The progress and outlook of bioelectrocatalysis for the production of chemicals, fuels, and materials

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Abstract

Bioelectrocatalysis is a green, sustainable, and efficient method to produce value-added chemicals, clean biofuels, and degradable materials. As an alternative approach to modern biomanufacturing technology, bioelectrocatalysis fully combines the merits of both biocatalysis and electrocatalysis to realize the green and efficient production of target products from electricity. Here we review the development status, discussing the current challenges, and looking toward the future development directions of bioelectrocatalysis. First, the structure, function, and modification methods of bioelectrocatalysis are detailed. Secondly, the mechanism of electron transfer, including mediated electron transfer and directed electron transfer, is described. Third, the impact of the electrode on bioelectrocatalysis was discussed. Fourth, the application of bioelectrocatalysis methods in the production of chemicals, biofuels, and materials are systematically analyzed and summarized. Finally, the Review details the future developments and perspectives on bioelectrocatalysis for electrosynthesis.

Introduction

Nature has evolved a diversity of proteins to perform a wide range of structural and catalytic functions. Oxidoreductases are a class of enzymes, accounting for approximately one-quarter of all known proteins, that catalyze redox reactions of two substrates; shuttling an electron(s) between the two substrates with the cofactor of the enzyme[1]. In recent decades, researchers have shown that oxidoreductase catalyzed reactions can be connected with electrodes, and electrochemical potential can be used to control the catalysis. Some whole living microbial cells which are capable of expressing oxidoreductases can also perform the electron transfer (electroactive microbial cell) and be used in electrochemical systems[2]. This connection has been proposed to open a new research field for the bioelectrocatalysis based manufacturing of chemicals, biofuels, and materials[3-6].

Both the isolated oxidoreductase and electroactive microbial cell catalyzed electrochemical oxidation or reduction are called bioelectrocatalysis[3]. Correspondingly, the isolated oxidoreductase and electroactive microbial cell are collectively referred to as bioelectrocatalysts. In traditional biocatalysis, the redox reactions require two substrates (electron donor and electron acceptor) and transfers electron(s) between them[7]. The electron transfer is performed by a variety of electron mediators such as nicotinamide adenine dinucleotide [NAD(H)] and its phosphoralted form [NADP(H)] or enzyme-bound cofactors including flavin mononucleotide (FMN), flavin adenine dinucleotide (FAD), heme and some other metal cofactor[7]. In comparison to traditional biocatalysis, bioelectrocatalysis integrates the catalytic function of bioelectrocatalysts with the electrode reaction[3,8]. The electrode substitutes the second substrate of the biocatalysts and can act as

either a source or a sink for electrons to support the bioelectrocatalytic reductive or oxidative reaction[5,9].

Bioelectrocatalysis combines the merits of both biocatalysis and electrocatalysis. The advantages of biocatalysis are high activity, high selectivity (including regioselectivity and stereoselectivity), and wide substrate scope[10]. For enzyme-based biocatalysis, enzymes are produced from renewable resources, and are biodegradable,nonhazardous and nontoxic. Enzymatic reactions are generally performed under mild conditions in an aqueous phase, and often do not request the functional-group activation, protection, and deprotection steps[11]. For the microbial cell-based biocatalysis, the diversified metabolic pathways provide the ability of microbial cell to produce a variety of products. The reducing equivalent can also be regenerated via the metabolic activities of the cell[12]. Electrochemical reactions can be utilized to safely provide the necessary redox equivalents for biocatalysis with the consumption of electricity[13] that can be produced from renewable resources (e.g., solar and wind)[6,14-16]. Accordingly, bioelectrocatalysis provides a good concept for sustainable green chemistry as it meets the 12 principles of green chemistry[11]. Bioelectrocatalysis has been widely employed for the construction of biosensor and biofuel cell devices[17,18]. Alternatively, the application of bioelectrocatalysis in the synthesis of desired chemicals biofuels and materials has gained interest recently[19]. For instance, the bioelectrochemical regeneration of redox cofactor has been widely used in CO_2 fixation[20-22], N_2 fixation[23-26], the synthesis of chiral compounds[27-30] and other products with high added-value[6,31-33].

The use of isolated oxidoreductases or electroactive microbial cells is well established for electrochemical biosensors, where the catalyst is immobilized onto the electrode surface[34].

Although this arrangement is useful for analytical purposes, it cannot meet the requirement of preparation of chemicals, biofuels, and materials. For isolated oxidoreductases, the long-term stability and the production rates are limited in these systems, because the efficiency of electron transfer is not high enough. Most enzymes have their active centers buried deep beneath the surface of the enzyme, which hinders the electrochemical communication between the bioelectrocatalysts and the electrode[35]. For electroactive microbial cells, the electron transfer rate is limited by the non-conductive nature of the cell membrane[36]. Some membrane enzymes, which have electrochemical communication with electrodes, are relatively buried in the cell membrane[37]. To make bioelectrocatalysis feasible for the preparation of biofuel, chemicals, and materials, the enhancement of electron transfer between the electrode surface and bioelectrocatalyst is the overriding issue in bioelectrocatalysis.

As a fast-growing research area, an abundance of Review articles on the topic of bioelectrocatalysis have been published in recent years. Most Review articles focu on either enzymatic[5,38-41] or microbial bioelectrocatalysis[16,42-47]and most are focused on energy or sensor applications. In order to more systematically and comprehensively show the progress of bioelectrocatalysis to the application of electrosynthesis, the contents related to enzymatic and microbial bioelectrocatalysis are two parallel subjects in this Review. Through the comparison of them, the differences between catalytic characteristics and application scope of these two bioelectrocatalysts are discussed in detail. Meanwhile, this Review presents the structural and functional features of bioelectrocatalyst, the mechanism of electron transfer, the impact of electrode, the applications of bioelectrocatalysis in the production of chemicals, biofuel and materials. Based on the summary of present research

progress, this Review points out that the combination of bioelectrocatalysis and synthetic biology, the design and application of new reaction medium and engineering application of bioelectrocatalysis in large scale are the research directions require more investigations in the futher. We expect to provide a useful reference for the future research efforts on the development of novel bioelectrocatalysts, the investigation of electron transfer mechanism, the design of bioelectrocatalytic systems, the bioelectrocatalytic production of more diverse products, and even the industrialization of bioelectrocatalysis.

Bioelectrocatalyst

Bioelectrocatalytic systems depend on the utilization of bioelectrocatalysts to catalyze the reactions. Accordingly, the bioelectrocatalyst is the functional core of the bioelectrocatalytic system[14]. The bioelectrocatalyst can be classified either as (i) an oxidoreductase or (ii) an electroactive microbial cell. With the development of protein engineering and metabolic engineering, the function of oxidoreductase and electroactive microbial cell can be effectively regulated and enhanced[5], which significantly improves the performance of bioelectrocatalysis system.

Oxidoreductase. The oxidoreductases used in bioelectrocatalysis can be classified in two ways based on the cofactor: metalloenzymes and non-metalloenzymes. The common metallocofactor motifs of metalloenzymes are: heme centers, iron center (Fe), iron-sulfur cluster (Fe-S), copper center (Cu), molybdenum centers (commonly called Moco), and tungsten centers (Wco). For the non-metalloenzymes, the cofactors include: flavin mononucleotide (FMN) or flavin adenine dinucleotide (FAD)and pyrroloquinoline quinone (PQQ)[5]. These oxidoreductases take advantage of the transformation of reduced and oxidized states of different cofactors to realize the electron transfer.

Catalytic function of oxidoreductase. The catalytic function of oxidoreductases depends on the function of their cofactors. The heme-containing enzymes are capable of performing electron transfer (cytochrome b and c)[48], catalyzing the reductive reaction of oxygen to water (cytochrome *c* oxidase)[49] and oxidizing different functional groups by molecular oxygen (monooxygenase P450)[50], and catalyzing the decomposition of peroxides (catalase and peroxidase)[51]. The typical Fe-S cluster-containing proteins are ferredoxin, hydrogenases, and nitrogenases. Ferredoxin is an electron carrier which shuttles electrons between electron donor and electron acceptor proteins (e.g., putidaredoxin mediated electron transfer between putidaredoxin reductase and P450cam[52]). In both NiFe and Fe-only hydrogenases, there are specific channels inside the protein to transport of H^+/H_2 to or from the active sites and the sites are wired to the surface for electron exchange with their redox partner by a conduit of Fe-S clusters[53]. Copper is a key cofactor which is involved in biological oxidation-reduction reactions and oxygen transport[54]. The copper-containing proteins participates in the transfer of electrons and oxygen, and finally catalysing the oxidative reaction. The redox potentials of multi-copper active sites are intricately linked to the substrate specificity of the protein and its ability to oxidize phenolic substrates, which is thermodynamically driven by the concomitant reduction of molecular oxygen[55]. The flavin (FAD or FMN) enzymes act as the electron shuttle between the substrate and either another electron carrier or to oxygen. Oxygenation reactions catalyzed by flavin oxidase include amongst others hydroxylation, epoxidations, Baeyer-Villiger oxidations and sulfoxidations with high regio- and/or enantioselectivity[56]. All PQQ-containing enzymes contain the bound PQQ cofactor along with or without hemegroup. The PQQ cofactor is coordinated with the apo-enzyme via Ca^{2+} ions and electrons are

transferred from the substrate via PQQ to the heme groups and then to the current collector. PQQ is present in glucose, aldose, glycerol, fructose and alcohol dehydrogenases.

Electrocative microbial cell. Contrary to oxidoreductases, whole microbial cells are able to catalyze a wider range of reactions, because the microbial cells act as miniature and self-reproducing bioreactors. As a more complex metabolic network exists inside the microbial cell, the reactions catalyzed by whole microbial cells are less specific toward substrates compared to isolated oxidoreductases[14], and the reduced equivalents can be regenerated through the metabolic activity of the cell. Some electroactive microbial cells have evolved unique electron transport mechanisms to realize electronic communication with electrodes.

Structural characteristics of electroactive microbial cell. The most well-studied electroactive microorganisms are *Geobacter sulfurreducens* and *Shewanella oneidensis* (**Figure 1a**, **1b,** and **1c**). *G. sulfurreducens* can employ electrically conductive pili (e-pili) which are a type IV pili composed of PilA protein to perform the direct interspecies electron transfer[57-59] (**Figure 1a**). The overlapping π-orbitals of aromatic amino acids which are packed 3 to 4 Å is the structural basis of the conductivity of e-pili and further promotes long-distance electron transport[60]. However, recent research puts different views on this issue (**Figure 1b**). Wang and coworkers[61] found that the conductive *G. sulfurreducens* filaments which are a polymerized chain of cytochrome OmcS are the real conduits for long-rang electron transport, not the pili composed by PilA protein. The function of PilA is to facilitate the secretion of OmcS outside the cells . The interface between adjacent subunits is extensive buried per subunit. The heme pairs of OmcS at the interface are parallel with approximately 4 Å edge-to-edge distance. This inter- subunit coordination and parallel stacking of heme promote the stability of the protein-protein

interface. Moreover, the closely stacked (< 4-6 Å) hemes form a continuous path to facilitate the electron transfer between OmcS monomers. In response to this conclusion, Lovley and coworkers [62,63] insist on the view that e-pili composed by PilA protein is the structure basis of long-range electron transport, because: 1. the long-range electron transport requires the formation of thick (> 50 μm) electrically conductive biofilm. The *omcS* gene deletion has no obvious impact on current production of biofilm[64]. 2. OmcS filaments do not participate in long-range electron transport as the expression of mutant pilin gene generates less conductive biofilm[65]; heterologous expression of the pilin genes in *G. sulfurreducens* yielded a strain expressing pili with low conductivity, but that expressed even more outer-surface OmcS[59]; *G. sulfurreducens* strain KN400 which expresses much less OmcS and much more PilA than wild type *G. sulfurreducens* generates higher current and much more conductive biofilms[64]. 3. e-pili expression is required for Fe(III) oxide reduction but there are substitutes, such as magnetite, for OmcS[66]. 4. observed phenotypes do not require OmcS filaments emanating at distance from the cell[67,68]. 5. there is no correlation between the expression level of PilA and the secretion of OmcS[69,70]. 6. the culture condition of Wang and coworkers' research is not good for the expression of e-pili. That is the reason for that PilA is barely detectable in their filament preparation. More in-depth research is required to further understand the mechanism of electron transport of *G. sulfurreducens*. For *S. oneidensis*, the apparent terminal cell-bound complex is MtrC It is a decaheam cytochrome protein which locates on the outside of the membrane and is able to donate electrons over a broad potential range (**Figure 1c**). Electrons are transported from the periplasm to MtrC through a transmembrane electron transfer module consisting of the transporting protein MtrA and incorporated a inside sheath protein MtrB[71]. Both

organisms also show the electron consumption activity at the cathode. For example, both *G. sulurreducens* and *S. oneidensis* can reduce fumarate to succinate with the consumption of electrons supplied by a cathode [72,73].

