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Review

Advanced Electroanalysis for Electrosynthesis

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Monica Brachi,[§] Wassim El Housseini,[§] Kevin Beaver, Rohit Jadhav, Ashwini Dantanarayana, Dylan G. Boucher, and Shelley D. Minteer*

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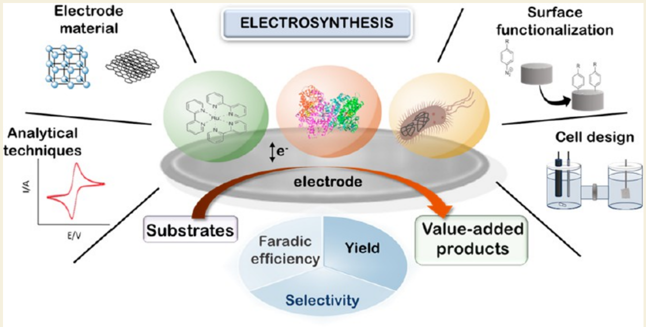

ABSTRACT: Electrosynthesis is a popular, environmentally friendly substitute for conventional organic methods. It involves using charge transfer to stimulate chemical reactions through the application of a potential or current between two electrodes. In addition to electrode materials and the type of reactor employed, the strategies for controlling potential and current have an impact on the yields, product distribution, and reaction mechanism. In this Review, recent advances related to electroanalysis applied in electrosynthesis were discussed. The first part of this study acts as a guide that emphasizes the foundations of electrosynthesis. These essentials include instrumentation, electrode selection, cell design, and electrosynthesis methodologies. Then, advances in electroanalytical techniques applied in organic, enzymatic, and microbial electrosynthesis are illustrated with specific cases studied in recent literature. To conclude, a discussion of future possibilities that intend to advance the academic and industrial areas is presented.

KEYWORDS: *Electroanalytical techniques, redox reactions, electrode materials, electrodes functionalization, electrochemical catalysts, reactors, organic electrosynthesis, enzymatic electrosynthesis, microbial electrosynthesis*

1. INTRODUCTION

The field of electrosynthesis is currently experiencing a period of revitalization as a result of advancements associated with the accessibility of sustainable electricity.[1−4] This resurgence is also driven by the growing utilization of robotic synthesis[5] and a renewed focus on elegant transformations achieved through efficient electrolysis systems without the need for additional reagents.[6] Furthermore, electrosynthesis is gaining momentum in the conversion of biomass into valuable commodity chemicals[7] and in the exploration of novel pathways for cross-coupling reactions.[8]

Electroanalysis is a specialized branch of electrochemistry that is dedicated to the advancement of novel techniques, methodologies, and electrode modifications for the purpose of conducting precise quantitative analytical investigations. Quantitative analytical investigations are employed in certain scenarios to sense and detect an analyte, such as glucose concentrations in blood. However, there has been notable progress in the development of electrochemical techniques in recent years. This progress has focused on studying electrocatalysis in fuel cells and electrolyzers, investigating intercalation in batteries, examining the interface between the electrode and the electrolyte, exploring corrosion and electroplating, and analyzing nanomaterial properties, including size, weight, shape, and surface area. Furthermore, advancements have been made in the field of materials and methodologies for in vivo analysis. Combinations of techniques for in situ or operando analysis have also been developed, along with novel electrodes modified chemically and biologically for the analysis of nonredox-active molecules. Additionally, significant enhancements have been made in voltammetric techniques, all of which have been integrated into these methodologies.

The purpose of this Review is to survey advances in electroanalytical techniques involved in electrosynthesis, including hybrid approaches that go beyond conventional electroanalytical methods. The initial focus of this Review is on the fundamental aspects of electrosynthetic reactions including choice of reactors, electrode materials, and a general overview about electroanalytical techniques. Then, recent

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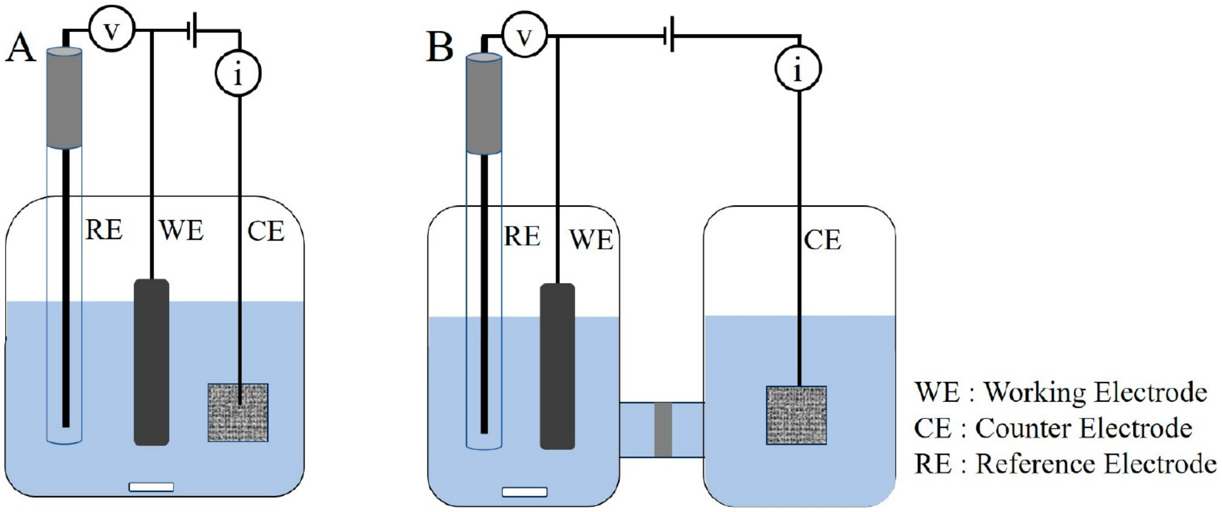


Figure 1. Batch electrochemical cells applied in electrosynthesis. (A) Undivided batch cell with three electrodes configuration. (B) H-cell reactor divided by a membrane with three electrodes configuration (working electrode, counter electrode, and reference electrode).

examples of applying electroanalytical techniques in organic, enzymatic, and microbial electrosynthesis are emphasized.

2. REACTORS CHOICE IN ELECTROCHEMICAL SYNTHESIS

In electrosynthesis, chronoamperometric (potentiostatic control) or chronopotentiometric (galvanostatic control) electrolysis can be performed. Potentiostatic electrolysis involves the application of a potential to the working electrode (WE) versus a reference electrode (RE). This results in the flow of a current between the working electrode (WE) and the counter electrode (CE).

A galvanostat is utilized to apply a consistent current to the cell while maintaining galvanostatic control.[6,9] In this particular scenario, the potential of the WE is permitted to float, thereby necessitating the use of only two electrodes.

For instance, in order to perform electrolysis on a big scale at a constant potential, one needs a potentiostat that is equipped with electronic control units that are able to keep potentials steady for lengthy periods of time. Because of the resistance of the solution, the potentiostat may apply a potential that is somewhat different from the potential that was meant to be applied. A reduction in the cell's electrical resistance is possible by taking these steps: minimizing the separation between electrode and choosing an electrolyte with a good conductivity.

Before proceeding with the construction of an electrosynthesis cell, it is necessary to make a deliberation regarding the choice between batch or flow electrolysis. Batch electrolysis is frequently employed for small-scale applications due to the easy availability of cells designed for this method. The implementation of effective mixing techniques, such as convection, is crucial for improving mass transfer rates and reducing reaction times. To achieve this, it is advised to make use of a magnetic stirrer or bubble inert gas into the solution. Moreover, the utilization of flow electrochemical reactors in chemical reactions has the potential to enhance mass transfer rates, thereby diminishing reaction time and enabling the execution of large-scale reactions utilizing equipment of smaller scale.[10−12] These reactors present several advantages compared to batch reactors, including enhanced mixing and thermal control capabilities, improved stability, and extended operational lifespan. Flow reactors operate by employing a pump to transfer substrate to the reaction vessel, where products are generated and subsequently expelled from the reaction vessel. Electrochemical flow reactors are commonly constructed using a variety of materials, including glass, polymers, stainless steel, and various metals.

As depicted in Figure 1, a divided (Figure 1A) or undivided electrochemical cell (Figure 1B) may be utilized in both batch and flow reactors. Both undivided and divided cells have their own set of benefits and drawbacks; the approach that is used in an experiment is determined by the specific circumstances of that experiment. In batch electrochemical reactors, the three-electrode configuration is typically used, whereas the two-electrode configuration is more common in flow electrochemical reactors. Recently, the introduction of multiphase reactors e.g., gas−liquid electrochemical reactors has gained a significant importance where gas diffusion electrodes (GDEs) are used as either the anode or cathode, for the electrochemical generation of reactants from gaseous substrates, such as O_2, CO_2, and H_2. This is because GDEs effectively address the limitations associated with mass transport based on solubility.[13]

The construction of undivided electrochemical cells is relatively simple. Nevertheless, caution must be taken to ensure that the counter electrode (CE) does not result in undesirable side reactions. Unwanted processes encompass the interference caused by the reaction of products formed at the CE with intermediates originating from the working electrode (WE) and the generation of byproducts. In situations where competing reactions occur and the utilization of a sacrificial counter electrode is not feasible, it is possible to employ a porous material or a nafion membrane to physically separate the cathode and anode. These materials facilitate the flow of current while effectively preventing the mixing of contents, thereby enabling the use of separate compartments for each electrode.[11] For reactions that take place in organic solvent, a methyl cellulose separator is used along with the according electrolyte and solvent. Alternately, agar may function as an acceptable replacement for methyl cellulose in reactions that take place in aqueous medium.[14]

The positioning and orientation of electrodes inside the cell are two other factors that must be considered regarding the cell's architecture. In a setup with two electrodes, it is essential to carefully consider the sizes and placements of the WE and the CE. These electrodes should have a surface area that is similar to

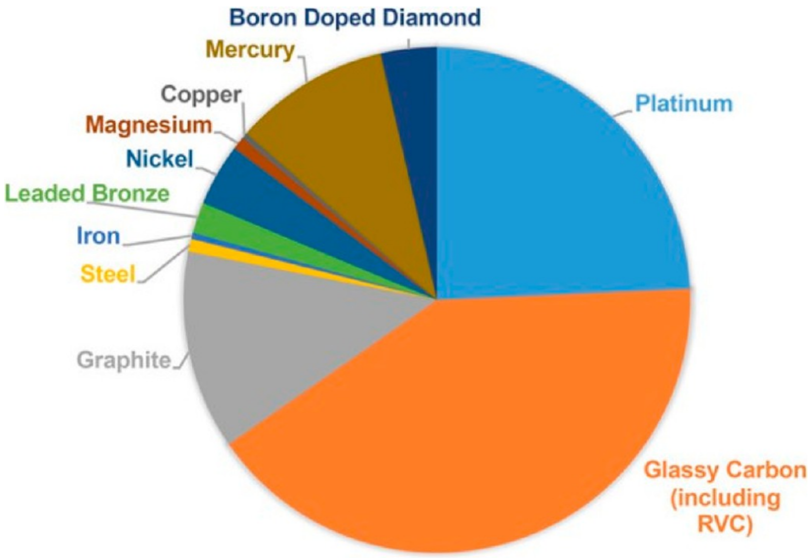


Figure 2. Cathode and anode electrode material occurrence in a survey of 915 synthetic electrochemical protocols published between 2000 and 2017.[16]

one another in order to maintain consistency in the cathode and anode reaction rates.

Furthermore, it is recommended to position the WE and CE in close proximity, while ensuring that an appropriate distance is maintained. This precaution is necessary to prevent any interference from electric fields between the electrodes, which may potentially impact the electrochemical reactions.[15] Additionally, it is important to note that when the distance between the electrodes is too close, there is a possibility of quenching the species of interest, such as electrogenerated catalysts, at the counter electrode (CE). In contrast, in a three-electrode configuration, it is necessary for all working electrode (WE) points to be equidistant from the reference electrode (RE). This ensures that the organization has a consistent level of potential. The CE is often placed in close proximity to the WE in order to maximize the conductance of the cell.[15]

3. ELECTRODE MATERIALS APPLIED IN ELECTROSYNTHESIS

It is essential to make an informed decision on the WE material in order to achieve the highest possible production yield and selectivity. The following is a list of many important qualities that working electrodes should possess: (1) a high level of conductivity, (2) a broad potential range that may be successfully employed, (3) a physical stability under varied circumstances, and (4) quick charge transfer. These are the characteristics of an ideal conducting material. In addition to this, it is of the utmost importance that the material used for the electrode is cost-effective and, if possible, free of any harmful characteristics.[16] In the majority of electrosynthetic applications, it is beneficial for the WE to possess a high surface area in order to promote increased mass transport. This is because a larger surface area makes it easier to increase mass transport. In contrast, it is important to emphasize that small electrode areas provide applications for electroanalytics within the framework of reaction pathways for synthesis.[17−19]

The frequency of the electrode materials, including carbon electrodes and metal electrodes, used in published synthetic electrochemical protocols between 2000 and 2017, is depicted in Figure 2. Following is a comprehensive overview of common electrode materials used in electrosynthesis.

3.1. Metal Electrodes

Metal electrodes possess several key characteristics that are commonly observed. These include the ability to facilitate rapid electron-transfer kinetics for a wide range of redox systems, as well as the presence of relatively broad anodic potential windows.

3.1.1. Platinum Electrodes. Platinum and its alloys are widely favored due to their durability, ease of maintenance, chemical and electrochemical stability, as well as their resistance to corrosion.[20,21] In aqueous environments, platinum (Pt) exhibits a significant overpotential for the process of oxygen evolution reaction.

However, the effectiveness of the process is impeded by the occurrence of H^+ reduction in the cathodic potentials. Platinum demonstrates considerable cathodic and anodic potential ranges in aprotic solvents. It is often utilized in electrosynthesis to oxidize organic substrates leading to the generation of carbon dioxide (CO_2).[22]

Notwithstanding these benefits, the extensive utilization of this technology is constrained by the exorbitant expenses involved and the detrimental effects of unintended adsorbates, like electrolytes or water itself, which lead to the poisoning of the electrode surface.[22]

3.1.2. Mercury Electrodes. Mercury electrodes hold significant historical value and are widely utilized at cathodic potentials due to their remarkable ability to generate a substantial overpotential for hydrogen evolution.[23] Furthermore, the utilization of liquid mercury offers a consistent and sustainable surface. Mercury has been employed in a range of electrosynthetic reactions, including intramolecular addition or cyclization, as well as dimerization.[3,24−28] Mercury electrodes have historically been employed in electroanalytical practices, specifically in applications such as stripping analysis and polarography.[29,30]

Nevertheless, it should be noted that the utilization of these electrodes is accompanied by significant toxicity concerns, as they possess high levels of toxicity. Additionally, their applicability is restricted to reductive potentials due to the susceptibility of mercury to oxidation. Consequently, there has been a recent phase-out of mercury electrodes.[17]

3.1.3. Silver Electrodes. The utilization of silver as a viable alternative to mercury has gained significant traction, primarily attributed to the catalytic properties exhibited by these electrodes. The high affinity that halides possess for silver facilitates the reduction of carbon−halogen bonds at positive potentials.[31] The utilization of these electrodes is predominantly limited to cathodic processes.[32] One example of a process in which the cathode material proved to be extremely essential to the outcome of the reaction is the electrochemical hydrodechlorination of 3,5,6-trichloropicolinic acid (3,5,6-T) (noted **1** in Figure 3).[33]

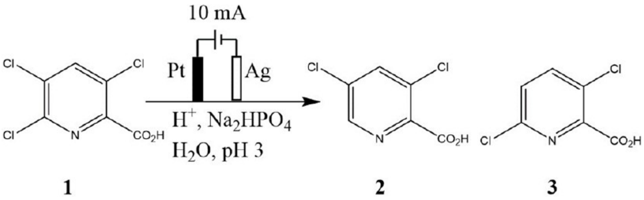


Figure 3. Study of the effect of electrode material on the electrochemical hydrodechlorination of 3,5,6-trichloropicolinic acid.

The reaction was a total failure regardless of whether copper, glassy carbon, or nickel cathodes were used. The production of any 3,5-dichloropicolinic acid (**2**) product, a chemical of substantial importance to the pharmaceutical industry, was only achieved by the application of silver. It was hypothesized that the utilization of copper and nickel would result in a smaller overpotential for proton reduction, which would make substrate reduction a more challenging task. It was determined that the electrocatalytic action of silver was responsible for the increased productivity of silver cathodes in comparison to glassy carbon cathodes.

3.1.4. Gold Nanoparticles. Gold nanoparticles (AuNPs) materials have garnered significant attention in the field of biocatalysis[34] and bioelectrochemistry applications, including enzyme immobilization for bioelectrocatalysis.[35] The 3D nanoporous network possesses a substantial surface area and exhibits the capability to accommodate a significant quantity of enzymes. AuNPs supports are increasingly being favored due to their favorable characteristics, such as excellent electrical conduction, chemical resistance, and simplicity of preparation. AuNPs can be produced through dealloying,[35,36] Au nanoparticle assembly,[37,38] or anodization.[39] Their structure is made up of continuous pores and ligaments. Adjusting the alloy composition and dealloying conditions allows for fine-tuning of the pore size to range from 10 to 700 nm.[35] AuNPS templates can also be modified for enzyme immobilization by adding self-assembled thiol monolayers, diazonium grafting, or electropolymerization.

3.1.5. Metal Organic Framework Electrodes. In addition to AuNPs, extensive research has been conducted on metal organic frameworks (MOFs) with the aim of exploring their extensive applications in catalysis.[40,41] The coordination networks known as MOFs are examples of porous and crystalline structures.[42] These networks are formed through the combination of metal ions or clusters with intermediate organic linkers.[43] Postsynthesis procedures allow for the change of MOFs' metal nodes and ligands, which enables the structures, pore sizes, and topologies of MOFs to be easily modified.[43] MOFs have garnered considerable attention owing to their notable characteristics, including their expansive surface area, ability to adjust pore size, and adaptable structure. In recent times, there have been notable advancements in the application of MOFs, wherein they have been employed for the purpose of immobilizing electrocatalytic enzymes[44,45] and facilitating drug delivery.[46]

3.1.6. Indium Tin Oxide Electrodes. Indium tin oxide (ITO) is one of the materials that has widely applied due to its notable characteristics, including its high electrical conductivity (0.05−0.1 m·S^{-1}), high optical transparency (85%), favorable physical and chemical properties, strong adhesion to various substrates, cost-effectiveness in preparation, low background response, and potential for miniaturization.[47]

Furthermore, the ITO electrode provides the added benefit of convenient modification through the utilization of linkers such as amines, allowing for the functionalization of the surface. Additionally, this material has proven to be highly effective in the fields of electroanalysis and biosensing.[48,49] Therefore, conducting a comprehensive examination of the recent progress made in the field of electroanalysis using ITO and assessing its current and potential capabilities for signal amplification holds considerable importance in expanding the scope and practicality of this material.[48,49]

One of the examples related to the application of ITO electrodes in bioelectrosynthesis was reported by Armstrong et al. The diffusion of an intermediate to the succeeding enzyme is frequently a stage in homogeneous enzymatic cascades that acts as a rate-limiting step. These problems with mass transfer are addressed and solved in nature by the confinement of enzymes, which shortens distances on sizes ranging from microns to nanometers. Armstrong et al. showed an ITO electrode that included confined enzymes, L-malate:Ferredoxin NADP$^+$ reductase (NADP$^+$/NADPH regeneration), and MaeB from *Escherichia coli* for CO_2 fixation to pyruvate to supply malate.[50] This system was integrated into a solar water splitting and CO_2 reduction device, and it produced a high faradaic efficiency at an applied electrolysis potential of 0.5 V vs SHE.

Despite possessing remarkable fundamental properties, electrodes based on ITO often exhibit a significant drawback in terms of their sluggish electron-transfer kinetics toward various electroactive molecules. This limitation, when compared to electrodes made of noble metals and glassy carbon, restricts the potential applications of ITO-based electrodes in the field of electroanalysis.

3.2. Carbon Materials

Carbon is a typical, low-cost electrode material that may be found in a variety of forms. It has a large potential window in aqueous conditions.[51,52] This category of electrodes includes different types materials e.g., glassy carbon, graphite, carbon felt, graphite, carbon nanotubes, and boron doped diamond.

3.2.1. Glassy Carbon. Glassy carbon (GC) is a material that is resistant to corrosion and oxidation in addition to being chemically stable.[53] GC electrodes made of reticulated vitreous carbon, also known as RVC, are composed of foamed glassy carbon. These electrodes feature an open honeycomb structure and an enhanced surface area.[54,55] In addition to the large applications of GC in electrosynthesis,[56] one of the examples related to the application of GC as WE in electrosynthesis is the hydrocarboxylation of imines in an electrochemical microflow reactor.[57]

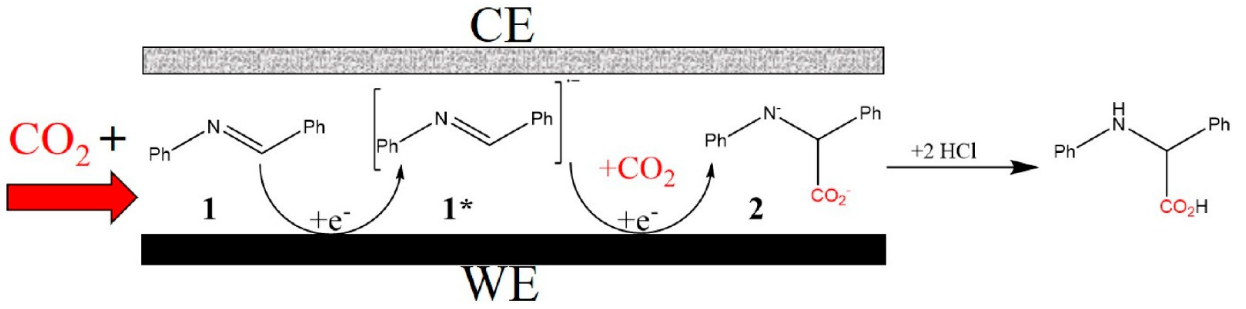


Figure 4. Application of GC electrode in electrochemical hydrocarboxylation of imines in a microflow reactor.

Qu et al. took into consideration cathode materials that had a large overpotential for the reduction of carbon dioxide (Figure 4). In this particular illustration, the overpotentials with different electrode materials, obtained from linear sweep voltammetry experiments, had a significant correlation with the yield. The maximum yield was achieved using glassy carbon (70%), which was followed by graphite (43%), then platinum (25%), and finally silver (0%). In order for the reaction to take place, the imine (**1**) first has to be reduced to a radical anion (**1***). Utilizing the interaction between radical anion (**1**) and CO_2 allows for the electrochemical synthesis of intermediate (**2**). The electrochemical carboxylation is accomplished without decomposition because of the quick acidification of unstable anion (**2**) in the flow operation. The charge efficiency and reaction yields are both impacted negatively by any competing direct CO_2 reduction.

3.2.2. Graphite. Graphite exhibits a lower cost and higher conductivity compared to glassy carbon. However, it possesses a reduced level of chemical stability and is more prone to chemical alteration.[16] The presence of stacked graphene planes gives rise to structural variations between the edge and basal planes, leading to discernible differences in their respective reactivities.[58] Alkanes exhibit a notable preference for the basal plane, whereas polar solutes demonstrate a pronounced preference for edge sites.[59]

3.2.3. Carbon Felt. Carbon felt, acting as a highly porous electrode,[60,61] is composed of an assortment of compressed carbon fibers. Carbon felt is chemically stable, conductive, and has extraordinarily high surface area per volume. For example, in enzymatic and microbial bioelectrocatalysis, using high-surface-area electrodes like carbon felt, capable of elevated loading of biocatalysts, is one way to increase the rate of reaction in electrochemical bioreactors.[62]

3.2.4. Carbon Nanotubes. Carbon nanotubes are largely applied in electrochemistry due to their high conductivity and large surface areas. These materials are mainly used in enzymatic bioelectrocatalysis. Placing a droplet of an enzyme and carbon nanotube suspension on the electrode's surface is an easy way to modify electrodes with carbon nanotubes and enzymes.[63] Modifying electrodes with carbon nanotubes can facilitate transfer of electrons between enzymes, like fructose dehydrogenase[64] and hydrogenase,[65] and electrodes, which results in effective biofuel cells.[66,67] Multiwalled carbon nanotubes were used by Jourdin et al. to improve the efficiency of a microbial system for carbon dioxide bioreduction.[68] These materials improved the rate of electron transfer between the electrode and microorganisms by 1.65 times and the rate of acetate production by 2.60 times when compared to graphite plate electrodes. In addition, for the electrocatalytic oxidation of 2,3-butanediol, Bulutoglu et al. immobilized alcohol dehydrogenase on multiwalled carbon nanotubes.[69]

3.3. Mesoporous Silica

Synthesis of mesoporous silicates (MPS) was initially documented for the first time in the year 1971, where a surge of MPS research soon followed.[70] In the wake of these further discoveries, there has been increasing research in the production of these materials, which currently include various MPS and functionalized substances that are able to contribute to immobilization.[71] MPS feature organized pore architectures, small sized pores size distributions, and a substantial surface area. Additionally, they are chemically changeable so that they may be functionalized in a manner of choice. In conjunction with their inherent chemical stability, these qualities place them in a position to be excellent candidates for use in bioelectrochemical reactors. The sol−gel and pore-template synthesis technologies are combined in the MPS synthesis process. In the process of pore formation, surfactants may play the role of structure-directing agents, particularly if they create micelles or microemulsions. A stable material is produced by the processes of hydrolysis and subsequent condensation of silanes around the template structures. After the template has been eliminated, the pores become visible, and the final structure is mesoporous.

These materials are typically applied in the immobilization of enzymatic bioelectrochemical catalysts for the synthesis of pure chemicals.[72,73]

Since their discovery, different MPS materials have been designed.[72,73] Silicates can be modified by functionalizing them with a variety of organic ligands[73,74] or by incorporating metals.[75,76] The use of functionalized MPS makes it possible to modify the material surface in order to achieve stable enzyme immobilization while keeping the activity of the catalyst intact. It is crucial to select an appropriate functionalized support; the enzyme must be securely attached to the support in order to minimize enzyme leaching, but it must also maintain sufficient flexibility to facilitate conformational variations. Carboxylates, amines, or thiols may be introduced into these mesoporous materials.

3.4. Modified Electrode Surface

In addition to what has been mentioned before about electrode modification, electrode surfaces have the potential to undergo modifications, either through chemical means (specifically covalent bonding)[77,78] or physical means (noncovalent interactions),[79] in order to enhance the reactivity of the electrode. This enhanced reactivity is achieved by facilitating the binding of analytes to the modified electrode. Several modification strategies for electrode surfaces have been reported, e.g., redox polymers, self-assembled monolayers

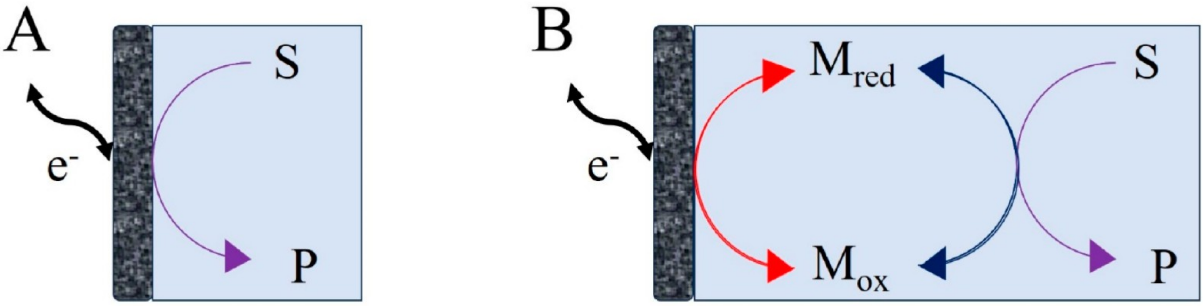


Figure 5. (A) Direct and (B) mediated electron transfer choices in electrochemical processes. (S): substrate, (P): product, and (M): redox mediator.

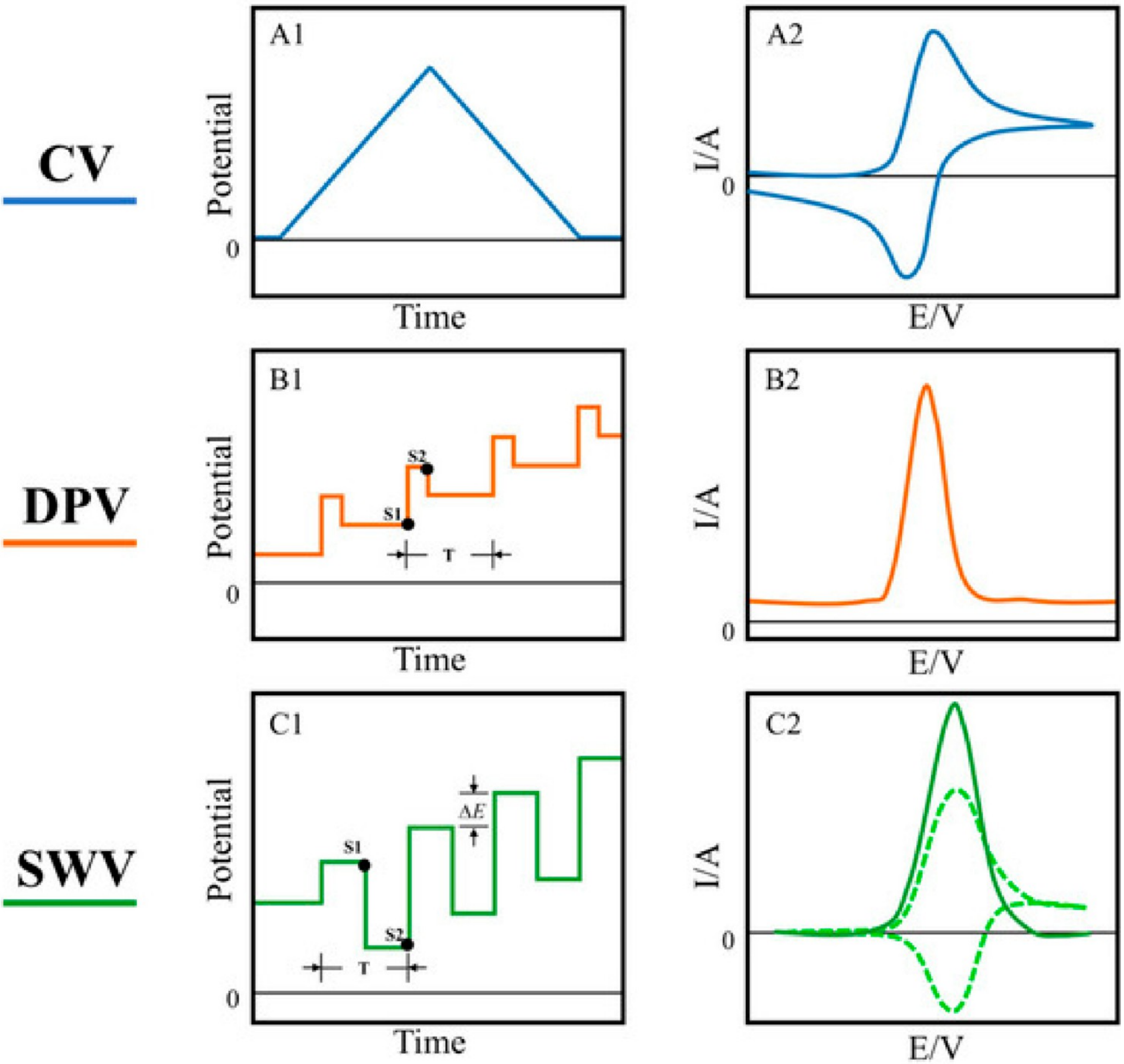


Figure 6. Representation of cyclic voltammetry (CV), differential pulse voltammetry (DPV), and square wave voltammetry (SWV) techniques. The potential (A1) and typical response current (A2) of CV; the potential waveform (B1) and voltammogram (B2) of DPV, where T is the waveform period and S1 and S2 are the two sampling points for the current; (C1) the typical potential waveform of SWV, where E is the potential increment and T is the potential period. The response current consists of forward (anodic current) and reverse (cathodic current) components (dashed line in (C2)), and the difference between these components results in a net current (solid line in (C2)).[96]

(SAMs), or even biofilms.[77,80,81] Furthermore, the utilization of metal alloys has been implemented in order to preserve the desired electrochemical characteristics while simultaneously reducing the occurrence of corrosion on the surface of the electrode.[82] Murray has extensively described the pathways of modification.[83]

The utilization of modified electrodes in synthetic processes has also encompassed the practice of asymmetric synthesis for organic compounds.[84,85] Nevertheless, the potential of utilizing modified electrodes in electrosynthesis remains largely untapped.[23]

In conclusion, when choosing an appropriate WE, it is crucial to take into account the overpotential of side reactions, such as hydrogen evolution, and the adsorption of molecules to the surface of the electrode. The latter scenario can present challenges in cases where adsorption is sufficiently strong to cause passivation of the electrode, resulting in the termination of electrolysis as unreacted substrate is unable to reach the electrode surface. In situations where adsorption is detrimental and it is not feasible to introduce a WE with a distinct composition, the inclusion of a redox mediator can effectively inhibit adsorption.[16]

4. ELECTROANALYTICAL TECHNIQUES APPLIED IN ELECTROSYNTHESIS

The diffusion layer is the region where most electrochemical reactions occur because of the heterogeneous character of these reactions. This layer is in proximity with the bulk solution. In addition to electron transfer at the electrode surface, in an electrochemical reaction, mass transfer occurs in two directions: from the bulk solution to the electrode surface (diffusion layer) and from the electrode surface to the bulk solution. In order to study the electrochemical phenomena occurring at the electrode surface and in the bulk solution, electroanalytical techniques can be employed. Electroanalytical methods may be broken down into (a) those that aim to alter the composition of the whole solution, and (b) those that focus just on the diffusion layer. The first category of methods, bulk electrolysis (or coulometry), is designed for large-scale, preparative processes.[86]

The second category consists of electroanalytical procedures, such as cyclic voltammetry (CV), that make use of low I/V (current over potential) conditions and do not affect bulk concentrations.

Before going deeply into electroanalytical techniques applied in electrosynthesis, it is important to differentiate between different modes of electron transfer that can be investigated in an electrochemical process.

Direct electron transfer (DET) allows molecules to transfer electrons on the electrode surface itself (Figure 5A). In contrast, in mediated electron transfer process (MET), a redox mediator "M" which is more easily oxidized or reduced than the substrate, serves as an electron-transfer shuttle from the heterogeneous electrode surface to the homogeneous dissolved substrates (Figure 5B).[87−90] This concept has several benefits. In many cases, the indirect approach improves reaction efficacy and chemoselectivity by avoiding unwanted side reactions. The mediator's simple homogeneous redox process eliminates kinetic inhibition of heterogeneous electron transfer, and structural modifications provide direct selectivity control.[91]

Intuitively, the best catalyst for a target reaction is the one that generates the largest current at the least negative potential (for reductions) or the most positive potential (for oxidations). The overpotential, η, is defined simply and precisely as the difference between the electrode potential, E, and a fixed potential, which is typically the standard potential for the reaction to be catalyzed. Another parameter characterizes the rate at which the catalyst can convert reactants to product and is best expressed by the turnover frequency (TOF), which is defined as the ratio of the number of molecules transformed to the number of catalyst molecules effectively used in this transformation per unit of time.

Below, we present a general overview about different electroanalytical methods applied in electrochemical synthesis.

4.1. Voltammetric Electroanalysis Techniques

Voltammetry is generally performed using a three-electrode configuration. It provides a specific voltage to a working electrode in order to produce the oxidation and reduction process of electroactive compounds, and it then samples the response current within a certain time range.[92] Common analytical techniques include cyclic voltammetry (CV),[93] differential pulse voltammetry (DPV),[94] and square wave voltammetry (SWV).[95] The selection of an appropriate voltammetric technique facilitates the study of the overall system, thereby enhancing the sensitivity and the selectivity.

4.1.1. Cyclic Voltammetry. Cyclic voltammetry is the predominant electrochemical technique utilized, wherein a triangular waveform potential scan is applied to a working electrode to measure the resulting current (Figure 6A1). The ongoing oxidation and reduction reactions of electroactive species in a solution are facilitated by the process of potential cycling at the working electrode.[97] As the applied potential on the working electrode approaches the equilibrium potential of the species in solution, there is a gradual increase in the response current and a corresponding decrease in the concentration of oxidized or reduced species on the electrode surface. A peak corresponding to oxidation or reduction is observed until the equilibrium is reached between the charge transfer and diffusion processes. (Figure 6A2)

By analyzing the shape of the voltammogram's peaks, one can obtain information regarding the reaction mechanism, such as the reversibility of the reaction, the redox potential, the reaction rate, and the concentration of the analyte.[98,99]

4.1.2. Pulse Voltammetry. Contemporary electrochemical methodologies encompass stripping analysis, a process that entails the concentration of the analyte onto or into the electrode's surface. Various forms of stripping analysis have been identified in the literature.[100,101] The contemporary iteration of stripping analysis initially emerged through the implementation of square wave and pulse polarography which evolved from fast linear sweep voltammetry.

Pulse voltammetry is a technique that can effectively mitigate background current interference and selectively isolate faradaic current. Pulse voltammetry involves the acquisition of periodic current measurements while a sequence of potential pulses is applied. At every possible stage, the gradual decrease of the capacitive current enables a more accurate evaluation of the faradaic current. DPV and SWV have emerged as highly sensitive pulse techniques that have gained significant traction in various applications.[15] In DPV technique, an initial potential is applied for a predetermined duration (Point S1 Figure 6B1). Subsequently, the potential is incrementally raised by several hundred millivolts and held at this level for a predetermined duration at the end of the pulse (Point S2 Figure 6B1). The potential is subsequently decreased to a level slightly higher than the initial baseline, and this procedure is iterated, resulting in the generation of a waveform that resembles a staircase. The current is assessed prior to the initiation of the pulse and once more toward its termination. This ultimately yields a voltammogram through the subtraction of currents obtained at each sampling point (Figure 6B2).

In the context of SWV, the potential is incrementally adjusted by a magnitude denoted for a duration of approximately few milliseconds (Point S1 Figure 6C1). Subsequently, the potential is adjusted in the opposite direction, but with an opposite sign, for an equivalent duration (Point S2 Figure 6C1). The waveform obtained, as depicted in Figure 6C1, consists of a square wave overlaid on a staircase waveform. The square wave typically exhibits an amplitude of 50/n millivolts.[102] The acquisition of a voltammogram for SWV involves the subtraction of the currents recorded at every sampling period, thereby eliminating nonfaradaic currents. The difference between the forward and the reverse currents (dashed curves Figure 6C2) corresponds to the net current (solid curve Figure 6C2).

In cases where there is an overlap of multiple cathodic or anodic peaks in a standard CV, the electrosynthetic community often uses DPV as a technique to enhance the resolution of these peaks.

Besides, one notable advantage of SWV in comparison to DPV is its enhanced speed and higher current output. In the meantime, SWV can be used to analyze reversible or quasi-reversible electrode processes. These characteristics make SWV a widely utilized technique in fundamental research and the analysis of biological compounds.[103,104]

By using voltammetric techniques to measure the redox potential of the components of a reaction, one may get insight into the different reactions that can occur at the electrodes as well as the ideal potential or current that has to be applied for the reaction that is desired. Besides, bulk electrolysis yields products on a larger scale, necessitating a substantially sizable electrode surface compared to the volume of solution. Below we present these two electroanalytical techniques.

4.2. Constant Current Electrolysis and Constant Potential Electrolysis Techniques

Constant current electrolysis (CCE) (Figure 7A1 and A2) typically makes use of a two-electrode configuration (Figure 7A2), with the exception of situations in which knowledge of the potential is essential.[15] A potential is applied to the WE in order to obtain the desired current. The value of this potential is governed by the thermodynamics of electron transport between the substrate and the electrode. At first, when there is an abundance of reactant, the reaction is restricted by electron transfer kinetics; but, when the concentration of the reactant declines, the reaction becomes limited by mass transfer.[15] Besides, when it is necessary, the WE potential will increase in order to keep the applied current constant. When currents are low, the rate at which electrons are transferred is slow, which in turn makes the electrolysis process take longer. On the other hand, high applied currents might have unintended consequences such as secondary reactions that may affect the substrate. The protection of the substrate is possible with the addition of an overcharge protector.[105] The electrolysis process is complete when there is a sudden change in the WE potential, which is caused by the consumption of all of the substrate (or almost all of it).

