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Artificial extracellular electron transfer engineering in nonclassical electroactive microorganisms for microbial electrochemical technologies

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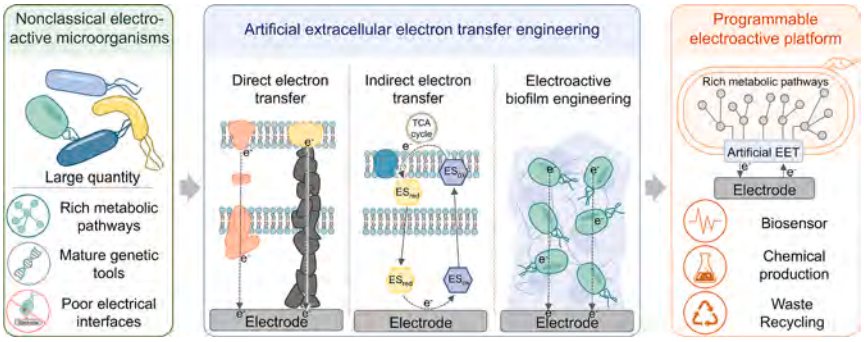
HIGHLIGHTS

- Artificial EET expands METs beyond naturally EAMs.
- DET, IET, and electroactive biofilms guide artificial EET construction.
- Host compatibility and redox alignment guide EET module selection.
- Functional electron flow is distinguished from apparent electroactivity.
- Engineered nonclassical EAMs enable biosensing, electrosynthesis, and remediation.

GRAPHICAL ABSTRACT



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ABSTRACT

Microbial electrochemical technologies (METs) couple microbial metabolism with extracellular electronic interfaces, but their broader application is limited by the small repertoire of naturally electroactive microorganisms. Nonclassical-electroactive microorganisms (nonclassical-EAMs) offer diverse metabolic functions, mature genetic tools, and environmental robustness, yet they usually lack efficient extracellular electron transfer (EET) pathways. This review critically examines artificial EET engineering as a strategy to convert function oriented nonclassical-EAMs for bioresource and environmental applications. We first summarize natural EET mechanisms as design templates, with an emphasis on cell-level direct and indirect electron transfer mechanisms as well as biofilm-level electron transfer mechanisms. We then compare three major engineering routes: genetically encoded or material-assisted direct electron transfer, endogenous or exogenous electron shuttles based indirect electron transfer, and electroactive biofilm engineering. Attention is given to host compatibility, redox-potential alignment, electron transfer directionality, metabolic coupling, and operational stability. Emerging applications in microbial biosensing, microbial electrosynthesis, carbon dioxide conversion, waste valorization, and pollutant remediation are discussed from the perspective of whether engineered electron flow is productively linked to target functions. Finally, we propose a demand-driven framework for selecting EET modules according to host physiology, application goals, electron-flow direction, and system constraints. Future progress will depend on quantitative electron-flow analysis, dynamic flux control, stable biofilm interfaces, and standardized

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performance metrics that distinguish functional electron transfer from apparent electroactivity. Artificial EET engineering of nonclassical-EAMs may expand METs into a more versatile platform for sustainable bioconversion and environmental biotechnology.

1. Introduction

Microbial electrochemical technologies (METs), including microbial fuel cells, microbial electrolysis cells, microbial electrosynthesis systems, and microbial electrochemical sensors, provide promising platforms for coupling microbial metabolism with extracellular electronic interfaces (Fan et al., 2024; Rabaey & Rozendal, 2010), thereby enabling wastewater treatment (Cao et al., 2021; Chen et al., 2026a; Chen et al., 2026b; Wang et al., 2016; Xiao et al., 2021; Yan et al., 2018), resource recovery (Logan & Rabaey, 2012), biosensing (Choi, 2022; Han et al., 2023; Li et al., 2025a), electro-fermentation (Tejedor-Sanz et al., 2022), and the sustainable production of value-added chemicals through microbial electrosynthesis (Li et al., 2025b; Xie et al., 2023). The biological basis of METs is a microbial metabolic process known as extracellular electron transfer (EET), through which electroactive microorganisms (EAMs) exchange electrons with extracellular electron acceptors or donors, including minerals and electrodes (Aiyer & Doyle, 2022; Shi et al., 2016). However, the performance and functional scope of METs remain constrained by the limited microbial resources currently available for efficient and controllable EET.