Some membrane-bound redox proteins are also involved in the electron transfer between microbial cells and electrode[74,75]. Rnf complexes (a membrane-bound NADH:ferredoxin oxidoreductase) are redox-driven ion pumps and have a membrane-bound, proton-translocating ferredoxin:NAD^+ oxidoreductase function which contributing to ATP synthesis. Rnf complexes have four flavin-containing cytoplasmatic multienzyme complexes from *Clostridia*, acetogens, and methanogens (**Figure 1d**).

Bioelectrocatalytic function of electroactive microbial cells. Both microbial electrosynthesis (MES) and electro-fermentation (EF) are capable of using electrochemical mechanisms to adjust microbial metabolic activities and produce targeted products. For the theoretical basis and practical application of MES and EF, an impactful Review from Ren and coworkers is a good reference[44].

A typical MES process utilizes autotrophic microbial cells as the bioelectrocatalyst with a cathode as the electron donor and certain substrates as electron acceptors for the target product synthesis[44]. EF is a unique process in which the metabolism balance of microbial cell can be shifted, even controlled, by electrode potential[76]. EF makes a microbial cell to be a micro reactor in which the biological and electrochemical process occurs simultaneously to generate electricity and other metabolic products. The electrodes can work as either electron sources or sink that impact the balance of the microbial metabolism and fermentation. During EF, microbes can be grouped into electrochemically active bacteria, exoelectrogens, electricigens, or anode-respiring bacteria based on their

electrochemical activities. Exoelectrogenic bacteria can produce electricity at anodes through electron generation during metal-reduction. Endoelectrogen microbes consume electric current at cathodes through oxidation of metals. These microbial cells have the capability to perform electron transfer via either direct use of membrane proteins, pili, filaments, cytochromes, or the secretion of electron mediators.

The modification of bioelecctrocatalysts. The bioelectrocatalysts, both oxidoreductases and electroactive microbial cells, have not evolved to adapt to the reactions in artificial bioelectrocatalytic systems. In order to improve the performance of bioelectrocatalytic system, the modification of bioelectrocatalysts are necessary to enhance the electron transfer and to improve the reaction efficiency.

The modification of oxidoredutase via protein engineering. The redox-active centers of oxidoreductase are often deeply buried in an insulating protein shell which hinders electrochemical communication between electrode and oxidoreductase. Furthermore, sophisticated control mechanisms regulate electron transfer with oxidoreductase to avoid the random electron transfer and further prevent the formation of radical formation and futile use of energy[77]. Protein engineering is an attractive solutions for breaking though the physiological constraints of oxidoreductases. Through the modification of the structure of oxidoreductases, especially the structure of active sites, protein engineering methods can be used to improve enzymatic properties and enhance the electrochemical performance of oxidoreductases. General methods in protein engineering include rational design, directed evolution, and their combination[1].

So far, several strategies have been used to modify the oxidoreductase and enhance their bioelectrochemical properties. 1) Truncation of oxidoreductase (**Figure 2a**)[78]. The active

site of an oxidoreductase is often buried inside the protein shell. By deleting the residues that are not essential for maintaining protein function and structure, researchers can make the active site to be exposed and place conducting support near the active site. 2) Surface modification (**Figure 2b**)[79-81]. Protein surface modification can facilitate the interaction between oxidoreductase and electrode (e.g., more stable and orientational immobilization of oxidoreductase on the electrode). 3) Active site mutation (**Figure 2c**)[82]. Introducing mutations in the active site or the region around the active site is another option to imporve electrochemical communication between oxidoreductase and electrode.

The modification of microbial cells via metabolic engineering. Metabolic engineering is an effective measurement to regulatory functions of the cell with the use of recombinant DNA technology and improve the cellular activities by manipulation of enzymatic transport. The potential applications of metabolic engineering span the entire spectrum of biotechnology and encompass the creation of new processes and products as well as improvement of existing processes[83]. Metabolic engineering presents the possibility to optimize the electron transport chains and modify or design production pathways of valuable compounds. Correspondingly, metabolic engineering is an effective method to enhance the electron transfer rate between electrodes and microbial cells and allow the microbial cells the ability to produce chemicals, biofuels, and materials with high added-value[84].

In order to enhance the cellular electron transport or improve the generation of targe product by the regulation of the cellular redox state, the target for metabolic engineering, including promising organism/substrate/product combination and key reactions that should be enhanced or eliminated, need to be identified. In general, two groups of techniques could be used to analyze and identify the target of metabolic engineering: omics-techniques

(quantitative and qualitative) and mathematical modeling (simulation and prediction)[84]. The omics-techniques was used in isolation, such as chromatographic methods to quantify metabolites[85] or proteomics analysis to investigate the effect of extracellular electron supply on the central metabolism[86]. Besides the omics methods, *in silico* modeling approaches is another option. Metabolic pathway analysis and flux balance analysis are very useful tools. These two methods can be used to test the network topology, essential reactions, and to compare the yields of different metabolic pathways[87]. The significance of the two methods for metabolic engineering is to investigate the impact of separating redox balance from the carbon balance and further study the application of microbial metabolism to produce target chemicals with external electron supply[84].

The electron transfer mechanism

The electron transfer between the bioelectrocatalysts and the electrodes links the biological and electrochemical processes. The electron transfer rate is the key determinant of the overall performance and efficiency of bioelectrochemical devices. In-depth study of electron transfer mechanisms is essential for the understanding of the structure-activity relationship of oxidoreductases and the structure-functional characteristics of the electron transport chain of electroactive microbial cells. Electron transfer mechanisms are also the theoretical basis for the design of bioelectrocatalytic reactor, electrode and reaction process.

Direct electron transfer (DET). Some oxidoreductases are able to undergo direct electron transfer with an electrode without the need of any electron mediator, which is referred to as direct electron transfer (DET) (**Figure 3a**). For DET, the distance between the redox cofactor of the oxidoreductase and electrode surface is critical. If the distance is shorter than 20 Å, DET can take place[88]. DET-type bioelectrocatalytic reactions are only observed

with very few redox enzymes and types of electrodes suitable for individual redox enzymes science the redox active centers of oxidoreductases are often deeply buried within the protein shell. A method usually used to minimize the distance between redox cofactor of oxidoreductase and electrode is the utilization of a unique docking motif to immobilize the protein in the desired orientation. For example, Lee and coworkers fused glucose dehydrogenase (GDH) with a gold-binding peptide (GBP). The GBP facilitated the orientation of GDH on the gold electrode surface and further decreased the distance between FAD cofactor of GDH and electrode to meet the requirement of DET[89]. Cross-linked hydrogels are another commonly used method to immobilize enzymes onto different electrode surfaces and can stabilize proteins through electrostatic interaction. Liu and coworkers immobilized three heme-proteins, including hemoglobin (Hb), myoglobin (Mb) and horseradish peroxidase (HRP) on edge-plane pyrolytic graphite electrodes by an agarose hydrogel, which allowed all 3 proteins to undergo fast DET[90]. Many reports have utilized pyrene to anchor proteins directly to a carbon electrode[91]. Hickey and coworkers reported an method to realize direct electrochemical communication with redox-active proteins based on linear poly(ethylenimine)(LPEI) that has been covalently modified with pyrene moieties (pyrene-LPEI). The pyrene-LPEI can be used to enable DET via the immobilization of many different oxidoreductases by cross-linking in the presence of multiwalled carbon nanotubes (MWCNTs) at carbon electrodes. They used this method to promote direct bioelectrocatalytic reduction of O_2 by laccase and N_2 by nitrogenase successfully[92]. Some microbial cells can also perform DET through the contact of cell membranes or conductive pili (mentioned above) with the electrode. DET of microorganisms requires that the cells have membrane-bound electron transport protein

relays that transfer electrons from the inside to the outside of the cells.. Some *Geobacter* and *Shewanella* strains can evolve electronically conducting molecular pili that allow the electroactive microbial cells to reach and utilize more distant solid electron acceptor. The conductive pili are connected to the membrane-bound cytochromes, via which the electron transfer to the outside of the cell is accomplished (**Figure 3c**)[93].

The medicated electron transfer (MET). Bioelectrocatalysts can utilize small electroactive molecules to perform mediated electron transfer between bioelectrocatalysts and electrodes (**Figure 3b** and **3d**). In practical application, the electronic properties of the mediator must match the type of target bioelectrocatalytic reaction. Foroxidative MET, the mediator reduction potential should be more positive than that of redox cofactor of the bioelectrocatalyst to enable spontaneous MET. For reductive MET, the reduction potential of the mediator should be more negative than that of the redox cofactor [5]. Extensive work with artificial redox mediators has been pursued to perform the electron transfer between bioelectrocatalysts and electrodes. Methyl viologen (MV)[94], anthraquinon-2,6-disulfonate (AQDS)[95] and neutral red (NR)[96] are usually be used as artificial electron transfer mediator. Some electroactive microbial cells can produce metabolites as natural electron mediators to transfer electrons[93]. For example, *S. oneidensis* MR-1 is capable of performing MET as well as DET. The flavin molecules secreted by *S. oneidensis* MR-1 enhanced the ability of its outer-membrane OmcS to transport electrons as redox cofactors/mediators.[97,98] Once a series of electron mediators with suitable electronic properties have been selected, they must next be screened for their ability to have electrochemical communication with the active site of enzyme as different oxidoreductase has different electron mediator preference based on their active site structures. For example, some research already demonstrated that

naphthoquinone derivatives can be used to perform efficient MET with one type of glucose oxidizing enzyme. However, another species did not exhibit any activity even if the enzymes have the same redox cofactor. This result reemphasizes the importance of cofactor accessibility by the electron mediator[99].

MET can also be utilized to perform the regeneration of nicotinamide coenzymes, $NAD(P)^+$/NAD(P)H. Nicotinamide coenzymes are of particular significance since 90% of known oxidoreductases need NAD(H)/ NADP(H) as coenzyme. Due to the high cost and large usage, the efficient and economical regeneration of nicotinamide coenzymes is of particular significance for industrial applications[100]. Some enzymes, such as lipoamide dehydrogenase, diaphorase, , the AMAPORS (artificial mediator accepting oxidoreductase), or ferredoxin $NAD(P)^+$ reductase (FNR), are involved in the MET based nicotinamide coenzyme regeneration system. These enzymes act as linking systems between one-electron mediators (such as cytochromes, FeS clusters, quinones and flavins) and two-electron mediators, making them suitable for electron transfer between the electrode and nicotinamide coenzyme and the regeneration of nicotinamide coenzymes. Some artificial electron mediators, such as viologens, cobalt sepulchrate, ferrocenes, flavins, and others, can also be used for this purpose[101].

Impact of electrode on bioelectrocatalysis

The properties of the interface between the bioelectrocatalysts and the electrodes determines the function and the reaction efficiency of the bioelectrocatalytic system. This interface can be adjusted in terms of chemical and topographical. The advancements of bioelectrocatalysis with regard to electrode materials and surface modification can be

attributed to the following properties: improved biocompatibility, improved adsorption of bioelectrocatalyts, increased electrochemically accessable surface area, enhanced electron exchange rate, enhanced mass transfer of substrate and products, and superior intrinsic conductivity, [102].

The bioelectrocatalyt-electrode interaction. The electrode chemistry and surface topography can influence the bioelectrocatalytic reaction at three different levels: 1. the adherence of bioelectrocatalyst including isolated oxidoreductases or electroactive microbial cells; 2. formation and structure of the biofilm; and 3. electron transfer between bioelectrocatalysts and electrode. The interface can be manipulated in terms of chemical and topographical features to better understand the interaction at nanometer and micrometer scales[102].