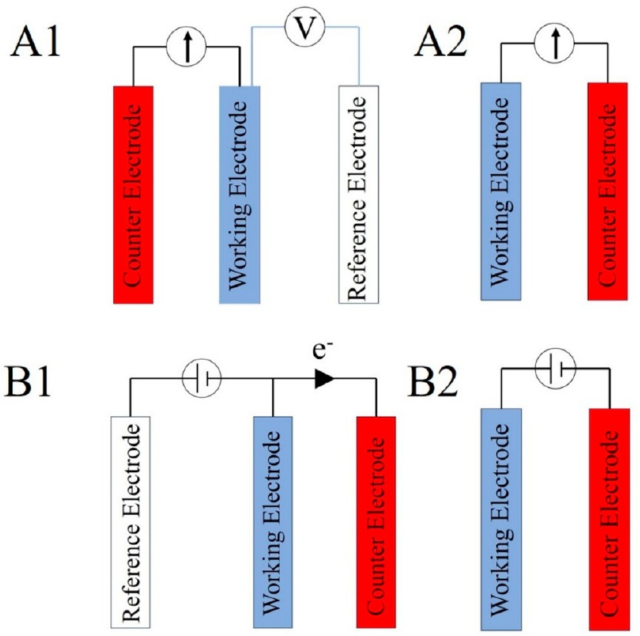


Figure 7. Schematic representation of CCE technique in a (A1) 3 electrode and (A2) 2 electrode configurations. Schematic representation of CPE technique in a (B1) 3 electrode and (B2) 2 electrode configurations.

Constant potential electrolysis (CPE) (Figure 7B1 and B2), in contrast to CCE, requires, in most cases, the inclusion of a reference electrode as part of a three-electrode arrangement in order to get an accurate measurement of WE potential (Figure 7B1).

A voltage is applied to the WE, and then a current response that is proportionate to the concentration of the substrate is detected (as well as the activity of the catalyst toward substrate conversion if a MET is applied). The current drops down exponentially as substrate is used up until it reaches a value that is considered to be the baseline. The potential applied in CPE corresponds to the potential of oxidation or reduction obtained from CV or DPV.[105]

The determination of the number of electrons (n) transferred in the process is one of the advantages of CPE over CCE. In addition, the application of a constant potential facilitates the maintenance of product selectivity. The integral of the chronoamperometric curve (I (A) vs t (s)) corresponds to the theoretical charge (Q in C) that is directly proportional to the number of electrons. Quantitative analysis of products can be obtained via electroanalytical techniques e.g., CV or spectroscopic ones e.g., UV−visible, LC-MS, GC-MS. Then, the ratio between the calculated charge that is directly proportional to the amount of product generated and the theoretical charge corresponds to the faradaic efficiency of the electrolysis.

The choice between methods may depend on the desired mechanistic information, the availability of materials, or the total cost. For example, in their study on electrochemically controlled polymerization processes, Peterson et al. highlighted the advantages of using CCE rather than CPE.[106]

In contrast to CPE, which led to uncontrolled polymerization, TEMPO-mediated anodic CCE in a divided cell was able to accomplish selective control of cationic vinyl polymerization (Figure 8). In this particular illustration, the polymerization process started by the oxidation of TEMPO. The resultant product then underwent a chain-transfer reaction with a dithiocarbamate agent, which led to the formation of intermediate **I**. TEMPO is regenerated as a byproduct of the mesolytic cleavage of **I**, which also results in the formation of the dithiocarbamate intermediate **II** and an oxocarbenium ion that is involved in the reversible addition−fragmentation chain transfer. The introduction of a cathodic current brings about the chain reaction's termination. It is hypothesized that this current will convert intermediate **II** into an anionic form and quench the cation that is responsible for the chain reaction's propagation. In this instance, CCE offered greater response control by making it possible for there to be changes in the WE potential in line with the changing concentration of $TEMPO^+$.

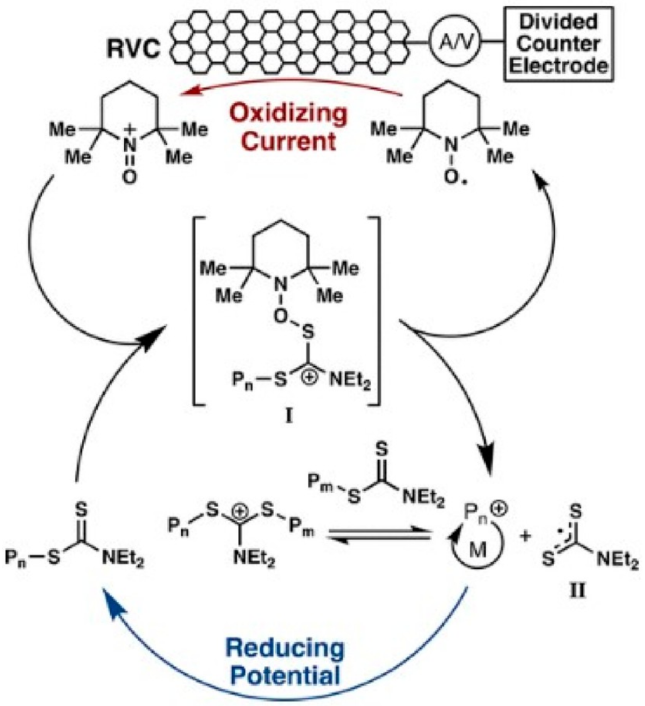


Figure 8. Mechanism of Tempo-mediated polymerization of vinyl ethers.[106] Reproduced from ref 106. Copyright 2018 American Chemical society.

More examples related to the application of CCE and CPE are added in sections 5.1.3 and 5.1.4.

5. RECENT EXAMPLES ABOUT THE APPLICATION OF ELECTROANALYTICAL TECHNIQUES IN ELECTROCHEMICAL SYNTHESIS

5.1. Organic Electrosynthesis

Organic electrosynthesis has been conducted for over 150 years. In 1849, Kolbe discovered the reaction that bears his name: Kolbe's synthesis. This is a dimerization of carboxylate anions at the anode. After two periods of intense experimental research (1920−1940 and 1970−1988), a better understanding of the most common reactionary mechanisms and limits of organic electrosynthesis processes was investigated. In addition to electrode materials and the reactor choice, the electrolyte/solvent choice in organic electrosynthesis is one of the factors to be considered.

The conductivity of the medium must be good, both in aqueous and nonaqueous environments. It is therefore necessary that the electrolytes are soluble and that they are sufficiently dissociated.[107] To do this, the dielectric constant (ε) of the solvent is an important parameter to be considered.[108−110] Baizer has classified solvents into three categories based on the dielectric constant: High ($\varepsilon > 60$), Medium ($20 < \varepsilon < 50$), and Low ($\varepsilon < 13$). In nonaqueous environments, the medium value of conductivity is suitable for organic electrosynthesis. Besides, it is important the analyte is soluble in the solvent.

There must be a distinction between protic and aprotic solvents.[109,111] The first category (such as water and alcohols) severely limits the area both in oxidation and reduction. In addition, they result primarily in protonation reactions. Water is a common medium, but its solvent power is limited to organic molecules. The addition of a miscible cosolvent is possible, but the influence of water will remain predominant on the reactionary mechanism.

It is also possible to conduct studies in dichloromethane or THF ($\varepsilon < 10$) with tetrabutylammonium salts. However, during electrolysis, the high potential difference at the reactor is often excessive, sometimes exceeding the capabilities of the apparatus and/or leading to a sharp heating of the solution that can reach the boiling point of the solvent.[111] Other solvents having high dielectric constants, such as tetramethylurea or hexamethylphosphoramide (HMPA), can be used. However, their toxicity and their purification problems gradually led to their abandonment. Others, such as propylene carbonate or *N*-methyl pyrrolidone, can cause parasitic reactions.

In conclusion, while choosing a solvent-electrolyte system, many factors have to be taken into consideration such as the conductivity with a medium or a high ε, the toxicity, the solvent purification, the potential range of the solvent, and the chemical stability of the system that provides stability for regenerated intermediates and products.[111,112] Below, we present some examples related to the application of electroanalysis in organic electrosynthesis.

5.1.1. Application of Voltammetric Techniques in Materials Synthesis and Characterization. As mentioned before, voltammetric techniques are compatible with a wide range of substances. The application of these techniques can be considered a crucial initial step that leads to the completion of the larger project. Below we present a classification of usage in the characterization and synthesis of compounds.

Application of CV in the Characterization and Synthesis of Organic Compounds and Polymers. The utility of CV in the characterization of xanthone, a bioactive compound, has been demonstrated, indicating that the presence of catechol is crucial for conferring effective scavenging properties against reactive species.[113] Besides, CV analysis conducted on amoxicillin in a methanol solvent exhibited quasi-reversible behavior. Additionally, the redox potential of amoxicillin was observed to undergo changes upon its interactions with metal ions and amino acids. These findings provide valuable guidance in the prescription of this antibiotic, cautioning against its concurrent use with certain metal ions and amino acids.[114−116] The electrochemical behavior of ibuprofen and its degradation was closely monitored using CV. It was observed that the behavior varied depending on the type of electrode used. For instance, adsorption was observed on a silver composite electrode, which provided valuable insights for the selection of an appropriate electrode for the removal of this pollutant.[117] In conjunction with electrochemical impedance spectroscopy (EIS), the utilization of CV has been determined to be highly advantageous in the characterization of redox processes for the purpose of designing biosensors. The behaviors of biosensors are influenced by diffusion and capacitance, which have the potential to provide valuable insights for the design of biosensors. The copolymerization of *N*-dodecylacrylamide (DDA) with ferrocene derivatives on ITO substrates has been found to exhibit favorable properties for use in catalytic and sensor devices.[118] In addition, the successful development and investigation of a polymer-modified electrode, specifically poly(vinylferrocenium) on platinum, was carried out using CV technique. This modification aims to enhance the electrode's applicability in the analysis of organic compounds, including adenine. The synthesis of the ruthenium complex film can be achieved through the process of electropolymerization, followed by its subsequent modification onto a platinum electrode. This

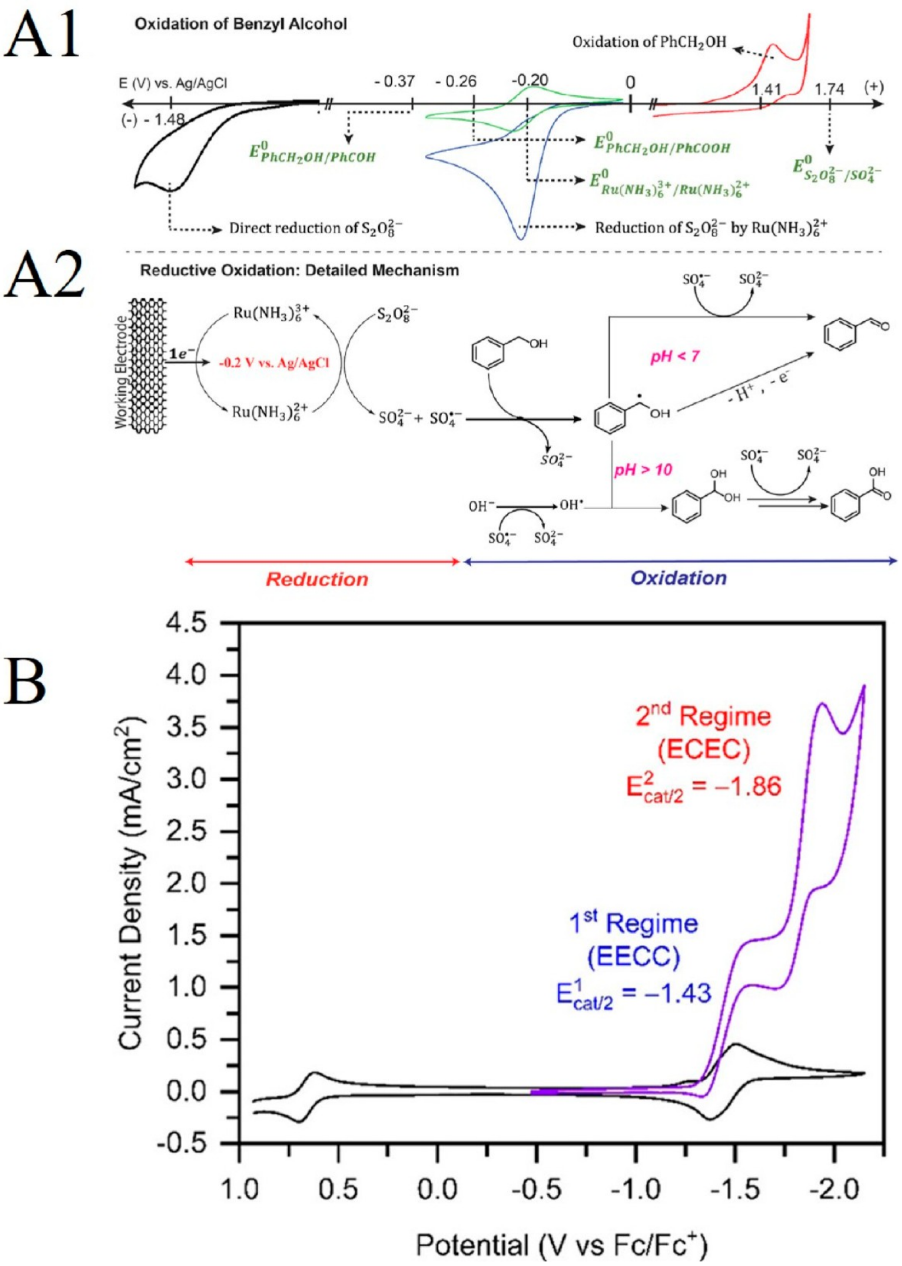


Figure 9. (A1) Voltammetric responses benzyl alcohol oxidation, $S_2O_8^{2-}$ reduction, and $Ru(NH_3)_6^{3+}$ redox mediation. (A2) Scheme representing the synthesis of oxidized products from $Ru(NH_3)_6^{3+}$ reduction depending on pH.[126] Reproduced from.[126] Copyright 2022 American Chemical Society. This work is openly licensed via CC-BY-NC-ND 4.0. (B) Cyclic voltammograms of $[Fe(tpyPY2Me)]^{2+}$ ($[Fe]^{2+}$): $^1[Fe]^{2+}$ scanned using Ar (black) and CO_2 (purple) in 1 M PhOH. CVs were conducted at 100 mV/s in an electrolyte of 0.10 M $TBAPF_6$ dissolved in CH_3CN. Two unique mechanistic pathways for $^1[Fe]^{2+}$ were proposed, demonstrated by the potential measurements where half of the maximum catalytic current ($E_{cat/2}$), and measured at −1.43 and −1.86 V vs Fc/Fc^+ and labeled in blue and red, respectively.[127] Reproduced from ref 127. Copyright 2022 American Chemical Society.

modified electrode serves as a sensor for the detection of hydrazine, and its characterization is conducted through CV.[119] The electroactivity of pyrrole allows for convenient monitoring of the behavior of different polypyrrole variants through electrochemical techniques, particularly CV. Consequently, extensive research on this topic has been conducted since the 1990s and continues to the present day.[120−122] CV can be effectively employed in the characterization of polyaniline to obtain insights into the kinetic behaviors of the dopant acid, which can provide valuable suggestions for film preparations.[122,123]

Recently, it has been also demonstrated that CV can be employed to electrograft molecules onto the surface of boron-doped diamond (BDD) without the need for a reducing agent e.g., methyl benzoic acid moieties. This method allows for the preparation of an ex situ working electrode for the determination of cadmium (Cd).[124] In addition, the electropolymerization of an electrode surface via CV with neutral red was reported by Arechederra et al. The electropolymerization was proceeded while sweeping the potential between 0.8 V and −0.8 V at 50 mV/s (for 30 scans) in the presence of neutral red and sodium nitrate and using phosphate buffer as electrolyte.[125] The

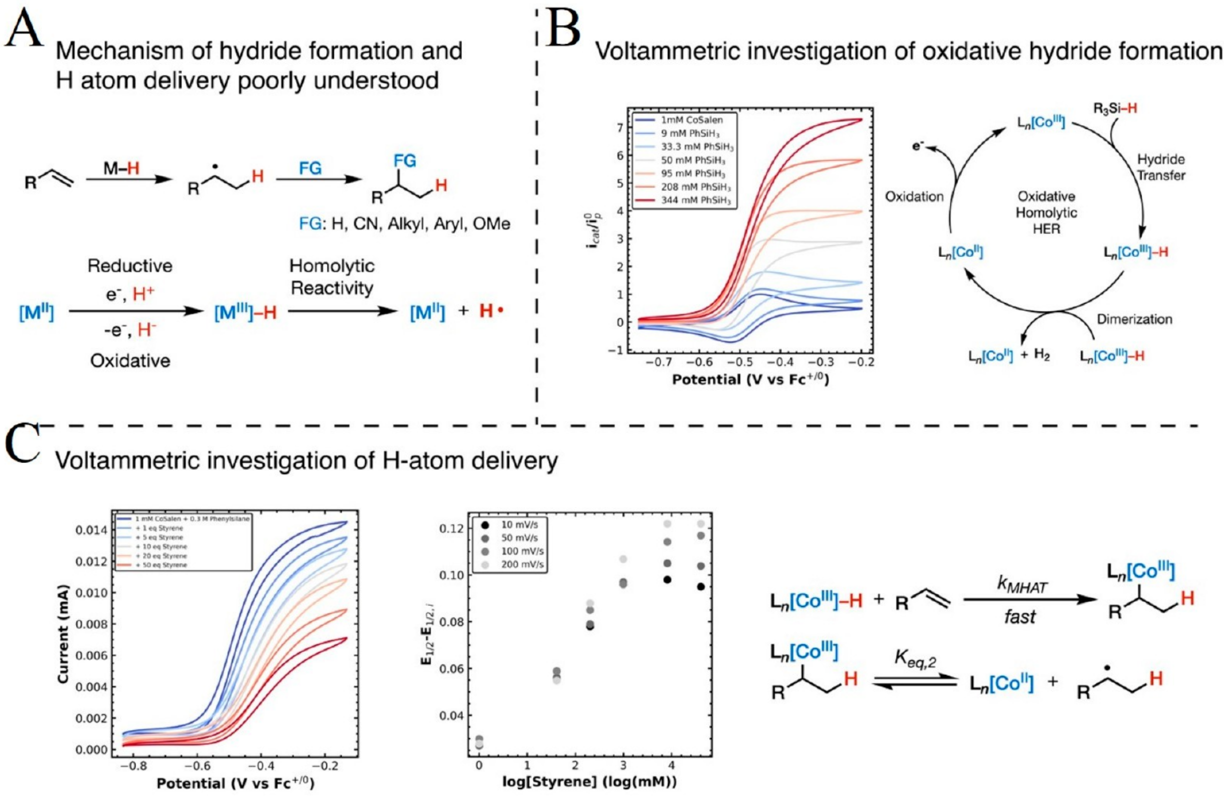


Figure 10. Summary of the voltammetric investigation of MHAT to alkene substrates. (A) Summary of the radical reactivity initiated by MHAT reactions with alkenes via the homolytic reactivity of metal hydrides. (B) Voltammetry of Co(II)(Salen) with increasing amounts of hydride donor, which illustrated ideal catalytic behavior. (C) Voltammetry of Co(II)(Salen) with increasing amounts of aryl alkene substrate; the shifting half-wave potential was indicative of a dynamic equilibrium between the neutral radical and metalated intermediates. Reproduced from ref 129. Copyright 2023 American Chemical society.

electropolymerized electrode was applied in both the regeneration of the NADH cofactor and its oxidation processes.

Hosseini et al. reported alcohol oxidation achieved through redox mediation where a reagent undergoes a reduction process to generate reactive species capable of oxidizing other substrates.[126] CV plays a crucial role in understanding the insight of electrocatalytic oxidation of alcohols through the reduction of peroxydisulfate by providing valuable information about the redox behavior of the mediator and its interaction with persulfate and alcohol substrates. With a DET, the direct reduction of $S_2O_8^{2-}$ occurs at −1.48 V (vs Ag/AgCl). On the other hand, the mediated reaction of $S_2O_8^{2-}$ catalyzed by $Ru(NH_3)_6^{3+}$ is possible at a lower potential. As shown in Figure 9A1, in the presence of the catalyst, a reduction current is observed at −0.2 V vs Fc/Fc^+. The addition of the substrate showed an increase of the current due to the electrocatalytic activity of the catalyst toward the reduction of $S_2O_8^{2-}$. At an electrolysis potential carried out at −0.2 V vs Fc/Fc^+, the generated $SO_4^{\bullet -}$ then produces benzyl aldehyde and benzoic acid, as shown in Figure 9A2.

By carefully analyzing the CV data and comparing experimental results with theoretical models, researchers can deduce the most probable reaction mechanisms governing the electrochemical process.[128] Head-Gordon and co-workers investigated iron-terpyridine complex i.e. $[Fe(tpyPY_2Me)]^{2+}$ ($[Fe]^{2+}$) as a homogeneous electrocatalyst for converting CO_2 into CO with turnover frequencies exceeding 100,000 s^{-1}.[127] The combination of theoretical and experimental studies reveals two distinct mechanistic pathways for the electrochemical CO_2 reduction catalyzed by $[Fe]^{2+}$, depending on the applied overpotential (Figure 9B). At low overpotentials, $[Fe]^{2+}$ undergoes a two-electron reduction. At higher negative applied potentials, an additional electron-transfer event occurs via either a stepwise or proton-coupled electron-transfer (PCET) pathway, leading to catalytic turnover from the monocationic iron complex ($[Fe]^+$) through an electrochemical−chemical−electrochemical−chemical (ECEC) mechanism.

Boucher et al. recently demonstrated the utility of cyclic voltammetry in understanding the mechanism of metal-hydride mediated hydrogen atom transfer (MHAT) reactions with aryl alkenes.[129] MHAT is a popular organic synthetic methodology for hydrofunctionalization and 1,3-isomerization of olefins (Figure 10A). MHAT utilizes the homolytic reactivity of transition metal hydrides to generate neutral radical species for capture by radical electrophiles. The highly transient nature of the metal hydride and organic radical intermediates has long hindered mechanistic understanding of these reactions. However, advances in electrosynthetic MHAT reactions have made an electroanalytical approach to mechanistic studies possible.

Voltammetry was used to establish the oxidative formation of the metal hydride from Co(II)(Salen) and hydride source, phenylsilane. In contrast to transition metal-catalyzed hydrogen evolution, which utilizes the reductive formation of a metal hydride from a proton and electron, synthetic MHAT typically operates by the catalytic oxidation of a hydride to a H atom. Voltammetry of the Co(II/III) couple of Co(Salen) illustrated canonical catalytic behavior upon addition of the hydride source

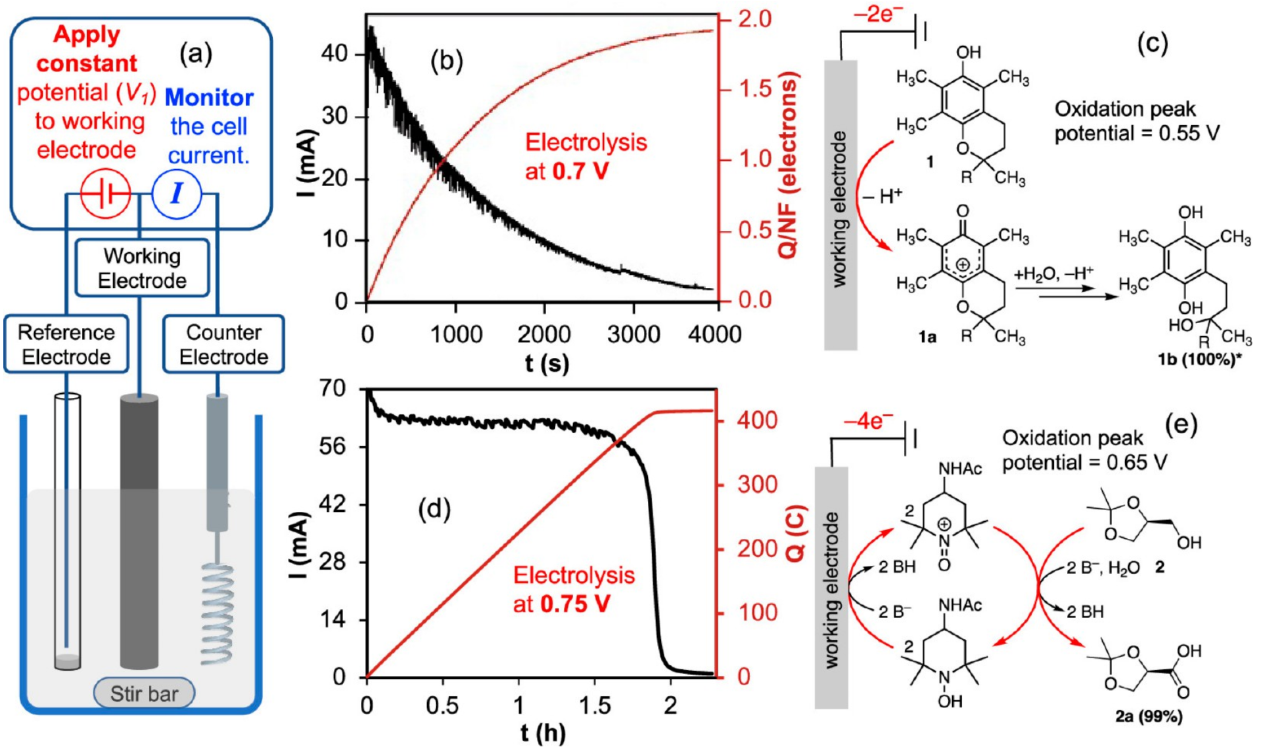


Figure 11. (a) Scheme of a cell with 3 electrode configuration. (b) Current (black trace) and theoretical charge (red trace) for the electrolysis of 13.2 mM vitamin E in CH_3CN at 0.7 V (vs Fc/Fc^+). (c) Vitamin E oxidation reaction.[144] (d) Current (black trace) and theoretical charge (red trace) for ACT-mediated electrolysis of solketal. 0.1 M solketal and 5 mol % ACT in aqueous $NaHCO_3$ solutions at 0.7 V (vs Ag/AgCl) are the reaction conditions. (e) Oxidation of solketal catalyzed by ACT.[146] Reproduced with permission from ref 147. Copyright 2021 American Chemical Society.

(Figure 10B), confirmed to be oxidative hydrogen evolution by gas chromatography. From the ideal S-shaped voltammograms the authors analyzed the catalytic plateau current as a function of hydride donor concentration to demonstrate that metal hydride formation was rate limiting and tunable based on ligand design.

Additionally, voltammetry was also used to study the reaction of the cobalt hydride with aryl alkene substrates. Diminished current and anodic shifts in the catalytic voltammograms upon addition of styrene derivatives indicated the formation of metal−alkyl intermediate in dynamic equilibrium with the neutral radical (Figure 10C), which was found to depend on the electronic structure of the styrene derivative. Variation in these shifts with scan rate suggested the primacy of the hydrometalated intermediate, which then decays to the neutral radical via reversible cobalt−carbon bond homolysis similar to the protected radicals in B_{12} and radical SAM enzymes. These mechanistic details help to explain the versatility of MHAT reactions in many types of transformations via both radical or metalated intermediates.

Finally, CV has been widely used in the analysis of specific electroactive compounds, such as quinones in natural rubbers, to gain a better understanding of their properties.[130] Hence, it is evident that CV will continue to be extensively employed in the field of organic synthesis and characterizations for the foreseeable future.

Application of CV in the Characterization and Synthesis of Metallic Nanoparticles, Metal Complexes, and Inorganic Materials. The study of metal−ligand electrochemical behavior can be effectively conducted using CV due to its ability to analyze electron transfer involved in complex formation. This has been demonstrated through simulations conducted at various ligand concentrations, and the determination of global formation constants was possible.

Recent research has provided evidence supporting the utilization of CV as a complementary technique to validate the findings of X-ray crystallography. This research specifically focuses on elucidating the behavior of copper-thiourea-halide complexes. Besides, the utilization of CV has demonstrated the utility of redox potential and reversibility in investigating metalloenzymes with molybdenum cofactor complexes as a model for elucidating their physiological function.[131]

CV can also serve as a method to track the behavior of activated iridium oxide film microelectrodes. This can be achieved by adjusting the scan rate during both the fabrication and animal implantation processes.[132]

In addition, the usage of a gold rotating disk electrode (Au RDE) has demonstrated its usefulness in assessing the surface coverage of silica microparticle monolayers. It has been observed that the adsorption strength increases with larger particle diameters and higher surface coverage. This insight provides valuable information regarding the deposition of different particles onto surfaces.[97] The characterization of the synthesized Pt−Ru catalysts and their catalytic activities in relation to fuel cells has been conducted using CV.[133] Besides, the functionalization of electrodes surface with active species has gained big importance (e.g., Rh complexes for the regeneration of the NADH cofactor[134] and platinum and gold nanoparticles (AuNPs) for hydrazine detection).[135]

These inorganic functionalized electrodes are in big competition with enzymatic systems where electrodes surfaces are modified with electrocatalytic enzymes (e.g., NAD^+ reductase for NADH regeneration,[136] ferredoxin $NADP^+$ reductase for NADPH regeneration),[137] and hydrogenase for

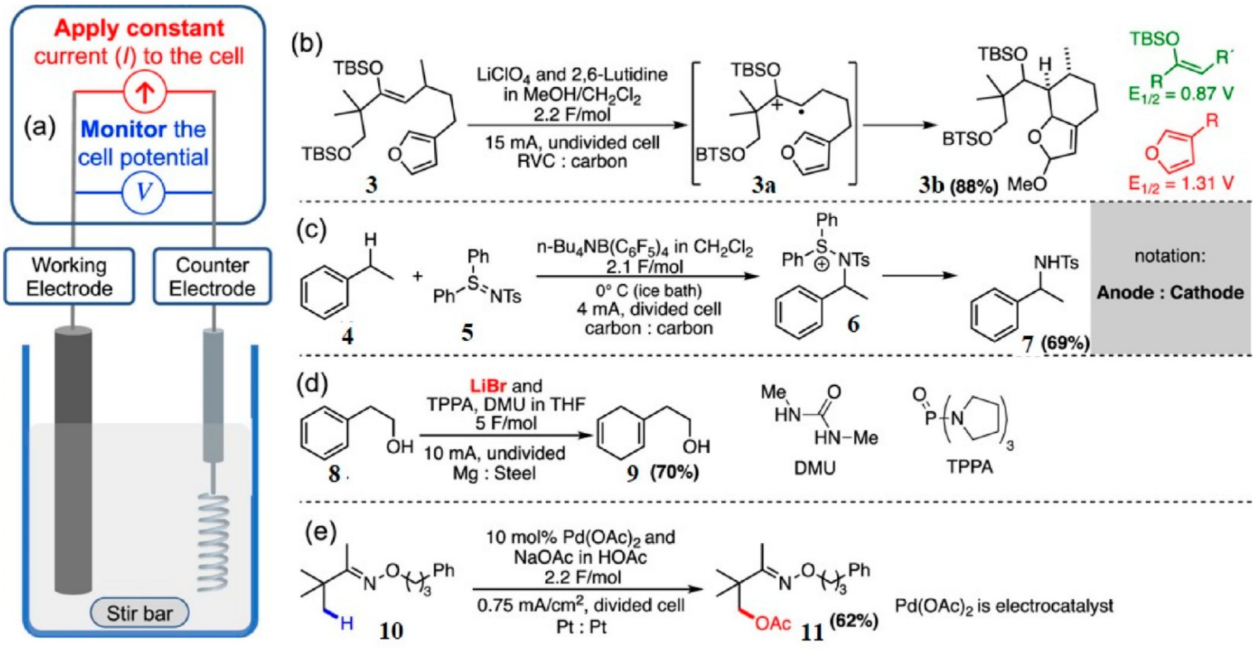


Figure 12. (a) Schematic cell configuration for 2-electrode CCE with examples (b−e). Refer to refs 149, 150, 107, and 151 for specific information regarding reactions (b), (c), (d), and (e) respectively. Individual yields are shown between parentheses. Reproduced ref 147. Copyright 2021 American Chemical Society.

hydrogen production and oxidation.[138] The choice between inorganic and enzymatic systems depends on the activity, stability, and economic viability of the process in which the catalyst is applied.

Application of SWV in Studying the Electrochemical Behavior of Chemicals. Sheng et al. reported the cathodic reduction peak during electrochemical allylic hydro-defluorination using ammonia as the hydrogen source.[139] The SWV analysis of cathodic reduction peak reveals the transfer of two electrons during the reduction process. Tang et al. investigated the formation of the redox species during the synthesis of Co(II) complexes of CoBr$_2$ and two *N*,*N*,*N*-tridentate ligands with different proportions.[140] Besides, Roesel et al. reported the electrochemical transformation of *o*-arylthiocarbamates to S-aryl derivatives using Newman−Kwart rearrangement.[141] The combination of experimental techniques and computational studies was employed to investigate the stepwise electron transfer processes, reactive intermediates, and the role of the electrocatalyst in promoting the rearrangement. The Hammett plot was constructed based on DPV analysis revealing that the oxidation of O- and S-aryl thiocarbamate generates radical cations.

5.1.2. Scanning Electrochemical Microscopy.

Scanning electrochemical microscopy (SECM) is a powerful and versatile electroanalytical technique that has gained popularity in various fields, including electrosynthesis.[142] SECM allows researchers to visualize and probe electrochemical processes at the micro- and nanoscale, providing valuable spatial information about electroactive species and redox reactions. In electrosynthesis, SECM offers unique capabilities to study the local electrochemical behavior on electrode surfaces, investigate catalytic activity, and optimize reaction conditions. SECM operates based on the principles of a feedback control system. A microelectrode probe is positioned close to the electrode surface of interest, and the distance between the probe and the surface is adjusted to maintain a constant current or potential difference. As the probe scans across the surface, it measures local electrochemical currents, generating data that can be used to construct maps of electrochemical activity. By scanning the probe in various patterns, researchers can obtain detailed images of electrochemical reactions with high spatial resolution.[143] In the context of electrosynthesis, SECM can provide spatial understanding of the reactant and product distribution, the influence of electrode morphology on catalytic activity, and the effects of different reaction conditions on the overall electrochemical performance. Chen and co-workers investigated the effect of acid treatment on Cu/Al alloy for the CO$_2$ reduction reaction.[143] The SECM investigation showed superior activity at acid-treated samples compared to flat catalytic surfaces. Moreover, SECM can be used to study the dynamics of electrode reactions in real time, offering insights into the kinetics of electrochemical transformations during electrosynthesis. Overall, the application of SECM in electrosynthesis opens new avenues for improving the design and implementation of sustainable electrochemical synthesis methods.

5.1.3. Organic Electrosynthesis with Constant Potential Electrolysis.

CPE is one of the techniques applied in electrosynthesis (Figure 11a). As shown in Figure 11b and 11c, one example of CPE is a process in which the substrate undergoes a direct electrolysis. The process involves the oxidation of vitamin E (noted **1**). The oxidation potential of **1** was determined by CV to be 0.55 V (versus Fc/Fc$^+$).[144] The working electrode potential for the CPE was carried out at 0.7 V (versus ref). The applied electrolysis potential is 150 mV more positive than the oxidation peak potential that was found by CV.[145,146] As can be seen in Figure 11b (the black trace), the current drops as the concentration of **1** is depleted. **1** is the only electroactive species at this applied potential. The theoretical charge is also shown (the red trace in Figure 11b). This charge reveals the consumption of 2e$^-$ per molecule. When the spent charge and the theoretical charge are identical, charge efficiency is calculated as 100%, since all oxidation currents are the

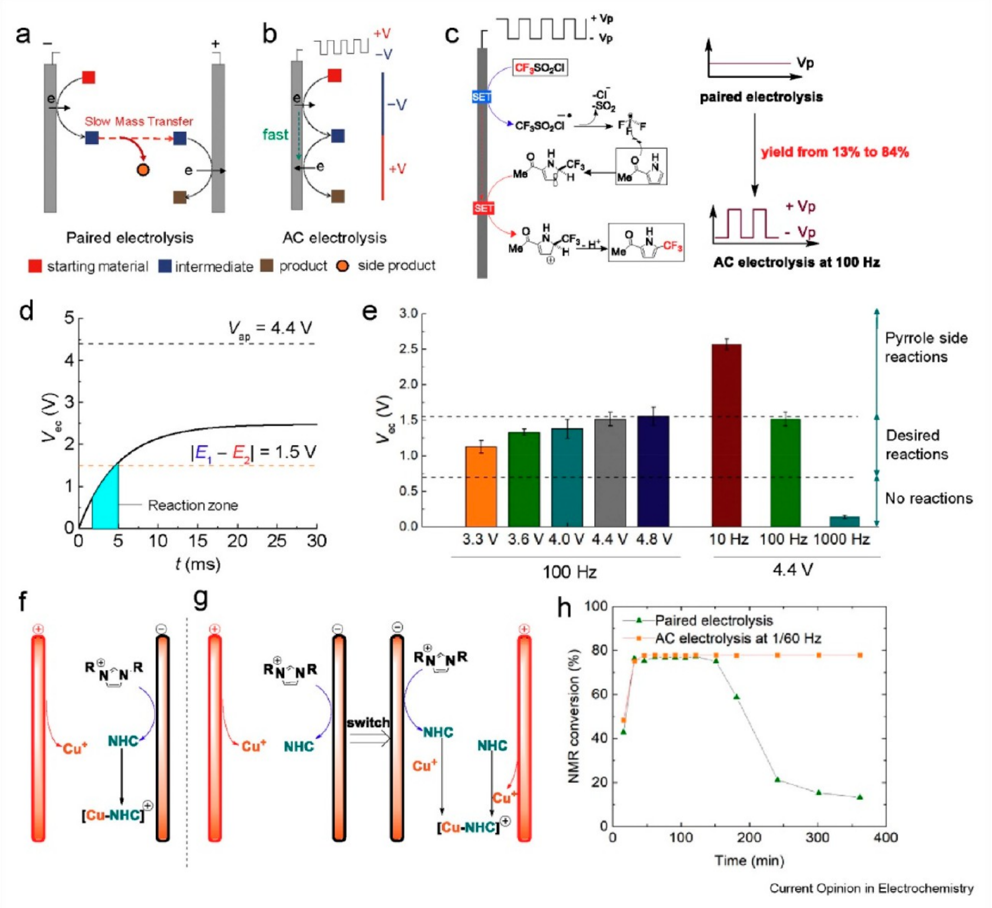


Figure 13. Schematics of (a) paired electrolysis and (b) AC electrolysis for a redox-opposite two-step sequential reaction. (c) Mechanism for the trifluoromethylation of 2-acetylpyrrole via AC electrolysis. (d) Theoretical modeling of the voltage available for electrochemical reactions (V_{ec}) in relation to the duration of the voltage pulse, t. The blue-highlighted region represents the reaction zone. (e) Predicted V_{ec} and potential reactions for trifluoromethylation of 2-acetylpyrrole at various AC voltage amplitudes and frequencies. Cu−N-heterocyclic carbene (Cu−NHC) complexes are synthesized via (f) paired electrolysis and (g) AC electrolysis, respectively. (h) Comparison of the long-term stability of a reaction with constant voltage versus 1/60 Hz AC voltage.[163] Reproduced with permission from ref 163. Copyright 2021 Elsevier.

consequence of the intended reaction. In other words, the desired reaction is responsible for the obtained oxidation current. In general, the charge efficiency of processes involving controlled potential electrolysis is rather high.

Besides, an example of a mediated electron transfer electrolysis reaction is the oxidation of an alcohol mediated by an aminoxyl radical.[146] Figure 11d depicts the electrolysis current and the theoretical charge traces (black and red, respectively) for the electrolysis of solketal (noted **2**) mediated by 4-acetamido-TEMPO. Figure 11e depicts the associated reaction scheme. The current behavior remains constant as far as the substrate **2** is in excess. However, a sudden decrease in current is observed upon substrate consumption. Under bulk electrolysis conditions, the faradaic efficiency is equal to 92%. While comparing the shapes of currents in direct versus mediated electrolysis, it appears the current in direct electrolysis decreases gradually as the concentration of substrate decreases; however, in mediated electrolysis case, the current decreases rapidly before the total consumption of the substrate. This means that the current of the mediated electrolysis is a function of the local concentration of substrate that is reacting with the mediator.

