehydrogenase. *Appl. Environ. Microbiol.* 79, 376–380
- Farid, M.a. *et al.* (2002) Alcohol production from starch by mixed cultures of *Aspergillus awamori* and immobilized *Saccharomyces cerevisiae* at different agitation speeds. *J. Basic Microbiol.* 42, 162–171
- Flynn, J.M. *et al.* (2010) Enabling unbalanced fermentations by using engineered electrode-interfaced bacteria. *MBio* 1, e00190
- Hongo, M. and Iwahara, M. (1979) Application of electro-energizing method to L-glutamic acid fermentation. *Agric. Biol. Chem.* 43, 2075–2081
- Kim, T.S. and Kim, B.H. (1988) Electron flow shift in *Clostridium acetobutylicum* fermentation by electrochemically introduced reducing equivalent. *Biotechnol. Lett.* 10, 123–128
- Schmitz, S. *et al.* (2015) Engineering mediator-based electroactivity in the obligate aerobic bacterium *Pseudomonas putida* KT2440. *Front. Microbiol.* 6, 284
- Pandit, A.V. and Mahadevan, R. (2011) In silico characterization of microbial electrosynthesis for metabolic engineering of biochemicals. *Microb. Cell Fact.* 10, 76
- Mathew, A.S. *et al.* (2015) Enhanced ethanol production via electrostatically accelerated fermentation of glucose using *Saccharomyces cerevisiae*. *Sci. Rep.* 5, 15713
- Hou, C.T. (2005) *Handbook of Industrial Biocatalysis*, CRC Press
- Park, D.H. and Zeikus, J.G. (1999) Utilization of electrically reduced neutral red by *Actinobacillus succinogenes*: physiological function of neutral red in membrane-driven fumarate reduction and energy conservation. *J. Bacteriol.* 181, 2403–2410

### Outstanding Questions

Can electrical current influence suspended microbial cells? Is the use of redox mediators mandatory?

What materials and electrode shapes will better suit the needs of fermentation processes?

Will the electron supply/draw be economical compared to using sugars/chemicals?

How can EF systems be integrated with existing fermenter equipment?

In a fermentative suspended culture, can EF increase cell count or activity?

Is EF a possible solution for redox and pH imbalances of industrial PMC-based fermentations?

In PMC, can EF lead to increased product purity in the broth? Can this be further improved by integrating separation/extraction membranes?

Would EF allow better control of fermentations of waste-streams?

Is EF a possible approach to gas (e.g., syngas) fermentations?

Can EF lower substrate purity requirements in traditional fermentations?