At nanometer scale, surface chemistry has crucial impact on both DET and MET between bioelectrocatalysts and electrodes. Especially for DET, it demands a build-up of direct and high efficiency electrical interaction between bioelectrocatalysts and electrode. The interaction is initiated by bioelectrocatalysts transport onto the electrode surface, then attachment or adhesion and finally electron transfer start-up. Attractive or repulsive molecular interactions at the single-enzyme or single-cell level play a decisive role in this process[103]. For isolated oxidoreductases, the enzyme-electrode interaction can be accomplished in four major ways[104]. 1. Electrostatic interaction. The electrostatic interaction involves tailoring protein binding to interfaces based on the opposite charges between the interface of electrode and enzyme. The electrostatic method has been used to facilitate the enzyme-electrode interaction on a variety of interfaces, including self-assembled monolayers (SAMs)[105] and hydrogels[92,106]. 2. Interaction induced by surface

energy. The energy-based interaction is also characteristically used with SAM interfaces. Modifying SAMs with high energy end-groups, such as hydroxyl and carboxyl functionalities, results in the selective adsorption of proteins to these high energy regions due to the affinity of protein to high energy surfaces[107,108]. 3. Covalent binding. Some bioelectrochemical devices utilize covalently bound enzyme at an interface to facilitate the enzyme-electrode interaction and electron transfer. A common way to achieve the covalent binding of the enzyme to the interface of electrode is cross-linking, which is a popular method of enzyme incorporation in a variety of polymers, including osmium redox polymer, tethered bilayer lipid membranes, and conductive organic polymers[104]. Polyamines, specifically poly-ethylenimines (PEI), are a group of polymers that have been shown to cross-link in the presence of enzyme which can assist in stabilization and/or immobilization of enzyme on electrode interface[109-111]. The formation of covalent bonds is another way to form enzyme-electrode interaction and facilitate DET. In Zhang and coworkers' research[112], the bilirubin oxidase was directionally immobilized on the gold wire electrode through the covalent bond between the cysteine residue and gold surface which enhanced the DET as the copper cofactor of bilirubin oxidase directly faces to the gold electrode. 4. Binding with nano material. Given the large specific surface area and their high surface free energy, nanoparticles can very strongly adsorb various kinds of proteins. Having comparable dimensions, the isolated oxidoreductase conjugated to nano particles maintains the natural conformation/structure and hence the functionality. The functionalized nano particles can be prepared via electrostatic adsorption, chemisorption of thiol derivations and specific affinity interactions[113]. For the nanoporous structures, its high surface area allows immobilized oxidoreductase access to a buffered solution, which can stabilize the

protein[104]. Additionally, the porous interfaces are ideal for mediated electron transfer and the mass transfer of substrates and products[41]. For electroactive microbial cells, hydrophilicity, an important electrode property that depends on hydrogen bonding and/or electrostatic and/or van der Walls forces, has been shown to promote bacteria adhesion[102,114]. Additionally, it has been shown that positively charged electrodes are more benificial to the formation of biofilm due to the fact that the surface of electroactive microbial cells usually has negative charge[115]. Beside the hydrophilicity of electrode, the combined effect of electroactive microbial cell surface properties and the nano-topography of the electrode is another important factor affecting the cell adhesion and biofilm development. Different electroactive microbial cells exhibit different cell surface morphologies, number of cellular appendages, surface charge density and polarizability[116,117]. Meanwhile, the nanoscale topography, especially the nano-roughness, can provide anchoring points for the electroactive microbial cell[118]. Moreover, the applied electrode potential can impact the specific adsorption of ions, surface charge, and the local electric field and the migration and adhesion properties of electroactive microbial cell. Finally, the comprehensive impact of nano-topography of electrode surface and the morphological characteristics of the cell surface ultimately determines the cell adhesion. After the adhesion, the electrode not only acts as a substratum for the electroactive microbial cell, but also involved in the metabolism of microbial cell via the electron exchange, especially the DET process[102].

The modification of electrode surfaces at the micrometer scale introduces more surface area for both the immobilization of the bioelectrocatalyst which facilitates DET and for abiotic coenzyme or electron mediator regeneration for MET. Switching from 2-D to 3-D

porous materials such as foam, felt, or fiber brushes is an effective method to increase the current density and production rate per volume of electrode, as the surface area of the electrode is significantly increased[102]. Moreover, the use of carbon scaffolds with large pores in micrometer scale with an interconnected framework can effectively avoid mass transfer limitations. Furthermore, the large pores (micrometer scale) allow bacteria to penetrate through the structure and colonize the biofilm internally[119]. In Santoro and coworkers' research[120], the surface properties including porosity, roughness and surface wettability of the polytetrafluoroethylene-treated carbon paper were analyzed. Their results exihibited a positive correlation between the number of bacteria attached and the surface porosity of the carbon paper electrode at the small scale (5-10 μm).

Electrode materials and modification. Bioelectrocatalysis is a coupled reaction between a redox reaction and an electrode reaction[121]. Consequently, the choice of the electrode material has a great impact on the performance of the bioelectrocatalysis system. An ideal electrode material should have many different properties, such as biocompatible surface, adequate specific surface area, strong chemical stability (includes corrosion resistance), excellent mechanical strength, high conductivity, scalable and manufacturable system, low-cost and low environmental impact[102,122]. Various electrodes have been developed, such as gas diffusion electrodes[123], graphite fiber brush anodes[124], carbon-based foams[125], carbon-based fleece[126], electrospun carbon nanofibers[127], stainless steel[128], etc. to meet the different requirements of various applications.

Although the development of new electrode materials has made great progress, the use of a single component still cannot meet the actual requirement in many cases. For example, stainless steel is the most promising material for microbial bioelectrocatalysis, because of

its low cost, good corrosion resistance, excellent electrical conductivity, and great scale-up potential. However, the passive layer on stainless steel leads to the low biocompatibility and further limits the electron exchange between the electroactive microbial cell and the electrode surface[129]. Electrode modification is an effective way to improve the performance of the electrode as it changes the physical and chemical properties to provide for better bioelectrocatalysts attachment and electron transfer. The usual modification methods include ammonia gas treatment[130]; chitosan[131,132], cyanuric chloride[133,134], 3-aminopropyltriethoxysilane[135], and melamine[136,137] to lead to better electron transfer. Thin layers of metal (e.g. Au, Pd, or Ni) coating[138] are effective to reduce the activation energy threshold energy of electron transfer and nanomaterial modification (e.g. carbon nanotubes, graphene, nanoparticles)[113,139] can offer an open, three-dimensional and conductive matrix for the enzyme attachment and electroactive microbial cell growth. In recent years, electrode modification methods based on redox polymer have received more and more attention. A redox polymer has a nonconductive backbone with redox side chains. These polymers are capable of shuttling electrons via self-exchange-based conduction. Most redox polymers have an outer sphere redox species (i.e. ferrocene or transition metal complexes) as the pendant species, but there are also many examples of organic redox pendants (i.e. viologens, 2,2,6,6-Tetramethylpiperidinyloxyl (TEMPO), and quinones)[140]. The osmium redox polymer modification has played an important role in photoelectrochemical system. Researchers used osmium redox-hydrogels based on poly(vinyl)imidazole-based polymer to entrap and immobilize photosystem II complex (PSII) onto the electrode. The obtained bioelectrochemical system gave rise to photocurrent densities of around 45 μA cm^{-2} [141,142]. In some other studies, an osmium redox

polymer was combined with a cobaltocene redox polymer for mediating photosystem I and hydrogenase for the photoelectrochemical production of hydrogen[143]. The combination of carbon nanotube (CNT) with polymers is beneficial, since CNT can improve the mechanical strength and electrical conductivity of the resulting polymer-CNT hybrids.The unique properties and geometry of polymer-CNT hybrids, provides a three-dimensional nanostructure with a large electroactive area[144].

Bioelectrocatalysis for the production of chemicals

Based on the wide substrate scope of bioelectrocatalysts, bioeletrocatalysis is able to be used to produce wide range of chemicals, especially fine chemicals. In recent years, the conception of electrobiorefining is proposed. The combination of biorefineries and (bio)electrochemical transformations further expanding the application space of bioelectrocatalysis

N_2 reduction and production of ammonia. The reduction of the chemically-inert dinitrogen (N_2), the major constituent of Earth's atmosphere, to more useful ammonia is a key step in the global biogeochemical N cycle[145]. Electrochemical ammonia production based on nitrogenase at room temperature under ambient pressure is an alternative technology to Haber-Bosch process. The synthesis of ammonia from N_2 by nitrogenase follows reaction (1) under optimal conditions (where ATP is adenosine triphosphate, ADP is adenosine diphosphate, and Pi is inorganic phosphate).

$$N_2 + 8H^+ + 16\,MgATP + 8e^- \rightarrow 2NH_3 + H_2 + 16MgADP + 16\,Pi \qquad (1)$$

Three distinct nitrogenase enzymes sharing similar characteristics have been found. They are distinguished by their metallocofactors (co): FeMo-co, FeFe-co, and VFe-co. The most

widely studied and understood nitrogenase enzyme contains the FeMo-co, and is known as MoFe nitrogenase. Minteer's group established an effective bioelectrocatalytic N_2 fixation system based on purified nitrogenase and MET in which nitrogenase catalyzed N_2 reduction was combined with enzymatic hydrogen oxidation (**Figure 4a**). Using a proton exchange membrane as a separator, a NH_3 producing nitrogenase cathodic compartment was coupled with a hydrogenase-based anodic compartment, where hydrogen was employed as the terminal electron donor. Methyl viologen was used as an electron mediator to shuttle electrons at both the anode and cathode. The coupling of this nitrogenase cathode with a hydrogenase bioanode that oxidize molecular hydrogen to provide electrons results in an enzymatic fuel cell that can produce NH_3 from H_2 and N_2 with simultaneously production ofelectrical current. The Faradaic efficiency of this N_2 reduction achieved 26.4%[24]. Their research realized bioelectrocatalytic N_2 fixation and ammonia production from H_2 and N_2 at ambient condition. In order to eliminate the need for the Fe-protein, Minteer et al. further developed a DET based bioelectrocatalytic N_2 fixation system[92]. They immobilized MoFe nitrogenase in a pyrene-LPEI hydrogel on a carbon electrode. This immobilization strategy showed the catalytic subunit of nitrogenase (MoFe protein) has the possibility to perform ATP-independent direct electroenzymatic reduction of N_2 to NH_3. This is of great significance for reducing the production cost of bioelectrocatalytic N_2 fixation.

Besides purified nitrogenase, some microbial cells can also perform N_2 fixation in a bioelectrocatalytic system. In comparison with nitrogenase-based N_2 fixation, microbial bioelectrocatalytic N_2 fixation has many advantages such as the microbes grow, reproduce, and are continually producing new nitrogenase enzymes[23]. The complicated process of

nitrogenase purification is no longer needed. More importantly, the nitrogenase which works inside the cell is more stable than the purified counterpart and is more suitable for the long-term N_2 fixation. In some studies, algal and cyanobacteria were utilized for nitrogen fixation and ammonia production. Leddy and Paschkewitz used a SA-1 mutant of *Anabaena variabilis* immobilized on a glassy carbon electrode with a hydrophobically modified Nafion film to electrochemically produce ammnia from N_2[146]. Another interesting research is from Chong Liu and coworkers[147]. In their research (**Figure 4b**), a H_2-oxidizing bacterium *Xanthobacter autotrophicus* in a hybrid inorganic-biological system was utilized to synthesize NH_3 from N_2 and H_2 generated from electrocatalytic water splitting at ambient conditions in a single reactor . In detail, a constant voltage was applied between a cobalt-phosphorus alloy hydrogen evolution cathode and a cobalt phosphate oxygen evolution anode for the water splitting and H_2 generation. The hydrogenase of *X. autotrophicus* oxidizes the generated H_2, fueling CO_2 reduction in the Calvin cycle and N_2 fixation by nitrogenase. The generated NH_3 can diffuse extracellularly. The *X. autotrophicus* cells can be used as electrogenerated biofertilizer and added to soils to improve the growth of Cherry Belle radish by up to approximately 1440% in terms of storage root mass. The achievement of this research is that the H_2 used in N_2 fixation can be renewably obtained from water splitting and can be utilized *in situ* as an electron mediator. The latest research of microbial bioelectrocatalytic N_2 fixation was reported by Ortiz-Medina and coworkers[148]. Their using anaerobic, single-chamber microbial electrolysis cell to realize the conversion from N_2 to NH_4^+. In the microbial electrolysis cell, the acetate oxidation of exoelectrogenic bacteria provides electrons for N_2 fixation. Then, the exoelectrogens transfer the electron to the anode during respiration. Then, the

electrons were driven to cathode by an external voltage to produce H_2 gas. H_2 can be consumed by the exoelectrogens to provide electrons for further N_2 fixation and/or converted to CH_4 by methanogens. NH_4^+ production rate is approaching 5.2×10^{-12} mol s^{-1} cm^{-2}. In the comparison with purified nitrogenase, a non-negligible disadvantage of microbial cell based N_2 fixation is the assimilation of NH_4^+. NH_4^+ is incorporated into amino acids by a cyclic pathway comprising glutamine synthetase and glutamate synthase (GS-GOGAT pathway) which is not conducive to the accumulation and secretion of NH_4^+. In practical research, the ammonium assimilation inhibitor needs to be added or the genes of GS-GOGAT pathway need to be knocked out[149].