In these two electrolysis examples, preventing side effect reactions was avoided by controlling the conditions, and this has led to high faradaic efficiency. The utilization of a three-electrode CPE configuration is beneficial in the context of electrocatalytic oxidation, particularly when the substrate exhibits a redox potential that is marginally higher than that of the catalyst.[87]

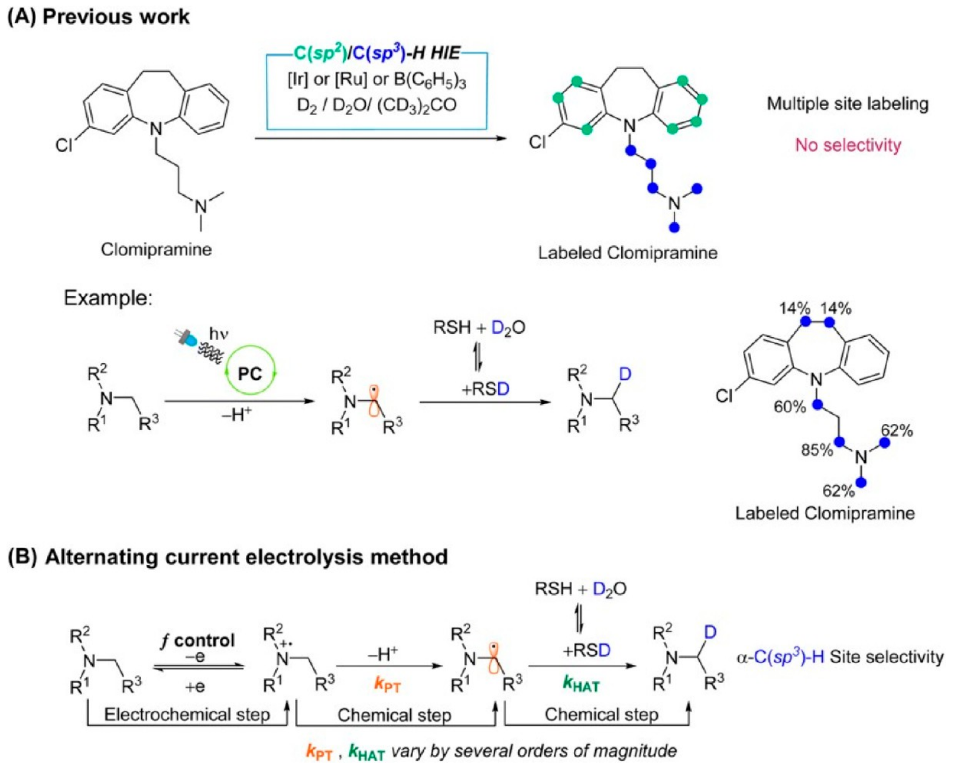
5.1.4. Organic Electrosynthesis with Constant Current Electrolysis (CCE).

The two-electrode CCE (Figure 12a) can be conducted using a variety of affordable and easily accessible power supplies capable of applying a DC. This includes the DC power sources commonly found in portable electronic devices.[148] Several affordable power devices on the market feature the capability to exhibit both current and potential values. This enables users to calculate the theoretical charge (Q).

While the precise control of the working electrode potential may not be possible, it is important to note that stopping the reaction after the passage of theoretical charge can serve as a means to achieve selectivity, particularly if there are significant differences between redox potentials of the reactants.[149] An example of this phenomenon can be observed in the oxidation of compound number **3**, which consists of two distinct functional groups exhibiting different redox potentials. (Figure 12b).

By allowing the reaction to proceed until 2.2 Faradays per mole of charge had been transferred, the functional group with a comparatively lower redox potential underwent oxidation,

Scheme 1[a]



[a](A) Illustration of previously reported methods for hydrogen isotope exchange (HIE) at C(sp^2)−H and C(sp^3)−H sites, with the D-labeling of -amine sites of clomipramine using a photocatalyst (PC) and Hydrogen Atom Transfer (HAT) thiol catalyst as an illustration. (B) AC electrolysis method for deuteration at −C(sp^3)−H sites that may achieve -amine site selectivity through AC-frequency.[166] Reproduced with permission from ref 166. Copyright 2023 Royal Society of Chemistry.

leading to a significant yield of the intended product.[149] Despite that, running the experiment at low current decreases the kinetics of the reaction, and high selectivity and faradaic efficiency were obtained after the consumption of the reactant (**3**).[9]

A noteworthy illustration is the notion of a "cation pool" in the context of oxidative functionalization of ethylbenzene derivatives (**4**). The objective of this reaction design is to generate a product or intermediate (**6**) with a positive charge, possessing a significantly elevated oxidation potential compared to the initial compound, thereby mitigating the risk of excessive oxidation (Figure 12c). The reactions were ended with a faradaic efficiency of 69% and a maximum product yield of 86% was attained.

In numerous cases, accurately determining the ending of electrolysis reactions is challenging due to the low current efficiency. Measuring the consumed charge or monitoring the cell potential may not provide precise results in such situations. In such cases, the reaction process can be monitored using thin-layer chromatography[149] or a spectroscopic technique[107] to accurately determine the end of the electrolysis (Figure 12d).

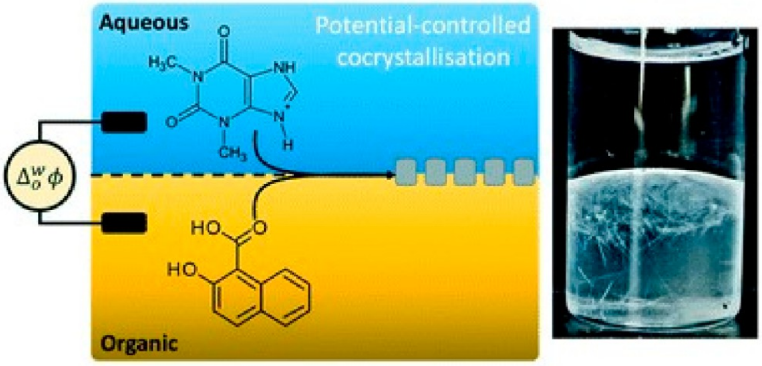
Figure 12e depicts a well-known strategy for characterizing secondary reactions and optimizing reaction conditions with a variation of the applied currents.[151] The impact of current level on reaction yield is well reported in the production of asymmetric biphenols through anodic C−C cross-coupling and electrochemical Birch reduction.[107,152] Notably, the current can be modified. However, it is important to recognize that the surface area of the electrode plays a crucial role in determining the efficacy of electron transfer via redox-active species. Ensuring the reproducibility of CCE reactions involves reporting the current density per unit area of the electrode.[153,154]

5.1.5. Electrolysis with Alternating Current (AC) for Organic Electrosynthesis.

Recent interest has been drawn to electrochemical organic synthesis due to its unique reactivity and environmentally friendly methodology. However, the use of paired electrolysis processes is restricted due to the sluggish mass transfer that occurs between two electrodes, which necessitates the presence of stable intermediates.[155−162] Due to the loss of intermediates during mass transfer, paired electrolysis typically results in low yields for reactions involving short-lived intermediates (Figure 13a). Recently, alternating current electrolysis has increased in interest, which eliminates this disadvantage of paired electrolysis. An alternating voltage is utilized to apply sequential driving force to the redox transformations of the substrates on the electrode (Figure 13b).

In this particular scenario, it is not necessary for intermediates to move between the two electrodes. As a result, short-lived intermediates are able to react instantly to the reversal in polarity between the electrodes. This advantage of AC electrolysis was demonstrated by Rodrigo et al.[164] with the example of trifluoromethylation of (hetero)arenes. This model reaction proceeds through a sequential mechanism. Initially, triflyl chloride undergoes reduction to generate a CF_3 radical. Subsequently, the highly reactive CF_3 radical reacts with aromatic systems, leading to the formation of a radical intermediate. Finally, this intermediate is oxidized to yield the desired product. (Figure 13c). The instability of radical intermediates formed by the addition of CF_3 radicals to (hetero)arenes, which lack aromaticity, leads to low yields of

Scheme 2. Schematic Illustration of the Cocrystallization Process of Caffeine and 1-Hydroxy-2-naphthoic Acid at the Aqueous–Organic Interface with a Monitored Potential[167a]



[a]Reproduced with permission from ref 167. Copyright 2021 Royal Society of Chemistry.

trifluoromethylated products when employing the paired electrolysis method. The trifluoromethylated product yield for the model substrate 2-acetylpyrrole, when a constant voltage was applied, was determined to be 13%. At the frequency of 100 Hz, which is considered optimal for AC, the oxidation of radical intermediates into the final product was found to be highly effective, resulting in an increased product yield of 84%. A numerical model was developed to analyze the voltage available for electrochemical reaction (V_{ec}) due to the significant impact of charge and discharge of the electrical double layer on the AC electrolysis process at high frequencies. The zone of reaction during each voltage pulse was determined (Figure 13d). In addition, they utilized this model to calculate the actual V_{ec} under various AC electrolysis voltage amplitudes and frequencies and to predict the possible reactions that would occur under these electrolysis conditions (Figure 13e). The predictions were highly consistent with the experimental findings and exhibited a high level of consistency with the experimental results. The electrogenerated intermediates, which are retained within the diffusion layer of an electrode, exhibit both effective and reproducible reactions. In their study, Schotten et al.[165] observed that the electrosynthesis of Cu–N-heterocyclic carbene complexes ($[Cu{-}NHC]^+$) using a constant voltage demonstrated inadequate reproducibility. The reduction of 1,3-Bis(2,4,6-trimethylphenyl)imidazolinium chloride (IMesHCl) to NHC is necessary for this reaction (Figure 13f). The limited reproducibility observed in paired electrolysis can be attributed to the electrodeposition of copper ions onto the cathode. Over time, these copper ions form dendritic structures of copper metal, which can cause short circuits during extended reaction periods. (Figure 13h). By employing AC electrolysis at a low frequency, the researchers successfully mitigated metal deposition and dendrite formation, thereby ensuring the long-term stability of the reaction. Furthermore, it has been observed that the reaction rate is often higher with AC voltage compared to a constant voltage. This can be attributed to the continuous replenishment of IMesHCl near the electrode surface during voltage pulses.

In a more recent study, Behera et al. presented an electrochemical method for facilitating hydrogen isotope exchange (HIE) at α-C(sp^3)–H amine sites.[166] Tetrahydroisoquinoline and pyrrolidine have been chosen as two representative substrates due to their distinct kinetics in proton transfer (PT) and hydrogen atom transfer (HAT) at the α-C(sp^3)–H amine sites. These differences are exploited to manipulate the outcome of the hydrogen isotope exchange (HIE) reaction under varying frequencies of applied alternating current (AC) (Scheme 1). The tetrahydroisoquinolines exhibited the greatest level of deuterium incorporation when subjected to direct current (DC) electrolysis conditions at 0 Hz, while the pyrrolidines demonstrated the highest deuterium incorporation at 0.5 Hz. The examination of the distribution of products and the incorporation of D isotopes at various frequencies indicates that the hydrogen isotope effect (HIE) of tetrahydroisoquinolines is constrained by the sluggish hydrogen atom transfer (HAT) process, while the HIE of pyrrolidines is constrained by the excessive oxidation of their α-amino radical intermediates. The potential utilization of AC-frequency-dependent HIE (Hydrogen Isotope Exchange) in amines holds promise for the selective labeling of α-amine sites within a single drug compound. This development has the potential to greatly influence the pharmaceutical sector.

5.1.6. Liquid–Liquid Electrochemical Extraction for the Synthesis of Controlled Sized Cocrystals. Controlling the growth of cocrystals is a significant goal in the pharmaceutical and medicinal chemistry. Herzog et al.[167] reported an electrochemical cocrystallization process that takes place at the interface between an aqueous and an organic phase. 1-hydroxy-2-naphthoic acid (1H2N) is soluble in 1,2-dicloroethane (DCE); however, caffeine is soluble in the aqueous phase (Scheme 2). The formation of a polymorphic cocrystal of caffeine:1-hydroxy-2-naphthoic acid was facilitated by favorable positive interfacial potentials. X-ray diffraction demonstrated that selective cocrystallization was achieved in this manner.

In this work, a potential was applied at the interface between the aqueous phase and the organic phase. The driving force present in this scenario led to a transfer of charge across the interface. As a consequence, cocrystal generation occurred at the interface. Besides, the idea was to figure out how the imposed potential difference at the oil–water interface affects the cocrystallization process.

The cocrystals were obtained and subjected to analysis using scanning electron microscopy (SEM). The findings of the study indicated that the most significant crystal sizes for saturated caffeine were observed when an intermediate potential difference was applied, resulting in crystals with big sizes. Conversely, cocrystals produced under the highest potential difference had small sizes.

This observation suggests that the potential difference has an impact on the mass transport of caffeine and consequently affects the size of the cocrystals.

Based on the obtained findings, it appears that the electrochemically controlled cocrystallization technique holds potential for broad applicability across various pharmaceutical compounds.

5.1.7. Application of Electroanalysis in Organic Industrial Electrosynthesis. Industrial electrochemistry is a highly diverse field that includes the synthesis of organic and inorganic compounds, the conversion and storage of energy, and the galvanic deposition of metals and alloys. Electrochemistry also plays a significant role in corrosion protection, sensor devices, and other technologies involving charge transfer at an electrified interface.

In today's world, research is rapidly becoming more specialized to address challenges that include several disciplines. Electrochemical surface technology has been more prevalent in recent years due to the fact that almost every commercial product now goes through some kind of surface treatment in order to improve its usefulness and characteristics.

Electrochemical engineering is a branch of chemical engineering that focuses on the creation and improvement of electrochemical processes by using the basic rules of electrochemistry and chemical engineering. Electrolysis of salt solutions, which results in the synthesis of chlorine and sodium hydroxide, as well as the generation of chlorate and hypochlorite, as well as the production of peroxodisulfate and hydrogen peroxide, are among the most common industrial processes in use today. Other processes that are theoretically possible include electrowinning and refining of metals such as aluminum and magnesium from molten salts or copper, nickel, and zinc from aqueous electrolytes. Additional examples of the significance of electrochemistry in industry today include the electro-organic synthesis of adiponitrile as a precursor to polyamide production (Nylon), electrochemical machining for low-impact structuring of hard metals, electroplating in the galvanic industry, and cataphoretic painting of automobile body panels.

An example of the application of organic electrosynthesis in industry is the "Monsanto process" that refers to the electrochemical hydrodimerization of acetonitrile to produce adiponitrile, a key intermediate in the synthesis of 6,6-nylon.[168] This process achieved a production volume of 340 thousand tons/year in 2010, establishing it as the most significant electrochemical process in the industrial sector. The initial batch process employed in 1965 involved the utilization of a divided cell featuring lead/lead oxide electrodes, alongside a catholyte mixture comprising 16% acetonitrile, 16% adiponitrile, 40% tetraethylammonium ethylsulfate, and 28% water.

A voltage of 12 V was applied to each cell, resulting in a cathode current density ranging from 400 to 600 mA/cm^2, which ultimately led to a selectivity of 92% in the production of adiponitrile. The yearly production of 900 t was achieved through the utilization of a cumulative cell current of 2870 A, while consuming 6700 kW-hours per ton. Following this, the process progressed through a number of phases of development, culminating in the present second-generation or "new Monsanto" method, which was introduced in 1984. This modernized technology enables similar selectivity and features an undivided flow cell with electrodes composed of carbon steel sheets and cadmium-plated surfaces. The mixture under consideration is a colloidal suspension of acetonitrile and adiponitrile in an aqueous medium, comprising 0.4% hexamethylene-bis(ethyldibutylammonium) and 15% disodium hydrogen phosphate. The distance between the electrodes has been decreased from 70 mm to 1.8 mm, resulting in a notable reduction in the required quantity of supporting electrolyte and enabling a simpler isolation process through straightforward extraction followed by distillation. This process has resulted in a significant reduction of 37% in energy consumption, with the current rate standing at 2500 kWh per ton.[169]

Another example of the application of electroanalytical techniques in industrial organic and inorganic electrosynthesis is the synthesis of metallic nanoparticles by "VITO company".[170] This company described a method for the synthesis of platinum group metal nanoparticles (NPs) using the gas diffusion electrocrystallization (GDEx) technique (Figure 14).

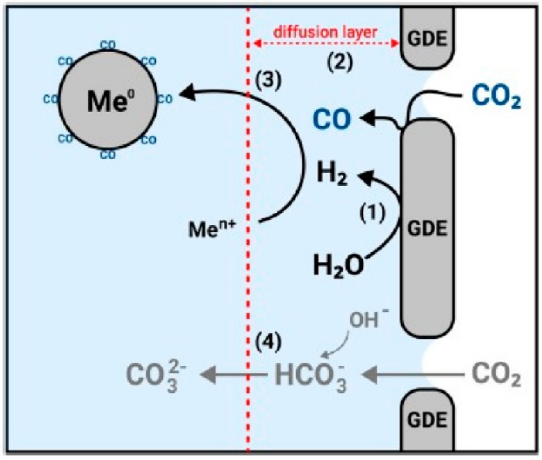


Figure 14. Gas-diffusion electrocrystallization (GDEx) electrochemical reactor schematic for the synthesis of PGM NPS. In GDEx, H_2 from the water reduction reaction (WRR) (1) functions as a reducing agent, and (2) CO from the CO_2 reduction reaction (CRR) regulates the size of elemental nanoparticles composed of metal ions in solution (3). (4) The ratio of unreacted CO2 to HCO_3^- and CO_3^{2-} is crucial for using up the OH^- produced by the WRR and CRR and ending the process.[170]

CO_2 and water undergo simultaneous electrochemical reduction at the triple-phase boundary of uncatalyzed gas-diffusion electrodes, yielding H_2 and CO. Both gases, but H_2 in particular, are reducing agents for water-soluble noble metal ions, resulting in the formation of nanoclusters of metal. CO can act as a capping agent as well. Additionally, the presence of CO_2 prevents the formation of metal hydroxides by stabilizing the pH. In this process, an electrolysis current of −30 mA/cm^2 was applied.

5.2. Bioelectrosynthesis

Although organic electrosynthesis offers a way to carry out several chemical reactions in an environmentally compatible way, such complex organic reactions can be equally carried out by biological catalysts in an even greener way. That is because biological catalysts, such as enzymes and microbes, work intrinsically at milder conditions than organic catalysts with very good selectivity and activity. One example, that will be illustrated further, is nitrogenase enzyme that can catalyze the production of ammonia and hydrogen at ambient conditions opposed to the industrial Haber-Bosch process that requires high temperature and pressure. Enzymes have developed the

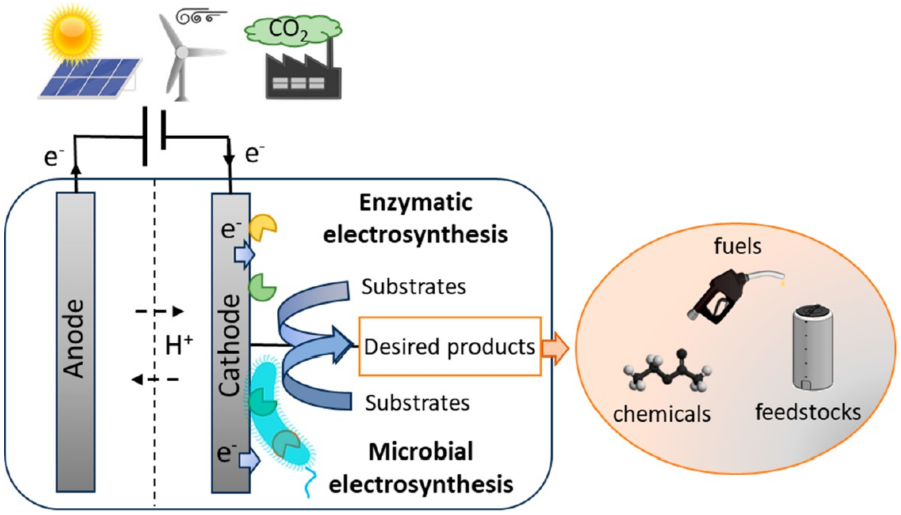


Figure 15. Bioelectrocatalytic synthetic systems.

ability to catalyze crucial chemical reactions such as formation of C−C bonds and nitrogen reduction, which are of high interest for industrial synthetic processes, as a result of billions of years of evolution. Some of these catalysts, designated as bioelectrochemical systems (BESs), are able to carry out electrochemical processes, transforming organic compounds into electrical energy and vice versa (Figure 15). A main distinction can be made on the typology of biocatalysts applied in BESs: enzymes and microbial cells.[171] Simpler than intact cells but more complex than individual enzymes, organelles (mitochondria and chloroplasts) are an additional class of biocatalysts that can be applied for electrochemical reactions. Inspired by nature, scientists have applied BESs for four main purposes: bioremediation, biosensing, the production of electrical energy, and the production of valuable chemicals, feedstocks and fuels. This last feature gave rise to the relatively new field of bioelectrosynthesis.[171] In the following sections, a description of the main electrochemical techniques applied to BESs, as well as a comprehensive discussion of the principles governing the functioning of biocatalysts and the factors crucial for their activity, will be treated. Recent advances in this field and examples of significant synthetic reactions by BESs will be summarized.

5.2.1. Electroanalytical Techniques for Characterization of Bioelectrochemical Systems.

Factors like geometric shape and surface area of the electrode, the ratio between the electrode area and volume of the electrolyte, and type of cell, can play a role in the BESs performance. The catalytic performances of BEs, resulting from structural and functional changes that impact biocatalyst activity as well as originating from various other factors such as electrode material, can be evaluated electrochemically.[172] Electrochemical techniques provide a large amount of information in real-time at low costs without the requirement for sample collection and off-line monitoring favoring a more automated and efficient process control.

Protein film voltammetry (PFV) is commonly applied to the study of redox proteins.[173] CV is the most popular voltammetric technique used in PFV. The current generated upon application of a potential provides kinetic and mechanistic information about the enzyme object of study. CV can offer insights on the type of electron transfer (ET) mechanism, direct or mediated, of both enzymes and electrogenic microbes.[174] CV can be used to detect the presence of membrane bound redox proteins responsible for ET in electroactive bacteria and their redox potential.[174] It can assess the redox activity of bacteria after immobilization at the electrode or after performing catalysis over a period of time. It can be used as well to detect the presence of secreted diffusible redox mediators responsible for mediated ET and establish their contribution in the overall ET. Such contribution can be determined by comparison of the redox response in CVs obtained from bacteria in the same growth medium (that possibly contains secreted redox mediators) and in newly filtered medium.[175] Diffusion limitations in the system and distintions between electrochemical interactions that occur at electrode surface and the bulk phase can be evaluated by CV by correlating linearity of peak current to various scan rates. CV can be used to determine the projected surface area of the electrode. This is one of the most important parameters that has to be taken into account when comparing the performances of different bioelectrode materials.[176] This is expressed by the Anson equation:

$$Q = 2nFACD^{1/2}\pi^{1/2}t^{1/2}$$

where Q is the total experimental charge obtained by integration of the peak area in a CV associated with a specific redox process, n is the number of electrons involved in the electrochemical reaction, F is the Faraday's constant (96 485 C mol^{-1}), A is the electrode surface area, C is the concentration of the redox active catalyst or mediator, D is the diffusivity, and t is the time. Though, the Anson equation is not representative of three-dimensional, nanostructured or highly porous electrodes or electrodes modified by biofilms. The adsorption of electrochemically active molecules and the capacitive charge that is created when microbial systems are used, can indeed also contribute to the total charge consumed at the electrode.[176] SWV, by reducing nonfaradaic contributions, allows a more accurate calculation of k_{ET} and electroactive surface coverage.[174]

Fourier-transformed AC voltammetry can be used to amplify the enzymatic response, in case of poor enzyme loading or orientation.[177] During CV, a large-amplitude sinusoidal wave is applied to the linear potential sweep and the spectrum obtained by Fourier transformation of the current can aid in distinguishing faradaic and nonfaradaic contributions. As an example, this technique has been helpful to separate the catalytic and electron transfer current contributions of [NiFe] hydrogenases.[178]

Chronoamperometry (CA) is probably the most important technique in bioelectrosynthesis since the applied potential enables the constant flow of electrons to the biocatalysts driving the redox equivalents necessary for substrate reduction. The selection of the potential value influences the bioconversion rates and production yield. The current density recorded by CA is a direct measure of the biocatalyst turnover and allows for determination of the heterogeneous k_{ET} for surface-confined bioelectrodes.[174] Amperometric $i-t$ curves generated by enzymatic activity as a function of substrate concentration allows for determination of the apparent K_M of a redox enzyme after fitting data to the Michaelis−Menten equation. In microbial BESs, CA is indeed very useful. When the working electrode is poised at a certain oxidation potential, microbes can use the electrode as the terminal electron acceptor. This configuration is useful for monitoring biofilm growth. The relative increase in current over time is directly correlated with the density of cells immobilized at the electrode as well as substrate utilization by bacteria.[172] Vice versa, when it is poised at the desired reduction potential, the electrons can be used by microbes for driving metabolic pathways involved in bioproduction. In this case, CA monitors the consumption of current by microorganisms. The efficiency of electron utilization in the transformative reaction, also defined as the percentage of electrons that is recovered as product, is the faradic or Coulombic efficiency (ε_C) calculated from the current output generated during CA. In particular, it can be expressed by

$$\varepsilon_c = FM_p\Delta e/\int idt$$

where F is Faraday's constant, M_p the moles of product(s), Δe is the difference in degree of reduction between the substrate and the product, and $\int i\,dt$ is the total charge obtained by integration of the current supplied or produced over time.[176]

Hydrodynamic electrochemistry, CV and CA, is often used to study enzyme kinetics removing mass transfer limitations. In this case, rotating disk electrodes are used to induce a controlled flux of fluid to the electrode surface and different aspects can be studied such as conformational changes, intramolecular electron transfer, or the binding/release of substrates/products from an enzyme active site.[179]

Apart from electrode material properties such as permittivity and conductivity, electrochemical impedance spectroscopy (EIS) can provide information about properties of the biocatalyst-electrochemical interface, kinetic rates of the reaction of interest, mass transfer reaction mechanisms.[172] Since EIS signals can be related to the polar nature of lipid membranes, proton gradients on the outer surface of bacterial cells, and membrane-associated ET reactions, EIS can also be employed for monitoring cell viability, density and physiology of microbial BEs.[172] The exchange current density, j_{ex}, is a reliable parameter for evaluating the performances of BESs. BESs characterized by high j_{ex} are able to exchange electrons and respond to a change in potential more rapidly. This reflects in higher production yields. It can be expressed by

$$j_{ex} = RT/nFR_{ct}$$

where F is Faraday's constant, R is the ideal gas constant (8.314 J K^{-1} mol^{-1}), T is the temperature, n is the number of electrons transferred, and R_{ct} is the charge transfer resistance that can be obtained by EIS.[180]

Electrochemical techniques can be associated with other methods to obtain additional and complementary informations useful to assess BESs. In spectroelectrochemistry, electrochemical techniques are coupled with different spectroscopic methods, such as UV−vis, IR, Raman, for studying ET mechanism in BESs although with important limitations. For instance, IR spectroscopy coupled with electrochemistry has been applied to the study of the stability and the catalytic activity of hydrogenases and nitrogenases.[174] Quartz crystal microbalance with dissipation monitoring (QCM-D) is a robust surface-sensitive technique to monitor changes in the resonance frequencies (Δf_n) and in the dissipation signals (ΔD_n) of the piezoelectric quartz crystal determined by the adsorption of molecules. When coupled with electrochemistry (EQCM-D), this technique can reveal electrochemical properties associated with changes in the film structures. For instance, the comparison between QCM-D measurements of the adsorbed enzymes with CV can give information on the amount of enzymatically active molecules to diagnose the causes of long-term activity loss. Similarly, it can determine the amount of favorably oriented enzymes in the film useful to maximize the ET.[181,182] The adsorption and electroactivity of hydrogenase and formate dehydrogenase have been studied with EQCM-D.[181] Lastly, QCM-D is a robust tool to monitor in real-time bacterial cell deposition on a surface and biofilm growth which are important factors for the creation of a more stable and durable microbial immobilization on the electrode and sustained production.[183]

5.2.2. Enzymatic Electrosynthesis. Extensive research has been conducted over the last decades on enzymatic systems for biosensors, energy conversion, biofuel cells, and biocapacitors. Enzymatic electrosynthesis (EES) refers to a process in which an energy input is driven to the enzymatic catalytic centers of single or multienzymes for the synthesis of value-added chemicals.[184] Direct electrical energy, light, and electrons deriving from either enzymatic or microbial catalysis, can be used as reducing equivalents for the synthesis of the desired products.[184] In EES, the driving force is provided by an electrode that acts as a terminal electron acceptor or electron donor. Although enzymes can generate products and byproducts at both anode and cathode compartments, EES is generally performed at the cathode where the consumption of external electrons drives the reduction of a substrate. In paired electrolysis, both the anode and cathode can participate in the formation of different products.[185]

Enzymatic reactions are usually performed in aqueous environment under mild conditions (room temperature, normal pressure, neutral pH). Some enzymes possess high chemo-, regio-, and stereoselectivities which often exceed that of chemical synthesis. They can achieve high turnover rates and current densities and the overpotential is generally lower compared to direct redox or molecular electrocatalysis.[186] Compared to microbial systems, enzymes allow the simplification of the process with limited side reactions and higher selectivity. For instance, the production of ethanol from CO_2 by microbial systems can lead to the concomitant synthesis of butanol and the reverse reaction can also simultaneously occur.[187] Moreover, the electron transfer process is facilitated due to the absence of cell membranes.[184]

Despite this, EES has some limitations. Enzymes perform a single reaction step thus converting a single substrate upon transfer of only one or two electrons. The intermediate compounds tend to accumulate during the biocatalytic transformation and can inhibit the downstream processing of the substrates. The process of production and purification of enzymes is complex, often requiring genetically modified microorganisms. Furthermore, many enzymes are fragile and have a limited lifetime (typically 7−10 days). Conformational changes in the protein native structure can occur as a result of local changes in environmental conditions (such as pH, temperature, and high voltages) requiring frequent electrode replacement.[171]

Oxidoreductases. EES involves a class of enzymes named oxidoreductases, which account for one-quarter of the known enzymes, that are capable of electron transfer from one molecule to another or to an electrode surface. They can be generally classified into metalloenzymes and nonmetalloenzymes. The reaction range of oxidoreductases is quite vast and includes oxygenation, dehydrogenation, disproportionation, single electron transfer, oxidative bond formation.[188] Dehydrogenases catalyze reversible oxide reduction reactions by removing or adding hydrogen atoms from a variety of substrates. Oxygenase converts alkanes to alcohols, olefins to epoxides, sulfides to sulfoxides, oxidizes aromatic compounds, and cleave carbon−carbon bonds. Reductases are another category of oxidoreductases that only catalyze reduction reactions, such as N_2 reduction to NH_3.[188]

The majority of oxidoreductases require stochiometric amounts of the cofactor that is responsible for the transfer of electrons and protons and in general for the catalytic function of the enzyme. Common metallocofactors include heme, iron center (Fe), iron−sulfur cluster (Fe−S), copper center (Cu), tungsten center (Wco), and molybdenum center (Moco). Nonmetallocofactors can be freely diffusing, such as nicotinamide adenine dinucleotide (NAD(H)) and nicotinamide adenine dinucleotide phosphate (NADP(H)), or enzyme-bound such as flavin adenine dinucleotide (FAD), flavin mononucleotide (FMN), pyrroloquinoline quinone (PQQ).[186] In heme-containing proteins, the prosthetic group is the porphyrin complex of iron(II)-heme or iron(III)-hemin, which is involved in electron transfer processes (cytochrome *b* and *c*),[189] reduction of oxygen to water (cytochrome c oxidase),[190] oxidation of different functional groups by molecular oxygen (P450 monooxygenase),[191] and decomposition of peroxides (peroxidase and catalase).[192] Fe−S clusters are composed of iron atoms bound with sulfur-containing ligands, the rhombic [2Fe−2S] and cubane [4Fe−4S] types are the most simple. Fe−S clusters are present in ferredoxin, hydrogenases, and nitrogenases.[193] Copper centers in proteins are responsible for the transferring of electrons and oxygen to perform various oxidation reactions. Three categories of copper centers can be distinguished: type 1 (T1) or blue copper, type 2 (T2) or normal copper, type 3 (T3) or coupled binuclear copper centers.[194] Laccase is a typical multicopper-containing enzyme which facilitates *n*-diphenylenediamine and phenol oxidation, with concomitant reduction of dioxygen. Flavoenzymes possess flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD), and they carry out various oxygenation reactions including hydroxylation, Baeyer−Villiger oxidations, epoxidations, and sulfoxidations.[195] PQQ-containing oxidoreductases include the quinoproteins whose active sites contain only PQQ, such as glycerol dehydrogenase and glucose dehydrogenase, as well as the quino(hemo)proteins that contain one or multiple heme groups such as fructose dehydrogenase and alcohol dehydrogenase. The oxidized form of the C5 carbonyl in the quinolone ring is highly reactive toward nucleophiles, like ammonia, amines, amino acids, and alcohols.[196]

Since cofactors are very expensive and labile, cofactor regeneration strategies are crucial for thermodynamically and kinetically facilitating product formation and broadening EES applications.

Cofactor Regeneration Strategies. The recycling of the cofactor allows the catalytic reaction to proceed with only small amounts of the cofactor initially added to the reaction medium. Aside from economic costs, continuous regeneration allows the reaction to move forward in the positive direction increasing the yields of generated products. Chemical, enzymatic, photochemical, and electrochemical methods have been developed for cofactor regeneration (Figure 16).

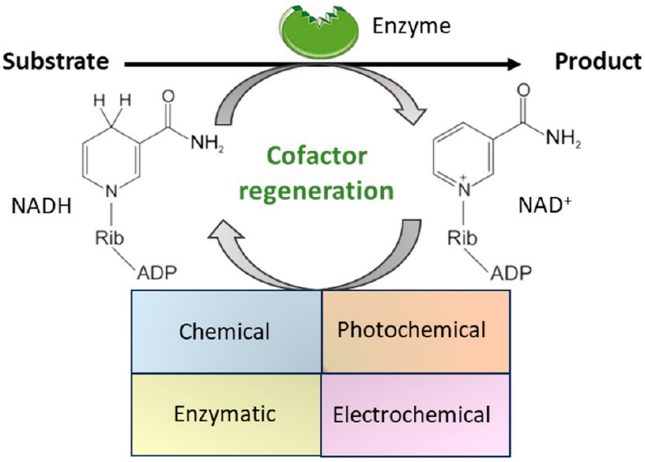


Figure 16. Overview of the main cofactor regeneration strategies.

Chemical regeneration of the cofactors can be achieved by using inorganic reducing agents such as sodium dithionite and sodium borohydride, oxidants such as O_2, or organometallic complexes such as $[Cp^*Rh(bpy)(H_2O)]^{2+}$.[197] The total turnover number (TTN), defined as the amount of product divided by the amount of cofactor, as well as the selectivity, is one or 2 orders of magnitude lower. Importantly, chemical agents can be harmful to enzymes causing their denaturation.[197]

In substrate-coupled reaction systems, only one enzyme is used for product formation while a sacrificial substrate, that is responsible for cofactor regeneration, can be externally added or in situ produced. For instance, H_2O_2 can be added or continuously supplied by electrochemical means in heme peroxidase reactions to regenerate the reactive compound I intermediate.[198] The sacrificial substrate can be harmful to the biocatalyst and its presence complicates successive separation procedures. In the previous example, the porphyrin unit in heme peroxidases is very sensitive to oxidative degradation by H_2O_2 so enzyme inhibition can often occur.[198]

In enzyme-coupled reaction systems, a second enzyme is used with the specific purpose of regenerating the cofactor. During the conversion of a cosubstrate into a coproduct, the cofactor is converted back into the desired form. Regeneration systems based on enzymes are selective and versatile as they afford a wide range of substrates. However, they suffer from possible inhibition effects and intrinsic instability, high cost, and complication of product isolation. The most common enzymes employed are diaphorase, lipoamide dehydrogenase (LipDH), ferredoxin NAD(P)$^+$ reductase, and AMAPORS (artificial mediator accepting oxidoreductases).[186] Other examples of enzymes less commonly used for NAD(P)H regeneration are formate dehydrogenase (FDH), glucose dehydrogenase (GDH), and glutamate dehydrogenase (GluDH).[197] Most of these enzymes require a redox mediator to transfer electrons. For instance, diaphorase together with methyl viologen (MV) was used for the reduction of cyclohexanone derivatives by ADH,[199] pyruvate by D-lactate dehydrogenase,[200] and the synthesis of (*R*)-mandelic acid by benzoylformate dehydrogenase.[201] Some enzymes instead do not require a redox mediator and they can solve this function via the establishment of a direct communication with the electrode. Hydrogenases belong to this group, and they have been applied for NADH regeneration for instance for the conversion of alpha-ketoglutarate to L-glutamate by L-glutamate dehydrogenase.[202]

Photochemical methods, based on the use of solar energy, are relatively new. They mimic the natural process observed in plants where the electrons are stored in NADH for the synthesis of carbohydrates. Mediators and photostabilizers are usually required to increase the efficiency of conversion in the visible region.[197]

Electrochemical methods have great attractiveness, as they are efficient and mass-free. The reducing equivalents in this case are electrons, the cheapest redox equivalents, which are provided by an electrode in a continuous process and they can be obtained from green and sustainable energy sources, such as solar, wind, water, and nuclear energy.[198] Electrochemical cofactor regeneration strategies can be roughly divided into two types: direct and indirect electron transfer.[203]

Regeneration of nicotinamide cofactors is relevant for industrial applications since ~80% of oxidoreductases need NAD(H)/NADP(H).[204] The direct reduction of NAD(P)$^+$ is a two-step process, the first involves the transfer of one electron to form a radical species. Then this radical can be protonated and a second electron can be transferred. Direct regeneration can be achieved by using metal electrodes, metal nanoparticles, metal oxides, and alloy-based materials. During direct reduction at the electrode, electrochemically inactive NAD dimers can be formed. Furthermore, the protonation step is not selective, and besides 1,4-NAD(P)H, which is the desired active form, also 1,6-NAD(P)H can be generated, which is not able to bind enzymes. The direct electrochemical oxidation of NADH requires high oxidation potentials (around 900 mV vs SHE) that can cause electrode fouling, passivation, and unwanted oxidation of side products. The method has a slow reaction speed and poor selectivity. It was successfully applied in the early stages of electroenzymatic synthesis for the conversion of alcohol to aldehyde and glucose to gluconic acid,[204] but for the above reasons is not generally used in practice with the exceptions of reactions that are oxidation-stable and do not form side-products. Indirect regeneration via the use of mediators, which can transfer two electrons in one step and dramatically decrease the overpotential, is thus usually preferred.

Rhodium-based mediators have been the first systems used for NAD$^+$ reduction, in particular, Rh(bpy)$_3$ was applied for the synthesis of cyclohexanol from cyclohexanone by ADH.[205] The incorporation of a pentamethyl cyclopentadienyl (Cp*) ligand, applied for the electroenzymatic synthesis of lactate from pyruvate by D-lactate dehydrogenase,[206] greatly improved the mediator selectivity (>99%) and then it found several applications for the synthesis of different products: the reduction of 4-phenyl-2-butanone to (*S*)-4-phenyl-2-butanol and the synthesis of (*R*)-phenylethanol by alcohol dehydrogenase.[207,208] Recently, electrochemical bulk regeneration of NADH for L-lactate dehydrogenase catalysis has been realized in a bipolar electroenzymatic system based on multiple glassy carbon beads containing a metal−organic framework (MOF) shell functionalized with [Rh(Cp*)(bpy)Cl]$^+$. In this system, mass transport limitations are overcome by mechanical stirring of the beads allowing regeneration of NADH with high turnover frequencies at the negatively polarized cathode while a sacrificial cosubstrate, ascorbic acid, is oxidized at the anodically polarized side. [Cp*Rh(phen)Cl$^+$] containing a 1,4-phenanthroline ligand has been shown to be effective for the synthesis of glutamate.[203] Polymers of dyes such as neutral red, methylene green, and azure B, are another class of mediators that can be applied to cofactor interconversion.

Common 2-electron redox mediators that have been applied for NADH oxidation in electroenzymatic systems are phenanthroline metal complexes, bipyridine complexes, phenazine methosulfate, 3,4-dihydroxybenzaldehyde.[203] The indirect methods involving the use of redox mediators are often combined with enzymatic methods. For instance, diaphorase has been used for NADH oxidation with methyl viologen (MV^{2+}), ferrocene, cobaltocene, quinone, and osmium-derived redox polymers.[186]

For flavin-containing enzymes, cofactor regeneration can be done by molecular oxygen. The principal drawback is the concomitant production of H_2O_2 that can negatively affect enzyme activity and stability in absence of catalase.[198] Electrochemistry is an elegant tool for cofactor regeneration via the use of mediating systems.[209] Polymer-bound ferrocene derivatives served as mediators in FAD regeneration for continuous processes with in situ product removal for the oxidation of *p*-cresol to *p*-hydroxybenzaldehyde by *p*-cresol methylenehydroxylase,[203] xylitol to l-xylose by galactose oxidase[210] and electrolysis of L-glycerin-3-phosphate to dihydroxyacetone phosphate by L-glycerin-3-phosphate oxidase.[207]

Although many regeneration systems have been successfully applied in various biocatalytic processes, they are still hard to scale up because of the relatively small surface area of the electrodes compared to large reaction volumes which lower the conversion rates. Clever design of the electrode-chamber structure and the improvement of electrode surface roughness and porosity are pivotal factors to increase the surface area of the electrode. Microfluidic control can be beneficial and solve the challenge.[203]

Electron Transfer between Enzyme and Electrode. In order to generate high current and power density, it is vital to increase efficiency of the biocatalyst electron transfer rate. Two main mechanisms, direct electron transfer (DET) and mediated electron transfer (MET) are distinguished. In DET, a physical contact between the redox active center of the enzyme (which can correspond to the catalytic center or be an electron donating/accepting site other than the catalytic center) and the electrode can be established. The distance between the redox active center and the electrode is critical and should be shorter than 14 Å to facilitate tunneling via DET according to Marcus theory.[211] Only a few oxidoreductases can perform DET, laccases, and peroxidases are the most studied and characterized. The correct orientation of the redox active sites on the electrode has a pivotal role for achieve effective DET.