36. Shin, H. *et al.* (2002) Electrically enhanced ethanol fermentation by *Clostridium thermocellum* and *Saccharomyces cerevisiae*. *Appl. Microbiol. Biotechnol.* 58, 476–481
37. Choi, O. *et al.* (2012) Butyrate production enhancement by *Clostridium tyrobutyricum* using electron mediators and a cathodic electron donor. *Biotechnol. Bioeng.* 109, 2494–2502
38. Schievano, A. *et al.* (2012) Two-stage vs single-stage thermophilic anaerobic digestion: comparison of energy production and biodegradation efficiencies. *Environ. Sci. Technol.* 46, 8502–8510
39. Battle-Vilanova, P. *et al.* (2015) Deciphering the electron transfer mechanisms for biogas upgrading to biomethane within a mixed culture biocathode. *RSC Adv.* 5, 52243–52251
40. Angenent, L.T. and Wrenn, B.A. (2008) Optimizing mixed-culture bioprocessing to convert wastes into bioenergy. In *In: Bioenergy* (2008 edn) (Wall, L. *et al.*, eds), pp. 179–194, ASM Press
41. Sen, B. *et al.* (2016) State of the art and future concept of food waste fermentation to bioenergy. *Renew. Sustain. Energy Rev.* 53, 547–557
42. Zhao, Z. *et al.* (2015) Potential for direct interspecies electron transfer in an electric-anaerobic system to increase methane production from sludge digestion. *Sci. Rep.* 5, 11094
43. Babu, M.L. *et al.* (2013) Bio-electrolytic conversion of acidogenic effluents to biohydrogen: an integration strategy for higher substrate conversion and product recovery. *Bioresour. Technol.* 133, 322–331
44. Modestra, J.A. *et al.* (2015) Electro-fermentation of real-field acidogenic spent wash effluents for additional biohydrogen production with simultaneous treatment in a microbial electrolysis cell. *Sep. Purif. Technol.* 150, 308–315
45. Park, D.H. *et al.* (1999) Microbial utilization of electrically reduced neutral red as the sole electron donor for growth and metabolite production. *Appl. Environ. Microbiol.* 65, 2912–2917
46. Hafez, H.M. Greenfield Ethanol Inc. Method and system for electro-assisted hydrogen production from organic material. US 20130217089 A1.
47. Selembo, P.A. *et al.* (2009) High hydrogen production from glycerol or glucose by electrohydrogenesis using microbial electrolysis cells. *Int. J. Hydrogen Energy* 34, 5373–5381
48. Dennis, P.G. *et al.* (2013) Dynamics of cathode-associated microbial communities and metabolite profiles in a glycerol-fed bioelectrochemical system. *Appl. Environ. Microbiol.* 79, 4008–4014
49. Zhou, M. *et al.* (2013) Carbon and electron fluxes during the electricity driven 1,3-propanediol biosynthesis from glycerol. *Environ. Sci. Technol.* 47, 11199–11205
50. Zhou, M. *et al.* (2015) Development of bioelectrocatalytic activity stimulates mixed-culture reduction of glycerol in a bioelectrochemical system. *Microb. Biotechnol.* 8, 483–489
51. Xafenias, N. *et al.* (2015) Electrochemical startup increases 1,3-propanediol titers in mixed-culture glycerol fermentations. *Process Biochem.* 50, 1499–1508
52. Steinbusch, K.J.J. *et al.* (2010) Bioelectrochemical ethanol production through mediated acetate reduction by mixed cultures. *Environ. Sci. Technol.* 44, 513–517
53. Van Eerten-Jansen, M.C.A.A. *et al.* (2013) Bioelectrochemical production of caproate and caprylate from acetate by mixed cultures. *ACS Sustain. Chem. Eng.* 1, 513–518
54. Van Hecke, W. *et al.* (2012) Integrated bioprocess for long-term continuous cultivation of *Clostridium acetobutylicum* coupled to pervaporation with PDMS composite membranes. *Bioresour. Technol.* 111, 368–377
55. Bechthold, I. *et al.* (2008) Succinic acid: a new platform chemical for biobased polymers from renewable resources. *Chem. Eng. Technol.* 31, 647–654
56. Andersen, S.J. *et al.* (2014) Electrolytic membrane extraction enables production of fine chemicals from biorefinery sidestreams. *Environ. Sci. Technol.* 48, 7135–7142
57. Gildemyn, S. *et al.* (2015) Integrated production, extraction, and concentration of acetic acid from CO<sub>2</sub> through microbial electrosynthesis. *Environ. Sci. Technol. Lett.* 2, 325–328
58. Xu, J. *et al.* (2015) In-line and selective phase separation of medium-chain carboxylic acids using membrane electrolysis. *Chem. Commun.* 51, 6847–6850
59. Hongo, M. *et al.* (1986) Novel method of lactic acid production by electro-dialysis fermentation. *Appl. Environ. Microbiol.* 52, 314–319
60. Pratt, S. *et al.* (2012) Inhibition by fatty acids during fermentation of pre-treated waste activated sludge. *J. Biotechnol.* 159, 38–43
61. Agler, M.T. *et al.* (2012) Chain elongation with reactor microbiomes: upgrading dilute ethanol to medium-chain carboxylates. *Energy Environ. Sci.* 5, 8189
62. Grootcholten, T.I.M. *et al.* (2013) Improving medium chain fatty acid productivity using chain elongation by reducing the hydraulic retention time in an upflow anaerobic filter. *Bioresour. Technol.* 136, 735–738
63. Choi, K. *et al.* (2013) In situ biphasic extractive fermentation for hexanoic acid production from sucrose by *Megasphaera elsdenii* NCIMB 702410. *Appl. Biochem. Biotechnol.* 171, 1094–1107
64. Roy, S. *et al.* (2016) Electro-stimulated microbial factory for value added product synthesis. *Bioresour. Technol.* <http://dx.doi.org/10.1016/j.biortech.2016.03.052>
65. Lee, J.W. *et al.* (2011) Microbial production of building block chemicals and polymers. *Curr. Opin. Biotechnol.* 22, 758–767
66. Sindhu, R. *et al.* (2011) Production and characterization of poly-3-hydroxybutyrate from crude glycerol by *Bacillus sphaericus* NII 0838 and improving its thermal properties by blending with other polymers. *Brazilian Arch. Biol. Technol.* 54, 783–794
67. Cok, B. *et al.* (2014) Succinic acid production derived from carbohydrates: an energy and greenhouse gas assessment of a platform chemical toward a bio-based economy. *Biofuels, Bioprod. Biorefining* 8, 16–29
68. Angenent, L.T. *et al.* (2004) Production of bioenergy and biochemicals from industrial and agricultural wastewater. *Trends Biotechnol.* 22, 477–485
69. Lee, P.C. *et al.* (1999) Succinic acid production by *Anaerobiospirillum succiniciproducens*: effects of the H<sub>2</sub>/CO<sub>2</sub> supply and glucose concentration. *Enzyme Microb. Technol.* 24, 549–554
70. Geertman, J.-M.A. *et al.* (2006) Engineering NADH metabolism in *Saccharomyces cerevisiae*: formate as an electron donor for glycerol production by anaerobic, glucose-limited chemostat cultures. *FEMS Yeast Res.* 6, 1193–1203
71. Liu, Y.-P. *et al.* (2008) Strategies of pH control and glucose-fed batch fermentation for production of succinic acid by *Actinobacillus succinogenes* CGMCC1593. *J. Chem. Technol. Biotechnol.* 83, 722–729
72. Lominadze, G.S. (1940) Electrofermentation of tea. *Sovetskie Subtropiki* 8, 27–28
73. Kim, T.S. *et al.* (1990) Petroleum desulfurization by *Desulfovibrio desulfuricans* M6 using electrochemically supplied reducing equivalent. *Biotechnol. Lett.* 12, 757–760
74. Suzuki, M. *et al.* (1986) Electrical stimulation of hybridoma cells producing monoclonal antibody to cAMP. *Biochim. Biophys. Acta - Mol. Cell Res.* 889, 149–155
75. Emde, R. and Schink, B. (1990) Enhanced propionate formation by *Propionibacterium freudenreichii* subsp. *freudenreichii* in a three-electrode amperometric culture system. *Appl. Environ. Microbiol.* 56, 2771–2776
76. Gregory, K.B. *et al.* (2004) Graphite electrodes as electron donors for anaerobic respiration. *Environ. Microbiol.* 6, 596–604
77. Araújo, O.Q.F. *et al.* (2004) Electrical stimulation of *Saccharomyces cerevisiae* cultures. *Bras. J. Microbiol.* 35, 97–103
78. Rozendal, R.A. *et al.* (2008) Towards practical implementation of bioelectrochemical wastewater treatment. *Trends in Biotechnology* 26, 450–459
79. Cheng, S. *et al.* (2009) Direct biological conversion of electrical current into methane by electromethanogenesis. *Environ. Sci. Technol.* 43, 3953–3958
80. Vasudevan, D. *et al.* (2014) Upgrading dilute ethanol from syngas fermentation to n-caproate with reactor microbiomes. *Bioresour. Technol.* 151, 378–382
81. Liu, K. *et al.* (2014) Mixed culture syngas fermentation and conversion of carboxylic acids into alcohols. *Bioresour. Technol.* 152, 337–346
82. Manzini, E. *et al.* (2015) Dark fermentation effectiveness as a key step for waste biomass refineries: influence of organic matter