Chiral chemicals. Chirality is a key factor in the efficacy of many drugs and agrochemicals, so the production of chiral intermediates with high optical puritiy has become increasingly important. Currently, single enantiomers can be produced via chemical or chemoenzymatic synthesis method[150]. Oxidoreductases are effective biocatalysts to catalyze asymmetric reactions and produce chiral chemicals[151]. One drawback of oxidoreductases is the requirement of expensive coenzymes (e.g., $NAD(P)^+$/NAD(P)H) and coenzyme regeneration systems[152]. As mentioned above, bioelectrocatalytic coenzyme regeneration methods get rid of the side products from the coenzyme as well as the substrate separation[153]. Recently, the application of bioelectrocatalytic coenzyme regeneration for the synthesis of the chiral chemicals has gained prominence due to the merits of using electricity as a clean power source and enzymes with high enantioselectivity as catalysts while minimizing the formation of byproduct or pollutant[14]. Some microbial cells, especially engineered *E.coli* cells, can also be used to prepare chiral chemicals in bioelectrocatalysis system. One limitation of whole-cell biosynthesis is *E. coli* does not

naturally perform electron transport. The consumption of coenzyme and asymmetric reaction take place in the cytoplasm. The reduction of electron mediator takes place around the electrode. Therefore, electron transport through penetrating the cell membrane is a critical problem that needs to be addressed.

Chiral alcohol. Alcohol dehydrogenases (ADHs) are capable of catalyzing the asymmetric reduction of prechiral carbonyls and aldehydes to produce chiral alcohols. In Hildebrand's research[154], ADH from *Lactobacillus brevis* was employed to reduce acetophenone to produce (*R*)-phenylethanol. The coenzyme, NADPH, was electrochemically regenerated with a rhodium complex as electron mediator. The reaction in buffer solution was optimized for high productivity (14 g L^{-1} d^{-1}) and enantioselectivity (>99%). Cytochrome P450 monooxygenases has the ability to hydroxylate saturated carbon atoms and can also be utilized to prepare chiral alcohols. Various research groups have demonstrated electrochemical response of P450 monooxygenases either via direct electrochemical communication between the heme-group and a cathode[155,156] or using mediated electron transfer[157,158]. Vilker and coworkers constructed a bioelectrochemical reactor in which the reducing equivalent to the cycle is supplied directly to the purified cytochrome CYP101 (P450cam) via its natural redox partner (putidaredoxin) using an antimony-doped tin oxide working electrode. The challenge of their research is the effective control of oxygen concentration. On one side, oxygen is an inevitable part of the P450 catalytic cycle. On the other side, oxygen also reacts quickly with the reduced components of the electron transport chain and is easily reduced on cathode. The oxygen dilemma not only lowers the current efficiencies of electroenzymatic processes but also yields reactive oxygen species that have to be dealt with in order to prevent the denature of enzyme. In order to fix this

problem, the required O_2 was produced at a Pt counter electrode through water electrolysis (**Figure 5a**). As a result, a continuous catalytic cycle was maintained for more than 5h and 2600 enzyme turnovers. The highest formation rate of product achieved 36 nmol of 5-exo-hydroxyamphor/nmol of P450cam per min[157,159]. In traditional biocatalysis systems, P450 monooxygenases can only use the low concentration of dissolved oxygen, which limits the reaction rate. Compared with traditional biocatalysis, the significance of this research is that the bi-functional counter electrode can be used to produce the indispensable substrate (O_2) for P450 monooxygenases catalyzed hydroxylation reaction which enhances the reaction efficiency.

The microbial cell can also be used in bioelectrocatalysis systems to produce chiral alcohols. As mentioned above, the electron shuttle to bridge the insulating out membrane is a critical problem which needs to be resolved. Sturm-Richter and coworkers heterologously expressed c-type cytochromes CymA, MtrA and STC from *Shewanella oneidensis* in *E coli* cell to construct a electron transport chain. With the addition of the electron transport chain, the electron transfer into the periplasm was accelerated by 183%[160]. This engineered *E. coli* cell with heterologous electron transport chain is a bioelectrocatalytic platform, which is capable of integrating different oxidoreductases via co-expression to synthesize a variety of products. The heterologous electron transport chain can effectively transport electrons to support the intracellular coenzyme regeneration and the redox reaction. On the basis of this work, Mayr and coworkers integrated a NADPH-dependent alcohol dehydrogenase from *Lactobacillus brevis* into this engineered *E. coli* platform to perform the asymmetric reduction of acetophenone and the synthesis of (*R*)-1-phenylethanol (**Figure 5b**)[161]. After integration of alcohol dehydrogenase, the engineered

E.coli exhibited good performance. The import of exogenous electrons via the electron transport chain effectively ensures the regeneration of NADPH. The maximum yields of 39.4% at a coulombic efficiency of 50.5% with ee_p > 99% was demonstrated at a rate of 83.5 μM/h.

Chiral epoxy compound. Asymmetric epoxidations are useful transformation process to prepare pharmaceuticals and fine chemicals[162]. The introduction of two C-O bonds in one reaction not only leads to the formation of up to two chiral centers, but also provides the possibility to produce a diversity of key intermediates due to the facile opening of the epoxide ring. An effective method for the preparation of chiral epoxy compounds is the utilization of an enzymatic epoxidation catalyst (flavin-dependent monooxygenases)[56]. Ruinatscha and coworkers established a scalable system for styrene monooxygenase (StyA) catalyzing the asymmetric (*S*)-epoxidation of styrene with high ee_p, current efficiencies, and space-time yields, [162]. The authors utilized highly porous reticulated vitreous carbon electrodes, which maximize volumetric surface area in a flow-through mode to rapidly regenerate the consumed $FADH_2$ cofactor required for StyA activity at -0.75 V vs. Ag/AgCl. Under optimized reaction condition, an average space-time yield of 0.35 g L^{-1} h^{-1} could be achieved during 2 h with a final (*S*)-styrene oxide yield of 75.2%. The ee_p of produced (*S*)-styrene oxide achieved 99.5%.

Chiral amino acid and derivatives. Amino acid oxidase (AOx) is an enzyme with FAD as redox center. The enzyme catalyzes the oxidation of amino acid to imino acid with the consumption of O_2. In living organisms, the generated byproduct, hydrogen peroxide, is decomposed by the catalase. Furthermore, since this enzyme reaction is known to proceed under equilibrium conditions in the absence of the catalase[163], the reverse reaction can be

electrochemically induced by choosing an electron mediator having the ability to reduce FAD in AOx. This enzyme can be used to perform deracemization, stereoinversion, and asymmetric synthesis of amino acids[30]. Kawabata and coworkers studied the electrochemical reduction of pyruvate to alanine with the use of an electrode on which both AOx and electron mediator are immobilized[164]. In detail, the authors chose 1-aminopropyl-1'-methyl-4,4'-dipyridinium iodide (ADPy) as electron mediator, D-amino acid oxidase (D-AOx) from *Porcine kidney* as bioelectrocatalysts. The immobilization of AOx and ADPy was conducted by cross-linking using glutaraldehyde. The electrochemical reduction of pyruvate was conducted at -0.7 V vs. Ag/AgCl with 30mM NH_4OH. After 10 h reaction, 8.9 mM D-alanine was generated. The enantiomer excess of generated D-alanine was higher than 99%. The Faradaic efficiency was higher than 97%. In Wu and Zhu's research, an enzymatic electrosynthesis cell and an a glucose-fueled enzymatic fuel cell were integrated into a self-powered enzymatic electrosynthesis system to produceL-3,4-Dihydroxyphenylalanine (L-DOPA, a drug for the treatment of Parkinson's disease). The maximum L-DOPA production rate of this hybrid bioelectrochemical system achieved 118.3 mg h^{-1} L^{-1} with a highest Coulombic efficiency of 90%[6].

Product of asymmetric reduction of olefins. Ene-reductases are able to catalyze the asymmetric hydrogenation of olefins and generate up to two stereogenic centers.. They are subdivided into four classes: enoate reductases, old yellow enzymes (OYEs), flavin-independent short-chain dehydrogenases/reductases (SDRs), and medium-chain dehydrogenases/reductases (MDRs). Ene-reductases require NAD(P)H as coenzyme for hydride donation[165]. For the OYE enzyme and enoate reductases family, electroenzymatic methods for coenzyme regeneration have been developed to support the asymmetric

reduction of olefins. In Simon and coworkers' research, the asymmetric synthesis of (2*R*)-2-methyl-3-phenylpropionate by an enoate reductase from *Clostridium tyrobutyricum* was combined with electrochemical regeneration of reduced methyl viologen ($MV^{\cdot+}$). The $MV^{\cdot+}$ was used electron mediator to transfer electrons from cathode to enoate reductase. After 80 h reaction, the conversion ratio of 80 mM substrate achieved 95%[166].

Chrial sulfoxides. Chloroperoxidase (CPO) is a versatile heme-dependent peroxidase that catalyzes a variety of reactions[167]. Under exclusion of halogen ions, the enzyme catalyzes among others, enantioselective epoxidations, selective hydroxylation of hydrocarbons, selective oxidations of primary alcohols, and enantioselective sulfoxidations. The products of the different sulfoxidation reaction can be used as insecticides, fungicides, and drugs for the treatment of allergies, actinodermatitis, medical conditions of the gastrointestinal tract, and as an antidote for alcohol intoxication[168]. However, CPO use in preparative or industrial-scale reaction has been hindered by instability towards hydrogen peroxide, which is the cosubstrate of CPO for both the hologenase and peroxidase reaction[169]. One effective method used to provide controlled addition of hydrogen peroxide without the formation of a byproduct is the electrochemical *in-situ* reduction of oxygen at a carbon electrode. The generated hydrogen peroxide could be consumed by CPO *in situ*. Consequently, the concentration of hydrogen peroxide can be controlled at a low level. In Lütz and coworkers' research[29], the CPO from *Caldariomyces fumago* was used to perform the asymmetric oxidation of thioanisole to produce (*R*)-methylphenylsulfoxide (**Figure 6**). In practical research, oxygen was bubbled into the electrolyte solution. The dissolved oxygen was reduced to hydrogen peroxide at -0.5 V vs. Ag/AgCl and *in situ* consumed by

CPO. The reaction was carried out on 300 mL scale with a productivity of 30 g L^{-1} d^{-1} and ee_p >98.5%.

Chiral amine. Chen et al. developed an upgraded bioelectrocatalytic N_2 fixation system. In this system, the end-product of N_2 fixation was no longer ammonia, but high-value-added chiral amine intermediates (**Figure 7**)[27]. A cathode was utilized to supply enough electrons to synchronously realize the regeneration of reduced $MV^{\cdot+}$ and NADH for the reductive reaction catalyzed by nitrogenase and *L*-alanine dehydrogenase respectively. The product of bioelectrocatalytic N_2 reduction, ammonia, was *in-situ* utilized by *L*-alanine dehydrogenase to generate alanine with the consumption of NADH. The generated alanine was utilized by ω-Transaminase as amino group donor to catalzye the amination of ketone substrate and the production of desired chiral amine intermediates. After 10 hours of reaction, the maximum concentration of 1-methyl-3-phenylpropylamine is 0.54 mM with the 27.6% highest Faradaic efficiency and >99% ee_p value of product. Based on the wide substrate scope and excellent enantioselectivity of ω-transaminase, the upgraded N_2 fixation system has great potential to produce a variety of chiral amine intermediates and finally to realize the conversion from chemically inert N_2 to amines with high added-value.