MET relies instead on the use of external soluble or immobilized redox mediators to shuttle electrons. Enzymes that possess a redox-active center buried inside a protein/glycoprotein shell, are generally unable to establish electrochemical communication with the electrode and require a redox mediator. Redox mediators display various structural and electronic properties and thus the choice of the most suitable ones depends upon the enzymatic system used. In general, their redox potential should match the target of the bioelectrocatalytic reaction.[186] For oxidative MET, the reduction potential of the mediator should be more positive than that of the enzymatic cofactor, while for reductive MET it is the opposite. Cofactor accessibility of the mediator is also important and dependent upon the active site structure.

In general, DET is simpler and more suitable for the synthesis of high-value chemicals that does not require high current densities, it also obviates stability and toxicity issues linked with leaking mediators. On the other side, the indirect electron transfer, by reaching higher current densities, is more feasible for scale-up industrial production.

Immobilization Methods of Enzymes. The immobilization of enzymes on the electrode surface is fundamental for EES design and operation. It reduces the physical distance between the electrode surface and the enzyme, simplifying diffusion, and allows high loading onto a matrix or surface. Moreover, it protects the enzyme from the surrounding environment (pH, temperature, solvents, etc.) thus increasing enzyme stability and reuse for applications.[171] Immobilization is crucial for the stability and efficiency of enzymatic cascades that involve freely diffusing intermediates. Moreover, for redox active enzymes capable of DET, it is fundamental to achieve the correct orientation of the enzyme on the electrode in order to provide efficient electron transfer. Some of the challenges that need to be overcome are conformational changes of the enzyme after immobilization that cause loss of enzyme activity. Different strategies have been employed over the years to fabricate enzyme-immobilized electrodes.

Adsorption is based on intermolecular forces such as van der Waals forces, dipole−dipole interactions, ionic interactions, and hydrogen bonding. Adsorption is also described as the preferred method to preserve enzyme structure.[212] However, it is not ideal for long-term operation, especially under stirring conditions, because the adsorbed enzymes tend to detach from the electrode surface over time. In order to increase the adsorption rate and selectivity, a substrate mimicking docking site, able to anchor a particular enzyme motif, can be created on the electrode. For instance, anthracene-modified multiwalled carbon nanotubes (MWCNTs) or naphtylene-modified single-walled carbon nanotubes (SWCNTs) were used to immobilize laccase.[213,214] Similarly, a pyrene-modified nickel complex was used to dock His-tagged nitrogenase.[215] Covalent bonding and entrapment are other two major categories of immobilization strategies. The first serves to create a stable bonding but requires knowledge of the structure of the enzyme to obtain specific attachment on the electrode without causing denaturation. Entrapment, which involves employing a cross-linked matrix like a polymeric hydrogel, can be used without selectivity to immobilize a wide range of enzymes. These matrices allow the diffusion of substrates and products while retaining the catalysts close to the electrode. Cross-linkers such as glutaraldehyde, carbodiimide, and maleimide are often used for entrapment.[216] Pyrene−LPEI is a versatile example of a polymer used for enzyme entrapment, the pyrene moieties establish $\pi-\pi$ stacking interactions with carbon-based electrodes.[217]

Electrode Modifications for Improving Enzyme Biocatalysis. The electrode physicochemical and morphological characteristics have a huge impact on biocatalysis. These can be specifically tailored to favor the adsorption of the biocatalysts, enhance the electron transfer rate, improve mass transport of the substrates and products, and increase the electroactive surface area and biocompatibility. Preferred requirements for electrode materials are high electrical conductivity and stability, low cost, corrosion resistance, and scalability. Carbon-based electrodes are the most widely used in combination with biocatalysts since they meet almost all the above requirements.[218] Gas-diffusion electrodes are useful for biocatalysis involving gaseous substrates such as dioxygen, dihydrogen, and carbon dioxide that are directly supplied from the gaseous phase to the reaction layer.[219]

The introduction of three-dimensional nanostructured electrodes, as well as the use of porous materials such as carbon-based foam, carbon felt, graphitic fiber brush, and electron spun carbon nanofibers, can significantly improve the current densities for the possibility to adsorb large amounts of biocatalysts overcoming the challenges linked with ohmic losses and low production yields. This feature is particularly useful in the case of biocatalysts capable of performing DET.[204] Chemical modification of the electrodes and their integration with nanomaterials, including nanoparticles and carbon nanotubes, are central for increasing the electrocatalytic performances of the biosystems. These materials have excellent conductivity and a high ratio of the effective surface area against the projected surface area. Nano-objects and enzymes have comparable sizes, so usually the natural conformation and thus functionality of the enzyme is preserved compared to immobilization on planar electrodes. The size of nanomaterials can be controlled with various manufacturing methods, and should be selected based on the size and hydrophobicity of the biocatalyst. Different enzyme entrapment strategies are based on matching the enzyme size with the pores of the hosting matrix. Moreover, the most utilized micro- and nanostructures are characterized by accelerated heterogeneous electron transfer kinetics due to the strengthening of the electric field by expansion of the electric double layer.[220] As an example, immobilization of glucose oxidase (GOx) in the pores of a mesoporous carbon matrix with a narrowing “bottleneck” pore structure by Kwon et al. showed both improved electron transfer and enzyme stability.[221] The pore-entrapment methodology was found to be beneficial in the efforts to immobilize enzymatic cascades on electrodes. Nano- and microstructured supports, such as silica microspheres, can favor the adsorption of CO_2 and thus increase CO_2 conversion rates by carbonic anhydrase (CA).[222]

The functionalization of electrodes with redox polymers has been largely employed for the entrapment of several oxidoreductases. Redox polymers are constituted by a non-conductive backbone with redox side pendant groups, they are capable of transferring electrons via self-exchange conduction. Naphthoquinone or benzylpropylviologen pendants have been grafted on the backbone of linear polyethylenimine, and cobaltocene pendants on poly(allylamine) backbone, to facilitate MET with enzymes or allow NAD+/NADH interconversion.[223−225] The polymeric microenvironment can protect oxygen-sensitive enzymes against oxygen damage and limit DET contributions by contaminant species.[196] Composite materials that combine carbon nanotubes with redox polymers can be beneficial to improve the mechanical properties and the conductivity of the polymers.

Protein Engineering Approaches. The modification of the enzymatic catalyst is often necessary to improve the performances of the bioelectrodes, in terms of electron transfer rate and reaction efficiency. The most common methods in protein engineering are rational design and directed evolution, which can also be combined. In rational design mutations are inserted in specific locations via site-directed mutagenesis, thus structural protein data, either experimental or theoretical, is necessary. Often the relationship between structure and function is difficult to predict, for instance, the resulting rate of electron transfer after modification. In directed evolution, a detailed crystallographic knowledge of the protein structure is instead not necessary. A mutant enzyme library is assembled and then screened successively to identify the protein with the desired properties.[226]

In general, protein engineering can be usefully applied to oxidoreductases for different purposes. Since in many oxidoreductases the cofactor is deeply buried inside of the insulating protein shell, truncation of some residues or portions of the shell that are not essential for protein function, can facilitate the electrical communication with the electrode by exposition of the redox active site. Usually, the C-terminal, the N-terminal, or loops are the regions more suitable for truncation. Furthermore, deletion of nonessential part of the enzyme, a process called downsizing, can increase the DET-type biocatalytic current densities because of the increased surface concentration of the effective enzyme on the support.[227] For instance, deletion of the heme 1*c* and 2*c* regions in formate dehydrogenase (FDH) caused a simplified and direct electron transfer pathway determining larger bioelectrocatalytic current densities compared to the native enzyme.[228] Deglycosylation is a prime example of a successful surface modification method to increase the transfer of electrons and promote DET.[196] Nonglycosylated horseradish peroxidase (HRP) generated by Gorton and co-workers effectively enhanced electron transfer more than 30 times that of wild-type HRP.[229]

Mutations that cause conformational changes can vary the formal potential of redox enzymes.[227] Mutation of the amino acid side chains that are coordinated with metallic cofactors, such as heme and copper clusters, can shift the formal potentials of the cofactors as well. In particular, axial ligands with a strong electron-donating character shifts the formal potential of the cofactor in the negative direction due to the stabilization of the cofactor oxidized state and vice versa. For instance, the substitution of the axial ligand methionine 467 in the type I copper site of BOD with glutamine (M467Q_BOD) caused a significant negative shift (approximately 0.23 V) in the DET-type bioelectrocatalytic reduction of O_2.[230] The modification or the introduction of specific residues can also vary the interaction with the mediator. Via directed evolution, high-redox-potential laccases with enhanced stability and activity toward redox mediators have been generated.[231]

Point mutations and iterative targeted mutagenesis have been directed to change the enzyme affinity for the cofactor or even its substrate preference. For instance, Chen et al. were able to modify the coenzyme specificity of 6-phosphogluconate dehydrogenase (6PGDH) from $NADP^+$ to NAD^+ by a rational design strategy after the identification of the residues involved in coenzyme binding. The maximum power density and current density were ~25% higher than the native enzyme.[232] Rational mutation of the enzyme binding pocket can alter the regio- and stereoselectivity of an enzyme. For instance, the modification of a large amino acid (Phe, Trp) in the active site of ene-reductases or alcohol dehydrogenases (ADH) and involved in the selective binding of the carbonyl group of the substrate in a *pro*-S or *pro*-R orientation, with smaller residues, can result in "flipped" substrate binding and thus reversed enantioselectivity.[233]

Modification of the protein surface can serve to create a specific anchoring site for a stable and oriented immobilization of the enzyme improving DET-type bioelectrocatalytic properties. For example, introduction of cysteine residues onto the enzymes can promote their specific attachment at thiol- and maleimide-functionalized electrodes by formation of (di)sulfide bonds.[234] Introducing a new peptide on either terminus of the enzyme can also serve this scope; for instance, a gold-binding peptide (GBP) has been introduced on glucose dehydrogenase enabling apparent DET and its spatially controlled immobilization on GNP arrays.[235]

The introduction of an electroactive domain into a native enzyme can be beneficial for achieving DET-bioelectrocatalysis. Cellobiose dehydrogenase has been fused with a smaller electrode-active cytochrome domain by a flexible polypeptide. The cytochrome domain acts as a built-in mediator, promoting the electron transfer from the catalytic domain to the electrode.[236] Engineering approaches with creation of fusion proteins have been conducted on flavodoxin[237] and FAD-GDH[238] for similar scopes.

Multienzymatic Cascades. Multienzymatic synthesis involves a series of reaction steps, that can be carried out by adding all enzymes either simultaneously (one-pot) or sequentially into a reaction vessel (Figure 17). Enzymatic cascades are abundant in nature, living organisms carry out a huge number of enzymatic reactions in a common reaction medium, the cytosol. Enzymatic cascade reactions have been reproduced in vitro for two main applications: the complete oxidation of the biofuels and value-added product formation. For this latter application, they are advantageous because they allow more complex synthetic schemes and traditional intermediate separation steps can be bypassed, saving time and reagents.[239] For an optimal functioning of the enzymatic cascade, it is necessary that all the components maintain their activity, and their colocalization in close proximity is important to avoid the diffusion of the intermediates away from the active sites in the bulk solution.[239] Substrate channeling strategies allow control over the mass transport of intermediates produced between enzymatic steps, allowing their fast and direct transport from one active site to another. The exposure to competing side reactions is also avoided and the enzymes are protected from accumulation of toxic intermediates. Moreover, the efficient

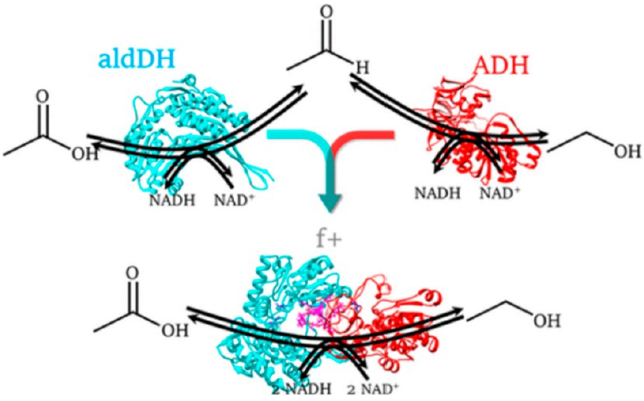


Figure 17. Reaction cascade for the reversible interconversion of ethanol and acetate is promoted by a fusion protein, f+, comprised of ADH and aldDH and a cationic linker that allows substrate channeling of acetaldehyde. Reproduced from ref 242. Copyright 2021 American Chemical Society. This work is openly licensed via CC-BY-NC-ND 4.0.

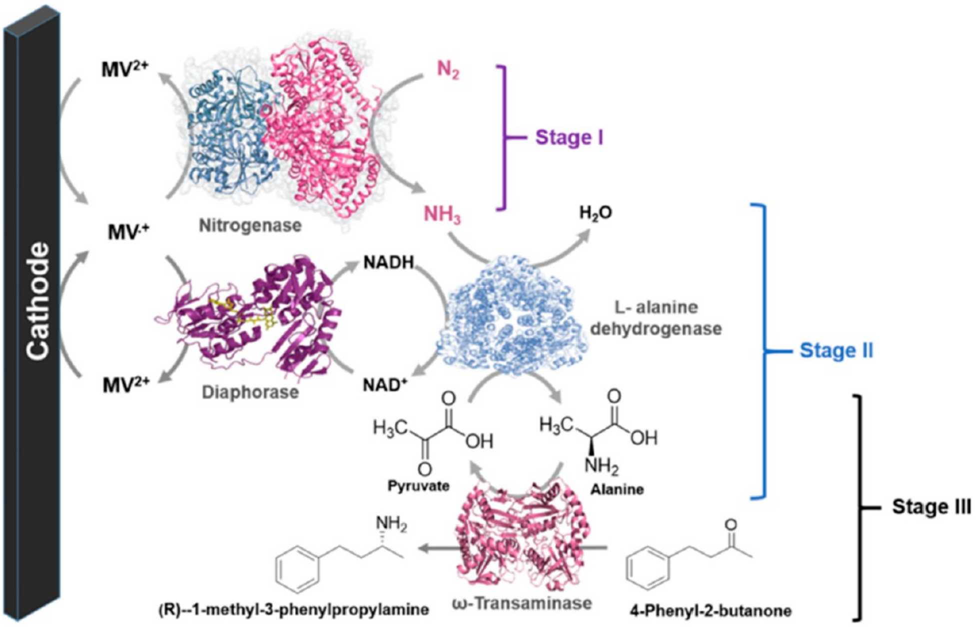


Figure 18. Scheme of the enzymatic N_2 fixation system comprising nitrogenase, L-alanine dehydrogenase, ω-transaminase, and diaphorase, used to produce a variety of chiral amines from N_2. Reproduced from ref 251. Copyright 2019 American Chemical Society.

processing of the reactants and the low concentration of the intermediates in the bulk solution thermodynamically favor the reaction in the forward direction. Therefore, reaction efficiencies and yields are increased.[240]

Proximity is a key factor for substrate channeling. The colocalization of the enzymatic components of the cascade has been realized by entrapment of the enzymatic constituents in micropores and microcapsules, or by construction of molecular (protein, nucleic acid, and polymer) scaffolds. The creation of these scaffolds has proven to increase the production yield of 1,2-propandiol, butyrate, succinate, glucaric acid, dihydrogen, and many more.[240] The spatially controlled organization of the enzymes on the electrode at the nanometer scale is crucial. The orientation of the active sites should also be taken into account in the design of a multienzymatic cascade.[240]

Some artificial hybrid cascades can be constructed by recombinant or fusion enzymes or by the combination of an organic catalyst, such as amino-TEMPO, with enzymatic catalysts to allow a simplification of the number of enzymes necessary and an improved catalytic performance.[241]

Bifunctional fusion proteins can increase catalytic turnover rates and substrate channeling capabilities. For example, a fusion protein composed of ADH and aldDH, involved in the two-step reversible conversion of ethanol and acetate, was designed to facilitate electrostatic substrate channeling by placing a cationic linker (thus with opposite charge than the intermediate) in the truncated termini of the proteins in close proximity to the active sites. The engineered fusion protein showed a 500-fold increased conversion efficiency compared to the unbound enzymes.[242]

Some examples of enzymatic cascades that have been applied for synthetic purposes will be illustrated in the next sections.

Enzymatic Nitrogen Fixation. Molecular nitrogen (N_2) is the most abundant component of Earth's atmosphere. N_2 fixation involves reducing stable, triple-bonded dinitrogen to yield reduced derivatives such as ammonia (NH_3). This reaction is the basis for the existence of all nitrogen-based biomolecules such as proteins, enzymes, and nucleic acids.[243] Ammonia is a fundamental nitrogen source required in agricultural fertilizers, medicaments, and daily life products. Currently, ammonia is produced predominantly using the Haber−Bosch process developed in the 20th century, which requires high pressures and temperatures and accounts for 1−2% of the global energy demand while producing ~3% of the global carbon emission.[244] Conversely, diazotrophic microorganisms can catalyze the ATP-dependent reduction of N_2 to metabolically active forms of nitrogen in ambient conditions with 75% efficiency due to the presence of nitrogenases.[245] Nitrogenases are the only enzymes known to reduce N_2 to NH_3 following the reaction in eq 1 (where ATP is adenosine triphosphate, ADP is adenosine diphosphate, and Pi is inorganic phosphate), and thus represent a very appealing area of research.

$$\begin{aligned} & N_2 + 8H^+ + 16MgATP + 8e^- \\ & \quad \rightarrow 2NH_3 + H_2 + 16MgADP + 16P_i \end{aligned} \tag{1}$$

MoFe nitrogenase is the most widely studied and well-known nitrogenase that contains two proteins, both a Fe and MoFe protein with a MoFe cofactor. Vanadium- or iron-only (VFe and FeFe) nitrogenases are two alternatives.[246] Minteer's group has coupled a MoFe nitrogenase- and Fe-protein-containing cathode with a hydrogenase-containing bioanode in a H_2-biofuel cell able to produce NH_3 from N_2.[247] In this system, the electrons deriving from H_2 oxidation flow in the cathode where methyl viologen (MV) acts as a mediator to transfer electrons to the Fe-protein and then electrons are passed from this latter to the MoFe cofactor for N_2 reduction with simultaneous production of electrical energy in the presence of MgATP. The electron transfer between the Fe-protein and the MoFe cofactor and the subsequent release of the inorganic phosphate is the limiting step of the catalytic reaction.[248] In the presence of a redox polymer with a formal potential more negative than that of the Fe-protein (−0.48 V vs SCE), the electron transfer from the mediator directly to the MoFe cofactor is possible circumventing the necessity of the Fe-protein and the consumption of ATP. The immobilization of the MoFe nitrogenase on an electrode functionalized by a poly(allylamine)poly(vinylamine) with cobaltocene pendants achieved the reduction of N_3^- and NO_2^- to NH_3 in a Fe-protein/ATP independent way.[217] Similarly, nitrogenase entrapped in a neutral red polymer achieved bioelectrochemical reduction of N_2, N_3^-, and NO_2^-.[249] Successively, the DET-promoted biocatalytic reduction of N_2 was achieved by cross-linking of carbon paper with nitrogenase enzyme using a hydrogel (pyrene-LPEI).[250] NH_3 alone is a low-value product and necessitates further catalysts, such as costly noble metal catalysts, for its incorporation in compounds with high added value. A multienzymatic cascade has been developed by Minteer et al. to transfer NH_3 to ketone substrates producing the corresponding chiral amines (Figure 18). The method used methyl viologen to shuttle electrons, along with a cascade of diaphorase, L-alanine dehydrogenase, and ω-transaminase.[251] L-Alanine dehydrogenase was able to produce alanine from pyruvate using the NH_3 generated by nitrogenase and then, ω-transaminase performed the transfer of the amino group from alanine to different ketones: 4-phenyl-2 butanone, 4-methyl methoxy phenyl acetone, phenoxyacetone, 2-pentanone, methoxyacetone, and 2-octanone, to produce the corresponding chiral amines.

The same concept of aminating α-keto acid to yield corresponding asymmetric products by an enzymatic cascade (nitrogenase, diaphorase, and leucine dehydrogenase) was coupled to fuel cell technology for the simultaneous production of electrical energy. The resulting enzymatic biofuel cell powered by H_2 oxidation produced chiral amino acids in enantiomeric excess and with high faradaic efficiency.

Enzymatic Electrosynthesis of H_2. Molecular hydrogen (H_2) is a clean and renewable energy carrier and industrial feedstock, with the potential of replacing fossil-fuels. It has high energy content per unit weight compare to other gaseous fuels (120 MJ/kg for H_2 vs 44 MJ/kg for gasoline). Additionally, it can be used for hydrogenation of a variety of products. Currently, it is produced by reforming fossil fuels. Biological hydrogen production catalyzed by hydrogenases is renewable and carbon neutral.[252] Hydrogenases are classified into [FeFe]- and [NiFe]-hydrogenases based on the identity of the metal in their catalytic clusters. [FeFe]-hydrogenase contains a [4Fe−4S] subcluster (called H cluster) bridged to a 2Fe subcluster via a bridging cysteine thiolate. In [NiFe]-hydrogenase one of the Fe ions of the 2Fe subcluster is bridged to a Ni atom, which in turn, coordinates with two additional cysteine thiolates.[253] The main drawback of using these enzymes for industrial applications is their deactivation in the presence of oxygen. Research has been lately focused on the characterization and implementation of oxygen-insensitive hydrogenases[254,255] or their modification to improve their O_2 tolerance.[256] As already mentioned, their entrapment in polymeric matrices can physically insulate and shield the enzyme from O_2 inhibition. In some cases, the redox polymer, such as the viologen-based redox polymer that was specifically designed by Plumeré et al., can act as a self-activated protection system by inducing O_2 reduction by transferring the electrons deriving from hydrogenases activity.[257] [FeFe]- and [NiFe]-hydrogenases can perform DET, and they have been immobilized on many different electrode materials: glass carbon electrodes,[258] carbon nanotubes,[259] and TiO_2 electrodes reaching faradaic efficiency up to ∼98% for hydrogen evolution.[260] Phototoelectrodes, such as a TiO_2-coated p-Si photocathode, can generate visible-light-driven photocurrents for the reduction of protons to H_2 with comparable faradaic efficiency.[261] Semiartificial photoelectrochemical systems were designed to wire photosystem II on anodes, for catalyzing water oxidation upon illumination coupled with a hydrogenase biocathode for H_2 production.[262,263]

Enzymatic CO_2 Conversion. CO_2 arises many environmental concerns due to its ever-increasing emissions in the world and atmospheric accumulation that plays a crucial role in global climate change. Bioconversion of CO_2 to produce useful chemicals and biofuels is considered a viable and sustainable approach to transition from the existing linear carbon economy into a circular one.[264] Partial hydrogenation of CO_2 has been accomplished by heterogeneous catalysis, electrocatalysis, and photocatalysis.[265] The selectivity of the enzymatic reactions can overcome issues related to competitive oxygen and proton reduction reactions that occur during electrocatalysis and thus has great attractiveness. The synthesis of formic acid from CO_2 using formate dehydrogenase (FDH) has been the most studied single-enzyme process. Formic acid has a commercial value as a chemical feedstock, hydrogen carrier, and fuel for fuel cells. Its production has been limited by the cost of the cofactor (NADH). Different cofactor regeneration systems have been successfully employed, such as neutral red with immobilized FDH.[266] Some NAD-independent FDH enzymes with electroactive metal centers such as Mo and W, have been isolated from anaerobic extremophiles with good reaction rates. W-Containing FDH enzymes overcome the need of NADH due to their [4Fe-4S] clusters in proximity of the enzyme surface, a feature that allows DET.[267] FDH has also been used in combination with carbonic anhydrase (CA) on cold rolled graphite powder by Srikanth et al.,[268] CA improved CO_2 solubilization and formic acid production. Reda and co-workers adsorbed the W-containing FDH on a pyrolytic graphite edge electrode observing DET-promoted CO_2 reduction to formate with 97% Faradaic efficiencies.[269] Nitrogenases, containing MoFe, FeFe, or VFe, may also facilitate reduction of CO_2. C−C bond formation from CO_2 with production of ethylene and propene was achieved by using a VFe nitrogenase from *Azotobacter vinelandii*.[270] The enzymatic conversion of CO_2 can be carried out also by carbon monoxide dehydrogenase leading to the formation of CO that can be further utilized for liquid hydrocarbon generation and acetic acid generation through Fischer−Tropsch and Monsanto Cativa processes, respectively.[271] Currently, the most common enzymatic system uses a cascade of three dehydrogenases for the complete reduction of CO_2 to methanol: formate dehydrogenase (FDH), formaldehyde dehydrogenase (FalDH), and alcohol dehydrogenase (ADH). Each step of the enzymatic cascade requires NADH as coenzyme for the 2-electron reduction.[271] Methanol can be directly used as an alternative fuel, as part of biodiesel production, or as gasoline additive upon conversion to dimethyl ether.[239] The current method of industrial production is gas-phase synthesis, which is costly. In the literature different methods for the coimmobilization of the three catalysts on different types of electrode materials have been reported, including encapsulation on silica gel-succinimidyl poly(ethylene glycol)[272] or on silica gel-alginate composites[273] to increase the biocompatibility. A three-layer architecture has been created by sequential entrapment of the three enzymes in a polymeric membrane by membrane fouling formation.[274] Sequential immobilization can be a suitable strategy for enzymatic cascades because it allows the reaction conditions for each step to be adjusted separately.

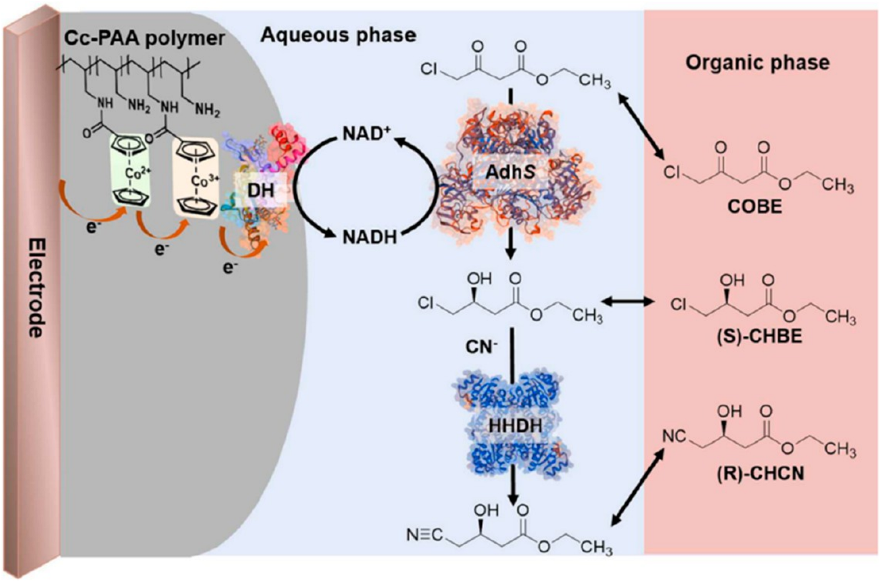


Figure 19. Scheme of the biphasic bioelectrocatalytic system for the preparation of β-hydroxy nitriles. Reproduced from ref 290. Copyright 2020 American Chemical Society.

The three enzymes have been immobilized also on single polystyrene microparticles.[275] The enzyme glutamate dehydrogenase has been then immobilized separately in separate microparticles for NADH regeneration. The collision among the two types of particles in the reaction solution was able to afford the *in situ* coenzyme regeneration.[275] The conversion from CO_2 to methanol can also be realized by photoelectrochemical methods. The three-member enzymatic cascade was integrated into a photochemical cell composed of a photoanode (Co-Pi/α-Fe_2O_3) and a photocathode ($BiFeO_3$) that was able to regenerate NADH for the production of methanol driven by solar energy.[276]

Enzymatic Electrosynthesis of Fine Chemicals. Chemical transformations, such as stereospecific reduction, cyclization, or C−H bond activation, are still troubling in molecular catalysis. In particular, chirality is a key factor in the efficacy of many drugs and agrochemicals. Enantiomers are traditionally produced by chemical or chemoenzymatic synthetic methods.[277] Enzymes have binding pockets with high enantioselectivity, and operate under mild conditions, thus their use is receiving increasing attention.

Hydroxylation of carbon−hydrogen bonds. Oxyfunctionalizations are chemical transformations involving the insertion of oxygen atoms into (nonactivated) C−H-, C−C-, C═C-bonds or onto heteroatoms, which are of significant interest for synthesizing asymmetrical alcohols or halides in the pharmaceutical industry. In traditional synthetic procedures, transition metal catalysts and stoichiometric amounts of reductant are necessary. These organic synthetic processes are still challenging because of the poor regioselectivity resulting in complex product mixtures and tedious isolation of the desired products.[278] Selective oxyfunctionalizations can be performed instead by monooxygenases, peroxygenases, and chloroperoxidase.[279]

Cytochrome P450 monooxygenase has been the center of attention for several decades now, it has great potential for industrial application since it oxidizes an impressive range of substrates, including α-substituted phenols, fatty acids, and steroids. It has thus great potential for the electrosynthetic preparation of drug metabolites in a controlled and mild manner.[280] The reactions can be promoted by both DET or MET and different immobilization and engineering strategies have been developed over the last years.[281,282]

Peroxygenases and chloroperoxidases have limited substrate range compared to cytochrome P450 monooxygenase but they are more easy to scale up since they are structurally simpler.[283] Their activity is driven by H_2O_2 and electrochemical and photoelectrochemical methods can support the controlled in situ production of H_2O_2 and favor their use in synthetic chemistry. For instance, a photoelectrochemical tandem cell consisting of a FeOOH/$BiVO_4$ photoanode and a graphitic carbon nitride/reduced graphene oxide powered by a Cu-(In,Ga)Se_2 solar cell with a light adsorber was developed for light-driven H_2O_2 generation for peroxygenase activity.[284]

Alkane monooxygenase (AlkB) from *Pseudomonas putida* was immobilized on a cathode of a biofuel cell and coupled with a hydrogenase bioanode to catalyze the selective terminal oxyfunctionalization of alkanes under mild conditions while simultaneously producing energy. The conversion of octane to 1-octanol was characterized by a high rate (690 nmol cm^{-2}).[285] Chen et al. employed an enzymatic cascade for the conversion of inert hydrocarbons to high-value imines. In successive steps, a variety of terminal alkanes were converted to alcohols by alkB, then to aldehydes by an engineered choline oxidase (AcO_6) and finally to imines by NADPH-dependent reductive aminase (NfRedAm) while using ferredoxin-NADP reductase and neutral red to regenerate NADPH.[286]

A glucose-powered enzymatic fuel cell was developed by Wu and Zhu, in which a tyrosinase enzyme was used to convert L-tyrosine to L-DOPA, which is used in the treatment of Parkinson's disease. By controlling the reduction potential of the working electrode, the overoxidation of the quinone can be avoided, which is otherwise difficult to control with other methods. In this system, the reducing equivalents were provided by a glucose dehydrogenase/$Fe(CN)_6$.[287]

Reduction of Carbonyls. The asymmetric reduction of carbonyls (ketone groups) catalyzed by carbonyl reductases such as ADH, is important for the production of chiral alcohols.[288] The reduction of ethyl 4-chloroacetoacetate to ethyl (*S*)-4-chloro-3-hydroxybutanoate catalyzed by ADH at a conversion rate close to 100% was obtained using cobaltocene-modified poly(allylamine) for NADH regeneration. The obtained product was further processed by halohydrin dehalogenase and converted to (*R*)-ethyl-4-cyano-3-hydroxybutyrate, which is an active ingredient of cholesterol-lowering drugs.[289]

The low solubility of organic compounds in the aqueous phase is a relevant issue in electrosynthesis. β-hydroxy nitriles, high-value precursors of statins, have been produced by the combined action of alcohol dehydrogenase from *Lactobacillus kefir* (AdhS) followed by halohydrin dehalogenase (HHDH) from *Agrobacterium radiobacter* by Minteer et al. in a biphasic system.[290] In this relevant study, the conversion ratio in the biphasic system, consisting of an aqueous buffered solution and methyl *tert*-butyl ether as organic phase, was 85% times higher than the one achieved in the single-phase system (Figure 19). Usually, in a biphasic system, the catalysts are contained in the aqueous phase while the organic phase acts as a substrate reservoir and end-product sink. These systems can overcome solubility issues for hydrophobic substrates and intermediates in water and favor the recovery of products in situ (by extraction of the organic phase), moreover, they can decrease the level of inhibitory compounds. Important considerations regarding the choice of organic solvent, in terms of physiochemical properties and biocompatibility, should be taken when using these systems with biological entities.[196]

Deracemization of enantiomeric mixtures can be controlled by enzyme selection and by control over the applied potential. Wan and Co realized the nanoconfinement of (*S*)-selective or (*R*)-selective ADH variants and ferredoxin NADP$^+$ reductase in a nanoporous ITO layer allowing the catalytic interconversion, in a bidirectional and controlled way, between secondary alcohol enantiomers and ketones by performing oxidative and reductive cycles.[291]

Reductive Amination of Carbonyls. Reductive amination of α-keto acids is one of the most useful and versatile methods for the preparation of chiral amines, which are ubiquitous in bioactive compounds, pharmaceuticals, and agrochemicals.[292] Enzymes responsible for the reductive amination of α-keto acids to L-amino acids are amino acid dehydrogenases that use free ammonium. An example is the production of L-glutamate by glutamate dehydrogenase, which reduces oxoglutarate via reductive amination. As already illustrated for other dehydrogenases, cofactor regeneration systems are essential for the sustainable production of the fine chemicals. The Armstrong group scaled-up the regeneration of the NADPH cofactor catalyzed by Ferredoxin NADP$^+$ reductase (FNR) from a 4 mL reactor volume and an electrode surface of 3.5 cm^2 to 500 mL volume with an electrode surface of 525 cm^2. The regeneration system was coupled to the reductive amination of 2-oxoglutarate catalyzed by L-glutamate dehydrogenase. Both enzymes were nanoconfined at the surface of a nanoporous ITO electrode. Results showed a total amination of 2-oxoglutarate with a faradaic efficiency of 100% approximately.[293] The method has the potential to be transferred from the laboratory scale to the industrial one, because of its simplicity that requires only the coimmobilization of the desired enzymes in the nanopores, low cost of electrode materials and possibility to scale-up the electron surface.

The synthesis of the products can be facilitated also by photoelectrochemical tandem assemblies. The enzymatic conversion of α-ketoglutarate to L-glutamate was boosted by the presence of an organometallic perovskite-based photovoltaic cell solar adsorber. Light adsorption improved the efficiency of water oxidation at the photoanode suppling electrons for NADH regeneration at the cathode (TTN was equal to 108,800 h^{-1}).[294] Minteer et al. employed leucine dehydrogenase in a H_2/α-keto acid enzymatic fuel cell with capability of producing L-norleucine, L-norvaline, L-valine, L-ter-leucine and L-cyclopropylglycine with high enantiomeric excess.[252]

Epoxidation and Reduction of Olefins. Opening of the epoxide ring and introduction of two C−O bonds are useful reactions for the synthesis of fine chemicals and pharmaceuticals as well as a diversity of key intermediates. Flavin-dependent monooxygenases can catalyze epoxidation reactions using molecular oxygen as oxidant. A commonly employed enzyme of this type is the FADH$_2$-dependent styrene monooxygenase from *Pseudomonas* sp. VLB120. It is composed by two parts, StyA is FADH$_2$-dependent and responsible for the epoxidation reaction while StyB for the transfer of reducing equivalents from NADH to StyA.[295] A flow-through system fabricated by the group of Schmid et al. on highly porous reticulated vitreous carbon electrodes was able to supply electrons for the reduction of FAD with a rate of 93 mM h^{-1} bypassing the need for NADH.[296]

The reduction of olefins by asymmetric hydrogenation is catalyzed by ene-reductases. Similarly to epoxidation, these reactions create two stereogenic centers on the substrate. Four main enzyme classes can be identified, all require NAD(P)H: old yellow enzymes (OYEs), enoate reductases, medium-chain dehydrogenases/reductases (MDRs), and flavin-independent short-chain dehydrogenase/reductases (SDRs).[297] Enoate reductase from *Clostridium tyrobutyricum* and OYE have been immobilized on carbon electrodes for the synthesis of (2*R*)-2-methyl-3-phenylpropionate and (*R*)-levodione, respectively.[196,298]

Recent Advancements in Bioinspired Mimetics. Bioinspired nanoenzymes refer to nanoscale materials that mimic the catalytic mechanism and exhibit similar properties of natural enzymes, in particular showing similar catalytic efficiency and reaction kinetics but increased stability.[299] The word nanozyme was first coined in 2004 to describe the ribonuclease-like activity of ligand functionalized gold nanoparticles.[300] Since most enzymes have metal-containing cofactors, the design of nanoenzymes with inorganic and organic active sites resembling the cofactor structure has become a new strategy for catalysis applications. PtNPs and CeNPs mimic catalase enzyme, while FeTe nanorods and FeS nanoneedles display peroxidase activities. PtNP-deposited MWCNTs have recently been adopted as a nanozyme platform for NADH oxidation with a recovery yield of bioactive NAD$^+$ close to 100%.[301] Inexpensive transition-metal-based nanoenzymes offer less mass activity and costs compared to noble metals. They can be incorporated into electrodes to develop efficient electrocatalysts with potentiality in large-scale commercial applications. These can be applied to oxygen evolution reaction (OER), oxygen reduction reaction (ORR), nitrogen reduction reaction (NRR), and hydrogen evolution reaction (HER).[300] Recently, nanospheres doped with transition metal oxides (Al−Co$_3$O$_4$) were applied for ammonia electrosynthesis. The urchin-like structure of the nanospheres limited the coalescence of gas bubbles, enabling better exposure of active sites to N$_2$.[302] Some nanostructures can mimic an enzymatic cascade reaction. For instance, a metallic organic framework (MOF) with a core−shell palladium nanoparticle catalyzed a two-step cascade for the production of the drug precursor 2-(4-aminobenzylidene)malononitrile from 4-nitrobenzaldehyde with 86% selectivity.[303] The presence of acid and basic active sites on different nanostructures has been employed by different research groups for deprotection−condensation cascade reaction sequences.[240]

Challenges and Perspectives of Enzymatic Bioelectrosynthesis. The field of bioelectrosynthesis is gaining significant interest and it is advancing rapidly for the possibility of developing eco-friendly and sustainable approaches for the production of low-value as well as high-value chemicals. A particular focus has been directed in the last years on CO_2 and N_2 fixation. Currently, the production rate of electroenzymatic synthesis is insufficient to meet the demands of practical applications. Issues linked with the long-term stability of the biocatalysts associated with their structural fragility, and oxygen sensitivity, need to be addressed in future for industrial scalability. Even though the current state of the art in electroenzymatic synthesis has demonstrated enormous potential applications for synthetic purposes, a number of significant obstacles could prevent its industrial application. First, the scope of the product is still constrained by enzymes. Specifically, the majority of electroenzymatic synthesis products are derived from a simple reaction catalyzed by a single enzyme; however, the synthesis of complex chemicals typically requires more complex synthetic pathways involving a series of catalytic reactions. The limited availability of biocatalysts is the primary cause of this issue, which could be resolved by identifying suitable enzymes or constructing versatile enzyme cascades. Investigating more highly active and thermostable enzymes can significantly improve the efficiency and durability of electroenzymatic synthesis systems. The modification of the biocatalysts, via genetic approaches, can be a powerful tool to overcome kinetic and thermodynamic constraints. It can be exploited to optimize the enzymatic activity and stability, to design novel enzymatic cascades and synthetic pathways. The selection of novel electrode materials and the development of effective immobilization strategies are of vital importance for improving the biocatalyst-electrode interactions. Also, the choice of suitable redox mediators is required to improve the performances of BESs and to obtain a fast and economical product synthesis. Integration of a coenzyme regeneration system into enzymatic synthesis processes is the prerequisite to render bioelectrosynthesis competitive with conventional electrosynthesis increasing production rate and faradic efficiency. The development of continuous flow reactors is desirable to increase yields of bioconversion due to a facilitated mass transport. Lastly, the employment of microbe-enzyme or chemical-enzyme hybrid systems, via the combination of the advantages of the different systems, can boost their future applicability.