- macromolecular composition and bioavailability. *Int. J. Energy Res.* 39, 1519–1527
83. Walcarius, A. *et al.* (2013) Nanomaterials for bio-functionalized electrodes: recent trends. *J. Mater. Chem.* 1, 4878–4908
84. Fiset, E. and Puig, S. (2015) Modified carbon electrodes: a new approach for bioelectrochemical systems. *J. Bioremediation Biodegrad.* 6, 1–2
85. Sleutels, T. *et al.* (2009) Improved performance of porous bioanodes in microbial electrolysis cells by enhancing mass and charge transport. *Int. J. Hydrogen Energy* 34, 9655–9661
86. Guo, K. *et al.* (2014) Flame oxidation of stainless steel felt enhances anodic biofilm formation and current output in bioelectrochemical systems. *Environ. Sci. Technol.* 48, 7151–7156
87. Cercado, B. *et al.* (2013) Biotic and abiotic characterization of bioanodes formed on oxidized carbon electrodes as a basis to predict their performance. *Biosens. Bioelectron.* 50, 373–381
88. Logan, B.E. (2010) Scaling up microbial fuel cells and other bioelectrochemical systems. *Appl. Microbiol. Biotechnol.* 85, 1665–1671
89. Krieg, T. *et al.* (2014) Reactor concepts for bioelectrochemical syntheses and energy conversion. *Trends Biotechnol.* 32, 645–655