Chemicals produced by electrobiorefining. Electrobiorefining is a relatively new concept proposed in recent years. It refers to a technological revolution of biorefineries by the addition of (bio)electrochemical transformations. For electrobiorefining, the microbially produced intermediates are converted to final product via the utilization of oxidative and reductive electroorganic reactions to that may serve as privileged building blocks[170]. Compared with the traditional biorefinery, their similarity is the electrobiorefinery also uses biomass as a source for the sustainable, ideally residue-free

production of different products. Their difference is the electrobiorefinery process has the extra mode of an electroorganic reaction. Electrobiorefineries are also different from the general microbial electrochemical technology. In microbial electrochemical technology, the metabolism of microbial cell is wired to the electrode. The electron exchange is based on extracellular electron transfer between microbial cell and electrode[171]. Forelectrobiorefinery, the microbial metabolism isonly indirectly influence by an electrochemical reaction. The full spatial separation of microbial and electrochemical conversion is the main feature of the electrobiorefining[170]. In practical application, the metabolic intermediate of the microbial cell (usually the precursor of final product) is upgraded to a final product via abiotic electroorganic reactionwith high energetic and chemical efficiency.

A classic example is from Matthiesen el. al.[172,173]. The target product of their research is trans-3-hexendioic acid, a important precursor of nylon-6,6 from muconic acid. They first constructed an engineered *Saccharomyces cerevisiae* strain via metabolic flux balance analysis and metabolic engineering modification. The engineered *S. cerevisiae* strain was able to convert glucose to muconic acid (muconic acid titer of 559.5 mg/L). The fermentation broth which contained muconic acid was subsequently hydrogenated in a three-electrode electrochemical cell. Electrocatalysis was preferred over conventional high-pressure hydrogenation as hydrogen is produced *in situ* by water splitting. In this configuration, muconic acid hydrogenation and hydrogen production take place simultaneously at the cathode, enabling seamless electrocatalytic hydrogenation.The muconic acid was electrocatalytically hydrogenated to 3-hexendioic acid in 94% yield and 100% Faradaic efficiency without any separation process. In this case, the microbial cells

and electrocatalysis system catalyzed two independent and synergistic processes. Because it does not involve electron transport between microbial cell and electrode, this process is more efficient and easier to implement in real-world applications. We can expect that the cheaper and more widely sourced cellulose, even lignocellulose, can be used as feedstock in this process after pretreatment. The electrobiorefinery has also been used in some other conversion process, such as glucose to methylsuccinic acid via *Aspergillus terreus* fermentation and electrochemical hydrogenation[174], and the fermented corn to drop-in fuel additive via microbiome fermentation and Kolbe electrolysis[175].

Bioelectrocatalysis for the production of biofuels

Biofuel is another important product of bioelectrocatalysis. Based on the diverse conversion function of bioelectrocatalyst, the cheap and readily available feedstocks, especially CO_2, are able to be converted to a variety of energy chemicals in the bioelectrocatalytic reaction system.

Hydrogen. Hydrogen is extensively used both as a chemical and fuel in various industrial processes. Hydrogenase can produce molecular hydrogen. Qian and coworkers used a mixture of clay and viologen polymer as a sandwich layer to immobilize hydrogenase to prepare hydrogenase modified electrodes. This modified electrode is efficient for electrochemical hydrogen evolution[176]. Some microbial cells which can express hydrogenase can also be immobilized on electrodes to perform hydrogen evolution. Tatsumi el al. used polycarbonate membranes to immobilize *Desulfovibrio vulgaris* whole cells on glassy carbon electrodes. Methyl viologen was used as a mediator to shuttle electrons between electrode and hydrogenase inside the cell to produce the catalytic currents for hydrogen evolution[177]. A more promising example was performed by Rozendal

et al[178]. Their research was based on a biocathode that was modified by selected mixed cultures of electroactive microorganisms. Through a three-phase biocathode startup procedure, acetate- and hydrogen–oxidizing bioanodes were turned into a hydrogen-producing biocathode by reversing the polarity of electrode. The biocathode produced 0.63 m^3 H_2/m^3 cathode liquid volume per day at a cathodic hydrogen efficiency of 49%.

CO_2 fixation based biofuels. Because of ever-increasing CO_2 emissions and environmental concerns, electrochemical CO_2 fixation to produce useful chemicals or biofuels is a prominent research direction in attempts to close the anthropogenic carbon cycle[20]. Formate dehydrogenase (FDH) is an enzyme which can catalyze the reversible oxidation of CO_2 to formic acid according to equation (2):

$$HCOO^- \leftrightarrow CO_2 + 2e^- + H^+ \qquad (2)$$

Many bacteria species are able to reduce CO_2 to muti-carbon organic molecules by directly accepting electrons from electrodes through CO_2 reducing microbial pathways, including the Calvin-Benson-Bassham-cycle, Wood-Ljungdahl pathway, reductive TCA cycle, reductive acetyl-CoA cycle, and acyl-CoA carboxylate pathway. It is important to emphasize that the emerging hybrid inorganic-biological system has significantly promoted the development of microbial cell-based CO_2 fixation and the production of biofuel in recent years[179-183]. In this system, the renewable electricity and electrode are used to realize the H_2 generation from renewable water splitting rather than natural gas. The generated H_2 acts as an electron donor after being oxidized by the hydrogenase of microbial cells and effectively facilities the CO_2 fixation and production of biofuels.

Formic acid. Formic acid is of commercial value as a chemical feedstock, an efficient carrier of hydrogen, and as a fuel for fuel cells[184]. Unfortunately, FDHs require an expensive coenzyme (i.e., NADH) alongside the CO_2 fixation reaction[185]. Therefore, the reduced equivalent supplementation is necessary to carry on the reaction effectively. Bioelectrocatalysis methods can be used to realize the coenzyme regeneration for FDHs. Srikanth et al. reported optimized potential (-0.8 V vs. Ag/AgCl) for CO_2 reduction to formic acid with 12.74 % Faradaic efficiency and a production rate of 225.81 mg L^{-1} h^{-1}.. Neutral red was employed as electron mediator to facilitate the regeneration of NADH. The FDH was immobilized on the graphite-based cathode to convert CO_2 to formic acid[186].

The work toward DET for the heterogeneous electroenzymatic reduction of CO_2 was done by Reda et al[187]. In their research, tungsten-containing FDH was adsorbed on glassy carbon. Using this enzyme electrode, the CO_2 reduction to formic acid was performed at reduction potentials below -0.8 V vs. Ag/AgCl to yield Faradaic efficiencies of 97% and higher.

Acetate and byutyrate. To date, acetate is the main product obtained by microbial electrosynthesis[188]. In Nevin and coworkers' research[189,190], they found some acetogenic bacteria including *Clostridium ljungdahlii*, *Moorella thermoacetica*, *Sporomusa* species and *Clostridium aceticum* were able to utilize the electrons derived from graphite electrodes to reduce CO_2 to acetate as the primary product and 2-oxobutyrate and formate as by-products with >80% efficiencies of the electrons consumed and recovered. Bajracharya et al. developed a stable and robust CO_2 reducing biocathode from a mixed culture inoculum to avoid the methane generation[191]. In their study, a selective enrichment was applied to samples of an anaerobic sludge to favor homoacetogenic activity suppressing the methanogens. Finally, the enriched mixed culture inoculum was

additionally supplemented by *Clostridium ljungdahlii* to ensure the presence of homoacetogens. Biomass growth and gradual acclimation to CO_2 electro-reduction accomplished a maximum acetate production rate of 400 mg $L_{catholyte}^{-1}$ d^{-1} at -1 V vs. Ag/AgCl. The accumulation of acetate achieved up to 7-10 g/L. Recently, Chong Liu's group developed a biocompatible biological-inorganic hybrid system that displayed high efficiency for electricity-driven CO_2 fixation[180]. In this system, water is split to O_2 by a cobalt phosphate anode and H_2 is produced by a cobalt-phosphorous alloy cathode. The generated H_2 is selectively consumed by hydrogenase of *Sporomusa ovata* and powers the reduction of CO_2 and acetate generation. Their research shows that the production of acetate is limited by the low solubility of H_2. Accordingly, a biocompatible perfluorocarbon nanoemulsion was used as a H_2 carrier to increase the throughput of CO_2 reduction. The production of acetate was increased by 190%. The average acetate titre of 6.4 g/L was achieved in 4 days with close to 100% Faradaic efficiency. The capability of microbial cells to produce longer chain organic compounds from CO_2 was already suggested[192]. Ganigué and coworkers' work shows the bioelectrochemical production of butyrate from CO_2 as a sole carbon source[193]. In their research, a 240 mL two-chambered H-type bioelectrochemical system was employed. A cathode made of commercial carbon cloth with an area of 9 cm^2 was used as a working electrode. The bioelectrochemical system was inoculated with enriched carboxydotrophic mixed culture from a syngas fermenting reactor, and pure CO_2 was bubbled into the reactor. The working voltage was set at -0.8 V vs. SHE. The highest concentration of butyrate was 20.2 mMC, with a maximum butyrate production of 1.82 mMC d^{-1}. The electrochemical characterization demonstrated that CO_2 reduction to butyrate was driven by hydrogen.

Alcohols. Bajracharya's group also used mixed microorganism culture to produce ethanol through CO_2 reduction[191]. In their research, the cathode potential was set at -0.9 V vs. Ag/AgCl to perform the CO_2 reduction and acetate production. When the accumulation of acetate reached higher than 1.5 g/L in batch operation, and the solution pH decreased to lower than 6, the products shifted to the production of ethanol. The highest accumulation of ethanol achieved 0.2 g/L. The hybrid inorganic-biological system can be used to produce various multi-carbon alcohols through the metabolism of different microbial cells. In Torella and coworkers' research[181], they perform CO_2 fixation in a triple-junction amorphous silicon solar cell with a cobalt phosphate oxygen evolution anode and a NiMoZn hydrogen evolution cathode. The water splitting takes place at the cobalt phosphate anode with H_2 production at NiMoZn cathode. CO_2 is continuously sparged into the metabolically engineered *Ralstonia eutropha* cell in which the polyhydroxybutyrate synthesis pathway is disrupted, and four genes were introduced into the cell to construct a pathway to redirect acetyl-CoA toward the synthesis of isopropanol. The H_2 was oxidized by the oxygen-tolerant hydrogenase to generate reduced coenzyme and ATP, and uses these to reduce CO_2 to 3-phosphoglycerate via the Calvin cycle. The generated 3-phosphoglcerate can be converted to isopropanol through the newly constructed metabolic pathway. In this system the engineered *R. eutropha* enabled the production of the isopropanol at up to 216 mg/L, and the highest bioelectrochemical fuel yield yet reported by >300%. This research is a successful example of the combination of bioelectrocatalysis and metabolic engineering. In their following research[182], the NiMoZn alloy cathode was substitute by the reactive oxygen species (ROS) resistant cobalt-phosphorus alloy cathode to inhibit the generation of ROS at cathode and relieve the toxicity of ROS to engineered

R. eutropha cell. After 6 days reaction, the isopropanol production achieved approximately 600 mg/L and the total concentration of isobutanol and 3-methyl-1-butanol achieved higher than 200 mg/mL.

Olefin and methane. Some studies show that nitrogenases, including MoFe, FeFe, and VFe nitrogenases, also can perform the CO_2 fixation. Hu and coworkers' research[194] demonstrated that both the MoFe- and FeFe-nitrogenases could be immobilized as polymer layer on an electrode and that electron transfer mediated by cobaltocene can drive CO_2 reduction to formic acid. Minteer and coworkers reported the electroenzymatic C-C bond formation from CO_2 catalyzed by VFe nitrogenase originated from *Azotobacter vinelandii*[21]. In their research, they employed two cobaltocene electron mediators (1,1'-dicarboxy-cobaltocenium and 1-carboxy-cobaltocenium). The bioelectrocatalytic VFe nitrogenase can form C-C bonds and reduce CO_2 to ethylene (C_2H_4) and propene (C_3H_6) bypassing the requirement of CO as the substrate The products were quantified after the passage of 4 C of charge at -0.86 V vs. SHE. 25 nmol C_2H_4 and 42 nmol C_3H_6 per μmol VFe were generated[21].

The hybrid bioinorganic platform can also be used to produce methane. In Nichols and coworkers' research[179], they developed an earth-abundant nanoparticulate nickel sulfide hydrogen evolving cathode. At an applied current of 7.5 mA, use of nanoparticulate nickel sulfide hydrogen evolving cathode and methanogenic bacterium *Methanosarcina barkeri* as the biocatalyst for CO_2 fixation and CH_4 production in 110 mL (4.3 mmol) of CH_4 over 7 days with a Faradaic efficiency of 74%. Moreover, the authors also demonstrated that the introduction of indium phosphide photocathodes and titanium dioxide photoanodes affords a fully solar-driven system for the CH_4 generation from water and CO_2. Their research

established compatible inorganic and biological components can synergistically couple light-harvesting and catalytic functions for solar-to-chemical conversion.