5.2.3. Microbial Electrosynthesis. Value-added natural products have been synthesized by microorganisms commercially for decades, in processes often referred to as fermentation, biomanufacturing, or bioprocessing. In addition, recombinant approaches (using heterologous expression) have been used to synthesize unnatural products in bacteria, perhaps the best example being recombinant insulin, which revolutionized diabetes treatment in the 1970s.[304] Recently, commercial industries have begun to adopt bioprocessing approaches in order to sustainably convert common wastes into useful products like fertilizers or fuels for transportation. Similar commercial advances have been prevalent combining both bacteria and electrochemistry. Potter was the first to identify current producing capabilities in microorganisms in 1911.[305] Since that moment, research into microbial electrochemistry gave rise to the new area of bioelectrochemistry based on the use of electroactive bacteria as biocatalysts. One of the main research focus has been the development of microbial fuel cells (MFCs) that harness electrical current from waste both in liquid and in solid form.[306] For example, pilot-scale studies have shown microbial fuel cells to be commercially feasible for sustainable brewery wastewater decontamination.[307] In addition, using the novel halophile *Salinivibrio* sp. EAGSL, which can thrive in 150 g L^{-1} sodium chloride, on a bioanode reinforced the idea of combining biosynthetic systems with microbial fuel cells, including a specialized subtype of these systems known as microbial desalination cells.[308,309] More recently, researchers have been focusing on the potential of microbial systems in electrosynthesis and their combination with MFCs. In microbial electrosynthesis intact microbial cells catalyze the synthesis of transportation fuels and value-added organic compounds. An electrical current is supplied or extracted from the microorganisms to sustain biochemical production. Some examples of reductive processes are the production of acetate from CO_2 and succinate from fumarate, while example of oxidative processes include production of ethanol from glycerol.[310] The merger of microbial electrochemical systems and biomanufacturing has already commenced.

The use of whole cells offers several compelling advantages compared to enzymatic systems: there is no need of enzyme production and isolation. Microbes naturally produce all enzyme necessary for a specific biochemical pathway (or even different pathways in parallel) and they naturally regulate enzyme production depending on the environmental conditions.[311] In this sense, a bacterial cell can be considered as a microreactor. Microbes are autoregenerating systems, in particular the enzymes can be produced on necessity in response to the cell's metabolic needs and the enzyme's degradation rate. Also, continuous cellular growth can replace the dead cells providing a constant supply of catalysts. Moreover, the enzymes are already in their natural and suitable environment and thus more stable.[308]

On the other hand, they also suffer from limitations: living cells constantly require nutrients and energy for survival. Substrate sensitivity is lower than isolated enzymes because multiple metabolic pathways are active simultaneously, thus using different substrates simultaneously. Also, competition between different metabolic pathways will inevitably lead to a loss of electrons and generation of unwanted byproducts, leading to low product selectivity. The isolation of the desired product will thus be more challenging.[171]

Extracellular Electron Transfer in Bacterial Cells. The ability of some microorganisms to acquire energy through electron transfer to or from extracellular compounds is termed extracellular electron transfer (EET). This process in nature regulates the biogeochemical cycling of various elements. The mechanisms of EET have been most intensely studied in *Shewanella oneidensis* and *Geobacter sulfurreducens*, two Gram-negative mesophilic bacteria that can be considered as model electroactive bacteria. EET occur via three main mechanisms in nature:[311]

DET is realized through either the expression of *c*-type cytochromes in the outer cellular membranes or long-distance electrical conduction by pili. Alternatively, EET can occur via MET determined by extracellular secretion of redox active compounds (flavins, quinones, etc.).

The protein machinery involved in the DET mechanism (Figure 20), which counts a series of periplasmic and outer-membrane proteins, has evolved as a strategy to overcome the necessity of these bacteria to reduce metals.[312] The transfer of electrons overcomes the impossibility of metals to cross the cellular membranes to reach the terminal reductases that are located in the cytosol or the inner membrane. In particular, the electrons deriving from metabolic activities and oxidation of substrates are transferred to the inner membrane, then they pass across the periplasm (sometimes via soluble electron acceptors located in the periplasm) and finally across the outer membrane to the extracellular electron acceptors.

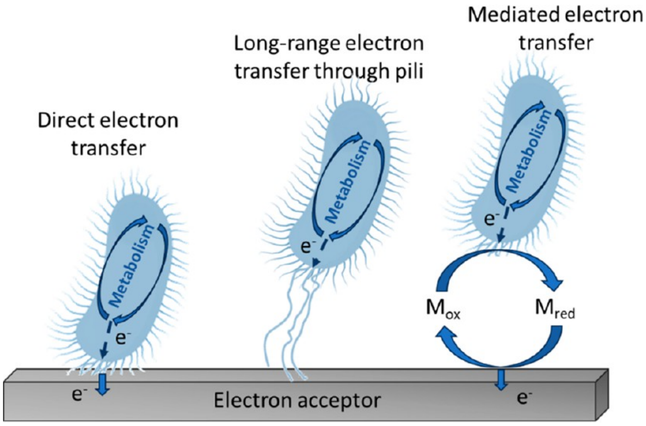


Figure 20. Principal extracellular electron transfer mechanisms from the cathode to microbes.

In *S. oneidensis*, DET is directed by the Mtr pathway which involves a series of proteins: CymA, MtrA, MtrB, MtrC, and OmcA, that are assembled to form a complex. CymA is a quinol dehydrogenase that is anchored to the inner membrane via a single α-helix. The electrons deriving from the oxidation of quinols to quinones are transferred to MtrA which is a decaheme *c*-cytochrome that forms, in combination with MtrB, aβ-barrel porin in the internal face of the outer membrane. This pore transfers electrons to the decaheme *c*-type cytochromes MtrC and OmcA, also localized in the outer membrane. MtrC-OmcA is the terminal reductase that transfers electrons to mineral oxides or the electrode. In *G. sulfurreducens*, DET is achieved by similar bounded-membrane cytochromes.[313]

The exact mechanism of ET via formation of conductive microbial nanowires or pili is still object of debate. Some authors have pointed out that these structures in *S. oneidensis* are just extensions of the outer cellular membrane with expressed porin−cytochrome complexes.[314] Conversely, these structures have been better characterized in *G. sulfurreducens.* They are called e-pili, a particular class of type IV pili that is the most common in microorganisms and contain PilA protein which facilitates the interspecies direct electron transfer.[315] Some authors sustain that other proteins present in IV pili structures, are responsible for the electron transport instead of PilA. In particular, a long chain of hexaheme cytochromes OmcS, that are interfaced and stabilized by stacking interactions, while the role of PilA is likely to promote the secretion of OmcS outside of the cell.[316] Still, the relative role of both OmcS and PilA proteins in ET is not completely unsolved since the deletion or downregulation of the OmcS gene had no impact on biofilm current production.[317] In general, the role of the conductive pili seems minor compared to the Mtr pathway, since the deletion of MtrA and MtrB abolished metal reduction ability in *S. oneidensis* and the double mutation of MtrC and OmcA resulted in a serious deficit in current production and ability to attach to the electrode surface.[318] The presence of conductive pili, also demonstrated in other species of bacteria including *Rhodopseudomonas palustris* RP2 strain[319] and *Desulfovibrio desulfuricans*,[320] offers an advantageous opportunity for increasing the electrode-microbe interface.

Gram-positive bacteria are generally considered nonelectroactive due to the presence of a thick peptidoglycan cell wall.[321] However, some peculiar electron transfer mechanisms have been evidenced in different bacterial species, such as the presence of c-type cytochromes in the cell wall (although generating a low current) in *Thermincola potens*,[322] or a flavin-based EET via flavinylated extracellular reductases in *Listeria monocytogenes.*[323] In general, their ability to perform EET is more limited compared to Gram-negative bacteria.

The bacterial secretion of endogenously produced redox mediators, such as flavins or quinones, is responsible for MET.[311] Also, some secreted metabolites, like H_2, formate, or sulfur species, can convey electrons.[324] MET is particularly developed during biofilm growth, because with increasing thickness of the biofilm a crescent number of bacteria are distanced from the electrode surface (>14 Å) causing a limitation of the ET. As compensation, bacteria start secreting soluble redox species to obviate the need for direct contact with the electron acceptor. *S. oneidensis* secretes mostly flavin molecules such as riboflavin (RF) and flavin mononucleotide (FMN) at the nanomolar scale.[325] Some studies have illustrated the relevance of these compounds in EET. For instance, the removal of the culture medium in a electrochemical cell containing *S. oneidensis MR- 1* and *MR-4* grown on a carbon electrode resulted in the loss of more than 70% of the current production, while replacement of the filtered medium in the same cell restored the current production to the initial level.[175] Secreted redox molecules can interact with cytochromes in the outer membranes. Okamoto et al. in 2014 demonstrated bifurcated direct electron transport process determined by the binding of FMN and RF to the MtrC and OmcA proteins respectively in *S. onedensis MR-1* cells grown on ITO electrode.[326] The effect was a significant shift of the redox potentials of these proteins in the positive direction, as a result of more favorable ET kinetics to the electrode. The existence of a specific FMN-binding domain in the cytochrome MtrC was then demonstrated.[327]

Some microbial species, although nonelectroactive, can secrete soluble redox-active metabolites that can serve as useful mediators for other bioelectrocatalytic systems. For instance, *Pseudomonas aeruginosa* is known to secrete at least five distinct phenazine derivatives. Naturally, these compounds promote quorum sensing favoring the communication between cells in response to environmental conditions such as the presence of competitive microbes.[328]

Electrode Modifications to Improve Microbial Interactions. The efficiency of bioelectrocatalysis and production rates are dependent upon the interactions between microbes and electrodes. The area of the electrode and the formation of a mature biofilm are primary factors.[329]

Biofilm growth on the electrode is of utmost importance for achieving high production rates. In nature, bacteria form biofilms, communities of sessile cells surrounded by extracellular polymeric substances (EPS) that consist of polysaccharides, proteins, lipids, and nucleic acids, in response to harsh environmental conditions. Biofilm formation depends on several factors, such as type of microorganism, environmental conditions, properties of the surface that is colonized. The formation of biofilm involves different stages.[330] In a first stage, planktonic cells continuously attach and detach from the electrode. Then some cells attach in an irreversible manner forming microcolonies that start to secrete components of the EPS, which supports the attachment of further cells. In the final step, the mature biofilm is composed of a complex architecture made of channels and pores that allows diffusion of nutrients, and the thickness is maintained constant by an equilibrium between bacteria growth and cell dispersal from the biofilm. While an increased number of cells in the biofilm can be beneficial for ET through the conduction via pili, an excessive thickness can cause mass transport limitation.[330]

The development of highly active electrode surfaces and biocompatibility are key factors for enhancing microbe-electrode interactions.

Three-dimensional electrodes with a porous structure, such as carbon felt, carbon cloth or carbon mesh, are the most employed for microbial electrocatalysis since they promote biomass growth with their a high surface area to volume ratio. In these materials, bacteria can colonize also the interior structure of the electrode which also favors the diffusion of culture media.[180] Pretreatments, for instance, gas or thermal treatment, can serve to modify electrode morphology and adjust porosity and roughness. Furthermore, carbon electrodes are versatile in terms of chemical functionalization.

Chemical modification by introduction of hydrophilic groups and positive charges can be beneficial for promoting cell adhesion of bacteria to the electrode via the establishment of hydrogen bonding, and electrostatic interactions as they were demonstrated to create better contact with c-type cytochromes.[331] Several strategies can be employed, including functionalization with metal oxide nanocomposites, conductive polymers, ethylenediamine, and diazonium salts.[332] The modification with metals is often beneficial to improve the conductivity of the electrode and ET kinetics or create positive charges. Graphene nanosheets modified with ITO and poly(allylamine hydrochloride) in a layer-by-layer mode generated elevated electrical currents.[333] The modification of carbon cloth with hematite nanocomposites was able to wire *S. oneidensis* providing great electricity output.[334]

The incorporation of carbon nanotubes and nanoparticles can greatly improve the surface area as well as the electrical communication. Redox polymers with grafted redox pendants, due to their intrinsic properties of favoring ET by self-exchange-based electron conduction, can improve the bioelectrode performances of a great factor. Osmium redox systems have been frequently used to wire microbial cells, including nonelectroactive bacteria.[335] Electrical connection between a gold electrode and membrane-bound pyrroloquinoline quinone (PQQ)-containing dehydrogenases in *Gluconobacter oxidans*, which are able to oxidize various substrates, was established using two osmium polymers.[336] Flow-through systems where the catholyte is continuously recirculated and forced to pass through the biofilm-covered electrode can be incredibly beneficial to promote the formation of a thick and heterogeneous biofilm as well as improve production yield.[337]

Mixed Cultures. Both pure and mixed cultures have been applied to MES. The growth of mixed cultures on the electrode has shown to be beneficial for bioelectrocatalysis and under same conditions, and mixed cultures often outperform pure cultures.[338] The presence of different species renders the biofilm more resilient to environmental stressors and fluctuations, making it more adaptable to different working conditions. In mixed cultures, syntrophic interactions between different organisms can be observed. In some cases, a metabolite produced by an organism is used as a substrate by the production strain. One microorganism can supply growth factors and vitamins that favor the growth of another microorganism or one strain can remove toxic products or scavenge oxygen that inhibits the growth of another.[339] Furthermore, the ET can be improved by the secretion of redox compounds or via the secretion of hydrogen and formate by the consortia.

For this reason, inoculum enrichment, where supplemental bacterial strains are added to the species involved in the biosynthesis, is often a strategy pursued in electrosynthetic processes. In other cases, the enrichment of an inoculum is carried out with suppression of a specific microorganism. Acetogens are often outcompeted by the presence of methanogens and various treatments are usually necessary for their elimination to improve acetate production, such as low pH, thermal shock,[340] short hydraulic retention time,[341] and addition of chemical inhibitors.[342] The main drawback of the use of a mixed culture is the loss of product specificity and the difficulty of obtaining a controlled and reproducible environment. Thus, understanding the role of the single species can aid in future industrial developments with the establishment of a defined mixed culture with appropriate characteristics.[343]

Recently, the use of yeast-bacteria and microalgae-bacteria consortia is gaining popularity for wastewater treatment. The symbiotic relationship improves the overall biomass and consequently wastewater removal efficiency while enriching it with valuable chemical and energy compounds[344] For instance, the use of yeast and bacterial cocultures could be effective for improving bioethanol production from lignocellulosic biomass while the exchange of nutrients and electrons in algal-bacterial consortia has shown to improve biohydrogen production.

Genetic Modification Strategies to Enhance Performance in Microbial Electrochemical Systems. Genetic modification of microbial cells has been utilized over the past decade, particularly to improve the EET efficiency of the microorganism itself as well as the electrical communication between bacterial cells and the electrode.[345] A variety of genetic modification tools are available to alter the genetic material, such as transplantation of a heterogeneous metabolic pathway with insertion of new genes into a host microorganism, the removal of unnecessary genes from the host, or replacement of existing genes of the host organism with new ones.[346]

The incorporation of novel genes expands the substrate scope that a particular microorganism can utilize.[347] To express novel genes in a host microorganism, an extrachromosomal genetic material called a plasmid vector is used.[348] Genes of interest can also be inserted into the bacterial chromosome using an integrating viral vector. The desired genes are first amplified using polymerase chain reaction (PCR). Next, these genes are cloned or joined to the plasmid or viral vectors using site-specific restriction endonucleases that cut the vectors at specific sites for the nucleotide sequence to bind to the vector.[346,348] The expression of downstream genes in a chromosome is regulated by gene promoters. These promoter regions can be modified to overexpress targeted genes.[348] For instance, overexpression of genes related to electron shuttling redox-active proteins such as the outer-membrane cytochromes, aids in enhancing the electron transfer capabilities of the modified bacteria.[349]

In recent years, genetic engineering strategies have been combined with microbial electrosynthesis to finetune the metabolic pathways responsible for the synthetic processes by increasing the production rate or by broadening the product spectrum increasing the product market value.[350] Despite the efforts made, there are still many shortcomings involved with genetic engineering, such as the low production rate of targeted products and limited knowledge about electron transfer kinetics between microbes and electrodes that prevents this technology from being industrially relevant.[351,352] Synergistic investigations of the EET pathways between the microbe-electrode interface and intracellular metabolism of the microorganisms is imperative to address the challenges associated with the current synthetic biology approaches for microbial electrosynthesis applications.[351] In this section, we will review some of the recent attempts made with genetic modification strategies to enhance performance in microbial electrosynthesis.

Intracellular electron carriers, particularly NADH, play a role in connecting the flux of electrons deriving from the oxidation of substrates to the outer membrane proteins or the extracellular electron shuttle responsible of the communication to the electrode (Figure 21). Tefft and TerAvest attempted to address the synergy between the electron transfer from the cathode to the intracellular metabolism of bacteria by leveraging the native EET pathway of *S. oneidensis* MR-1 and coupling that to an intracellular reduction reaction.[353] Electrons were able to flow from the electrode to the Mtr proteins native to *S. oneidensis* MR-1 and from these to the quinol pool of the inner membrane, finally from quinones to NAD^+ to generate NADH. However, this electron flow is not thermodynamically feasible since the quinone pool has a much more positive redox potential than NAD^+ ($\Delta E°_{\text{menaquinone/menaquinol}}$ = −0.08 V vs SHE, $\Delta E°_{\text{NAD+/NADH}}$ = −0.32 V vs SHE).[354] Under normal conditions, NADH dehydrogenase will catalyze the electron transfer from NADH to quinone and maintain the proton motive force (PMF) to conserve energy.[355] However, NADH dehydrogenase is also capable of catalyzing the reverse reaction using the energy from the proton motive force (PMF).[356] Based on this knowledge, the researchers genetically engineered *S. oneidensis* MR-1 by heterologously expressing a light-driven proton pump, proteorhodopsin, to generate PMF. The researchers hypothesized that the generated PMF will drive NADH dehydrogenase to catalyze the reduction of NAD^+. They also expressed a NADH-dependent enzyme, butanediol dehydrogenase (Bdh), to promote the concomitant reduction of acetoin to 2,3-butanediol. In presence of the substrate, they observed the production of ~0.5 mM 2,3-butanediol over 6 days showing the efficacy of the engineered electrosynthetic system. To eliminate the electron flow to nonspecific reactions, such as hydrogen production by hydrogenases, the researchers removed the native hydrogenases. By doing so, they observed a decrease in the reduction current and almost a 2-fold increase in 2,3-butanediol production. This research gives a fundamental understanding of cathode driven NADH-dependent intracellular reduction reactions in *S. oneidensis* MR-1.

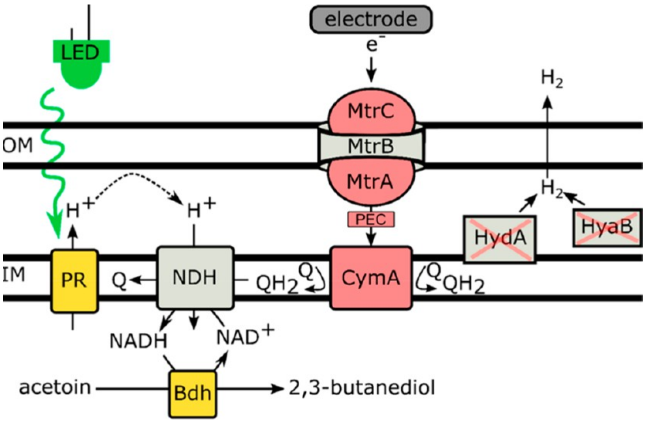


Figure 21. Engineered electron transfer pathway in *Shewanella oneidensis* MR-1 to transfer electrons from the electrode to intracellular NAD^+. Reproduced from ref 353. Copyright 2019 American Chemical Society.

Bioelectrosynthesis of ammonia from nitrogen is another industrially valuable application of genetically modified microorganisms. As for now, the majority of ammonia is produced industrially using the Haber-Bosch process that is environmentally detrimental and costly.[357] This encourages the use of diazotrophic microorganisms that can fix nitrogen using nitrogenase enzymes under ambient conditions with good faradaic efficiencies. Moving along with this idea, Dong et al. engineered a nondiazotrophic cyanobacterium, *Synechococcus elongatus* PCC 7942 strain, by incorporating a modified nitrogenase gene cluster into its genome (Figure 22). The engineered strain was employed in a bioelectrochemical system in the presence of a diffusible mediator, methyl viologen (MV), for the production of ammonia. Since adequate electrons were supplied for the turnover of nitrogenase, the engineered *S. elongatus* PCC 7942 produced 21 times more ammonia than the amount produced by the photosynthesis-driven fixation of nitrogen with a faradaic efficiency of about 7%.[358]

Another strategy is the incorporation of electron conduits to enhance the electron flux in native electroactive bacteria or creatation of electric channels in non electrogenic bacteria. In a follow-up study, Dong et al. upgraded the bioelectrochemical nitrogen-fixation system to overcome the electron transfer barrier represented by the outer membrane of the cyanobacteria. The researchers further genetically modified *S. elongatus* PCC7942 with the outer membrane cytochrome protein OmcS which is native to *Geobacter* spp.[359] The outcome was an increased electron permeability for transmembrane electron transfer while eliminating the challenges linked with the use of diffusible redox mediators such as MV. These challenges include cellular toxicity, mediator deactivation, energy loss during electron transfer, inefficient diffusion through the membranes, and contamination of the mediators.[360] The upgraded system resulted in a 13-fold increment of ammonia production compared to the previous study, with an approximate faradaic efficiency of 23%.[359]

Microbial Electrocatalytic H_2 Production. H_2 production can be pursued at both bioanodes and biocathodes. At anodes bacteria transfer electrons during oxidation of substrates, and these electrons then flow in the external electrical circuit to the cathodic compartment where H_2 evolution occurs by water reduction. Thus, a cathode potential greater than −0.414 V and a substrate, commonly provided by wastewater, are needed in the electrolysis cell to achieve H_2 bioconversion. Industrial and food processing wastewater, domestic wastewater, swine wastewater, fermentation effluents, and winery wastewater have been proposed for H_2 evolution.[361] The H_2 production rate reached 0.9 m^3_{H2} m^{-3} d^{-1} in a 4 L dual chamber cell

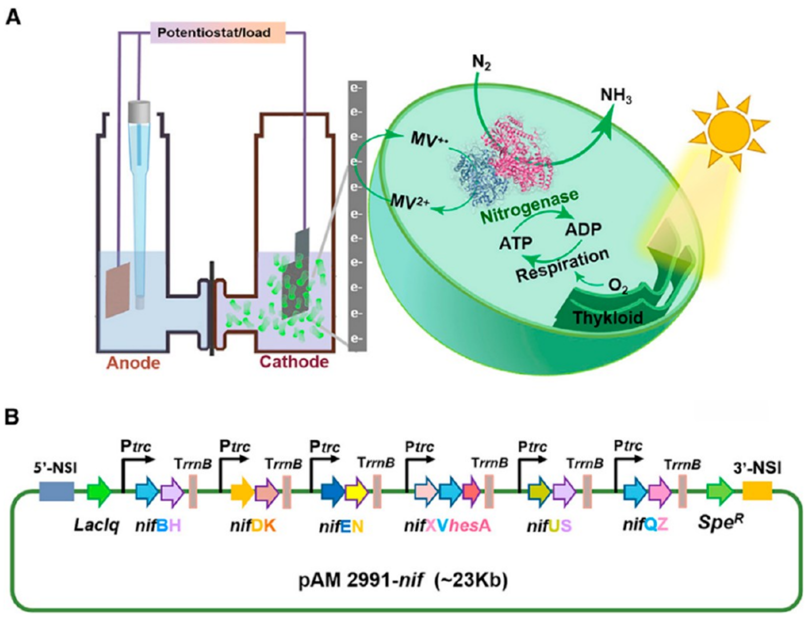


Figure 22. (A) Schematic representation of the bioelectrochemical nitrogen fixation system. (B) Construction and design of the nitrogenase gene cluster. Reproduced with permission from ref 358. Copyright 2021 Elsevier.

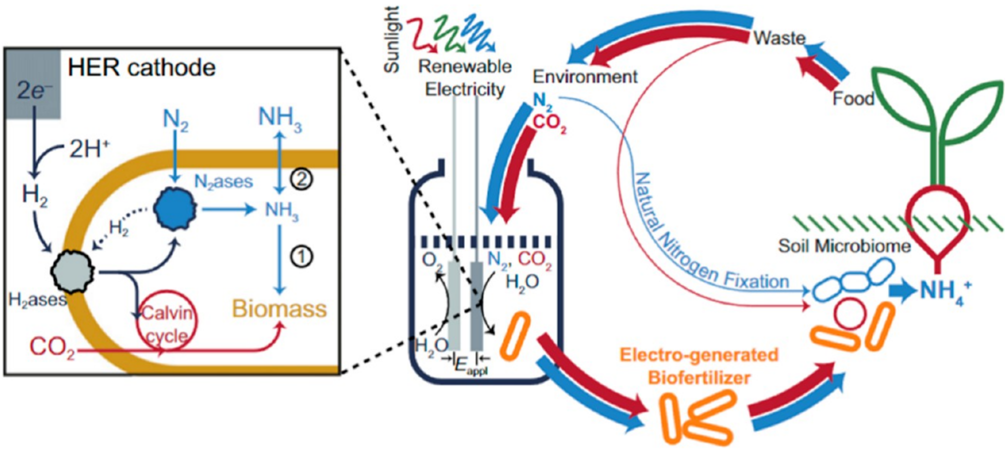


Figure 23. Scheme of the ambient nitrogen reduction cycle employed by Liu et al.[369] *X. autotrophicus* oxidizes the H2 generated by water splitting while reducing CO_2 and N_2. The generated NH3 can be released in the soil to improve plant growth. Reproduced with permission from ref 369. Copyright 2012 Royal Society of Chemistry.

containing an enriched bioanode community continuously fed (for 100 days) with acetate under saline conditions, revealing as auspicious application for the treatment of saline effluents combined with the production of H_2.[362]

In the cathodic mode, bacteria that express hydrogenases or nitrogenases are used at the cathodic chamber to catalyze proton reduction to H_2. Nitrogenases can produce H_2 as a byproduct of nitrogen fixation and they can function as hydrogenases in nitrogen-limitating conditions.[363] *Geobacter sulfurreducens*, *Desulfovibrio* spp., and *Rhodobacter capsulatus* spp., often enriched by *Proteobacteria*, are able to catalyze hydrogen production at cathode potentials below −0.8 V vs Ag/AgCl.[364] Some authors used *Desulfovibrio bulgaris* as a catalyst for achieving H_2 evolution via proton reduction using methylviologen as electron shuttle.[365] Photoautotrophic H_2 production up to 328 mL L^{-1} day^{-1} has been achieved with *Rhodobacter sphaeroides* grown on a biocathode in the presence of CO_2 as the sole carbon source under illumination and an applied potential of −0.9 V vs Ag/AgCl.[366]

Microbial Electrocatalytic N_2 Fixation. Electrosynthesis of ammonia from N_2 is gaining popularity, along with electroenzymatic synthesis, because it is more sustainable compared to the current industrial method which is costly and damaging to the environment. Nitrogen-fixing microorganisms, such as algae and cyanobacteria, are used in the cathodic chamber where hydrogen (either produced biotically or abiotically) and N_2 are converted to ammonia. The application of autotrophic nitrogen-fixing microbes for synthetic purposes is a recent concept that was introduced by Rago and co-workers in 2019.[367] In this case, nitrogen fixation was used for biomass production by a mixed microbial community. Different other studies then have applied this concept to the synthesis of ammonia. The cyanobacterial genus *Anabaena* has been immobilized on indium tin oxide (ITO) modified with polyvinylamine and ferredoxin acted as electron shuttle to mediate the electron transfer between the electrode and the nitrogenase.[368] The use of microorganisms can be advantageous compared to isolated enzymes because the nitrogenases, aside of being continuously regenerated, are protected from O_2 by natural mechanisms evolved by the microorganisms. In the previous example, the nitrogenase is protected from O_2 by formation of a heterocyst inside the cyanobacterium. Interestingly, a study by Liu et al. showed how a H_2-oxidizing bacterium, *Xanthobacter autotrophicus*, can be coupled with N_2 and CO_2 reduction driven by electrocatalytic water splitting at ambient conditions (Figure 23).[369] The produced ammonia, with the addition of a glutamate synthetase inhibitor, is released in the extracellular space, thus this microbe can be used in the soil as in situ producing biofertilizers to support the growth of radishes.

The use of the diazotroph *Pseudomonas stutzeri* A1501, at the cathode allowed ammonia production without the need of inhibitors or genetical engineering, nevertheless, the presence of an inhibitor of ammonia uptake was necessary to obtain a 30% increase in ammonia production.[370] As already pointed out, genetic engineering can be useful to circumvent inhibitory feedback mechanisms and increase ammonia production by heterologous expression.[358]

Microbial Electrosynthesis of Methane. Electrochemical production of methane is nowadays considered an appealing option. Methanogenic conversion of hydrogen to methane implies an energy loss of ~15% thus it has received less consideration than hydrogen production. However, methane can be stored and transported easily and integrated rapidly into the existing infrastructures.[342] Electromethanogenesis is determined by bacteria that can reduce HCO_3^-/CO_2 at the cathode. It is a process that is not particularly difficult to obtain, methane is often produced as a byproduct in other microbial synthetic processes because its production is thermodynamically favorable ($\Delta G = -130$ kJ for methane formation vs $\Delta G = -55$ kJ for acetate production).[371] In many systems, that are for instance employed for H_2 evolution, methanogenesis needs to be suppressed. The first example of electromethanogenesis from CO_2 reduction was reported in 2009 by Cheng et al. using a biocathode with *Methanobacterium palustre* with Coulombic efficiency of 96% at −1 V vs Ag/AgCl.[372] Both DET and MET have been used for electromethanogenesis, in some cases associated with wastewater treatment. A microbial community originating from brewery waste produced a mixture of methane, acetate, and hydrogen at a graphite cathode poised at −590 mV and methane was the dominant product with rates of 1−7 mM day^{-1} (for a 75 mL reactor). This is an example of how electrosynthesis can lead to the formation of different valuable products simultaneously.[373] Villano et al. showed that the rate of EET is dependent upon the cathode potential, in particular, a 20-fold increase in methane production was observed when shifting the potential from −0.65 V to −0.9 V vs SHE.[374] Although the bacterial culture used in this study was electroactive and capable of DET, the researchers supposed that the abiotic production of hydrogen that is prominent at more negative potential has a relevant role in indirectly promoting methane generation via MET. Recently, novel 3D-printed cathodes, consisting of carbon aerogel coated with a NiMo-alloy, were used for H_2 evolution and indirectly favor electromethanogenesis.[375]

Microbial Electrosynthesis of Acetate. Acetogenic microorganisms, or acetogens, count more than 100 species of anaerobes capable of employing the Wood−Ljungdahl pathway to convert CO or CO_2 to acetyl-CoA, which is an important intermediate in bacterial metabolism. The synthesis then is directed to multicarbon organic products, mainly acetate but also 2,3-butanediol, butyrate, ethanol, and butanol.[376] Acetate is the useful starting material for vinyl acetate products (such as polyvinyl acetates) that enter in the composition of paints, glue/adhesives, and industrial polymers. It accounts for a global production of around 10 million tons/year, typically achieved by petrochemical methods.[376] Different gaseous sources can be used which are vastly available: CO_2 captured from industrial flue gases, CO_2 streams from ethanol fermentation and breweries, and biogas from anaerobic digesters.[377] It is the main product of *Acetobacterium woodii* but this is unable to consume current, the species that are able to produce acetate using electrons are a limited subgroup of acetogens. Among them, some strains of *Sporomusa* and *Clostridium* spp. produce acetate, 2-oxobutyrate, and formate by CO_2 reduction when the cathode is poised at −0.4 V vs SHE.[378] In the first studies, productivity was quite low and not sufficient for economic implementation but then, research has been focusing on developing new cathode materials and improving bacterial attachment to the electrode with better results. For instance, the design of materials with a hierarchical porosity, such as the one employed by Flexer et al.[379] and by Cui et al.[380] significantly improved the rate of microbial acetate production. In the first case, the hierarchical structure was made by reticulated vitreous carbon covered by CNTs that were directly grown from the electrode by chemical vapor deposition (NanoWeb-RVC) and electrophoretic deposition (EPD-3D).[379] In the second example, the structure was made by an iron-oxide formed on carbon felt by a one-step process of pyrolysis of ferrocene using a microwave.[380] These techniques have the potential to be scaled-up because they are relatively simple and fast. The highly porous structure and extensive biofilm formed on these electrodes allowed acetate production at high rates.

A microbial community constituted by *Methanobacterium* spp. and *Acetobacterium* spp. was able to produce acetate over 150 days at −0.59 V vs SHE up to 17.25 mM, a value 100 times higher than the one obtained with a single culture.[381] The improvement can be attributed both to the continuous sparging of CO_2 and an optimal biofilm formed by the mixed culture.

Microbial Electrosynthesis for the Production of Alcohols and Volatile Fatty Acids. The reduction of CO_2 to large (>3C) carbon chain molecules, alcohols and carboxylic acids, is challenging because requires multiple proton-coupled electron transfer reactions.[382]

Short-chain fatty acids (SCFAs) (<6 carbon atoms) such as acetic, isovaleric, propionic, butyric, and isobutyric acid, can be used as synthetic building blocks in the manufacturing of bioplastics, resins, inks, paints, beverage additives, coatings, adhesive agents, plasticizers as well as chemical feedstocks and pharmaceutical products.[383] Their production is currently obtained by petrochemical routes. Bacteria synthesize fatty acids using acetyl-CoA with the consumption of ATP and NAD(P)H. The biological production of volatile fatty acids (VF) from various carbon sources mainly relies on anaerobic fermentative processes by *Acetobacter*, *Moorela*, *Clostridium*, and *Kluyveromyces*.[384] Microbial electrosynthesis can overcome the thermodynamic limitations of traditional fermentative processes and increase the production of fatty acids by electrical energy supply. Initially, studies mostly employed pure sugars as initial substrates such as glucose, and xylose, which resulted in high production yield and a simple purification process. But an increasing number of studies are now focusing on MES from organic-rich wastes, such as sludge, for ethical and environmental reasons.[384]

Among VFA, acetic acid is the principal product. The production of 17 g L^{-1} acetic acid by *M. thermoacetica* from fermentation of hydrolysates obtained by steam pretreatment of lignocellulosic biomass[385] and the production of 30.98 g L^{-1} acetic acid from cellulose using *Clostridium lentocellum* SG6,[386] were obtained. The strategies already discussed in the previous sections, relative to the use of porous electrodes and genetic engineering, are valid also in this case. The production of acetic acid was increased for instance by the overespression of PPQ-dependent alcohol dehydrogenase and an acetic acid exporter.[387,388]

Recently, MES of longer chain carboxylates up to six carbon atoms with higher value than acetate: propionate, isopropanol, butyrate, and caproate, has been reported from CO_2 by microorganisms from various biological habitats. Vassilev et co-workers filled the cathodic chamber with graphite granules that were colonized by a microbial community dominated by *Clostridium* sp., obtaining carboxylic acids and alcohols chains up to six carbon atoms chain. The use of granular carbon materials is advantageous for fluidic systems because they can be kept in movement increasing mass transfer and they can be continuously recharged by contact with a polarized collector.[330]

Medium-long fatty acids (MCFAs) with 6−12 carbon atoms can be used as antimicrobial agents, flavor additives, and precursors of biofuels.[371] The synthesis of MCFAs proceeds via a chain elongation reaction based on reverse β-oxidation (RBO) rather than the Wood-Ljungdahl pathway. In the RBO pathway, an acetyl-CoA interacts with SCFAs to extend the carbon chain length by two carbons for each cycle.[371] The electrochemical production of caproate and small amounts of enanthate and valerate was first demonstrated by Jiang et al. using a carbon felt electrode and ethanol as substrate.[389] In this case, the application of an electrical current increased the selectivity for caproate production from 32.3%, obtained with just ethanol as electron donor, to 91.5%. This outcome is important as it shows how current-driven production can be economically profitable lowering the costs associated with ethanol consumption and alleviating the environmental impact. In another study, the continuous circulation of the catholyte in a flow-through system, using a biofilm grown on carbon felt, allowed continuous chain elongation reactions improving the synthesis of caproate.[337]

The microbial electrosynthesis of alcohols, in particular ethanol, is very attractive for its potential replacement for gasoline. To date, different biomasses have been investigated for the production of bioethanol, including starch, sugars, and lignocellulosic biomasses.[390] MES of bioalcohols relies on microbial reduction of VFA, mostly acetate and butyrate.[391] Also, in this case, cathodic production can be associated with fuel cell technology and applied to wastewater treatment without requiring an electrical supply. *Saccharomyces cerevisiae* electrochemically produced 11.52 g L^{-1} of ethanol from CO_2 in 40 h in a bioelectro-Fenton system driven by a microbial fuel cell.[392] The addition of tungstate allowed improved biocatalysis of tungsten-containing aldehyde ferredoxin oxidoreductases (AORs) and tungsten-containing formate dehydrogenase (FDH) that are involved in the Wood-Ljungdahl pathway in *S. ovata* and it shows how the optimization of the composition of the growth medium can have a huge impact on electrosynthesis. The production of ethanol increased to 13.2 ± 1.2 mM with the addition of tungstate (compared to 1.5 ± 1.0 mM without tungstate), and the levels of acetate, 1-propanol, and 1-butanol were simultaneously increased.[393] The presence of redox mediators can also enhance the electrosynthesis. The addition of neutral red increased the production of ethanol by *E. coli* and *K. pneumoniae* by >90% compared to the control in absence of mediator.[394] The use of gas diffusion electrodes, by improving the CO_2 solubility and bioavailability, can also have a big impact for bioconversion. Srikanth et al. employed a gas diffusion electrode with a selective mixed culture, the CO_2 was initially converted into SCFAs (formate and acetate) and in a second time, to butanol and ethanol with high Coulombic efficiencies. After 60 days of operation, ethanol was the major product while after 90 days, butanol became the major product.[395]

NADH is an essential cofactor for the synthesis of acetone, butanol, and ethanol. Improving cofactor availability thus can be a useful strategy. For instance, by increasing the concentration of electron carriers, such as reduced neutral red, the production of butanol by *Clostridium beijerinckii* IB4 was increased significantly as a result of increased ATP levels and increased NADH/NAD ratio. The authors noted that the application of an electrical current stimulated bacterial growth and shortened the fermentation time.[396] Another powerful tool is genetic engineering, which can enhance or inhibit determinate metabolic pathways. A strain of *E. coli* able to convert butanol from supplemented butyrate was developed by removal of undesired genes. This strain optimized for butyrate metabolism then was cocultured with another genetically modified strain equipped with endogenous *atoDA* and with *Clostridium adhE2* genes for the in situ production of butyrate, yielding 5.5 g L^{-1} of butanol from glucose in 24 h.[397] The inhibition of methanogenesis is often necessary to increase the production of alcohol leading to more efficient CO_2 conversion. Enrichment procedures with inoculum pretreatment and specific operation conditions and additives can be used for this purpose. One method is heating the mixed culture inoculum to 90 °C for 1 h to eliminate the heat-tolerant methanogens, and then regrowing the medium in sodium 2-bromoethanesulfonate (NaBES), a methanogenesis inhibitor.[398]

Microbial Production of Bioplastics and Biomaterials. Biogenic materials (polysaccharides, polyamines, polyphospahtes and polyesters) are generally more biocompatible and more biodegradable compared to synthetic materials and they can be produced via more economical and sustainable ways. They have diverse biological functions, they are involved in energy storage, attachment on surfaces, and biofilm formation and the biosynthetic pathways involve multiple enzymes.

Bacterial polymers have found several medical as well as industrial applications. Xanthan gum is a bacterial biopolymer that has been used extensively in industry. Recombinant collagen, as well as alginate and hyaluronate have been used for tissue engineering because of their similarities with extracellular matrices of natural tissues and for drug delivery. Polysaccharides are used as antigens for the preparation of conjugate vaccines, while polyamides such as poly(γ-D-glutamic acid) can replace synthetic flocculants (polyacrylamide and polyaluminum chloride) in wastewater treatment.

Recently, the attention has been focused on the production of polyhydroxyalkanoates (PHAs). These are linear polyesters that are synthesized into hydrophobic spherical inclusions, the most common is Polyhydroxybutyrate (PHB). They can be used for high-value medical materials (platforms for enzyme immobilization, drug carriers, tissue engineering scaffolds) as well as for low-vale bioplastics. Their relevance is thus enormous given the current concerns linked to the huge negative environmental impact of plastics.

Roughly 40% of all prokaryotic strains are able to produce hydroxyalkanoate (HA) monomers and more than 150 HA are currently known, these are accumulated in the cell under

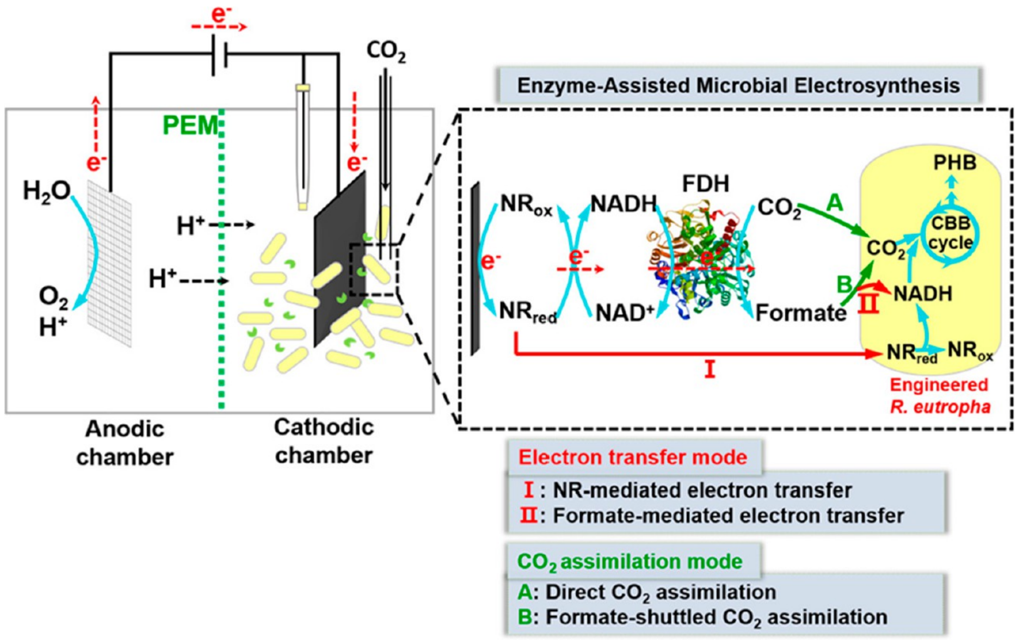


Figure 24. Scheme of a MES system for the reduction of CO_2 and synthesis of poly(3-hydroxybutyrate) (PHB). Neutral red (NR) and FDH enhance the formation of intracellular reducing equivalents for efficient CO_2 bioreduction and PHB biosynthesis in C. necator. CO_2 fixation in the microorganism is enhanced by the overexpression of Rubisco enzyme. Reproduced from ref 403. Copyright 2018 American Chemical Society.

nitrogen-limiting conditions as a form of carbon stock.[399] The drawback of microbial PHA synthesis is the necessity of cell lysis for their extraction. VFA are the precursors of PHA, thus they can be obtained by CO_2 reduction as a primary source. Acetate is converted to acetyl-CoA, then two acetyl-CoA are condensed by 3-ketothiolase (PhaA) enzyme forming acetoacetyl-CoA. Acetoacetyl-CoA reductase (PhaB) reduces acetoacetyl-CoA to (*R*)-3-hydroxybutyryl-CoA, which can be finally incorporated into the PHB polymer by PHA synthase (PhaC).[400] Butyrate can be directly reduced to (*R*)-3-hydroxybutyryl-CoA. The conversion of PHB from CO_2 was realized in two steps by Pepè Sciarria et al.[401] In the first step, CO_2 was reduced to acetate and butyrate by *Clostridium* spp. (73% conversion at −0.8 V vs SHE), then the sludge obtained after a separation and concentration step was used as inoculum for the production of PHB. Extensive research has focused on genetic engineering to create efficient cell factories for the production of specific biomaterials. The removal of genes linked with the synthesis of endotoxins in Gram-negative bacteria has been pursued to ensure the safety of the final products.[402] Heterologous expression of ribulose-1,5- bisphosphate carboxylase/oxygenase (Rubisco) in *Cupriavidus necator* (formerly known as *Ralstonia eutropha*) was carried out to facilitate the efficiency of CO_2 reduction and PHB production (Figure 24). The enzyme, via the Calvin−Benson−Bassham (CBB) cycle, allowed the conversion of CO_2 to 3-phosphate glyceraldehyde that is then directed to the synthesis of acetyl-CoA. The efficiency of the process was augmented by application of a potential (−0.6 V vs Ag/AgCl) at the cathode modified with neutral red and FDH enzyme to enhance the assimilation of CO_2 by *C. necator*.[403] This example shows how the integration of microbial and enzymatic catalysts can boost the overall synthetic process.

Future Directions of Microbial Electrosynthesis. Although microbial electrosynthesis (MES) has not yet undergone a surge in the mainstream industry, microbial electrosynthesis is emerging as a novel green technology with great potential for the production of renewable biofuels and commodity chemicals, using little energy input provided by a poised cathode. Energy can be provided by renewable sources such as solar, wind, and wave energy, and the production process can be linked with MFCs for wastewater treatment. Future studies should implore combining biocathodes with bioanodes as renewable sources of electrons, and both of these chambers can be optimized to synthesize a wide range of value-added molecules. An assortment of products can be generated from biorecycling of CO_2. Despite the advances in the field and its great potential, the technology is still far from entering the market, and optimization is required for commercialization. A deep understanding of microbial metabolic and EET pathways is critical. The generation of engineered strains with synthetic metabolic pathways is fundamental to help the cells to target specific wastes and more efficiently synthesize products and establish extracellular electron transfer in otherwise nonelectrogenic strains. It will be vital to embrace interdisciplinary science to scale-up these MES processes. Cathode materials that enhance microbe-electrode interface and chamber design can improve production rates. Mechanical engineering advances must be incorporated to design inexpensive materials and setups for MES systems.[404] It will also be imperative to establish new materials strategies that are compatible with living biocatalysts like intact bacterial cells, a growing field known as living electronics.[405] Still in the realm of academia, the Bio Pilot Plant at Leibniz-HKI (Germany), headed by Miriam A. Rosenbaum, is a promising example of a microbial electrosynthesis hub that the industrial sector should use as a model as MES technologies become more mainstream.[406] Further, government entities are beginning to set the example for MES adoption; the National Renewable Energy Laboratory (Colorado, USA) has recently focused attention on microbes as economically effective means of carbon dioxide reduction to value-added chemicals like ethanol, formate, or acetate.[407] Nevertheless, focusing on the small subset of well-known MES systems is one of the classic arguments against the adoption of MES technologies. It is estimated that 75% of MES studies focus only on acetate biosynthesis,[408] when acetate is one of a countless set of bacterial metabolites. Therefore, it is imperative to promote the variety inherent to microbial electrochemical systems because the potential of MES is virtually limitless, not only in target products but also the biodiversity of microorganisms and extremophiles.

6. CONCLUSION

In this Review, we examined electroanalytical techniques and electrochemical processes as instruments for manufacturing unique and valuable products by using simple reactions and reactors. These processes have a little impact on the environment and can be carried out at a cost that is affordable.

When working with organic chemicals to generate high value products, chemists and biochemists often find themselves in precarious positions. It is not simple to meet requirements such as lowering the activation energy required, increasing the electrocatalytic activity, and maintaining a low operating temperature. Voltammetric techniques such as cyclic voltammetry, differential pulse voltammetry, and square wave voltammetry are the infrastructure for the beginning of a big project. It is possible to carry out electrochemical synthesis under benign environmental conditions, at moderate or low temperatures and pressures, with simple and inexpensive facilities; all that is required is proper control of the working electrode potential, the cell voltage (especially in the cases of paired electrolysis), or the cell current that is applied to the terminals of the electrolytic cell reactor.

The galvanostatic mode is the one that is used the most in industrial synthesis. After that, the potential will automatically shift to the compound that has the lowest positive oxidation potential (anode) or the lowest negative reduction potential (cathode). Because of this, it is possible to investigate the chemistry of oxidation (or reduction) for a wide range of different substances, virtually under the same circumstances and without the need of testing a variety of different redox reagents. The description of practical applications of electrochemical routes for organic, enzymatic, and microbial electrosynthesis, on scales ranging from the laboratory to the factory floor, were investigated in this review. The electron transfer process within a chemical reaction is shown, after a brief description of suitable operating conditions and appropriate electrochemical equipment, to be capable of converting functional groups via mechanisms and reactive intermediates that are kinetically and environmentally more favorable than those of conventional methods.

It is believed that, with advances in electroanalysis, soon electrosynthesis will occupy a prestigious place in science and engineering, contributing to the specific, efficient, and widespread commercialization of chemicals. This will be possible because it will eliminate the use of dangerous reagents and environmental pollutants, cut down on waste production, work at or near room temperature, and use recent advances in electrochemistry, specifically in the areas of microreactor engineering, fluid-flow electrochemistry, nanoelectrochemistry, and bipolar electrochemistry.

■ ASSOCIATED CONTENT

Data Availability Statement

The data underlying this study are available in the published article.

■ AUTHOR INFORMATION

Corresponding Author

Shelley D. Minteer − *Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States; Kummer Institute Center for Resource Sustainability, Missouri University of Science and Technology, Rolla, Missouri 65409, United States;* orcid.org/0000-0002-5788-2249; Email: minteer@chem.utah.edu, shelley.minteer@mst.edu

Authors

Monica Brachi − *Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States*
Wassim El Housseini − *Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States*
Kevin Beaver − *Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States*
Rohit Jadhav − *Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States;* orcid.org/0000-0001-6377-7253
Ashwini Dantanarayana − *Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States*
Dylan G. Boucher − *Department of Chemistry, University of Utah, Salt Lake City, Utah 84112, United States*

Complete contact information is available at: https://pubs.acs.org/10.1021/acsorginorgau.3c00051

Author Contributions

[§]M.B. and W.E.H. contributed equally to this work. CRediT: **Monica Brachi** conceptualization, writing-original draft, writing-review & editing; **Wassim El Housseini** conceptualization, visualization, writing-original draft, writing-review & editing; **Kevin Beaver** writing-original draft, writing-review & editing; **Rohit Gajanan Jadhav** writing-original draft; **Ashwini Dantanarayana** writing-original draft; **Dylan G Boucher** writing-original draft; **Shelley D. Minteer** conceptualization, funding acquisition, writing-review & editing.

Notes

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■ REFERENCES

(1) Yan, M.; Kawamata, Y.; Baran, P. S. Synthetic Organic Electrochemical Methods since 2000: On the Verge of a Renaissance. *Chem. Rev.* **2017**, *117* (21), 13230−13319.
(2) Pletcher, D. Organic Electrosynthesis-A Road to Greater Application. A Mini Review. *Electrochem. commun.* **2018**, *88*, 1−4.
(3) Hammerich, O.; Speiser, B. *Organic Electrochemistry: Revised and Expanded*; CRC Press, 2015.
(4) Zirbes, M.; Waldvogel, S. R. Electro-Conversion as Sustainable Method for the Fine Chemical Production from the Biopolymer Lignin. *Curr. Opin. Green Sustain. Chem.* **2018**, *14*, 19−25.
(5) Ley, S. V.; Fitzpatrick, D. E.; Myers, R. M.; Battilocchio, C.; Ingham, R. J. Machine-Assisted Organic Synthesis. *Angew. Chemie Int. Ed.* **2015**, *54* (35), 10122−10136.
(6) Shatskiy, A.; Lundberg, H.; Kärkäs, M. D. Organic Electrosynthesis: Applications in Complex Molecule Synthesis. *ChemElectroChem.* **2019**, *6* (16), 4067−4092.
(7) Dai, J.-J.; Huang, Y.-B.; Fang, C.; Guo, Q.-X.; Fu, Y. Electrochemical Synthesis of Adiponitrile from the Renewable Raw Material Glutamic Acid. *ChemSusChem* **2012**, *5* (4), 617−620.

(8) Tang, S.; Liu, Y.; Lei, A. Electrochemical Oxidative Cross-Coupling with Hydrogen Evolution: A Green and Sustainable Way for Bond Formation. *Chem.* **2018**, *4* (1), 27−45.

(9) Nguyen, B. H.; Perkins, R. J.; Smith, J. A.; Moeller, K. D. Photovoltaic-Driven Organic Electrosynthesis and Efforts toward More Sustainable Oxidation Reactions. *Beilstein J. Org. Chem.* **2015**, *11* (1), 280−287.

(10) Gütz, C.; Stenglein, A.; Waldvogel, S. R. Highly Modular Flow Cell for Electroorganic Synthesis. *Org. Process Res. Dev.* **2017**, *21* (5), 771−778.

(11) Blanco, D. E.; Prasad, P. A.; Dunningan, K.; Modestino, M. A. Insights into Membrane-Separated Organic Electrosynthesis: The Case of Adiponitrile Electrochemical Production. *React. Chem. Eng.* **2020**, *5* (1), 136−144.

(12) Pletcher, D. Electrolysis Cells for Laboratory Organic Synthesis. *Curr. Opin. Electrochem.* **2020**, *24*, 1−5.

(13) Stöckl, M.; Lange, T.; Izadi, P.; Bolat, S.; Teetz, N.; Harnisch, F.; Holtmann, D. Application of Gas Diffusion Electrodes in Bioeconomy: An Update. *Biotechnol. Bioeng.* **2023**, *120* (6), 1465−1477.

(14) Martin, E. T.; McGuire, C. M.; Mubarak, M. S.; Peters, D. G. Electroreductive Remediation of Halogenated Environmental Pollutants. *Chem. Rev.* **2016**, *116* (24), 15198−15234.

(15) Bard, A. J.; Faulkner, L. R. *Electrochemical Methods: Fundamentals and Applications*, 2nd; Wiley: New York 2001.

(16) Heard, D. M.; Lennox, A. J. J. Electrode Materials in Modern Organic Electrochemistry. *Angew. Chemie Int. Ed.* **2020**, *59* (43), 18866−18884.

(17) Heineman, W. R. *Laboratory Techniques in Electroanalytical Chemistry*; CRC Press, 2018.

(18) Andrieux, C. P.; Garreau, D.; Hapiot, P.; Pinson, J.; Savéant, J. M. Fast Sweep Cyclic Voltammetry at Ultra-Microelectrodes: Evaluation of the Method for Fast Electron-Transfer Kinetic Measurements. *J. Electroanal. Chem. interfacial Electrochem.* **1988**, *243* (2), 321−335.

(19) Wipf, D. O.; Wightman, R. M. Submicrosecond Measurements with Cyclic Voltammetry. *Anal. Chem.* **1988**, *60* (22), 2460−2464.

(20) Stamenkovic, V. R.; Mun, B. S.; Arenz, M.; Mayrhofer, K. J. J.; Lucas, C. A.; Wang, G.; Ross, P. N.; Markovic, N. M. Trends in Electrocatalysis on Extended and Nanoscale Pt-Bimetallic Alloy Surfaces. *Nat. Mater.* **2007**, *6* (3), 241−247.

(21) Esakki Karthik, P.; Alessandri, I.; Sengeni, A. A Review on Electrodes Used in Electroorganic Synthesis and the Significance of Coupled Electrocatalytic Reactions. *J. Electrochem. Soc.* **2020**, *167* (12), 125503.

(22) Lamy, C. Electrocatalytic Oxidation of Organic Compounds on Noble Metals in Aqueous Solution. *Electrochim. Acta* **1984**, *29* (11), 1581−1588.

(23) Scholz, F.; others. *Electroanalytical Methods*; Springer, 2010; Vol. *1*.

(24) Batanero, B.; Pérez, M. J.; Barba, F. Electrosynthesis of 3-Chloro-4-Substituted-Coumarins. *J. Electroanal. Chem.* **1999**, *469* (2), 201−205.

(25) Claus, H.; Schäfer, H. 3, 3-Dichlorotetrahydrofurans by Reductive Addition of 3-Bromo 1, 1, 1-Trichloroalkanes to Carbonyl Compounds. *Tetrahedron Lett.* **1985**, *26* (40), 4899−4902.

(26) Anderson, J. D.; Baizer, M. M.; Petrovich, J. P. Electrolytic Reductive Coupling. XIII. 1 Intramolecular Reductive Coupling. Electrohydrocyclization2. *J. Org. Chem.* **1966**, *31* (12), 3890−3897.

(27) Little, R. D.; Fox, D. P.; Van Hijfte, L.; Dannecker, R.; Sowell, G.; Wolin, R. L.; Moens, L.; Baizer, M. M. Electroreductive Cyclization. Ketones and Aldehydes Tethered to. Alpha.,. Beta.-Unsaturated Esters (Nitriles). Fundamental Investigations. *J. Org. Chem.* **1988**, *53* (10), 2287−2294.

(28) Rabjohn, N.; Flasch, G. W., Jr Kolbe Electrosynthesis of Alkanes with Multiple Quaternary Carbon Atoms. *J. Org. Chem.* **1981**, *46* (20), 4082−4083.

(29) Ariño, C.; Serrano, N.; Diaz-Cruz, J. M.; Esteban, M. Voltammetric Determination of Metal Ions beyond Mercury Electrodes. A Review. *Anal. Chim. Acta* **2017**, *990*, 11−53.

(30) Mikkelsen, Ø.; Schrøder, K. H. Amalgam Electrodes for Electroanalysis. *Electroanal. An Int. J. Devoted to Fundam. Pract. Asp. Electroanal.* **2003**, *15* (8), 679−687.

(31) Isse, A. A.; Falciola, L.; Mussini, P. R.; Gennaro, A. Relevance of Electron Transfer Mechanism in Electrocatalysis: The Reduction of Organic Halides at Silver Electrodes. *Chem. Commun.* **2006**, No. 3, 344−346.

(32) Shain, I.; Perone, S. P. Application of Stripping Analysis to the Determination of Iodide with Silver Microelectrodes. *Anal. Chem.* **1961**, *33* (3), 325−329.

(33) Xu, Y.; Ding, X.; Ma, H.; Chu, Y.; Ma, C. Selective Hydrodechlorination of 3, 5, 6-Trichloropicolinic Acid at an Activated Silver Cathode: Synthesis of 3, 5-Dichloropicolinic Acid. *Electrochim. Acta* **2015**, *151*, 284−288.

(34) Xiao, X.; Siepenkoetter, T.; Whelan, R.; Salaj-Kosla, U.; Magner, E. A Continuous Fluidic Bioreactor Utilising Electrodeposited Silica for Lipase Immobilisation onto Nanoporous Gold. *J. Electroanal. Chem.* **2018**, *812*, 180−185.

(35) Scanlon, M. D.; Salaj-Kosla, U.; Belochapkine, S.; MacAodha, D.; Leech, D.; Ding, Y.; Magner, E. Characterization of Nanoporous Gold Electrodes for Bioelectrochemical Applications. *Langmuir* **2012**, *28* (4), 2251−2261.

(36) Ding, Y.; Kim, Y.-J.; Erlebacher, J. Nanoporous Gold Leaf:"-Ancient Technology"/Advanced Material. *Adv. Mater.* **2004**, *16* (21), 1897−1900.

(37) Matsumura, H.; Ortiz, R.; Ludwig, R.; Igarashi, K.; Samejima, M.; Gorton, L. Direct Electrochemistry of Phanerochaete Chrysosporium Cellobiose Dehydrogenase Covalently Attached onto Gold Nanoparticle Modified Solid Gold Electrodes. *Langmuir* **2012**, *28* (29), 10925−10933.

(38) Murata, K.; Kajiya, K.; Nakamura, N.; Ohno, H. Direct Electrochemistry of Bilirubin Oxidase on Three-Dimensional Gold Nanoparticle Electrodes and Its Application in a Biofuel Cell. *energy Environ. Sci.* **2009**, *2* (12), 1280−1285.

(39) Nishio, K.; Masuda, H. Anodization of Gold in Oxalate Solution to Form a Nanoporous Black Film. *Angew. Chem.* **2011**, *123* (7), 1641−1645.

(40) Sumida, K.; Rogow, D. L.; Mason, J. A.; McDonald, T. M.; Bloch, E. D.; Herm, Z. R.; Bae, T.-H.; Long, J. R. Carbon Dioxide Capture in Metal-Organic Frameworks. *Chem. Rev.* **2012**, *112* (2), 724−781.

(41) Zhang, T.; Lin, W. Metal-Organic Frameworks for Artificial Photosynthesis and Photocatalysis. *Chem. Soc. Rev.* **2014**, *43* (16), 5982−5993.

(42) Ou, S.; Wu, C.-D. Rational Construction of Metal-Organic Frameworks for Heterogeneous Catalysis. *Inorg. Chem. Front.* **2014**, *1* (10), 721−734.

(43) Li, J.-R.; Sculley, J.; Zhou, H.-C. Metal-Organic Frameworks for Separations. *Chem. Rev.* **2012**, *112* (2), 869−932.

(44) Mehta, J.; Bhardwaj, N.; Bhardwaj, S. K.; Kim, K.-H.; Deep, A. Recent Advances in Enzyme Immobilization Techniques: Metal-Organic Frameworks as Novel Substrates. *Coord. Chem. Rev.* **2016**, *322*, 30−40.

(45) Raja, D. S.; Liu, W.-L.; Huang, H.-Y.; Lin, C.-H. Immobilization of Protein on Nanoporous Metal-Organic Framework Materials. *Comments Inorg. Chem.* **2015**, *35* (6), 331−349.

(46) Wu, M.-X.; Yang, Y.-W. Metal-Organic Framework (MOF)-Based Drug/Cargo Delivery and Cancer Therapy. *Adv. Mater.* **2017**, *29* (23), 1606134.

(47) Armstrong, N. R.; Lin, A. W. C.; Fujihira, M.; Kuwana, T. Electrochemical and Surface Characteristics of Tin Oxide and Indium Oxide Electrodes. *Anal. Chem.* **1976**, *48* (4), 741−750.

(48) Bahadir, E. B.; Sezgintürk, M. K. Label-Free, ITO-Based Immunosensor for the Detection of a Cancer Biomarker: Receptor for Activated C Kinase 1. *Analyst* **2016**, *141* (19), 5618−5626.

(49) Choi, C. K.; Kihm, K. D.; English, A. E. Optoelectric Biosensor Using Indium-Tin-Oxide Electrodes. *Opt. Lett.* **2007**, *32* (11), 1405−1407.

(50) Morello, G.; Siritanaratkul, B.; Megarity, C. F.; Armstrong, F. A. Efficient Electrocatalytic CO2 Fixation by Nanoconfined Enzymes via a

C3-to-C4 Reaction That Is Favored over H2 Production. *ACS Catal.* **2019**, *9* (12), 11255−11262.

(51) Coustan, L.; Belanger, D. Electrochemical Activity of Platinum, Gold and Glassy Carbon Electrodes in Water-in-Salt Electrolyte. *J. Electroanal. Chem.* **2019**, *854*, 113538.

(52) Coustan, L.; Shul, G.; Belanger, D. Electrochemical Behavior of Platinum, Gold and Glassy Carbon Electrodes in Water-in-Salt Electrolyte. *Electrochem. commun.* **2017**, *77*, 89−92.

(53) Cowlard, F. C.; Lewis, J. C. Vitreous Carbon—A New Form of Carbon. *J. Mater. Sci.* **1967**, *2*, 507−512.

(54) Friedrich, J. M.; Ponce-de-León, C.; Reade, G. W.; Walsh, F. C. Reticulated Vitreous Carbon as an Electrode Material. *J. Electroanal. Chem.* **2004**, *561*, 203−217.

(55) Wang, J. Reticulated Vitreous Carbon—a New Versatile Electrode Material. *Electrochim. Acta* **1981**, *26* (12), 1721−1726.

(56) Harris, P. J. F. Fullerene-Related Structure of Commercial Glassy Carbons. *Philos. Mag.* **2004**, *84* (29), 3159−3167.

(57) Qu, Y.; Tsuneishi, C.; Tateno, H.; Matsumura, Y.; Atobe, M. Green Synthesis of α-Amino Acids by Electrochemical Carboxylation of Imines in a Flow Microreactor. *React. Chem. Eng.* **2017**, *2* (6), 871−875.

(58) Velický, M.; Toth, P. S.; Woods, C. R.; Novoselov, K. S.; Dryfe, R. A. W. Electrochemistry of the Basal Plane versus Edge Plane of Graphite Revisited. *J. Phys. Chem. C* **2019**, *123* (18), 11677−11685.

(59) Kinoshita, K. *Carbon: Electrochemical and Physicochemical Properties*; John Wiley Sons: New York, 1988.

(60) Carta, R.; Palmas, S.; Polcaro, A. M.; Tola, G. Behaviour of a Carbon Felt Flow by Electrodes Part I: Mass Transfer Characteristics. *J. Appl. Electrochem.* **1991**, *21*, 793−798.

(61) Oren, Y.; Soffer, A. Graphite Felt as an Efficient Porous Electrode for Impurity Removal and Recovery of Metals. *Electrochim. Acta* **1983**, *28* (11), 1649−1654.

(62) Mano, N. Recent Advances in High Surface Area Electrodes for Bioelectrochemical Applications. *Curr. Opin. Electrochem.* **2020**, *19*, 8−13.

(63) Yates, N. D. J.; Fascione, M. A.; Parkin, A. Methodologies for "Wiring" Redox Proteins/Enzymes to Electrode Surfaces. *Chem.—Eur. J.* **2018**, *24* (47), 12164−12182.

(64) Kano, K.; Ikeda, T. Fundamentals and Practices of Mediated Bioelectrocatalysis. *Anal. Sci.* **2000**, *16* (10), 1013−1021.

(65) Lojou, E. Hydrogenases as Catalysts for Fuel Cells: Strategies for Efficient Immobilization at Electrode Interfaces. *Electrochim. Acta* **2011**, *56* (28), 10385−10397.

(66) Gross, A. J.; Chen, X.; Giroud, F.; Abreu, C.; Le Goff, A.; Holzinger, M.; Cosnier, S. A High Power Buckypaper Biofuel Cell: Exploiting 1, 10-Phenanthroline-5, 6-Dione with FAD-Dependent Dehydrogenase for Catalytically-Powerful Glucose Oxidation. *Acs Catal.* **2017**, *7* (7), 4408−4416.

(67) Sim, H. J.; Lee, D. Y.; Kim, H.; Choi, Y.-B.; Kim, H.-H.; Baughman, R. H.; Kim, S. J. Stretchable Fiber Biofuel Cell by Rewrapping Multiwalled Carbon Nanotube Sheets. *Nano Lett.* **2018**, *18* (8), 5272−5278.

(68) Jourdin, L.; Freguia, S.; Donose, B. C.; Chen, J.; Wallace, G. G.; Keller, J.; Flexer, V. A Novel Carbon Nanotube Modified Scaffold as an Efficient Biocathode Material for Improved Microbial Electrosynthesis. *J. Mater. Chem. A* **2014**, *2* (32), 13093−13102.

(69) Bulutoglu, B.; Macazo, F. C.; Bale, J.; King, N.; Baker, D.; Minteer, S. D.; Banta, S. Multimerization of an Alcohol Dehydrogenase by Fusion to a Designed Self-Assembling Protein Results in Enhanced Bioelectrocatalytic Operational Stability. *ACS Appl. Mater. Interfaces* **2019**, *11* (22), 20022−20028.

(70) Chiola, V.; Ritsko, J. E.; Vanderpool, C. D. Process for Producing Low-Bulk Density Silica. US Patent, 3556725, 1971.

(71) Beck, J. S.; Vartuli, J. C.; Roth, W. J.; Leonowicz, M. E.; Kresge, C. T.; Schmitt, K. D.; Chu, C. T. W.; Olson, D. H.; Sheppard, E. W.; McCullen, S. B.; et al. A New Family of Mesoporous Molecular Sieves Prepared with Liquid Crystal Templates. *J. Am. Chem. Soc.* **1992**, *114* (27), 10834−10843.

(72) Wan, Y.; Zhao. On the Controllable Soft-Templating Approach to Mesoporous Silicates. *Chem. Rev.* **2007**, *107* (7), 2821−2860.

(73) Hoffmann, F.; Cornelius, M.; Morell, J.; Fröba, M. Silica-Based Mesoporous Organic-Inorganic Hybrid Materials. *Angew. Chemie Int. Ed.* **2006**, *45* (20), 3216−3251.

(74) Zhao, D.; Feng, J.; Huo, Q.; Melosh, N.; Fredrickson, G. H.; Chmelka, B. F.; Stucky, G. D. Triblock Copolymer Syntheses of Mesoporous Silica with Periodic 50 to 300 Angstrom Pores. *Science (80-.).* **1998**, *279* (5350), 548−552.

(75) Secundo, F.; Roda, G.; Vittorini, M.; Ungureanu, A.; Dragoi, B.; Dumitriu, E. Effect of Chemical Composition of SBA-15 on the Adsorption and Catalytic Activity of α-Chymotrypsin. *J. Mater. Chem.* **2011**, *21* (39), 15619−15628.

(76) Gaffney, D.; Cooney, J.; Magner, E. Modification of Mesoporous Silicates for Immobilization of Enzymes. *Top. Catal.* **2012**, *55*, 1101−1106.

(77) Murray, R. W. Chemically Modified Electrodes. *Acc. Chem. Res.* **1980**, *1*, 135.

(78) Murray, R. W. Chemically Modified Electrodes for Electrocatalysis. *Philos. Trans. R. Soc. London. Ser. A, Math. Phys. Sci.* **1981**, *302* (1468), 253−265.

(79) Ata, M. S.; Poon, R.; Syed, A. M.; Milne, J.; Zhitomirsky, I. New Developments in Non-Covalent Surface Modification, Dispersion and Electrophoretic Deposition of Carbon Nanotubes. *Carbon N. Y.* **2018**, *130*, 584−598.

(80) Snell, K. D.; Keenan, A. G. Surface Modified Electrodes. *Chem. Soc. Rev.* **1979**, *8* (2), 259−282.

(81) Zen, J.-M.; Senthil Kumar, A.; Tsai, D.-M. Recent Updates of Chemically Modified Electrodes in Analytical Chemistry. *Electroanal. An Int. J. Devoted to Fundam. Pract. Asp. Electroanal.* **2003**, *15* (13), 1073−1087.

(82) Gütz, C.; Selt, M.; Bänziger, M.; Bucher, C.; Römelt, C.; Hecken, N.; Gallou, F.; Galvão, T. R.; Waldvogel, S. R. A Novel Cathode Material for Cathodic Dehalogenation of 1, 1-Dibromo Cyclopropane Derivatives. *Chem.—Eur. J.* **2015**, *21* (40), 13878−13882.

(83) Murray, R. W.; Ewing, A. G.; Durst, R. A. Chemically Modified Electrodes. Molecular Design for Electroanalysis. *Anal. Chem.* **1987**, *59* (5), 379A−390A.

(84) Wattanakit, C. Chiral Metals as Electrodes. *Curr. Opin. Electrochem.* **2018**, *7*, 54−60.

(85) Ghosh, M.; Shinde, V. S.; Rueping, M. A Review of Asymmetric Synthetic Organic Electrochemistry and Electrocatalysis: Concepts, Applications, Recent Developments and Future Directions. *Beilstein J. Org. Chem.* **2019**, *15* (1), 2710−2746.

(86) Jörissen, J. Practical Aspects of Preparative Scale Electrolysis. *Encycl. Electrochem.* **2007**, DOI: 10.1002/9783527610426.bard080002.

(87) Francke, R.; Little, R. D. Redox Catalysis in Organic Electrosynthesis: Basic Principles and Recent Developments. *Chem. Soc. Rev.* **2014**, *43* (8), 2492−2521.

(88) Costentin, C.; Savéant, J.-M. Concepts and Tools for Mechanism and Selectivity Analysis in Synthetic Organic Electrochemistry. *Proc. Natl. Acad. Sci. U. S. A.* **2019**, *116* (23), 11147−11152.

(89) Oh, Y.; Hu, X. Organic Molecules as Mediators and Catalysts for Photocatalytic and Electrocatalytic CO2 Reduction. *Chem. Soc. Rev.* **2013**, *42* (6), 2253−2261.

(90) Amatore, C.; Cammoun, C.; Jutand, A. Electrochemical Recycling of Benzoquinone in the Pd/Benzoquinone-Catalyzed Heck-Type Reactions from Arenes. *Adv. Synth. Catal.* **2007**, *349* (3), 292−296.

(91) Nutting, J. E.; Rafiee, M.; Stahl, S. S. Tetramethylpiperidine N-Oxyl (TEMPO), Phthalimide N-Oxyl (PINO), and Related N-Oxyl Species: Electrochemical Properties and Their Use in Electrocatalytic Reactions. *Chem. Rev.* **2018**, *118* (9), 4834−4885.

(92) Achterberg, E. P. Laboratory Techniques in Electroanalytical Chemistry. *TrAC-Trends Anal. Chem.* **1996**, *15* (10), 550.

(93) Kissinger, P. T.; Heineman, W. R. Cyclic Voltammetry. *J. Chem. Educ.* **1983**, *60* (9), 702.

(94) Lovrić, M.; Osteryoung, J. Theory of Differential Normal Pulse Voltammetry. *Electrochim. Acta* **1982**, *27* (7), 963−968.

(95) Osteryoung, J. G.; Osteryoung, R. A. Square Wave Voltammetry. *Anal. Chem.* **1985**, *57* (1), 101−110.

(96) Liu, J.; Xu, Y.; Liu, S.; Yu, S.; Yu, Z.; Low, S. S. Application and Progress of Chemometrics in Voltammetric Biosensing. *Biosensors* **2022**, *12* (7), 494.

(97) Nosek, M.; Batys, P.; Skoczek, M.ł; Weroński, P. ł. Cyclic Voltammetry Characterization of Microparticle Monolayers. *Electrochim. Acta* **2014**, *133*, 241−246.

(98) Gosser, D. K. *Cyclic Voltammetry: Simulation and Analysis of Reaction Mechanisms*. VCH: New York, 1993; p 43.

(99) Evans, D. H.; O'Connell, K. M.; Petersen, R. A.; Kelly, M. J. Cyclic Voltammetry. *J.chem.educ.* **1983**, *60*, 290−293.

(100) Kalvoda, R.; Kopanica, M. Adsorptive Stripping Voltammetry in Trace Analysis. *Pure Appl. Chem.* **1989**, *61* (1), 97−112.

(101) Farghaly, O. A.; Hameed, R. S. A.; Abu-Nawwas, A.-A. H. Analytical Application Using Modern Electrochemical Techniques. *Int. J. Electrochem. Sci.* **2014**, *9* (6), 3287−3318.

(102) Kissinger, P.; Heineman, W. R. *Laboratory Techniques in Electroanalytical Chemistry, Revised and Expanded*; CRC Press, 2018.

(103) Gulaboski, R.; Mirčeski, V.; Bogeski, I.; Hoth, M. Protein Film Voltammetry: Electrochemical Enzymatic Spectroscopy. A Review on Recent Progress. *J. Solid State Electrochem.* **2012**, *16*, 2315−2328.

(104) Pohanka, M.; Bandouchova, H.; Sobotka, J.; Sedlackova, J.; Soukupova, I.; Pikula, J. Ferric Reducing Antioxidant Power and Square Wave Voltammetry for Assay of Low Molecular Weight Antioxidants in Blood Plasma: Performance and Comparison of Methods. *Sensors* **2009**, *9* (11), 9094−9103.

(105) Moeller, K. D. Using Physical Organic Chemistry to Shape the Course of Electrochemical Reactions. *Chem. Rev.* **2018**, *118* (9), 4817−4833.

(106) Peterson, B. M.; Lin, S.; Fors, B. P. Electrochemically Controlled Cationic Polymerization of Vinyl Ethers. *J. Am. Chem. Soc.* **2018**, *140* (6), 2076−2079.

(107) Peters, B. K.; Rodriguez, K. X.; Reisberg, S. H.; Beil, S. B.; Hickey, D. P.; Kawamata, Y.; Collins, M.; Starr, J.; Chen, L.; Udyavara, S.; et al. Scalable and Safe Synthetic Organic Electroreduction Inspired by Li-Ion Battery Chemistry. *Science (80-.).* **2019**, *363* (6429), 838−845.

(108) Dahm, C. E.; Peters, D. G. Electrochemical Reduction of Tetraalkylammonium Tetrafluoroborates at Carbon Cathodes in Dimethylformamide. *J. Electroanal. Chem.* **1996**, *402* (1−2), 91−96.

(109) Fuchigami, T.; Atobe, M.; Inagi, S. New Methodology of Organic Electrochemical Synthesis. *Fundam. Appl. Org. Electrochem.* **2014**, 129−186.

(110) Schulz, L.; Waldvogel, S. R. Solvent Control in Electro-Organic Synthesis. *Synlett* **2019**, *30* (03), 275−286.

(111) Izutsu, K. *Electrochemistry in Nonaqueous Solutions*; John Wiley & Sons, 2009.

(112) Elgrishi, N.; Rountree, K. J.; McCarthy, B. D.; Rountree, E. S.; Eisenhart, T. T.; Dempsey, J. L. A Practical Beginner's Guide to Cyclic Voltammetry. *J. Chem. Educ.* **2018**, *95* (2), 197−206.

(113) Santos, C. M. M.; Garcia, M. B. Q.; Silva, A. M. S.; Santus, R.; Morlière, P.; Fernandes, E. Electrochemical Characterization of Bioactive Hydroxyxanthones by Cyclic Voltammetry. *Tetrahedron Lett.* **2013**, *54* (1), 85−90.

(114) Auguste, A. F. T.; Quand-Meme, G. C.; Ollo, K.; Mohamed, B.; placide Sadia, S.; Ibrahima, S.; Lassiné, O. Electrochemical Oxidation of Amoxicillin in Its Commercial Formulation on Thermally Prepared RuO 2/Ti. *J. Electrochem. Sci. Technol.* **2016**, *7* (1), 82−89.

(115) Gnamba, C. Q.-M.; Appia, F. T. A.; Loba, E. M. H.; Sanogo, I.; Ouattara, L. Electrochemical Oxidation of Amoxicillin in Its Pharmaceutical Formulation at Boron Doped Diamond (BDD) Electrode. *J. Electrochem. Sci. Eng.* **2015**, *5* (2), 129−143.

(116) Orata, D.; Amir, Y.; Nineza, C.; Mukabi, M. Electrochemical Characterization of Amoxycillin, a Broad Spectrum Antibiotic on a Bentonite Host Matrix, Using Cyclic Voltammetry. *J. Appl. Chem.* **2014**, *7* (5), 50−58 others.

(117) Remes, A. I.; Ihoş, M.; Manea, F. Electrochemical Characterization of Some Electrode Materials for Pharmaceutically Active Compounds Degradation. *Chem. Bull. Politeh. Univ. Timisoara* **2010**, *55* (2), 152−155.

(118) Aoki, K.; Tokuda, K.; Matsuda, H. Hydrodynamic Voltammetry at Channel Electrodes: Part IX. Edge Effects at Rectangular Channel Flow Microelectrodes. *J. Electroanal. Chem. interfacial Electrochem.* **1987**, *217* (1), 33−47.

(119) Brown, K. L.; Hou, X.; Banks, O.; Krueger, K. A.; Hinson, J.; Peaslee, G. F.; DeYoung, P. A.; Alger, S. M.; Benzer, J.; Neils, T. L. Characterization of Tris (5-Amino-1, 10-Phenanthroline) Ruthenium (II/III) Polymer Films Using Cyclic Voltammetry and Rutherford Backscattering Spectrometry. *Int. J. Chem.* **2011**, *3* (4), 12.

(120) Walton, D. J.; Hall, C. E.; Chyla, A. Characterization of Poly (Pyrroles) by Cyclic Voltammetry. *Analyst* **1992**, *117* (8), 1305−1311.

(121) Sharma, P. K.; Gupta, G.; Singh, V. V.; Tripathi, B. K.; Pandey, P.; Boopathi, M.; Singh, B.; Vijayaraghavan, R. Synthesis and Characterization of Polypyrrole by Cyclic Voltammetry at Different Scan Rate and Its Use in Electrochemical Reduction of the Simulant of Nerve Agents. *Synth. Met.* **2010**, *160* (23−24), 2631−2637.

(122) Pruneanu, S.; Veress, E.; Marian, I.; Oniciu, L. Characterization of Polyaniline by Cyclic Voltammetry and UV-Vis Absorption Spectroscopy. *J. Mater. Sci.* **1999**, *34*, 2733−2739.

(123) Kuk, S. K.; Gopinath, K.; Singh, R. K.; Kim, T. D.; Lee, Y.; Choi, W. S.; Lee, J. K.; Park, C. B. NADH-Free Electroenzymatic Reduction of CO2 by Conductive Hydrogel-Conjugated Formate Dehydrogenase. *ACS Catal.* **2019**, *9* (6), 5584−5589.

(124) Innuphat, C.; Chooto, P. Determination of Trace Levels of Cd (II) in Tap Water Samples by Anodic Stripping Voltammetry with an Electrografted Boron-Doped Diamond Electrode. *ScienceAsia* **2017**, *43* (1), 33−41.

(125) Arechederra, M. N.; Addo, P. K.; Minteer, S. D. Poly (Neutral Red) as a NAD+ Reduction Catalyst and a NADH Oxidation Catalyst: Towards the Development of a Rechargeable Biobattery. *Electrochim. Acta* **2011**, *56* (3), 1585−1590.

(126) Hosseini, S.; Janusz, J. N.; Tanwar, M.; Pendergast, A. D.; Neurock, M.; White, H. S. Oxidation by Reduction: Efficient and Selective Oxidation of Alcohols by the Electrocatalytic Reduction of Peroxydisulfate. *J. Am. Chem. Soc.* **2022**, *144* (46), 21103−21115.

(127) Loipersberger, M.; Derrick, J. S.; Chang, C. J.; Head-Gordon, M. Deciphering Distinct Overpotential-Dependent Pathways for Electrochemical CO2 Reduction Catalyzed by an Iron-Terpyridine Complex. *Inorg. Chem.* **2022**, *61* (18), 6919−6933.

(128) Hoar, B. B.; Zhang, W.; Xu, S.; Deeba, R.; Costentin, C.; Gu, Q.; Liu, C. Electrochemical Mechanistic Analysis from Cyclic Voltammograms Based on Deep Learning. *ACS Meas. Sci. Au* **2022**, *2* (6), 595−604.

(129) Boucher, D. G.; Pendergast, A. D.; Wu, X.; Nguyen, Z. A.; Jadhav, R. G.; Lin, S.; White, H. S.; Minteer, S. D. Unraveling Hydrogen Atom Transfer Mechanisms with Voltammetry: Oxidative Formation and Reactivity of Cobalt Hydride. *J. Am. Chem. Soc.* **2023**, *145* (32), 17665−17677.