Bioelectrocatalysis for the production of material

Biomaterial, especially biodegradeable plastic, is an important product of bioelectrocatalysis. Currently, the massive using of plastic causes a great environmental impact. This problem could be partially solved by the production and using of recyclable and biodegradable plastic. However, nowadays, only a small amount of these plastic are effectively recycled[195]. Consequently, there is a need for alternative, sustainable, and more biodegradable/renewable plastics.

Production of bioplastics. Polyhydroxyalkanoates (PHAs) are sustainable and biodegradable polyesters that are produced by different microorganisms[196]. Polyhydroxybutyrate (PHB) is the most common type of PHA, which is accumulated in some microbial cells under nitrogen nutrient-limited conditions as carbon and energy stocks[197]. PHAs have similar physical properties to those of polyethylene. Therefore, it can be used to replace the common plastics in several applications[195]. Volatile fatty acids (VFA) are the direct metabolic precursors of PHA[198]. In microbial electrosynthesis systems, CO_2 can be reduced to short fatty acids (acetate and butyrate) and further be converted to PHAs through corresponding metabolic pathway.

Sciarria et al. used a two-step method to realize the conversion from CO_2 to PHB[195]. In the first step, CO_2 was reduced to acetate and butyrate with *Clostridium* spp. as the bioelectrocatalyst. Their result showed that 73% CO_2 was converted to acetate and butyrate. Then the generated acetate and butyrate were separated and concentrated by liquid

membrane extraction. In the second step, the activated sludge was used as inoculum feed. The microbial cells in the active sludge are able to use acetate and butyrate as a carbon source to synthesize PHB. In the pathway for the production of PHB, acetate is converted to acetyl-CoA, and two acetyl-CoA was condensed by 3 ketothiolase (PhaA) to form acetoacetyl-CoA. The acetoacetyl-CoA is reduced by acetoacetyl-CoA reductase (PhaB) to form (*R*)-3-hydroxybutyryl-CoA. Finally, (*R*)-3-hydroxybutyryl-CoA can be incorporated into the polymer as PHB by PHA synthase (PhaC). For butyrate, it can be reduced to (*R*)-3-hydroxybutyryl-CoA directly and then go into the PHB synthesis pathway. Finally, 0.41 kg of carbon as PHA were obtained per 1 kg of carbon as CO_2 inlet. Chen and coworkers[32] utilized metabolic engineering methods to introduce the ribulose-1,5-bisphosphate carboxylase/oxygenase (Rubisco) into the wild-type *Ralstonia eutropha* and allow the *R. eutropha* cell the ability of CO_2 fixation (**Figure 8a**). Formate dehydrogenase catalyzed the reduction of CO_2 to formate in the cathodic chamber. Formate can go through the cell wall of *R. eutropha* as an electron carrier to transfer electrons between electrode and *R. eutropha* cell. To enhance the formation of formate, neutral red (NR) was used to regenerate NADH. At the same time, NR is used as an electron mediator to deliver electron from cathode into *R. eutropha* to facilitate the efficiency of CO_2 reduction and PHB production. CO_2 diffused into the *R.eutropha* cell and was captured by Rubisco. Through the Calvin-Benson-Bassham (CBB) cycle, the CO_2 was fixed and converted to 3-phosphate glyceraldehyde and further converted to acetyl-CoA through the glycolysis pathway and pyruvate dehydrogenase complex. The generated acetyl-CoA can be used as a substrate for the synthesis of PHB. Upon application of the cathode potential at -0.6 V vs. Ag/AgCl and the metabolic engineering modified *R. eutropha*, the final concentration of PHB achieved

485 ± 13 mg/L. Besides using microbial cells as bioelectrocatalysts, purified enzymes can also be used as the bioelectrocatalyst to realize the *in vitro* production of PHB (**Figure 8b**). Alkotaini and coworkers constructed an *in vitro* PHB synthesis pathway which composed by acetyl-CoA synthase (Acs), PhaA, PhaB and PhaC with acetate as initial substrate[31]. The BVP-LPEI modified glassy carbon cathode effectively supplied electrons for the PhaB and yielded 1.6 mg in a 5 mL reaction mixture. These two research examples demonstrate the advantage of the combination of bioelectrocatalysis and metabolic engineering for complex transformation process and high value-added product production.

Summary and further perspectives

In this Review, we discuss the application of bioelectrocatalysis for the production of chemicals, biofuels, and material. Bioelectrocatalysts have unique structural features. For oxidoreductases, they usually contain cofactors which play critical role in the electron transfer through their redox state changes, and electrochemical method can be used for cofactor regeneration. For microbial cells, they can utilize conductive pili or pilus-like nanowire, the apparent terminal cell-bound complex, or secreted secondary metabolites as electron mediators to perform the electron transfer. Protein engineering and metabolic engineering are effective methods to improve the bioelectrocatalytic properties of bioelectrocatalysts. MET and DET are two main electron transport mechanisms and are also two principles in the design of bioelectrocatalytic systems. The development of electrode material and modification method improved the performance of bioelectrocatalysis devices. Recently, bioelectrocatalysis has been widely used in the production of chemicals, biofuels, and bioplastics. While the field of bioelectrocatalysis

has made great progress, there are still many problems need to be addressed. We think the following areas will require more investigation:

The combination of bioelectrocatalysis and synthetic biology. Synthetic biology is an interdisciplinary branch of biology, chemistry, and engineering that combines the investigative nature of biology with engineering design principles[199]. Most efforts in synthetic biology have been focused on the cost-competitive production of new drugs, natural products, biochemicals, and bioenergy[200]. In both *in vivo* and *in vitro* synthetic biology systems, oxidoreductases usually catalyze the key step of the conversion process of target products with the consumption of reduced equivalent[201-204]. Correspondingly, the reduced equivalent balancing and regeneration is a critical issue as the insufficient supply of reduced equivalent would slow down, even stop the entire system[205]. people can conceive to use bioelectrocatalytical reduced equivalent regeneration method. For *in vivo* synthetic biology systems, an electron transfer pathway could be introduced into the cell, e.g., MtrCAB pathway of *S. oneidensis* MR-1 to make the cell able to obtain electrons from electrode via DET. Additionally, the coenzyme preference of oxidoreductase composing the synthetic pathway can be reversed from natural coenzyme (NAD(P)/NAD(P)H) to a synthesized small-sized biomimetic coenzyme via protein engineering. Engineered oxidoreductases with the specificities on biomimetic nicotinamide coenzymes and losing the ability to utilize natural coenzyme can be used to develop bioorthogonal redox systems *in vivo* with the expression of nucleotide transporter[206]. The combination of bioelectrocatalytic coenzyme regeneration and bioorthogonal redox system have great application potential as the supply of external electrons and utilization of the biomimetic coenzyme (reduced by electrode) would make the artificial synthetic pathway thoroughly

rid the cell from dependence on endogenous coenzyme and perform the conversion function without disturbing the natural redox balance of the cell. This result is more conducive to long-term cell survival and efficient production of target produces. For *in vitro* synthetic biology system, the utilization of cheaper and more stable bomimetic coenzymes and bioelectrocatalytic coenzyme regeneration method would effectively reduce costs, simplify product separation process, and extend the system operating time.

The design and application of new reaction medium. In general condition, aqueous reaction mediums are used in bioelectrocatalytic systems. In the bioelectrocatalytic production of chemicals, the adding of organic substrate and the generation of the product can change the dielectric constant. Additionally, aqueous solutions limits the substrate and final product concentration due to their low maximum solubility. The aqueous-organic two-phase system should be an effective method to resolve the problem. In this system, the second phase of organic solvent is used to decouple the cofactor or mediator from substrate and product. With this method, it is highly possible to increase the concentration of substrate and product as the aqueous phase is continuously supplied with fresh substrate and while generated products would be extracted *in situ*[154]. Some beneficial attempts have been carried out[154,207,208].

The engineering application of bioelectrocatalysis in large scale. Although bioelectrocatalysis has promising potential and some great breakthroughs have been achieved, it is still far from entering the market. Biocatalysis which has been applied in industrial production is a good frame of reference to explain this problem. The characteristics of biocatalytic reactions, including momentum transfer, heat transfer, mass transfer and reaction process, are similar to that of traditional chemical reactions. Due to

the good technical compatibility with chemical engineering, biocatalysis can indiscriminately use the existing technology of chemical engineering (e.g. reactor design and scaling-up, reaction process design, purification method of product, assessment criterion system and cost accounting) with some minor modifications. However, the surface dependence of electron transfer of bioelectrocatalysis has to be coupled with three dimensional bioprocesses including bioreaction, mixing, substrate supply and gas transfer in liquid reaction medium[209]. As the reaction characteristic of bioelectrocatalysis is distinct from that of biocatalysis and chemical catalysis, the existing mature theories, standards and technical methods of biocatalysis and chemical catalysis are not fit to bioelectrocatalysis. Consequently, There is no corresponding standards for the benchmarking of bioelectrochemical processes versus established processes[210]. This drawback limits the engineered application of bioelectrocatalysis. Therefore, the theories, standards, and technical methods which apply to the engineered application of bioelectrocatalysis are in urgent need. Some beneficial attempts have been carried out recently [211].

The bioelectrocatalytic reactor is the core component of the bioelectrocatalysis system. In the lab scale, the main reactor is an H-cell, a two-chamber glass reactor. The major disadvantage of the H-cell is the high electrical internal resistance which limits the possibility of scaling it up to a pilot or industrial scale[212]. Single chamber reactors are relatively easy to be scaled-up. However, an enzyme-toxic or cell-toxic product can be produced on the counter electrode. The stirred tank reactor with good gassing and mixing is another option. Unfortunately, this reactor still has some drawbacks such as difficulty in scale-up, unequally distributed electrical field, and the inhibition of shear forces on biofilm growth[212,213]. Correspondingly, the design and development of a wide variety of new types

of bioelectrocatalysis reactors are required. In this field, Holtmann and coworkers have carried out fruitful works. They systematically summarized current bioelectrocatalytic reactors[211], developed a scalable bubble column reactor and scaled-up it to 50 L pilot scale[212,214]. The scale-up of reactor is another field which is essential for the engineered application bioelectrocatalysis. Several large scale tests have been carried out in bioelectrochemistry[209,215]. However, these reactors were not designed by using rational scale-up methods but empirical methods. The empirical method is mainly trial and error experiments with larger electrodes and the effect of scale-up is not satisfactory[216,217]. So far, the rational scale-up method of bioelectrocatalysis has not been shown. Consequently, it is urgently needed as the rational method is indispensable to allow better characterization, modeling, optimization, and comparison of different bioelectrocatalysis systems[214].

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Competing interests

The authors declare no competing interests.

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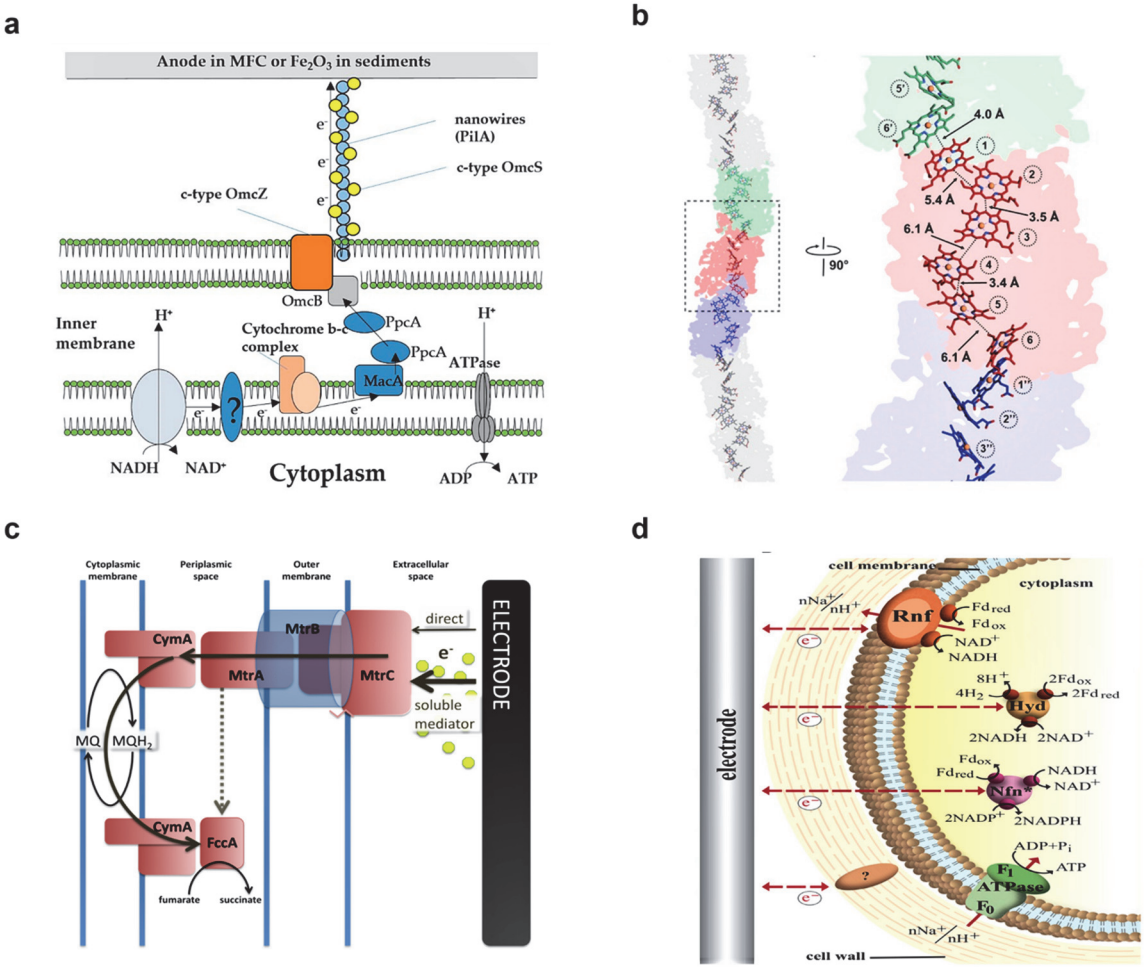


Figure 1. The structural basis of electroactive microbial cells for electron transfer. a, The electron transfer mechanism and conductive pili structure of *Geobacter sulfurreducens*. MFC: microbial fuel cell; OMS (OmcS, OmcZ and OmcB): C-type cytochromes; PpcA: triheme periplasmic cytochrome; MacA: diheme c-type cytochrome. Reprinted with permission from ref 57. Copyright 2019 MDPI. **b**, The structure of *G. sulfurreducens* nanowires reveals closely stacked hemes in an OmcS filament. Reprinted with permission from ref 61. Copyright 2019 Elsevier. **c**, The electron transfer mechanism and conductive membrane structure of *Shewanella oneidensis*. MtrA and MtrC: multiheme c-type cytochromes; MtrB: non-heme outer membrane β-barrel that connects MtrA and MtrC; CymA: cytoplasmic membrane associated quinol oxidase; FccA: fumarate reductase; MQ:

menaquinones. Reprinted with permission from ref 72. Copyright 2011 PLOS. **d**, The electron transfer mechanism and conductive membrane structure of some clostridia, acetogens, and methanogens. Hyd and Nfn: soluble electron-bifurcating complex; Rnf complex: membrane-bound Fd:NAD^+ oxidoreductase; Fd:ferredoxin. Reprinted with permission from ref 74. Copyright 2015 Frontiers.

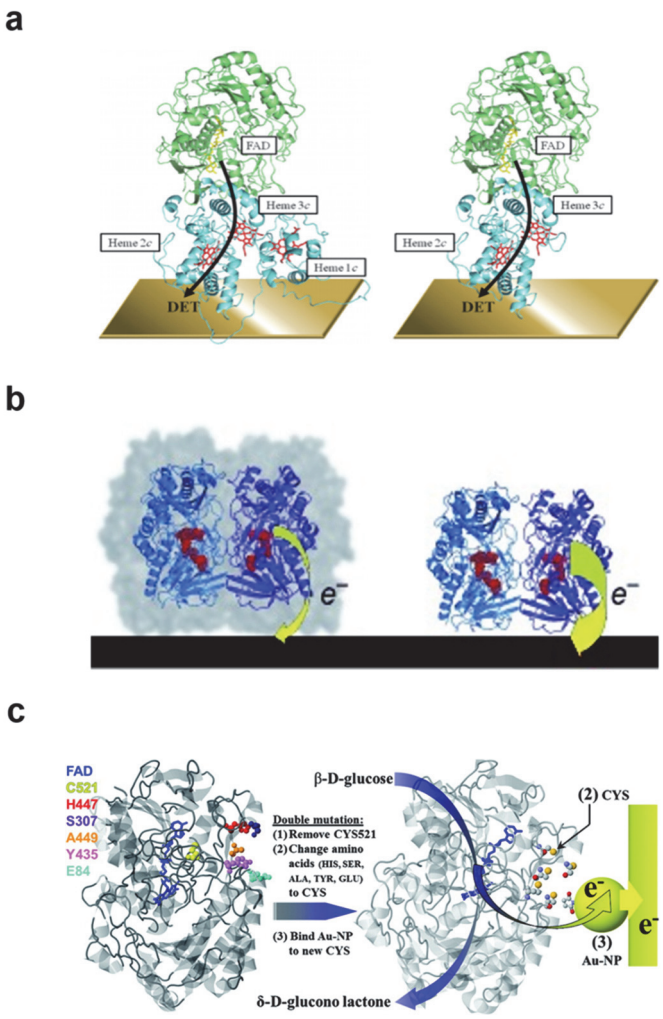


Figure 2. Three modification strategies of oxidoreductase to enhance the electron transfer. a, Truncation of oxidoreductase. The deletion of heme 1c binding domain which does not involve in the electron transfer pathway facilitates electron transfer. FAD: flavin adenine dinucleotide; DET: directed electron transfer.Reprinted with the permission from ref 78. Copyright 2018 J-STAGE. **b**, Surface modification. Deglycosylation of glucose oxidase decreases the distance between active site and electrode and facilitates the electron transfer. Reprinted with the permission from ref 79. Copyright 2009 John Wiley and Sons. **c**, Active site mutation. Genetic modification of a glucose oxidase to display a free thiol group near its active site which facilitates the site-specific attachment of a maleimide-

modified gold nanoparticle and enable direct electrical communication. Au-NP: gold nanoparticle; CYP: cytochromes P450. Reprinted with the permission from ref 82.

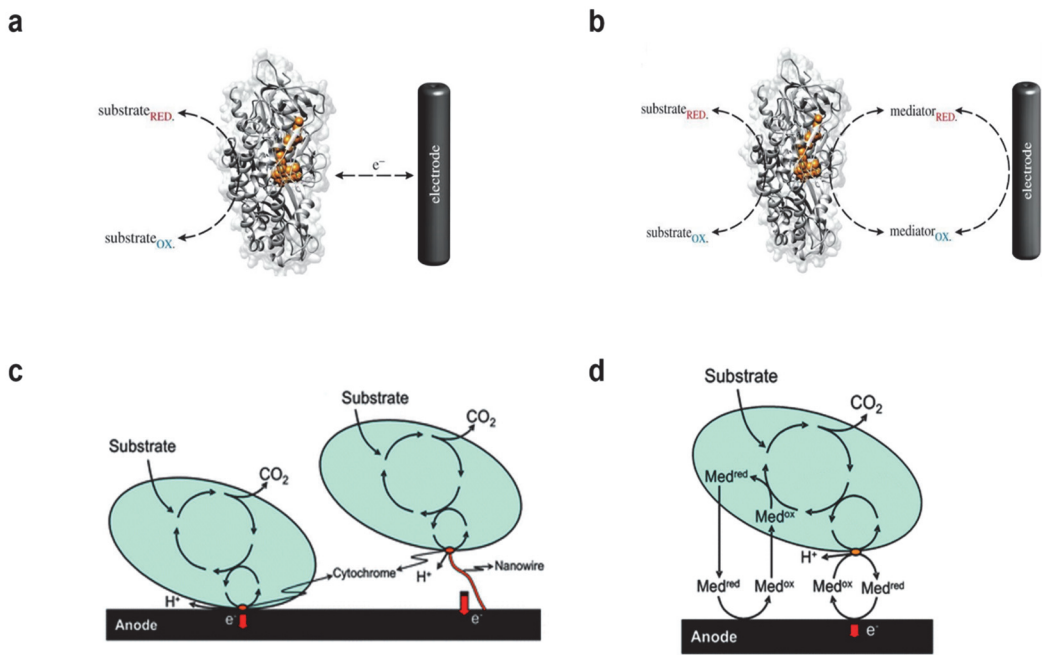


Figure 3. The MET and DET of oxidoreductase and electroactive microbial cell. a, DET of bioelectrochemical oxidation or reduction in a substrate by an oxidoreductase. Reprinted with the permission from ref 5. Copyright 2017 Royal Society. **b**, MET of bioelectrochemical oxidation or reduction of a substrate by an oxidoreductase. Reprinted with the permission from ref 5. Copyright 2017 Royal Society. **c**, illustration of the DET via membrane bound cytochromes or electronically conducting nanowires. Reprinted with the permission from ref 93. Copyright 2007 Royal Society of Chemistry. **d**, schematic illustration of MET via microbial metabolites. Two possible mechanisms have been proposed: shuttling via outer cell membrane cytochromes and via periplasmatic or cytoplasmatic redox couples. Reprinted with the permission from ref 93. Copyright 2007 Royal Society of Chemistry.

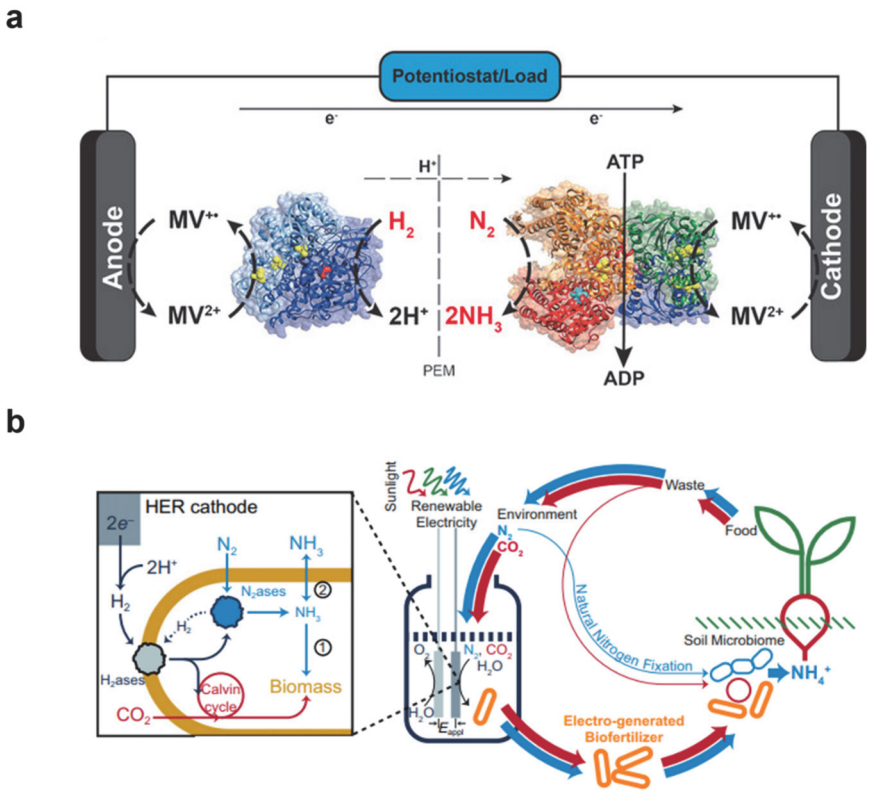


Figure 4. The bioelectrocatalytic N_2 fixation based on isolated nitrogenase and microbial cell. a, Compartmentalization of hydrogenase and nitrogenase Fe/MoFe proteins by the use of a proton exchange membrane (PEM) leads to a configuration that is able to utilize methyl viologen as the electron mediator in both chambers and simultaneously produces NH_3 and electrical energy from H_2 and N_2 at room temperature and ambient pressure. Reprinted with the permission from ref 24. Copyright 2017 John Wiley and Sons. **b**, Schematic of the electroaugmented nitrogen cycle. A constant voltage (E_{appl}) is applied between CoPi OER and Co-P HER electrode for water splitting. Hydrogenase of *X. autotrophicus* oxidizes the H_2, fueling CO_2 reduction in the Calvin cycle and N_2 fixation by nigrogenase. The generated NH_3 is typically incorporated into biomass (pathway 1), but can also diffuse extracellularly by inhibiting biomass formation (pathway 2). *X. autotrophicus* forms an electrogenerated biofertilizer that can be added to soil to

improve plant growth. Red pathway indicates carbon cycling; blue pathways indicate nitrogen cycling. CoPi: oxidic cobalt phosphate; OER: oxygen evolution reaction; HER: hydrogen evolution reaction.Reprinted with the permission from ref 147. Copyright 2017 National Academy of Science.