(130) Cabral, M. F.; Pedrosa, V. A.; Suffredini, H. B.; Moreno, R.; Mattoso, L. H. C.; Gonçalves, P. S.; Machado, S. A. S. Characterization of Conductive Natural Rubber by Cyclic Voltammetry and Electrochemical Impedance Spectroscopy. *Zaštita Mater.* **2006**, *47* (4), 41−45.

(131) Knittl, E. T.; Rusakov, D. A.; Korotkova, E. I.; Dorozhko, E. V.; Voronova, O. A.; Plotnikov, E. V.; Topaloglu-Sozuer, I.; Linert, W. Characterization of a Novel Dioxomolybdenum Complex by Cyclic Voltammetry. *Anal. Lett.* **2015**, *48* (15), 2369−2379.

(132) Hu, Z.; Troyk, P. R.; Cogan, S. F. Comprehensive Cyclic Voltammetry Characterization of AIROF Microelectrodes. In *2005 IEEE Engineering in Medicine and Biology 27th Annual Conference*; 2006; pp 5246−5249.

(133) Roth, C.; Martz, N.; Hahn, F.; Léger, J.-M.; Lamy, C.; Fuess, H. Characterization of Differently Synthesized Pt-Ru Fuel Cell Catalysts by Cyclic Voltammetry, FTIR Spectroscopy, and in Single Cells. *J. Electrochem. Soc.* **2002**, *149* (11), No. E433.

(134) Zhang, L.; Etienne, M.; Vilà, N.; Le, T. X. H.; Kohring, G.-W.; Walcarius, A. Electrocatalytic Biosynthesis Using a Bucky Paper Functionalized by [Cp*Rh(Bpy)Cl]+ and a Renewable Enzymatic Layer. *ChemCatChem.* **2018**, *10* (18), 4067−4073.

(135) Koçak, Ç. C.; Altin, A.; Aslicsen, B.; Koçak, S. Electrochemical Preparation and Characterization of Gold and Platinum Nanoparticles Modified Poly (Taurine) Film Electrode and Its Application to Hydrazine Determination. *Int. J. Electrochem. Sci.* **2016**, *11* (1), 233−249.

(136) Zhao, X.; Cleary, S. E.; Zor, C.; Grobert, N.; Reeve, H. A.; Vincent, K. A. Chemo-Bio Catalysis Using Carbon Supports: Application in H 2-Driven Cofactor Recycling †. *Chem. Sci.* **2021**, *12*, 8105−8114.

(137) Megarity, C. F.; Siritanaratkul, B.; Cheng, B.; Morello, G.; Wan, L.; Sills, A. J.; Heath, R. S.; Turner, N. J.; Armstrong, F. A. Electrified Nanoconfined Biocatalysis with Rapid Cofactor Recycling. *ChemCatChem.* **2019**, *11* (23), 5662−5670.

(138) Mertens, R.; Greiner, L.; van den Ban, E. C. D.; Haaker, H. B. C. M.; Liese, A. Practical Applications of Hydrogenase I from Pyrococcus Furiosus for NADPH Generation and Regeneration. *J. Mol. Catal. B Enzym.* **2003**, *24*, 39−52.

(139) Sheng, J.; Wu, N.; Liu, X.; Liu, F.; Liu, S.; Ding, W.; Liu, C.; Cheng, X. Electrochemical Allylic Hydrodefluorination Reaction Using Gaseous Ammonia as Hydrogen Source. *Chin. J. Org. Chem.* **2020**, *40* (11), 3873.

(140) Tang, T.; Sandford, C.; Minteer, S. D.; Sigman, M. S. Analyzing Mechanisms in Co (I) Redox Catalysis Using a Pattern Recognition Platform. *Chem. Sci.* **2021**, *12* (13), 4771−4778.

(141) Roesel, A. F.; Ugandi, M.; Huyen, N. T. T.; Májek, M.; Broese, T.; Roemelt, M.; Francke, R. Electrochemically Catalyzed Newman-Kwart Rearrangement: Mechanism, Structure-Reactivity Relationship, and Parallels to Photoredox Catalysis. *J. Org. Chem.* **2020**, *85* (12), 8029−8044.

(142) Santana Santos, C.; Jaato, B. N.; Sanjuán, I.; Schuhmann, W.; Andronescu, C. Operando Scanning Electrochemical Probe Microscopy during Electrocatalysis. *Chem. Rev.* **2023**, *123* (8), 4972−5019.

(143) Salverda, A.; Abner, S.; Mena-Morcillo, E.; Zimmer, A.; Elsayed, A.; Chen, A. Electrochemical, Scanning Electrochemical Microscopic, and In Situ Electrochemical Fourier Transform Infrared Studies of CO2 Reduction at Porous Copper Surfaces. *J. Phys. Chem. C* **2023**, *127* (15), 7151−7161.

(144) Williams, L. L.; Webster, R. D. Electrochemically Controlled Chemically Reversible Transformation of α-Tocopherol (Vitamin E) into Its Phenoxonium Cation. *J. Am. Chem. Soc.* **2004**, *126* (39), 12441−12450.

(145) Goes, S. L.; Mayer, M. N.; Nutting, J. E.; Hoober-Burkhardt, L. E.; Stahl, S. S.; Rafiee, M. Deriving the Turnover Frequency of Aminoxyl-Catalyzed Alcohol Oxidation by Chronoamperometry: An Introduction to Organic Electrocatalysis. *J. Chem. Educ.* **2021**, *98* (2), 600−606.

(146) Rafiee, M.; Konz, Z. M.; Graaf, M. D.; Koolman, H. F.; Stahl, S. S. Electrochemical Oxidation of Alcohols and Aldehydes to Carboxylic Acids Catalyzed by 4-Acetamido-TEMPO: An Alternative to "Anelli" and "Pinnick" Oxidations. *ACS Catal.* **2018**, *8* (7), 6738−6744.

(147) Rafiee, M.; Mayer, M. N.; Punchihewa, B. T.; Mumau, M. R. Constant Potential and Constant Current Electrolysis: An Introduction and Comparison of Different Techniques for Organic Electrosynthesis. *J. Org. Chem.* **2021**, *86* (22), 15866−15874.

(148) Frankowski, K. J.; Liu, R.; Milligan, G. L.; Moeller, K. D.; Aubé, J. Practical Electrochemical Anodic Oxidation of Polycyclic Lactams for Late Stage Functionalization. *Angew. Chemie Int. Ed.* **2015**, *54* (36), 10555−10558.

(149) Mihelcic, J.; Moeller, K. D. Anodic Cyclization Reactions: The Total Synthesis of Alliacol A. *J. Am. Chem. Soc.* **2003**, *125* (1), 36−37.

(150) Yoshida, J.; Shimizu, A.; Hayashi, R. Electrogenerated Cationic Reactive Intermediates: The Pool Method and Further Advances. *Chem. Rev.* **2018**, *118* (9), 4702−4730.

(151) Yang, Q.-L.; Li, Y.-Q.; Ma, C.; Fang, P.; Zhang, X.-J.; Mei, T.-S. Palladium-Catalyzed C (Sp3)— H Oxygenation via Electrochemical Oxidation. *J. Am. Chem. Soc.* **2017**, *139* (8), 3293−3298.

(152) Wiebe, A.; Riehl, B.; Lips, S.; Franke, R.; Waldvogel, S. R. Unexpected High Robustness of Electrochemical Cross-Coupling for a Broad Range of Current Density. *Sci. Adv.* **2017**, *3* (10), No. eaao3920.

(153) Hilt, G. Basic Strategies and Types of Applications in Organic Electrochemistry. *ChemElectroChem.* **2020**, *7* (2), 395−405.

(154) Trasatti, S.; Petrii, O. A. Real Surface Area Measurements in Electrochemistry. *Pure Appl. Chem.* **1991**, *63* (5), 711−734.

(155) Huang, H.; Yuan, G.; Li, X.; Jiang, H. Electrochemical Synthesis of Amides: Direct Transformation of Methyl Ketones with Formamides. *Tetrahedron Lett.* **2013**, *54* (52), 7156−7159.

(156) Hilt, G. Convergent Paired Electrolysis for the Three-Component Synthesis of Protected Homoallylic Alcohols. *Angew. Chemie Int. Ed.* **2003**, *42* (15), 1720−1721.

(157) Senboku, H.; Nagakura, K.; Fukuhara, T.; Hara, S. Three-Component Coupling Reaction of Benzylic Halides, Carbon Dioxide, and N, N-Dimethylformamide by Using Paired Electrolysis: Sacrificial Anode-Free Efficient Electrochemical Carboxylation of Benzylic Halides. *Tetrahedron* **2015**, *71* (23), 3850−3856.

(158) Batanero, B.; Barba, F.; Sánchez-Sánchez, C. M.; Aldaz, A. Paired Electrosynthesis of Cyanoacetic Acid. *J. Org. Chem.* **2004**, *69* (7), 2423−2426.

(159) Kärkäs, M. D. Electrochemical Strategies for C-H Functionalization and C-N Bond Formation. *Chem. Soc. Rev.* **2018**, *47* (15), 5786−5865.

(160) Habibi, D.; Pakravan, N.; Nematollahi, D. The Green and Convergent Paired Diels-Alder Electro-Synthetic Reaction of 1, 4-Hydroquinone with 1, 2-Bis (Bromomethyl) Benzene. *Electrochem. commun.* **2014**, *49*, 65−69.

(161) Ishifune, M.; Yamashita, H.; Matsuda, M.; Ishida, H.; Yamashita, N.; Kera, Y.; Kashimura, S.; Masuda, H.; Murase, H. Electroreduction of Aliphatic Esters Using New Paired Electrolysis Systems. *Electrochim. Acta* **2001**, *46* (20−21), 3259−3264.

(162) Yang, N.; Yu, S.; Macpherson, J. V.; Einaga, Y.; Zhao, H.; Zhao, G.; Swain, G. M.; Jiang, X. Conductive Diamond: Synthesis, Properties, and Electrochemical Applications. *Chem. Soc. Rev.* **2019**, *48* (1), 157−204.

(163) Rodrigo, S.; Gunasekera, D.; Mahajan, J. P.; Luo, L. Alternating Current Electrolysis for Organic Synthesis. *Curr. Opin. Electrochem.* **2021**, *28*, 100712.

(164) Rodrigo, S.; Um, C.; Mixdorf, J. C.; Gunasekera, D.; Nguyen, H. M.; Luo, L. Alternating Current Electrolysis for Organic Electrosynthesis: Trifluoromethylation of (Hetero) Arenes. *Org. Lett.* **2020**, *22* (17), 6719−6723.

(165) Schotten, C.; Taylor, C. J.; Bourne, R. A.; Chamberlain, T. W.; Nguyen, B. N.; Kapur, N.; Willans, C. E. Alternating Polarity for Enhanced Electrochemical Synthesis. *React. Chem. Eng.* **2021**, *6* (1), 147−151.

(166) Behera, N.; Gunasekera, D.; Mahajan, J.; Frimpong, J.; Liu, Z.-F.; Luo, L. Electrochemical Hydrogen Isotope Exchange of Amines Controlled by Alternating Current Frequency. *Faraday Discuss.* **2023**, *247*, 45.

(167) Kaliszczak, M.; Durand, P.; Wenger, E.; Dossot, M.; Jones, F.; Arrigan, D. W. M.; Herzog, G. Electrochemically Controlled Cocrystallisation of Caffeine: 1-Hydroxy-2-Naphthoic Acid. *CrystEngComm* **2021**, *24* (1), 48−51.

(168) Danly, D. E. Development and Commercialization of the Monsanto Electrochemical Adiponitrile Process. *J. Electrochem. Soc.* **1984**, *131* (10), 435C.

(169) Pletcher, D.; Walsh, F. C. Organic Electrosynthesis. In *Industrial Electrochemistry*; Springer, 1993; pp 294−330.

(170) Martinez-Mora, O.; Pozo, G.; Leon-Fernandez, L. F.; Fransaer, J.; Dominguez-Benetton, X. Synthesis of Platinum Group Metal Nanoparticles Assisted by CO 2 Reduction and H 2 Cogeneration at Gas-Diffusion Electrodes. *RSC Sustain.* **2023**, *1* (3), 454−458.

(171) Freguia, S.; Virdis, B.; Harnisch, F.; Keller, J. Bioelectrochemical Systems: Microbial versus Enzymatic Catalysis. *Electrochim. Acta* **2012**, *82*, 165−174.

(172) Turick, C. E.; Shimpalee, S.; Satjaritanun, P.; Weidner, J.; Greenway, S. Convenient Non-Invasive Electrochemical Techniques to Monitor Microbial Processes: Current State and Perspectives. *Appl. Microbiol. Biotechnol.* **2019**, *103* (20), 8327−8338.

(173) Léger, C.; Elliott, S. J.; Hoke, K. R.; Jeuken, L. J. C.; Jones, A. K.; Armstrong, F. A. Enzyme Electrokinetics: Using Protein Film Voltammetry to Investigate Redox Enzymes and Their Mechanisms. *Biochemistry* **2003**, *42* (29), 8653−8662.

(174) Bedendi, G.; De Moura Torquato, L. D.; Webb, S.; Cadoux, C.; Kulkarni, A.; Sahin, S.; Maroni, P.; Milton, R. D.; Grattieri, M. Enzymatic and Microbial Electrochemistry: Approaches and Methods. *ACS Meas. Sci. Au* **2022**, *2* (6), 517−541.

(175) Marsili, E.; Baron, D. B.; Shikhare, I. D.; Coursolle, D.; Gralnick, J. A.; Bond, D. R. Shewanella Secretes Flavins That Mediate Extracellular Electron Transfer. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (10), 3968−3973.

(176) Finkelstein, J.; Swartz, J.; Koffas, M. Bioelectrosynthesis Systems. *Curr. Opin. Biotechnol.* **2022**, *74*, 211−219.

(177) Adamson, H.; Bond, A. M.; Parkin, A. Probing Biological Redox Chemistry with Large Amplitude Fourier Transformed Ac Voltammetry. *Chem. Commun.* **2017**, *53* (69), 9519−9533.

(178) Adamson, H.; Robinson, M.; Wright, J. J.; Flanagan, L. A.; Walton, J.; Elton, D.; Gavaghan, D. J.; Bond, A. M.; Roessler, M. M.; Parkin, A. Retuning the Catalytic Bias and Overpotential of a [NiFe]-Hydrogenase via a Single Amino Acid Exchange at the Electron Entry/Exit Site. *J. Am. Chem. Soc.* **2017**, *139* (31), 10677−10686.

(179) Armstrong, F. A.; Camba, R.; Heering, H. A.; Hirst, J.; Jeuken, L. J. C.; Jones, A. K.; Léger, C.; McEvoy, J. P. Fast Voltammetric Studies of the Kinetics and Energetics of Coupled Electron-Transfer Reactions in Proteins. *Faraday Discuss.* **2000**, *116*, 191−203.

(180) Sharma, M.; Alvarez-Gallego, Y.; Achouak, W.; Pant, D.; Sarma, P. M.; Dominguez-Benetton, X. Electrode Material Properties for Designing Effective Microbial Electrosynthesis Systems. *J. Mater. Chem. A* **2019**, *7* (42), 24420−24436.

(181) Badiani, V. M.; Cobb, S. J.; Wagner, A.; Oliveira, A. R.; Zacarias, S.; Pereira, I. A. C.; Reisner, E. Elucidating Film Loss and the Role of Hydrogen Bonding of Adsorbed Redox Enzymes by Electrochemical Quartz Crystal Microbalance Analysis. *ACS Catal.* **2022**, *12* (3), 1886−1897.

(182) Singh, K.; Blanford, C. F. Electrochemical Quartz Crystal Microbalance with Dissipation Monitoring: A Technique to Optimize Enzyme Use in Bioelectrocatalysis. *ChemCatChem.* **2014**, *6* (4), 921−929.

(183) Olsson, A. L. J.; Van der Mei, H. C.; Busscher, H. J.; Sharma, P. K. Influence of Cell Surface Appendages on the Bacterium- Substratum Interface Measured Real-Time Using QCM-D. *Langmuir* **2009**, *25* (3), 1627−1632.

(184) Wu, R.; Ma, C.; Zhu, Z. Enzymatic Electrosynthesis as an Emerging Electrochemical Synthesis Platform. *Curr. Opin. Electrochem.* **2020**, *19*, 1−7.

(185) Martínez, N. P.; Isaacs, M.; Nanda, K. K. Paired Electrolysis for Simultaneous Generation of Synthetic Fuels and Chemicals. *New J. Chem.* **2020**, *44* (15), 5617−5637.

(186) Boucher, D.; Carroll, E.; Nguyen, Z.; Jadhav, R.; Simoska, O.; Beaver, K.; Minteer, S. D. Bioelectrocatalytic Synthesis: Concepts and Applications. *Angew. Chem.* **2023**, *135*, No. e202307780.

(187) Dominguez-Benetton, X.; Srikanth, S.; Satyawali, Y.; Vanbroekhoven, K.; Pant, D. Enzymatic Electrosynthesis: An Overview on the Progress in Enzyme-Electrodes for the Production of Electricity, Fuels and Chemicals. *J. Microb. Biochem. Technol. S* **2013**, *6*, 007.

(188) Schmitz, L. M.; Rosenthal, K.; Lütz, S. Enzyme-Based Electrobiotechnological Synthesis. *Bioelectrosynthesis* **2017**, *167*, 87−134.

(189) Mayo, S. L.; Ellis, W. R., Jr; Crutchley, R. J.; Gray, H. B. Long-Range Electron Transfer in Heme Proteins. *Science (80-.).* **1986**, *233* (4767), 948−952.

(190) Collman, J. P.; Devaraj, N. K.; Decréau, R. A.; Yang, Y.; Yan, Y.-L.; Ebina, W.; Eberspacher, T. A.; Chidsey, C. E. D. A Cytochrome c Oxidase Model Catalyzes Oxygen to Water Reduction under Rate-Limiting Electron Flux. *Science (80-.).* **2007**, *315* (5818), 1565−1568.

(191) Urlacher, V. B.; Eiben, S. Cytochrome P450 Monooxygenases: Perspectives for Synthetic Application. *Trends Biotechnol.* **2006**, *24* (7), 324−330.

(192) Vlasits, J.; Jakopitsch, C.; Bernroitner, M.; Zamocky, M.; Furtmüller, P. G.; Obinger, C. Mechanisms of Catalase Activity of Heme Peroxidases. *Arch. Biochem. Biophys.* **2010**, *500* (1), 74−81.

(193) Meyer, J. Iron-Sulfur Protein Folds, Iron-Sulfur Chemistry, and Evolution. *JBIC J. Biol. Inorg. Chem.* **2008**, *13* (2), 157−170.

(194) Solomon, E. I.; Sundaram, U. M.; Machonkin, T. E. Multicopper Oxidases and Oxygenases. *Chem. Rev.* **1996**, *96* (7), 2563−2606.

(195) Van Berkel, W. J. H.; Kamerbeek, N. M.; Fraaije, M. Flavoprotein Monooxygenases, a Diverse Class of Oxidative Biocatalysts. *J. Biotechnol.* **2006**, *124* (4), 670−689.

(196) Chen, H.; Simoska, O.; Lim, K.; Grattieri, M.; Yuan, M.; Dong, F.; Lee, Y. S.; Beaver, K.; Weliwatte, S.; Gaffney, E. M.; et al. Fundamentals, Applications, and Future Directions of Bioelectrocatalysis. *Chem. Rev.* **2020**, *120* (23), 12903−12993.

(197) Immanuel, S.; Sivasubramanian, R.; Gul, R.; Dar, M. A. Recent Progress and Perspectives on Electrochemical Regeneration of Reduced Nicotinamide Adenine Dinucleotide (NADH). *Chem.—Asian J.* **2020**, *15* (24), 4256−4270.

(198) Kohlmann, C.; Märkle, W.; Lütz, S. Electroenzymatic Synthesis. *J. Mol. Catal. B Enzym.* **2008**, *51* (3−4), 57−72.

(199) Kashiwagi, Y.; Yanagisawa, Y.; Shibayama, N.; Nakahara, K.; Kurashima, F.; Anzai, J.; Osa, T. Preparative, Electroenzymatic Reduction of Ketones on an All Components-Immobilized Graphite Felt Electrode. *Electrochim. Acta* **1997**, *42* (13−14), 2267−2270.

(200) Kang, Y.-W.; Kang, C.; Hong, J.-S.; Yun, S.-E. Optimization of the Mediated Electrocatalytic Reduction of NAD+ by Cyclic Voltammetry and Construction of Electrochemically Driven Enzyme Bioreactor. *Biotechnol. Lett.* **2001**, *23*, 599−604.

(201) Kim, M.-H.; Yun, S.-E. Construction of an Electro-Enzymatic Bioreactor for the Production of (R)-Mandelate from Benzoylformate. *Biotechnol. Lett.* **2004**, *26*, 21−26.

(202) Cantet, J.; Bergel, A.; Comtat, M. Coupling of the Electroenzymatic Reduction of NAD+ with a Synthesis Reaction. *Enzyme Microb. Technol.* **1996**, *18* (1), 72−79.

(203) Li, Y.; Liu, G.; Kong, W.; Zhang, S.; Bao, Y.; Zhao, H.; Wang, L.; Zhou, L.; Jiang, Y. Electrocatalytic NAD (P) H Regeneration for Biosynthesis. *Green Chem. Eng.* **2023**, DOI: 10.1016/j.gce.2023.02.001.

(204) Chen, H.; Dong, F.; Minteer, S. D. The Progress and Outlook of Bioelectrocatalysis for the Production of Chemicals, Fuels and Materials. *Nat. Catal.* **2020**, *3* (3), 225−244.

(205) Wienkamp, R.; Steckhan, E. Indirect Electrochemical Regeneration of NADH by a Bipyridinerhodium (I) Complex as Electron-transfer Agent. *Angew. Chem., Int. Ed. Engl.* **1982**, *21* (10), 782−783.

(206) Ruppert, R.; Herrmann, S.; Steckhan, E. Efficient Indirect Electrochemical In-Situ Regeneration of NADH :Electrochemically Driven Enzymatic Reduction of Pyruvate Catalyzed by d-Ldh. *Tetrahedron Lett.* **1987**, *28* (52), 6583−6586.

(207) Steckhan, E. Electroenzymatic Synthesis. In *Electrochemistry V*; Springer, 1994; pp 83−111.

(208) Zhang, C.; Zhang, X.; Fu, Y.; Zhang, L.; Kuhn, A. Metal-Organic Framework Functionalized Bipolar Electrodes for Bulk Electroenzymatic Synthesis. *J. Catal.* **2023**, *421*, 95−100.

(209) Hill, H. A. O.; Oliver, B. N.; Page, D. J.; Hopper, D. J. The Enzyme-Catalysed Electrochemical Conversion of p-Cresol into p-Hydroxybenzaldehyde. *J. Chem. Soc. Chem. Commun.* **1985**, No. 21, 1469−1471.

(210) Petersen, A.; Steckhan, E. Continuous Indirect Electrochemical Regeneration of Galactose Oxidase. *Bioorg. Med. Chem.* **1999**, *7* (10), 2203−2208.

(211) Page, C. C.; Moser, C. C.; Chen, X.; Dutton, P. L. Natural Engineering Principles of Electron Tunnelling in Biological Oxidation-Reduction. *Nature* **1999**, *402* (6757), 47−52.

(212) Jesionowski, T.; Zdarta, J.; Krajewska, B. Enzyme Immobilization by Adsorption: A Review. *Adsorption* **2014**, *20*, 801−821.

(213) Meredith, M. T.; Minson, M.; Hickey, D.; Artyushkova, K.; Glatzhofer, D. T.; Minteer, S. D. Anthracene-Modified Multi-Walled

Carbon Nanotubes as Direct Electron Transfer Scaffolds for Enzymatic Oxygen Reduction. *Acs Catal.* **2011**, *1* (12), 1683−1690.

(214) Karaśkiewicz, M.; Nazaruk, E.; Żelechowska, K.; Biernat, J. F.; Rogalski, J.; Bilewicz, R. Fully Enzymatic Mediatorless Fuel Cell with Efficient Naphthylated Carbon Nanotube-Laccase Composite Cathodes. *Electrochem. commun.* **2012**, *20*, 124−127.

(215) Patel, J.; Cai, R.; Milton, R.; Chen, H.; Minteer, S. D. Pyrene-Based Noncovalent Immobilization of Nitrogenase on Carbon Surfaces. *ChemBioChem.* **2020**, *21* (12), 1729−1732.

(216) Nguyen, H. H.; Kim, M. An Overview of Techniques in Enzyme Immobilization. *Appl. Sci. Converg. Technol.* **2017**, *26* (6), 157−163.

(217) Hickey, D. P.; Cai, R.; Yang, Z.-Y.; Grunau, K.; Einsle, O.; Seefeldt, L. C.; Minteer, S. D. Establishing a Thermodynamic Landscape for the Active Site of Mo-Dependent Nitrogenase. *J. Am. Chem. Soc.* **2019**, *141* (43), 17150−17157.

(218) Mazurenko, eI; De Poulpiquet, A.; Lojou, E. Recent Developments in High Surface Area Bioelectrodes for Enzymatic Fuel Cells. *Curr. Opin. Electrochem.* **2017**, *5* (1), 74−84.

(219) So, K.; Sakai, K.; Kano, K. Gas Diffusion Bioelectrodes. *Curr. Opin. Electrochem.* **2017**, *5* (1), 173−182.

(220) Kitazumi, Y.; Shirai, O.; Yamamoto, M.; Kano, K. Numerical Simulation of Diffuse Double Layer around Microporous Electrodes Based on the Poisson-Boltzmann Equation. *Electrochim. Acta* **2013**, *112*, 171−175.

(221) Kwon, K. Y.; Youn, J.; Kim, J. H.; Park, Y.; Jeon, C.; Kim, B. C.; Kwon, Y.; Zhao, X.; Wang, P.; Sang, B. I.; et al. Nanoscale Enzyme Reactors in Mesoporous Carbon for Improved Performance and Lifetime of Biosensors and Biofuel Cells. *Biosens. Bioelectron.* **2010**, *26* (2), 655−660.

(222) Zhai, T.; Wang, C.; Gu, F.; Meng, Z.; Liu, W.; Wang, Y. Dopamine/Polyethylenimine-Modified Silica for Enzyme Immobilization and Strengthening of Enzymatic CO2 Conversion. *ACS Sustain. Chem. Eng.* **2020**, *8* (40), 15250−15257.

(223) Milton, R. D.; Hickey, D. P.; Abdellaoui, S.; Lim, K.; Wu, F.; Tan, B.; Minteer, S. D. Rational Design of Quinones for High Power Density Biofuel Cells. *Chem. Sci.* **2015**, *6* (8), 4867−4875.

(224) Yuan, M.; Kummer, M. J.; Milton, R. D.; Quah, T.; Minteer, S. D. Efficient NADH Regeneration by a Redox Polymer-Immobilized Enzymatic System. *ACS Catal.* **2019**, *9*, 5486−5495.

(225) Quah, T.; Milton, R. D.; Abdellaoui, S.; Minteer, S. D. Bioelectrocatalytic NAD+/NADH Inter-Conversion: Transformation of an Enzymatic Fuel Cell into an Enzymatic Redox Flow Battery. *Chem. Commun.* **2017**, *53* (60), 8411−8414.

(226) Wong, T. S.; Schwaneberg, U. Protein Engineering in Bioelectrocatalysis. *Curr. Opin. Biotechnol.* **2003**, *14* (6), 590−596.

(227) Adachi, T.; Kitazumi, Y.; Shirai, O.; Kano, K. Recent Progress in Applications of Enzymatic Bioelectrocatalysis. *Catalysts* **2020**, *10* (12), 1413.

(228) Kaida, Y.; Hibino, Y.; Kitazumi, Y.; Shirai, O.; Kano, K. Ultimate Downsizing of D-Fructose Dehydrogenase for Improving the Performance of Direct Electron Transfer-Type Bioelectrocatalysis. *Electrochem. commun.* **2019**, *98*, 101−105.

(229) Lindgren, A.; Tanaka, M.; Ruzgas, T.; Gorton, L.; Gazaryan, I.; Ishimori, K.; Morishima, I. Direct Electron Transfer Catalysed by Recombinant Forms of Horseradish Peroxidase: Insight into the Mechanism. *Electrochem. commun.* **1999**, *1* (5), 171−175.

(230) Kamitaka, Y.; Tsujimura, S.; Kataoka, K.; Sakurai, T.; Ikeda, T.; Kano, K. Effects of Axial Ligand Mutation of the Type I Copper Site in Bilirubin Oxidase on Direct Electron Transfer-Type Bioelectrocatalytic Reduction of Dioxygen. *J. Electroanal. Chem.* **2007**, *601* (1−2), 119−124.

(231) Mateljak, I.; Monza, E.; Lucas, M. F.; Guallar, V.; Aleksejeva, O.; Ludwig, R.; Leech, D.; Shleev, S.; Alcalde, M. Increasing Redox Potential, Redox Mediator Activity, and Stability in a Fungal Laccase by Computer-Guided Mutagenesis and Directed Evolution. *ACS Catal.* **2019**, *9* (5), 4561−4572.

(232) Chen, H.; Zhu, Z.; Huang, R.; Zhang, Y.-H. P. Coenzyme Engineering of a Hyperthermophilic 6-Phosphogluconate Dehydrogenase from NADP+ to NAD+ with Its Application to Biobatteries. *Sci. Rep.* **2016**, *6* (1), 36311.

(233) Ding, Y.; Perez-Ortiz, G.; Peate, J.; Barry, S. M. Redesigning Enzymes for Biocatalysis: Exploiting Structural Understanding for Improved Selectivity. *Front. Mol. Biosci.* **2022**, *9*, 908285.

(234) Ferapontova, E.; Schmengler, K.; Börchers, T.; Ruzgas, T.; Gorton, L. Effect of Cysteine Mutations on Direct Electron Transfer of Horseradish Peroxidase on Gold. *Biosens. Bioelectron.* **2002**, *17* (11−12), 953−963.

(235) Lee, Y. S.; Baek, S.; Lee, H.; Reginald, S. S.; Kim, Y.; Kang, H.; Choi, I.-G.; Chang, I. S. Construction of Uniform Monolayer-and Orientation-Tunable Enzyme Electrode by a Synthetic Glucose Dehydrogenase without Electron-Transfer Subunit via Optimized Site-Specific Gold-Binding Peptide Capable of Direct Electron Transfer. *ACS Appl. Mater. Interfaces* **2018**, *10* (34), 28615−28626.

(236) Bollella, P.; Ludwig, R.; Gorton, L. Cellobiose Dehydrogenase: Insights on the Nanostructuration of Electrodes for Improved Development of Biosensors and Biofuel Cells. *Appl. Mater. Today* **2017**, *9*, 319−332.

(237) Gilardi, G.; Meharenna, Y. T.; Tsotsou, G. E.; Sadeghi, S. J.; Fairhead, M.; Giannini, S. Molecular Lego: Design of Molecular Assemblies of P450 Enzymes for Nanobiotechnology. *Biosens. Bioelectron.* **2002**, *17* (1−2), 133−145.

(238) Ito, K.; Okuda-Shimazaki, J.; Mori, K.; Kojima, K.; Tsugawa, W.; Ikebukuro, K.; Lin, C.-E.; La Belle, J.; Yoshida, H.; Sode, K. Designer Fungus FAD Glucose Dehydrogenase Capable of Direct Electron Transfer. *Biosens. Bioelectron.* **2019**, *123*, 114−123.

(239) Lee, Y. S.; Lim, K.; Minteer, S. D. Cascaded Biocatalysis and Bioelectrocatalysis: Overview and Recent Advances. *Annu. Rev. Phys. Chem.* **2021**, *72*, 467−488.

(240) Wheeldon, I.; Minteer, S. D.; Banta, S.; Barton, S. C.; Atanassov, P.; Sigman, M. Substrate Channelling as an Approach to Cascade Reactions. *Nat. Chem.* **2016**, *8* (4), 299−309.

(241) Hickey, D. P.; McCammant, M. S.; Giroud, F.; Sigman, M. S.; Minteer, S. D. Hybrid Enzymatic and Organic Electrocatalytic Cascade for the Complete Oxidation of Glycerol. *J. Am. Chem. Soc.* **2014**, *136* (45), 15917−15920.

(242) Kummer, M. J.; Lee, Y. S.; Yuan, M.; Alkotaini, B.; Zhao, J.; Blumenthal, E.; Minteer, S. D. Substrate Channeling by a Rationally Designed Fusion Protein in a Biocatalytic Cascade. *JACS Au* **2021**, *1* (8), 1187−1197.

(243) Jia, H.-P.; Quadrelli, E. A. Mechanistic Aspects of Dinitrogen Cleavage and Hydrogenation to Produce Ammonia in Catalysis and Organometallic Chemistry: Relevance of Metal Hydride Bonds and Dihydrogen. *Chem. Soc. Rev.* **2014**, *43* (2), 547−564.

(244) Chen, J. G.; Crooks, R. M.; Seefeldt, L. C.; Bren, K. L.; Bullock, R. M.; Darensbourg, M. Y.; Holland, P. L.; Hoffman, B.; Janik, M. J.; Jones, A. K.; et al. Beyond Fossil Fuel-Driven Nitrogen Transformations. *Science (80-.).* **2018**, *360* (6391), No. eaar6611.

(245) Burgess, B. K.; Lowe, D. J. Mechanism of Molybdenum Nitrogenase. *Chem. Rev.* **1996**, *96* (7), 2983−3012.

(246) Cadoux, C.; Milton, R. D. Recent Enzymatic Electrochemistry for Reductive Reactions. *ChemElectroChem.* **2020**, *7* (9), 1974−1986.

(247) Milton, R. D.; Cai, R.; Abdellaoui, S.; Leech, D.; De Lacey, A. L.; Pita, M.; Minteer, S. D. Bioelectrochemical Haber-Bosch Process: An Ammonia-producing H2/N2 Fuel Cell. *Angew. Chemie Int. Ed.* **2017**, *56* (10), 2680−2683.

(248) Badalyan, A.; Yang, Z.-Y.; Seefeldt, L. C. A Voltammetric Study of Nitrogenase Catalysis Using Electron Transfer Mediators. *ACS Catal.* **2019**, *9* (2), 1366−1372.

(249) Lee, Y. S.; Ruff, A.; Cai, R.; Lim, K.; Schuhmann, W.; Minteer, S. D. Electroenzymatic Nitrogen Fixation Using a MoFe Protein System Immobilized in an Organic Redox Polymer. *Angew. Chemie Int. Ed.* **2020**, *59* (38), 16511−16516.

(250) Hickey, D. P.; Lim, K.; Cai, R.; Patterson, A. R.; Yuan, M.; Sahin, S.; Abdellaoui, S.; Minteer, S. D. Pyrene Hydrogel for Promoting Direct Bioelectrochemistry: ATP-Independent Electroenzymatic Reduction of N 2. *Chem. Sci.* **2018**, *9* (23), 5172−5177.

(251) Chen, H.; Cai, R.; Patel, J.; Dong, F.; Chen, H.; Minteer, S. D. Upgraded Bioelectrocatalytic N2 Fixation: From N2 to Chiral Amine Intermediates. *J. Am. Chem. Soc.* **2019**, *141* (12), 4963−4971.

(252) Lee, H.-S.; Vermaas, W. F. J.; Rittmann, B. E. Biological Hydrogen Production: Prospects and Challenges. *Trends Biotechnol.* **2010**, *28* (5), 262−271.

(253) Peters, J. W.; Schut, G. J.; Boyd, E. S.; Mulder, D. W.; Shepard, E. M.; Broderick, J. B.; King, P. W.; Adams, M. W. W. [FeFe]-and [NiFe]-Hydrogenase Diversity, Mechanism, and Maturation. *Biochim. Biophys. Acta (BBA)-Molecular Cell Res.* **2015**, *1853* (6), 1350−1369.

(254) Fritsch, J.; Scheerer, P.; Frielingsdorf, S.; Kroschinsky, S.; Friedrich, B.; Lenz, O.; Spahn, C. M. T. The Crystal Structure of an Oxygen-Tolerant Hydrogenase Uncovers a Novel Iron-Sulphur Centre. *Nature* **2011**, *479* (7372), 249−252.

(255) Volbeda, A.; Darnault, C.; Parkin, A.; Sargent, F.; Armstrong, F. A.; Fontecilla-Camps, J. C. Crystal Structure of the O2-Tolerant Membrane-Bound Hydrogenase 1 from Escherichia Coli in Complex with Its Cognate Cytochrome B. *Structure* **2013**, *21* (1), 184−190.

(256) Del Barrio, M.; Guendon, C.; Kpebe, A.; Baffert, C.; Fourmond, V.; Brugna, M.; Léger, C. Valine-to-Cysteine Mutation Further Increases the Oxygen Tolerance of Escherichia Coli NiFe Hydrogenase Hyd-1. *ACS Catal.* **2019**, *9* (5), 4084−4088.

(257) Szczesny, J.; Marković, N.; Conzuelo, F.; Zacarias, S.; Pereira, I. A. C.; Lubitz, W.; Plumeré, N.; Schuhmann, W.; Ruff, A. A Gas Breathing Hydrogen/Air Biofuel Cell Comprising a Redox Polymer/Hydrogenase-Based Bioanode. *Nat. Commun.* **2018**, *9* (1), 4715.

(258) Qian, D.-J.; Nakamura, C.; Wenk, S.; Wakayama, T.; Zorin, N.; Miyake, J. Electrochemical Hydrogen Evolution by Use of a Glass Carbon Electrode Sandwiched with Clay, Poly (Butylviologen) and Hydrogenase. *Mater. Lett.* **2003**, *57* (5−6), 1130−1134.

(259) McDonald, T. J.; Svedruzic, D.; Kim, Y.-H.; Blackburn, J. L.; Zhang, S. B.; King, P. W.; Heben, M. J. Wiring-up Hydrogenase with Single-Walled Carbon Nanotubes. *Nano Lett.* **2007**, *7* (11), 3528−3534.

(260) Morra, S.; Valetti, F.; Sarasso, V.; Castrignanò, S.; Sadeghi, S. J.; Gilardi, G. Hydrogen Production at High Faradaic Efficiency by a Bio-Electrode Based on TiO2 Adsorption of a New [FeFe]-Hydrogenase from Clostridium Perfringens. *Bioelectrochemistry* **2015**, *106*, 258−262.

(261) Lee, C.; Park, H. S.; Fontecilla-Camps, J. C.; Reisner, E. Photoelectrochemical H2 Evolution with a Hydrogenase Immobilized on a TiO2-protected Silicon Electrode. *Angew. Chemie Int. Ed.* **2016**, *55* (20), 5971−5974.

(262) Mersch, D.; Lee, C.-Y.; Zhang, J. Z.; Brinkert, K.; Fontecilla-Camps, J. C.; Rutherford, A. W.; Reisner, E. Wiring of Photosystem II to Hydrogenase for Photoelectrochemical Water Splitting. *J. Am. Chem. Soc.* **2015**, *137* (26), 8541−8549.

(263) Sokol, K. P.; Robinson, W. E.; Warnan, J.; Kornienko, N.; Nowaczyk, M. M.; Ruff, A.; Zhang, J. Z.; Reisner, E. Bias-Free Photoelectrochemical Water Splitting with Photosystem II on a Dye-Sensitized Photoanode Wired to Hydrogenase. *Nat. Energy* **2018**, *3* (11), 944−951.

(264) Chiranjeevi, P.; Bulut, M.; Breugelmans, T.; Patil, S. A.; Pant, D. Current Trends in Enzymatic Electrosynthesis for CO2 Reduction. *Curr. Opin. Green Sustain. Chem.* **2019**, *16*, 65−70.

(265) Qiao, J.; Liu, Y.; Hong, F.; Zhang, J. A Review of Catalysts for the Electroreduction of Carbon Dioxide to Produce Low-Carbon Fuels. *Chem. Soc. Rev.* **2014**, *43* (2), 631−675.

(266) Jayathilake, B. S.; Bhattacharya, S.; Vaidehi, N.; Narayanan, S. R. Efficient and Selective Electrochemically Driven Enzyme-Catalyzed Reduction of Carbon Dioxide to Formate Using Formate Dehydrogenase and an Artificial Cofactor. *Acc. Chem. Res.* **2019**, *52* (3), 676−685.

(267) Maia, L. B.; Moura, I.; Moura, J. J. G. Molybdenum and Tungsten-Containing Formate Dehydrogenases: Aiming to Inspire a Catalyst for Carbon Dioxide Utilization. *Inorg. Chim. Acta* **2017**, *455*, 350−363.

(268) Srikanth, S.; Alvarez-Gallego, Y.; Vanbroekhoven, K.; Pant, D. Enzymatic Electrosynthesis of Formic Acid through Carbon Dioxide Reduction in a Bioelectrochemical System: Effect of Immobilization and Carbonic Anhydrase Addition. *ChemPhysChem* **2017**, *18* (22), 3174−3181.

(269) Reda, T.; Plugge, C. M.; Abram, N. J.; Hirst, J. Reversible Interconversion of Carbon Dioxide and Formate by an Electroactive Enzyme. *Proc. Natl. Acad. Sci. U. S. A.* **2008**, *105* (31), 10654−10658.

(270) Cai, R.; Milton, R. D.; Abdellaoui, S.; Park, T.; Patel, J.; Alkotaini, B.; Minteer, S. D. Electroenzymatic C-C Bond Formation from CO2. *J. Am. Chem. Soc.* **2018**, *140* (15), 5041−5044.

(271) Mohan, S. V.; Pandey, A.; Varjani, S. *Biomass, Biofuels, Biochemicals: Microbial Electrochemical Technology: Sustainable Platform for Fuels, Chemicals and Remediation*; Elsevier, 2018.

(272) Wu, H.; Huang, S.; Jiang, Z. Effects of Modification of Silica Gel and ADH on Enzyme Activity for Enzymatic Conversion of CO2 to Methanol. *Catal. today* **2004**, *98* (4), 545−552.

(273) Xu, S.; Lu, Y.; Li, J.; Jiang, Z.; Wu, H. Efficient Conversion of CO2 to Methanol Catalyzed by Three Dehydrogenases Co-Encapsulated in an Alginate- Silica (ALG- SiO2) Hybrid Gel. *Ind. Eng. Chem. Res.* **2006**, *45* (13), 4567−4573.

(274) Luo, J.; Meyer, A. S.; Mateiu, R. V.; Pinelo, M. Cascade Catalysis in Membranes with Enzyme Immobilization for Multi-Enzymatic Conversion of CO2 to Methanol. *N. Biotechnol.* **2015**, *32* (3), 319−327.

(275) El-Zahab, B.; Donnelly, D.; Wang, P. Particle-tethered NADH for Production of Methanol from CO2 Catalyzed by Coimmobilized Enzymes. *Biotechnol. Bioeng.* **2008**, *99* (3), 508−514.

(276) Kuk, S. K.; Singh, R. K.; Nam, D. H.; Singh, R.; Lee, J. K.; Park, C. B. Photoelectrochemical Reduction of Carbon Dioxide to Methanol through a Highly Efficient Enzyme Cascade. *Angewandte Chemie - International Edition.* **2017**, *56*, 3827−3832.

(277) Patel, R. N. Biocatalytic Synthesis of Intermediates for the Synthesis of Chiral Drug Substances. *Curr. Opin. Biotechnol.* **2001**, *12* (6), 587−604.

(278) Betori, R. C.; May, C. M.; Scheidt, K. A. Combined Photoredox/Enzymatic C- H Benzylic Hydroxylations. *Angew. Chemie Int. Ed.* **2019**, *58* (46), 16490−16494.

(279) Chakrabarty, S.; Wang, Y.; Perkins, J. C.; Narayan, A. R. H. Scalable Biocatalytic C-H Oxyfunctionalization Reactions. *Chem. Soc. Rev.* **2020**, *49* (22), 8137−8155.

(280) Urlacher, V. B.; Girhard, M. Cytochrome P450 Monooxygenases: An Update on Perspectives for Synthetic Application. *Trends Biotechnol.* **2012**, *30* (1), 26−36.

(281) Sugihara, N.; Ogoma, Y.; Abe, K.; Kondo, Y.; Akaike, T. Immobilization of Cytochrome P-450 and Electrochemical Control of Its Activity. *Polym. Adv. Technol.* **1998**, *9* (5), 307−313.

(282) Fantuzzi, A.; Fairhead, M.; Gilardi, G. Direct Electrochemistry of Immobilized Human Cytochrome P450 2E1. *J. Am. Chem. Soc.* **2004**, *126* (16), 5040−5041.

(283) Sigmund, M.-C.; Poelarends, G. J. Current State and Future Perspectives of Engineered and Artificial Peroxygenases for the Oxyfunctionalization of Organic Molecules. *Nat. Catal.* **2020**, *3* (9), 690−702.

(284) Choi, D. S.; Lee, H.; Tieves, F.; Lee, Y. W.; Son, E. J.; Zhang, W.; Shin, B.; Hollmann, F.; Park, C. B. Bias-Free in Situ H2O2 Generation in a Photovoltaic-Photoelectrochemical Tandem Cell for Biocatalytic Oxyfunctionalization. *ACS Catal.* **2019**, *9* (11), 10562−10566.

(285) Yuan, M.; Abdellaoui, S.; Chen, H.; Kummer, M. J.; Malapit, C. A.; You, C.; Minteer, S. D. Selective Electroenzymatic Oxyfunctionalization by Alkane Monooxygenase in a Biofuel Cell. *Angew. Chem.* **2020**, *132* (23), 9054−9058.

(286) Chen, H.; Tang, T.; Malapit, C. A.; Lee, Y. S.; Prater, M. B.; Weliwatte, N. S.; Minteer, S. D. One-Pot Bioelectrocatalytic Conversion of Chemically Inert Hydrocarbons to Imines. *J. Am. Chem. Soc.* **2022**, *144* (9), 4047−4056.

(287) Wu, R.; Zhu, Z. Self-Powered Enzymatic Electrosynthesis of L-3, 4-Dihydroxyphenylalanine in a Hybrid Bioelectrochemical System. *ACS Sustain. Chem. Eng.* **2018**, *6* (10), 12593−12597.

(288) Goldberg, K.; Schroer, K.; Lütz, S.; Liese, A. Biocatalytic Ketone Reduction—a Powerful Tool for the Production of Chiral Alcohols—Part I: Processes with Isolated Enzymes. *Appl. Microbiol. Biotechnol.* **2007**, *76*, 237−248.

(289) Müller, M. Chemoenzymatic Synthesis of Building Blocks for Statin Side Chains. *Angew. Chemie Int. Ed.* **2005**, *44* (3), 362−365.

(290) Dong, F.; Chen, H.; Malapit, C. A.; Prater, M. B.; Li, M.; Yuan, M.; Lim, K.; Minteer, S. D. Biphasic Bioelectrocatalytic Synthesis of Chiral β-Hydroxy Nitriles. *J. Am. Chem. Soc.* **2020**, *142* (18), 8374−8382.

(291) Wan, L.; Heath, R. S.; Megarity, C. F.; Sills, A. J.; Herold, R. A.; Turner, N. J.; Armstrong, F. A. Exploiting Bidirectional Electrocatalysis by a Nanoconfined Enzyme Cascade to Drive and Control Enantioselective Reactions. *ACS Catal.* **2021**, *11* (11), 6526−6533.

(292) Tripathi, R. P.; Verma, S. S.; Pandey, J.; Tiwari, V. K. Recent Development on Catalytic Reductive Amination and Applications. *Curr. Org. Chem.* **2008**, *12* (13), 1093−1115.

(293) Cheng, B.; Wan, L.; Armstrong, F. A. Progress in Scaling up and Streamlining a Nanoconfined, Enzyme-Catalyzed Electrochemical Nicotinamide Recycling System for Biocatalytic Synthesis. *ChemElectroChem.* **2020**, *7*, 4672−4678.

(294) Lee, Y. W.; Boonmongkolras, P.; Son, E. J.; Kim, J.; Lee, S. H.; Kuk, S. K.; Ko, J. W.; Shin, B.; Park, C. B. Unbiased Biocatalytic Solar-to-Chemical Conversion by FeOOH/BiVO4/Perovskite Tandem Structure. *Nat. Commun.* **2018**, *9* (1), 4208.

(295) Otto, K.; Hofstetter, K.; Röthlisberger, M.; Witholt, B.; Schmid, A. Biochemical Characterization of StyAB from Pseudomonas Sp. Strain VLB120 as a Two-Component Flavin-Diffusible Monooxygenase. *J. Bacteriol.* **2004**, *186* (16), 5292−5302.

(296) Ruinatscha, R.; Buehler, K.; Schmid, A. Development of a High Performance Electrochemical Cofactor Regeneration Module and Its Application to the Continuous Reduction of FAD. *J. Mol. Catal. B Enzym.* **2014**, *103*, 100−105.

(297) Toogood, H. S.; Knaus, T.; Scrutton, N. S. Alternative Hydride Sources for Ene-Reductases: Current Trends. *ChemCatChem.* **2014**, *6* (4), 951−954.

(298) Son, E. J.; Lee, S. H.; Kuk, S. K.; Pesic, M.; Choi, D. S.; Ko, J. W.; Kim, K.; Hollmann, F.; Park, C. B. Carbon Nanotube-Graphitic Carbon Nitride Hybrid Films for Flavoenzyme-Catalyzed Photoelectrochemical Cells. *Adv. Funct. Mater.* **2018**, *28* (24), 1705232.

(299) Gao, L.; Zhuang, J.; Nie, L.; Zhang, J.; Zhang, Y.; Gu, N.; Wang, T.; Feng, J.; Yang, D.; Perrett, S.; et al. Intrinsic Peroxidase-like Activity of Ferromagnetic Nanoparticles. *Nat. Nanotechnol.* **2007**, *2* (9), 577−583.

(300) Mishra, P.; Lee, J.; Kumar, D.; Louro, R. O.; Costa, N.; Pathania, D.; Kumar, S.; Lee, J.; Singh, L. Engineered Nanoenzymes with Multifunctional Properties for Next-generation Biological and Environmental Applications. *Adv. Funct. Mater.* **2022**, *32* (8), 2108650.

(301) Song, H.; Ma, C.; Wang, L.; Zhu, Z. Platinum Nanoparticle-Deposited Multi-Walled Carbon Nanotubes as a NADH Oxidase Mimic: Characterization and Applications. *Nanoscale* **2020**, *12* (37), 19284−19292.

(302) Lv, X.-W.; Liu, Y.; Hao, R.; Tian, W.; Yuan, Z.-Y. Urchin-like Al-Doped Co3O4 Nanospheres Rich in Surface Oxygen Vacancies Enable Efficient Ammonia Electrosynthesis. *ACS Appl. Mater. Interfaces* **2020**, *12* (15), 17502−17508.

(303) Zhao, M.; Deng, K.; He, L.; Liu, Y.; Li, G.; Zhao, H.; Tang, Z. Core-Shell Palladium Nanoparticle@ Metal-Organic Frameworks as Multifunctional Catalysts for Cascade Reactions. *J. Am. Chem. Soc.* **2014**, *136* (5), 1738−1741.

(304) Sandow, J.; Landgraf, W.; Becker, R.; Seipke, G. Equivalent Recombinant Human Insulin Preparations and Their Place in Therapy. *Eur. Endocrinol.* **2014**, *11* (1), 10.

(305) Potter, M. C. Electrical Effects Accompanying the Decomposition of Organic Compounds. *Proc. R. Soc. London. Ser. b* **1911**, *84* (571), 260−276.

(306) Choudhury, P.; Uday, U. S. P.; Mahata, N.; Tiwari, O. N.; Ray, R. N.; Bandyopadhyay, T. K.; Bhunia, B. Performance Improvement of Microbial Fuel Cells for Waste Water Treatment along with Value Addition: A Review on Past Achievements and Recent Perspectives. *Renew. Sustain. Energy Rev.* **2017**, *79*, 372−389.

(307) Niju, S.; Priyadharshini, K. A Review on Microbial Fuel Cell Technology for Brewery Industry Wastewater Treatment-From Fundamentals to Pilot Scale Studies. *Environ. Prog. Sustain. Energy* **2023**, DOI: 10.1002/ep.14191.

(308) Dannys, E.; Green, T.; Wettlaufer, A.; Madhurnathakam, C. M. R.; Elkamel, A. Wastewater Treatment with Microbial Fuel Cells: A Design and Feasibility Study for Scale-up in Microbreweries. *J. Bioprocess Biotech* **2016**, *6*, 1000267.

(309) Imoro, A. Z.; Mensah, M.; Buamah, R. Developments in the Microbial Desalination Cell Technology: A Review. *Water-Energy Nexus* **2021**, *4*, 76−87.

(310) Rabaey, K.; Girguis, P.; Nielsen, L. K. Metabolic and Practical Considerations on Microbial Electrosynthesis. *Curr. Opin. Biotechnol.* **2011**, *22* (3), 371−377.

(311) Yang, Y.; Xu, M.; Guo, J.; Sun, G. Bacterial Extracellular Electron Transfer in Bioelectrochemical Systems. *Process Biochem.* **2012**, *47* (12), 1707−1714.

(312) Shi, L.; Dong, H.; Reguera, G.; Beyenal, H.; Lu, A.; Liu, J.; Yu, H.-Q.; Fredrickson, J. K. Extracellular Electron Transfer Mechanisms between Microorganisms and Minerals. *Nat. Rev. Microbiol.* **2016**, *14* (10), 651−662.

(313) Shi, L.; Richardson, D. J.; Wang, Z.; Kerisit, S. N.; Rosso, K. M.; Zachara, J. M.; Fredrickson, J. K. The Roles of Outer Membrane Cytochromes of Shewanella and Geobacter in Extracellular Electron Transfer. *Environ. Microbiol. Rep.* **2009**, *1* (4), 220−227.

(314) Pirbadian, S.; Barchinger, S. E.; Leung, K. M.; Byun, H. S.; Jangir, Y.; Bouhenni, R. A.; Reed, S. B.; Romine, M. F.; Saffarini, D. A.; Shi, L.; et al. Shewanella Oneidensis MR-1 Nanowires Are Outer Membrane and Periplasmic Extensions of the Extracellular Electron Transport Components. *Proc. Natl. Acad. Sci. U. S. A.* **2014**, *111* (35), 12883−12888.

(315) Tan, Y.; Adhikari, R. Y.; Malvankar, N. S.; Ward, J. E.; Nevin, K. P.; Woodard, T. L.; Smith, J. A.; Snoeyenbos-West, O. L.; Franks, A. E.; Tuominen, M. T. The Low Conductivity of Geobacter Uraniireducens Pili Suggests a Diversity of Extracellular Electron Transfer Mechanisms in the Genus Geobacter. *Front. Microbiol.* **2016**, *7*, 980.

(316) Wang, F.; Gu, Y.; O'Brien, J. P.; Sophia, M. Y.; Yalcin, S. E.; Srikanth, V.; Shen, C.; Vu, D.; Ing, N. L.; Hochbaum, A. I. Structure of Microbial Nanowires Reveals Stacked Hemes That Transport Electrons over Micrometers. *Cell* **2019**, *177* (2), 361−369.

(317) Malvankar, N. S.; Tuominen, M. T.; Lovley, D. R. Lack of Cytochrome Involvement in Long-Range Electron Transport through Conductive Biofilms and Nanowires of Geobacter Sulfurreducens. *Energy Environ. Sci.* **2012**, *5* (9), 8651−8659.

(318) Bretschger, O.; Obraztsova, A.; Sturm, C. A.; Chang, I. S.; Gorby, Y. A.; Reed, S. B.; Culley, D. E.; Reardon, C. L.; Barua, S.; Romine, M. F.; et al. Current Production and Metal Oxide Reduction by Shewanella Oneidensis MR-1 Wild Type and Mutants. *Appl. Environ. Microbiol.* **2007**, *73* (21), 7003−7012.

(319) Venkidusamy, K.; Megharaj, M.; Schröder, U.; Karouta, F.; Mohan, S. V.; Naidu, R. Electron Transport through Electrically Conductive Nanofilaments in Rhodopseudomonas Palustris Strain RP2. *RSC Adv.* **2015**, *5* (122), 100790−100798.

(320) Eaktasang, N.; Kang, C. S.; Lim, H.; Kwean, O. S.; Cho, S.; Kim, Y.; Kim, H. S. Production of Electrically-Conductive Nanoscale Filaments by Sulfate-Reducing Bacteria in the Microbial Fuel Cell. *Bioresour. Technol.* **2016**, *210*, 61−67.

(321) Ehrlich, H. L. Are Gram-positive Bacteria Capable of Electron Transfer across Their Cell Wall without an Externally Available Electron Shuttle? *Geobiology* **2008**, *6* (3), 220−224.

(322) Carlson, H. K.; Iavarone, A. T.; Gorur, A.; Yeo, B. S.; Tran, R.; Melnyk, R. A.; Mathies, R. A.; Auer, M.; Coates, J. D. Surface Multiheme C-Type Cytochromes from Thermincola Potens and Implications for Respiratory Metal Reduction by Gram-Positive Bacteria. *Proc. Natl. Acad. Sci. U. S. A.* **2012**, *109* (5), 1702−1707.

(323) Light, S. H.; Su, L.; Rivera-Lugo, R.; Cornejo, J. A.; Louie, A.; Iavarone, A. T.; Ajo-Franklin, C. M.; Portnoy, D. A. A Flavin-Based Extracellular Electron Transfer Mechanism in Diverse Gram-Positive Bacteria. *Nature* **2018**, *562* (7725), 140−144.

(324) Stams, A. J. M.; De Bok, F. A. M.; Plugge, C. M.; Van Eekert, M. H. A.; Dolfing, J.; Schraa, G. Exocellular Electron Transfer in Anaerobic Microbial Communities. *Environ. Microbiol.* **2006**, *8* (3), 371−382.

(325) Kotloski, N. J.; Gralnick, J. A. Flavin Electron Shuttles Dominate Extracellular Electron Transfer by Shewanella Oneidensis. *mBio* **2013**, *4*, E00553-12.

(326) Okamoto, A.; Hashimoto, K.; Nealson, K. H.; Nakamura, R. Rate Enhancement of Bacterial Extracellular Electron Transport Involves Bound Flavin Semiquinones. *Proc. Natl. Acad. Sci. U. S. A.* **2013**, *110* (19), 7856−7861.

(327) Clarke, T. A.; Edwards, M. J.; Gates, A. J.; Hall, A.; White, G. F.; Bradley, J.; Reardon, C. L.; Shi, L.; Beliaev, A. S.; Marshall, M. J.; et al. Structure of a Bacterial Cell Surface Decaheme Electron Conduit. *Proc. Natl. Acad. Sci. U. S. A.* **2011**, *108* (23), 9384−9389.

(328) Bosire, E. M.; Rosenbaum, M. A. Electrochemical Potential Influences Phenazine Production, Electron Transfer and Consequently Electric Current Generation by Pseudomonas Aeruginosa. *Front. Microbiol.* **2017**, *8*, 892.

(329) Mier, A. A.; Olvera-Vargas, H.; Mejía-López, M.; Longoria, A.; Verea, L.; Sebastian, P. J.; Arias, D. M. A Review of Recent Advances in Electrode Materials for Emerging Bioelectrochemical Systems: From Biofilm-Bearing Anodes to Specialized Cathodes. *Chemosphere* **2021**, *283*, 131138.

(330) Vassilev, I.; Dessì, P.; Puig, S.; Kokko, M. Cathodic Biofilms-A Prerequisite for Microbial Electrosynthesis. *Bioresour. Technol.* **2022**, *348*, 126788.

(331) Guo, K.; Freguia, S.; Dennis, P. G.; Chen, X.; Donose, B. C.; Keller, J.; Gooding, J. J.; Rabaey, K. Effects of Surface Charge and Hydrophobicity on Anodic Biofilm Formation, Community Composition, and Current Generation in Bioelectrochemical Systems. *Environ. Sci. Technol.* **2013**, *47* (13), 7563−7570.

(332) Li, C.; Cheng, S. Functional Group Surface Modifications for Enhancing the Formation and Performance of Exoelectrogenic Biofilms on the Anode of a Bioelectrochemical System. *Crit. Rev. Biotechnol.* **2019**, *39* (8), 1015−1030.

(333) Zhu, Y.; Ji, J.; Ren, J.; Yao, C.; Ge, L. Conductive Multilayered Polyelectrolyte Films Improved Performance in Microbial Fuel Cells (MFCs). *Colloids Surfaces A Physicochem. Eng. Asp.* **2014**, *455*, 92−96.

(334) Zhou, S.; Tang, J.; Yuan, Y. Conduction-Band Edge Dependence of Carbon-Coated Hematite Stimulated Extracellular Electron Transfer of Shewanella Oneidensis in Bioelectrochemical Systems. *Bioelectrochemistry* **2015**, *102*, 29−34.

(335) Hasan, K.; Patil, S. A.; Leech, D.; Hägerhäll, C.; Gorton, L. Electrochemical Communication between Microbial Cells and Electrodes via Osmium Redox Systems. *Biochem. Soc. Trans.* **2012**, *40* (6), 1330−1335.

(336) Vostiar, I.; Ferapontova, E. E.; Gorton, L. Electrical "Wiring" of Viable Gluconobacter Oxydans Cells with a Flexible Osmium-Redox Polyelectrolyte. *Electrochem. commun.* **2004**, *6* (7), 621−626.

(337) Jourdin, L.; Raes, S. M. T.; Buisman, C. J. N.; Strik, D. P. Critical Biofilm Growth throughout Unmodified Carbon Felts Allows Continuous Bioelectrochemical Chain Elongation from CO2 up to Caproate at High Current Density. *Front. Energy Res.* **2018**, *6*, 7.

(338) Hengsbach, J.-N.; Sabel-Becker, B.; Ulber, R.; Holtmann, D. Microbial Electrosynthesis of Methane and Acetate—Comparison of Pure and Mixed Cultures. *Appl. Microbiol. Biotechnol.* **2022**, *106* (12), 4427−4443.

(339) Elias, S.; Banin, E. Multi-Species Biofilms: Living with Friendly Neighbors. *FEMS Microbiol. Rev.* **2012**, *36* (5), 990−1004.

(340) Steinbusch, K. J. J.; Arvaniti, E.; Hamelers, H. V. M.; Buisman, C. J. N. Selective Inhibition of Methanogenesis to Enhance Ethanol and N-Butyrate Production through Acetate Reduction in Mixed Culture Fermentation. *Bioresour. Technol.* **2009**, *100* (13), 3261−3267.

(341) Si, B.; Li, J.; Li, B.; Zhu, Z.; Shen, R.; Zhang, Y.; Liu, Z. The Role of Hydraulic Retention Time on Controlling Methanogenesis and Homoacetogenesis in Biohydrogen Production Using Upflow Anaerobic Sludge Blanket (UASB) Reactor and Packed Bed Reactor (PBR). *Int. J. Hydrogen Energy* **2015**, *40* (35), 11414−11421.

(342) Jadhav, D. A.; Chendake, A. D.; Schievano, A.; Pant, D. Suppressing Methanogens and Enriching Electrogens in Bioelectrochemical Systems. *Bioresour. Technol.* **2019**, *277*, 148−156.

(343) Deutzmann, J. S.; Spormann, A. M. Enhanced Microbial Electrosynthesis by Using Defined Co-Cultures. *ISME J.* **2017**, *11* (3), 704−714.

(344) Fuentes, J. L.; Garbayo, I.; Cuaresma, M.; Montero, Z.; González-del-Valle, M.; Vílchez, C. Impact of Microalgae-Bacteria Interactions on the Production of Algal Biomass and Associated Compounds. *Mar. Drugs* **2016**, *14* (5), 100.

(345) Kracke, F.; Lai, B.; Yu, S.; Krömer, J. O. Balancing Cellular Redox Metabolism in Microbial Electrosynthesis and Electro Fermentation-a Chance for Metabolic Engineering. *Metab. Eng.* **2018**, *45*, 109−120.

(346) Surti, P.; Kailasa, S. K.; Mungray, A. K. Genetic Engineering Strategies for Performance Enhancement of Bioelectrochemical Systems: A Review. *Sustain. Energy Technol. Assessments* **2021**, *47*, 101332.

(347) Li, F.; Li, Y.; Sun, L.; Li, X.; Yin, C.; An, X.; Chen, X.; Tian, Y.; Song, H. Engineering Shewanella Oneidensis Enables Xylose-Fed Microbial Fuel Cell. *Biotechnol. Biofuels* **2017**, *10*, 1−10.

(348) Yan, Q.; Fong, S. S. Challenges and Advances for Genetic Engineering of Non-Model Bacteria and Uses in Consolidated Bioprocessing. *Front. Microbiol.* **2017**, *8*, 2060.

(349) TerAvest, M. A.; Ajo-Franklin, C. M. Transforming Exoelectrogens for Biotechnology Using Synthetic Biology. *Biotechnol. Bioeng.* **2016**, *113* (4), 687−697.

(350) Shin, H. J.; Jung, K. A.; Nam, C. W.; Park, J. M. A Genetic Approach for Microbial Electrosynthesis System as Biocommodities Production Platform. *Bioresour. Technol.* **2017**, *245*, 1421−1429.

(351) Al-Mamun, A.; Ahmed, W.; Jafary, T.; Nayak, J. K.; Al-Nuaimi, A.; Sana, A. Recent Advances in Microbial Electrosynthesis System: Metabolic Investigation and Process Optimization. *Biochem. Eng. J.* **2023**, *196*, 108928.

(352) Jiang, Y.; Zeng, R. J. Expanding the Product Spectrum of Value Added Chemicals in Microbial Electrosynthesis through Integrated Process Design—a Review. *Bioresour. Technol.* **2018**, *269*, 503−512.

(353) Tefft, N. M.; TerAvest, M. A. Reversing an Extracellular Electron Transfer Pathway for Electrode-Driven Acetoin Reduction. *ACS Synth. Biol.* **2019**, *8* (7), 1590−1600.

(354) Unden, G.; Bongaerts, J. Alternative Respiratory Pathways of Escherichia Coli: Energetics and Transcriptional Regulation in Response to Electron Acceptors. *Biochim. Biophys. Acta (BBA)-Bioenergetics* **1997**, *1320* (3), 217−234.

(355) Pinchuk, G. E.; Hill, E. A.; Geydebrekht, O. V.; De Ingeniis, J.; Zhang, X.; Osterman, A.; Scott, J. H.; Reed, S. B.; Romine, M. F.; Konopka, A. E.; et al. Constraint-Based Model of Shewanella Oneidensis MR-1 Metabolism: A Tool for Data Analysis and Hypothesis Generation. *PLoS Comput. Biol.* **2010**, *6* (6), No. e1000822.

(356) Spero, M. A.; Aylward, F. O.; Currie, C. R.; Donohue, T. J. Phylogenomic Analysis and Predicted Physiological Role of the Proton-Translocating NADH: Quinone Oxidoreductase (Complex I) across Bacteria. *MBio* **2015**, *6* (2), 10−1128.

(357) Smil, V. Detonator of the Population Explosion. *Nature* **1999**, *400* (6743), 415.

(358) Dong, F.; Lee, Y. S.; Gaffney, E. M.; Grattieri, M.; Haddadin, H.; Minteer, S. D.; Chen, H. An Engineered, Non-Diazotrophic Cyanobacterium and Its Application in Bioelectrochemical Nitrogen Fixation. *Cell Reports Phys. Sci.* **2021**, *2* (6), 100444.

(359) Dong, F.; Lee, Y. S.; Gaffney, E. M.; Liou, W.; Minteer, S. D. Engineering Cyanobacterium with Transmembrane Electron Transfer Ability for Bioelectrochemical Nitrogen Fixation. *ACS Catal.* **2021**, *11* (21), 13169−13179.

(360) Xiao, X.; Yu, H.-Q. Molecular Mechanisms of Microbial Transmembrane Electron Transfer of Electrochemically Active Bacteria. *Curr. Opin. Chem. Biol.* **2020**, *59*, 104−110.

(361) Kadier, A.; Simayi, Y.; Kalil, M. S.; Abdeshahian, P.; Hamid, A. A. A Review of the Substrates Used in Microbial Electrolysis Cells

(MECs) for Producing Sustainable and Clean Hydrogen Gas. *Renew. Energy* **2014**, *71*, 466−472.

(362) Carmona-Martínez, A. A.; Trably, E.; Milferstedt, K.; Lacroix, R.; Etcheverry, L.; Bernet, N. Long-Term Continuous Production of H2 in a Microbial Electrolysis Cell (MEC) Treating Saline Wastewater. *Water Res.* **2015**, *81*, 149−156.

(363) Mishra, A. K.; Kaushik, M. S.; Tiwari, D. N. Nitrogenase and Hydrogenase: Enzymes for Nitrogen Fixation and Hydrogen Production in Cyanobacteria. In *Cyanobacteria*; Elsevier, 2019; pp 173−191.

(364) Vignais, P. M.; Billoud, B. Occurrence, Classification, and Biological Function of Hydrogenases: An Overview. *Chem. Rev.* **2007**, *107* (10), 4206−4272.

(365) Lojou, E.; Durand, M. C.; Dolla, A.; Bianco, P. Hydrogenase Activity Control at Desulfovibrio Vulgaris Cell-coated Carbon Electrodes: Biochemical and Chemical Factors Influencing the Mediated Bioelectrocatalysis. *Electroanal. An Int. J. Devoted to Fundam. Pract. Asp. Electroanal.* **2002**, *14* (13), 913−922.

(366) Li, S.; Sakuntala, M.; Song, Y. E.; Heo, J.; Kim, M.; Lee, S. Y.; Kim, M.-S.; Oh, Y.-K.; Kim, J. R. Photoautotrophic Hydrogen Production of Rhodobacter Sphaeroides in a Microbial Electrosynthesis Cell. *Bioresour. Technol.* **2021**, *320*, 124333.

(367) Rago, L.; Zecchin, S.; Villa, F.; Goglio, A.; Corsini, A.; Cavalca, L.; Schievano, A. Bioelectrochemical Nitrogen Fixation (e-BNF): Electro-Stimulation of Enriched Biofilm Communities Drives Autotrophic Nitrogen and Carbon Fixation. *Bioelectrochemistry* **2019**, *125*, 105−115.

(368) Knoche, K. L.; Aoyama, E.; Hasan, K.; Minteer, S. D. Role of Nitrogenase and Ferredoxin in the Mechanism of Bioelectrocatalytic Nitrogen Fixation by the Cyanobacteria Anabaena Variabilis SA-1 Mutant Immobilized on Indium Tin Oxide (ITO) Electrodes. *Electrochim. Acta* **2017**, *232*, 396−403.

(369) Liu, C.; Sakimoto, K. K.; Colón, B. C.; Silver, P. A.; Nocera, D. G. Ambient Nitrogen Reduction Cycle Using a Hybrid Inorganic−Biological System. *Proc. Natl. Acad. Sci.* **2017**, *114* (25), 6450−6455.

(370) Chen, S.; Jing, X.; Yan, Y.; Huang, S.; Liu, X.; Chen, P.; Zhou, S. Bioelectrochemical Fixation of Nitrogen to Extracellular Ammonium by Pseudomonas Stutzeri. *Appl. Environ. Microbiol.* **2021**, *87* (5), No. e01998-20.

(371) Chu, N.; Hao, W.; Wu, Q.; Liang, Q.; Jiang, Y.; Liang, P.; Ren, Z. J.; Zeng, R. J. Microbial Electrosynthesis for Producing Medium Chain Fatty Acids. *Engineering* **2022**, *16*, 141−153.

(372) Cheng, S.; Xing, D.; Call, D. F.; Logan, B. E. Direct Biological Conversion of Electrical Current into Methane by Electromethanogenesis. *Environ. Sci. Technol.* **2009**, *43* (10), 3953−3958.

(373) Marshall, C. W.; Ross, D. E.; Fichot, E. B.; Norman, R. S.; May, H. D. Electrosynthesis of Commodity Chemicals by an Autotrophic Microbial Community. *Appl. Environ. Microbiol.* **2012**, *78* (23), 8412−8420.

(374) Villano, M.; Aulenta, F.; Ciucci, C.; Ferri, T.; Giuliano, A.; Majone, M. Bioelectrochemical Reduction of CO2 to CH4 via Direct and Indirect Extracellular Electron Transfer by a Hydrogenophilic Methanogenic Culture. *Bioresour. Technol.* **2010**, *101* (9), 3085−3090.

(375) Kracke, F.; Deutzmann, J. S.; Jayathilake, B. S.; Pang, S. H.; Chandrasekaran, S.; Baker, S. E.; Spormann, A. M. Efficient Hydrogen Delivery for Microbial Electrosynthesis via 3D-Printed Cathodes. *Front. Microbiol.* **2021**, *12*, 696473.

(376) Schiel-Bengelsdorf, B.; Dürre, P. Pathway Engineering and Synthetic Biology Using Acetogens. *FEBS Lett.* **2012**, *586* (15), 2191−2198.

(377) Roy, M.; Yadav, R.; Chiranjeevi, P.; Patil, S. A. Direct Utilization of Industrial Carbon Dioxide with Low Impurities for Acetate Production via Microbial Electrosynthesis. *Bioresour. Technol.* **2021**, *320*, 124289.

(378) Nevin, K. P.; Hensley, S. A.; Franks, A. E.; Summers, Z. M.; Ou, J.; Woodard, T. L.; Snoeyenbos-West, O. L.; Lovley, D. R. Electrosynthesis of Organic Compounds from Carbon Dioxide Is Catalyzed by a Diversity of Acetogenic Microorganisms. *Appl. Environ. Microbiol.* **2011**, *77* (9), 2882−2886.

(379) Flexer, V.; Jourdin, L. Purposely Designed Hierarchical Porous Electrodes for High Rate Microbial Electrosynthesis of Acetate from Carbon Dioxide. *Acc. Chem. Res.* **2020**, *53* (2), 311−321.

(380) Cui, M.; Nie, H.; Zhang, T.; Lovley, D.; Russell, T. P. Three-Dimensional Hierarchical Metal Oxide-Carbon Electrode Materials for Highly Efficient Microbial Electrosynthesis. *Sustain. Energy Fuels* **2017**, *1* (5), 1171−1176.

(381) Marshall, C. W.; Ross, D. E.; Fichot, E. B.; Norman, R. S.; May, H. D. Long-Term Operation of Microbial Electrosynthesis Systems Improves Acetate Production by Autotrophic Microbiomes. *Environ. Sci. Technol.* **2013**, *47* (11), 6023−6029.

(382) Weekes, D. M.; Salvatore, D. A.; Reyes, A.; Huang, A.; Berlinguette, C. P. Electrolytic CO2 Reduction in a Flow Cell. *Acc. Chem. Res.* **2018**, *51* (4), 910−918.

(383) Lee, W. S.; Chua, A. S. M.; Yeoh, H. K.; Ngoh, G. C. A Review of the Production and Applications of Waste-Derived Volatile Fatty Acids. *Chem. Eng. J.* **2014**, *235*, 83−99.

(384) Bhatia, S. K.; Yang, Y.-H. Microbial Production of Volatile Fatty Acids: Current Status and Future Perspectives. *Rev. Environ. Sci. Bio/Technology* **2017**, *16*, 327−345.

(385) Ehsanipour, M.; Suko, A. V.; Bura, R. Fermentation of Lignocellulosic Sugars to Acetic Acid by Moorella Thermoacetica. *J. Ind. Microbiol. Biotechnol.* **2016**, *43* (6), 807−816.

(386) Ravinder, T.; Ramesh, B.; Seenayya, G.; Reddy, G. Fermentative Production of Acetic Acid from Various Pure and Natural Cellulosic Materials by Clostridium Lentocellum SG6. *World J. Microbiol. Biotechnol.* **2000**, *16*, 507−512.

(387) Nakano, S.; Fukaya, M.; Horinouchi, S. Putative ABC Transporter Responsible for Acetic Acid Resistance in Acetobacter Aceti. *Appl. Environ. Microbiol.* **2006**, *72* (1), 497−505.

(388) Wu, X.; Yao, H.; Cao, L.; Zheng, Z.; Chen, X.; Zhang, M.; Wei, Z.; Cheng, J.; Jiang, S.; Pan, L.; et al. Improving Acetic Acid Production by Over-Expressing PQQ-ADH in Acetobacter Pasteurianus. *Front. Microbiol.* **2017**, *8*, 1713.

(389) Jiang, Y.; Chu, N.; Qian, D.-K.; Zeng, R. J. Microbial Electrochemical Stimulation of Caproate Production from Ethanol and Carbon Dioxide. *Bioresour. Technol.* **2020**, *295*, 122266.

(390) Zabed, H.; Sahu, J. N.; Suely, A.; Boyce, A. N.; Faruq, G. Bioethanol Production from Renewable Sources: Current Perspectives and Technological Progress. *Renew. Sustain. Energy Rev.* **2017**, *71*, 475−501.

(391) Quraishi, M.; Wani, K.; Pandit, S.; Gupta, P. K.; Rai, A. K.; Lahiri, D.; Jadhav, D. A.; Ray, R. R.; Jung, S. P.; Thakur, V. K.; et al. Valorisation of CO2 into Value-Added Products via Microbial Electrosynthesis (MES) and Electro-Fermentation Technology. *Fermentation* **2021**, *7* (4), 291.

(392) Birjandi, N.; Younesi, H.; Ghoreyshi, A. A.; Rahimnejad, M. Electricity Generation, Ethanol Fermentation and Enhanced Glucose Degradation in a Bio-electro-Fenton System Driven by a Microbial Fuel Cell. *J. Chem. Technol. Biotechnol.* **2016**, *91* (6), 1868−1876.

(393) Ammam, F.; Tremblay, P.-L.; Lizak, D. M.; Zhang, T. Effect of Tungstate on Acetate and Ethanol Production by the Electrosynthetic Bacterium Sporomusa Ovata. *Biotechnol. Biofuels* **2016**, *9*, 1−10.

(394) Harrington, T. D.; Mohamed, A.; Tran, V. N.; Biria, S.; Gargouri, M.; Park, J.-J.; Gang, D. R.; Beyenal, H. Neutral Red-Mediated Microbial Electrosynthesis by Escherichia Coli, Klebsiella Pneumoniae, and Zymomonas Mobilis. *Bioresour. Technol.* **2015**, *195*, 57−65.

(395) Srikanth, S.; Singh, D.; Vanbroekhoven, K.; Pant, D.; Kumar, M.; Puri, S. K.; Ramakumar, S. S. V. Electro-Biocatalytic Conversion of Carbon Dioxide to Alcohols Using Gas Diffusion Electrode. *Bioresour. Technol.* **2018**, *265*, 45−51.

(396) He, A.-Y.; Yin, C.-Y.; Xu, H.; Kong, X.-P.; Xue, J.-W.; Zhu, J.; Jiang, M.; Wu, H. Enhanced Butanol Production in a Microbial Electrolysis Cell by Clostridium Beijerinckii IB4. *Bioprocess Biosyst. Eng.* **2016**, *39*, 245−254.

(397) Saini, M.; Chen, M. H.; Chiang, C.-J.; Chao, Y.-P. Potential Production Platform of N-Butanol in Escherichia Coli. *Metab. Eng.* **2015**, *27*, 76−82.

(398) Bajracharya, S.; Yuliasni, R.; Vanbroekhoven, K.; Buisman, C. J. N.; Strik, D. P.; Pant, D. Long-Term Operation of Microbial Electrosynthesis Cell Reducing CO2 to Multi-Carbon Chemicals with a Mixed Culture Avoiding Methanogenesis. *Bioelectrochemistry* **2017**, *113*, 26−34.

(399) Koller, M.; Mukherjee, A.; Obruca, S.; Zinn, M. Polyhydroxyalkanoates (PHA): Microbial Synthesis of Natural Polyesters. In *Microbial Production of High-Value Products*; Springer, 2022; pp 185−236.

(400) Lee, S. Y. Bacterial Polyhydroxyalkanoates. *Biotechnol. Bioeng.* **1996**, *49* (1), 1−14.

(401) Pepè Sciarria, T.; Batlle-Vilanova, P.; Colombo, B.; Scaglia, B.; Balaguer, M. D.; Colprim, J.; Puig, S.; Adani, F. Bio-Electrorecycling of Carbon Dioxide into Bioplastics. *Green Chem.* **2018**, *20* (17), 4058−4066.

(402) Taguchi, S.; Ooi, T.; Mizuno, K.; Matsusaki, H. Advances and Needs for Endotoxin-Free Production Strains. *Appl. Microbiol. Biotechnol.* **2015**, *99*, 9349−9360.

(403) Chen, X.; Cao, Y.; Li, F.; Tian, Y.; Song, H. Enzyme-Assisted Microbial Electrosynthesis of Poly (3-Hydroxybutyrate) via CO2 Bioreduction by Engineered Ralstonia Eutropha. *ACS Catal.* **2018**, *8* (5), 4429−4437.

(404) Das, S.; Diels, L.; Pant, D.; Patil, S. A.; Ghangrekar, M. M. Microbial Electrosynthesis: A Way towards the Production of Electro-Commodities through Carbon Sequestration with Microbes as Biocatalysts. *J. Electrochem. Soc.* **2020**, *167* (15), 155510.

(405) Armstrong, R. Towards the Microbial Home: An Overview of Developments in Next-generation Sustainable Architecture. *Microb. Biotechnol.* **2023**, *16* (6), 1112−1130.

(406) Boto, S. T.; Bardl, B.; Harnisch, F.; Rosenbaum, M. A. Microbial Electrosynthesis with Clostridium Ljungdahlii Benefits from Hydrogen Electron Mediation and Permits a Greater Variety of Products. *Green Chem.* **2023**, *25* (11), 4375−4386.

(407) Huang, Z.; Grim, R. G.; Schaidle, J. A.; Tao, L. The Economic Outlook for Converting CO 2 and Electrons to Molecules. *Energy Environ. Sci.* **2021**, *14* (7), 3664−3678.

(408) Jourdin, L.; Burdyny, T. Microbial Electrosynthesis: Where Do We Go from Here? *Trends Biotechnol.* **2021**, *39* (4), 359−369.