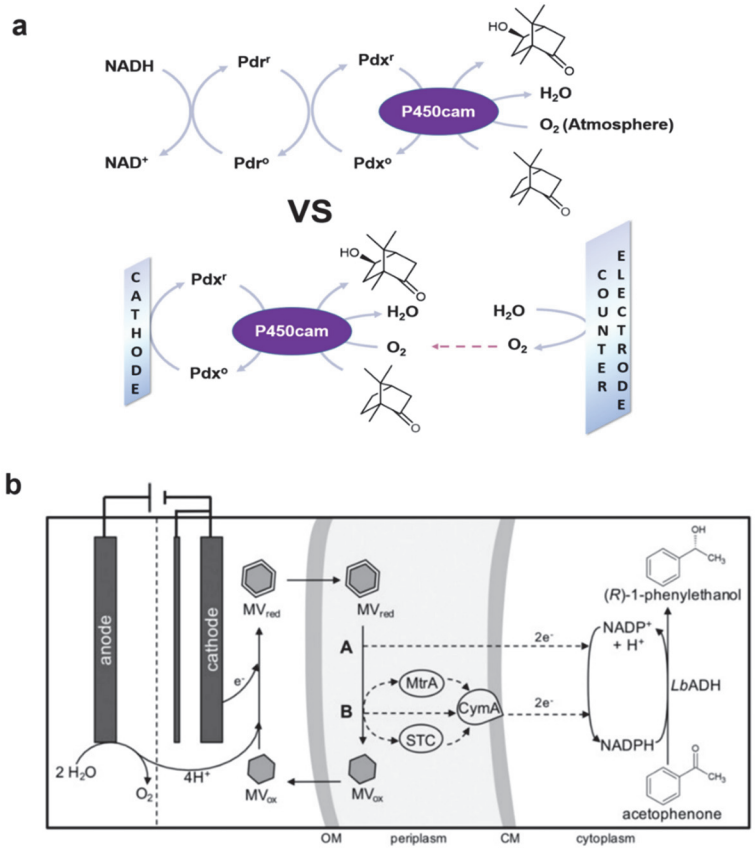


Figure 5. The bioelectrochemical preparation of chiral alcohol based on isolated P450cam and engineered *E.coli* cell. a, Natural (upper) and direct-electrode-driven (lower) catalytic cycles for camphor hydroxylation by the P450cam system. Pdr^r: reduced putidaredoxin reductase; Pdr^o: oxidized putidaredoxin reductase; Pdx^r: reduced putidaredoxin; Pdx^o: oxidized putidaredoxin. Reprinted with the permission from ref 157 with some modification. Copyright 1997 National Academy of Sciences. **b**, Microbial electrosynthesis of chiral alcohols with *E. coli*. The cytoplasmatic NADPH-pool is linked to the cathode by using extracellular electron transfer through methyl viologen as mediator and further periplasmatic cytochromes. In the cytoplasm, the enantioselective reduction takes place. OM: outer membrane; CM: cytoplasmic membrane. Reprinted with the permission from ref 161 with some modification. Copyright 2019 John Wiley and Sons.

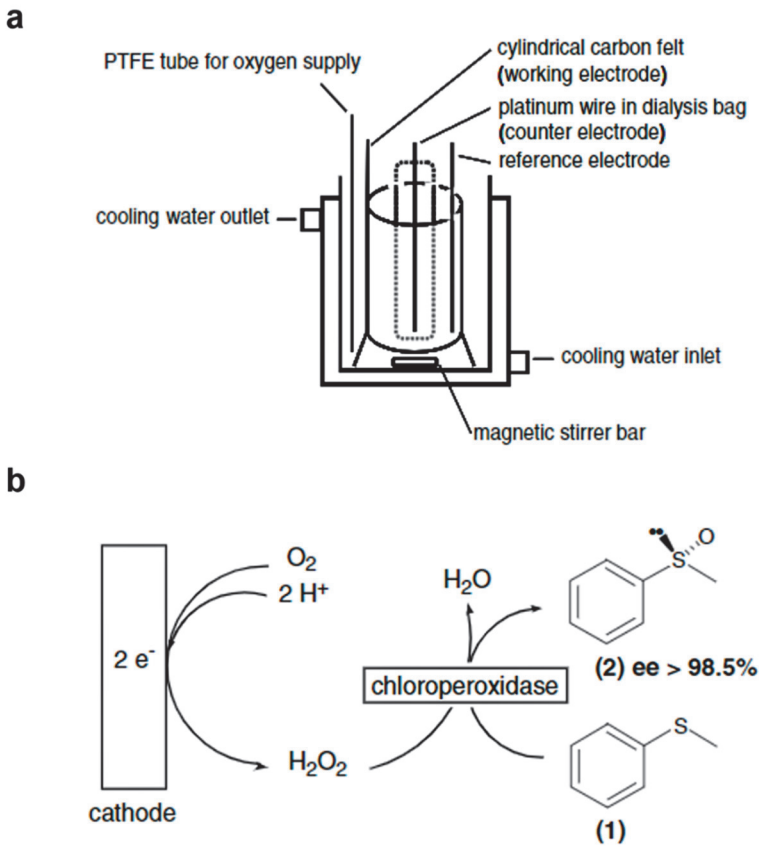
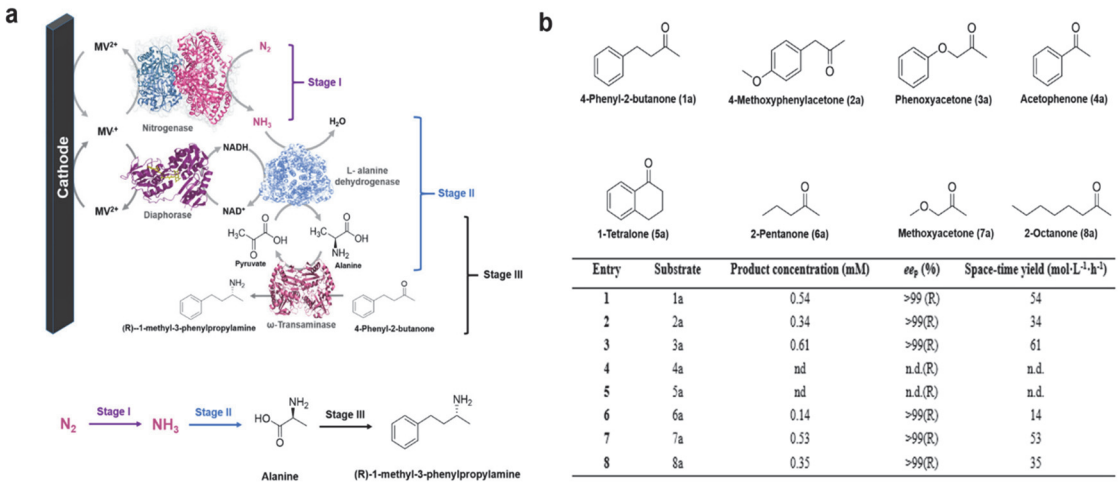


Figure 6. The bioelectrochemical cell design and reaction process of (*R*)-methylphenylsulfoxide preparation. **a**, Scheme of the bioelectrochemical cell. **b**, Electroenzymatic oxidation of thioanisole (*1*) to (*R*)-methylphenylsulfoxide (*2*) catalyzed by chloroperoxidase. Reprinted with the permission from ref 29. Copyright 2004 Elsevier.



Entry	Substrate	Product concentration (mM)	ee_p (%)	Space-time yield (mol·L^{-1}·h^{-1})
1	1a	0.54	>99 (R)	54
2	2a	0.34	>99(R)	34
3	3a	0.61	>99(R)	61
4	4a	nd	n.d.(R)	n.d.
5	5a	nd	n.d.(R)	n.d.
6	6a	0.14	>99(R)	14
7	7a	0.53	>99(R)	53
8	8a	0.35	>99(R)	35

Figure 7. The upgraded bioelectrocatalytic N_2 fixation. a,Schematic representation of the upgraded bioelectrocatalytic N_2 fixation system and the conversion route from N_2 to the chiral amine intermediate. **b**, asymmetric amination of various prochiral ketones catalyzed by the upgraded N_2 fixation system . Reprinted with the permission from ref 27. Copyright 2019 American Chemical Society.

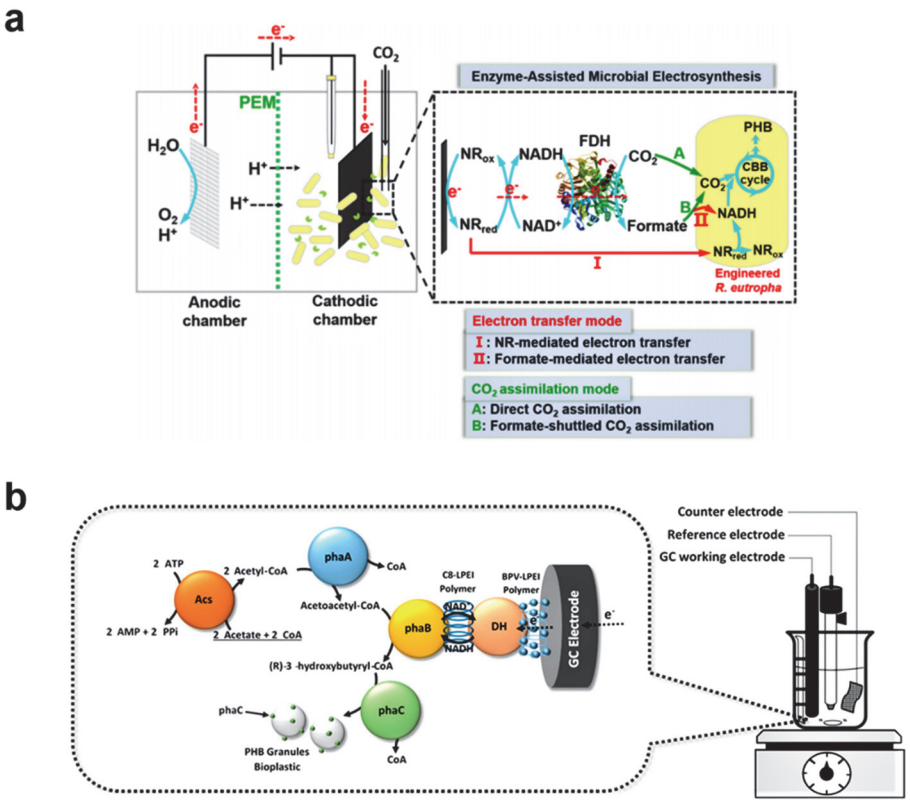


Figure 8. Sustainable bioelectrosynthesis of the bioplastic polyhydroxybutyrate (PHB). a, Schematic of the FDH-assisted microbial electrosynthesis (MES) system to reduce CO_2 for the synthesis of PHB. FDH: formate dehydrogenase; CBB: Calvin-Benson-Bassham cycle. NR: neutral red. Reprinted with the permission from ref 32. Copyright 2018 American Chemical Society. **b**, representation of the bioelectrocatalytic conversion of acetate into PHB. The reaction is mediated by four enzymatic reactions and an additional one on the modified electrode for the NADH regeneration. Acs: acetyl-CoA synthase, phaA: β-ketothiolase, phaB: acetoacetyl-CoA reductase, phaC: PHB synthase, DH: diaphorase. C8-LPEI: octyl-linear polyethylenimine; BPV-LPEI: *N*-benzyl-*N*-propyl-4,4'-bipyridimium-modified linear polyethylenimine, benzylpropylviologen. Reprinted with the permission from ref 31. Copyright 2018 American Chemical Society.