To date, the development of METs has relied largely on naturally occurring EAMs, particularly model EAMs such as *Shewanella* and *Geobacter* (Logan et al., 2019). These EAMs have provided fundamental insights into natural EET mechanisms and underpinned the development of diverse METs. Nevertheless, many EAMs still show limitations in substrate utilization, product synthesis, environmental adaptability, or genetic accessibility (Harnisch et al., 2024). Moreover, to date only a relatively limited number of microorganisms have been systematically characterized as EAMs, which restricts the biological diversity available for the development of METs (Logan et al., 2019; Paquete et al., 2022). Although isolating new EAMs from target environments can directly expand EAM diversity, it is often time-consuming due to the extensive characterization required to elucidate their EET and metabolic pathways (Szydlowski et al., 2022; Yuan et al., 2014). Therefore, next-generation METs require not only the exploration of natural EAMs, but also the expansion of electroactivity into microorganisms selected for specific functional tasks.

Nonclassical electroactive microorganisms (nonclassical-EAMs) are microorganisms that are not typically classified as classical EAMs and whose native EET capability has not been clearly demonstrated or is relatively weak, thereby limiting efficient electron transfer, such as *Escherichia coli* (Kundu et al., 2025), *Citrobacter freundii* (Xiao et al., 2021), *S. aureus* (Zhang et al., 2023b), *Clostridium intestinale* (Liu et al., 2024). However, many nonclassical-EAMs possess diverse metabolic capabilities, mature genetic tools, well-characterized regulatory networks and established industrial or environmental application backgrounds (d'Espaux et al., 2017; Pontrelli et al., 2018). They can degrade pollutants, synthesize value-added chemicals, and tolerate harsh environmental conditions (Kim et al., 2025; Nielsen & Keasling, 2016). This creates a central mismatch: nonclassical-EAMs offer desirable metabolic functions but cannot be efficiently connected to electrodes. Artificial EET engineering provides a promising solution by enabling nonclassical-EAMs to establish efficient electronic connections with electrodes. Recent advances in synthetic biology and conductive materials have enabled artificial EET construction in diverse nonclassical-EAMs (Atkinson et al., 2022; Kundu et al., 2025; Wang et al., 2026). In this context, the key challenge is not simply to increase current output, but to determine whether engineered electron flow can be efficiently, directionally, and stably coupled to target metabolic or environmental functions.

Several previous reviews have summarized natural EET mechanisms, electroactive biofilms, EAM enrichment, and general MET applications (Harnisch et al., 2024; You et al., 2023; Zhang et al., 2023a; Zhang et al., 2024a). However, few have critically examined how artificial EET can be rationally installed in nonclassical-EAMs and whether the resulting electron flow can be functionally coupled to specific applications. This review focuses on artificial EET engineering in nonclassical-EAMs by summarizing natural EET mechanisms as design templates, comparing direct electron transfer (DET), electron shuttle-mediated indirect electron transfer (IET), and electroactive biofilm engineering, and discussing their applications in microbial biosensing, microbial electrosynthesis, carbon dioxide conversion, waste valorization, and pollutant remediation. Finally, key research needs are identified, including demand-driven strategy selection, functional electron-flow analysis, dynamic flux control, stable biofilm interfaces, and standardized performance evaluation.

2. Natural EET mechanisms as design templates for artificial EET engineering

For artificial EET engineering in nonclassical-EAMs, natural EET mechanisms provide a blueprint for understanding how electrons are transferred from intracellular metabolism to extracellular interfaces. In this section, we examine how metabolism-derived electrons are routed across the cell membrane, how electroactive biofilms maintain interfacial electron transfer, and how these mechanisms can be translated into general engineering principles for constructing artificial EET in nonclassical-EAMs.

2.1. Cell-level EET templates

At the cellular level, natural EET provides two major templates for artificial EET construction: DET and IET. DET involves electron exchange through direct physical or electronic contact between cells and extracellular electron acceptors or donors, whereas IET relies on diffusible electron shuttles that shuttle electrons between cells and extracellular redox interfaces. DET is mediated mainly by redox proteins and conductive cellular structures that establish EET pathways between cells and extracellular interfaces (Fig. 1**A**). In model EAMs such as *Shewanella oneidensis* and *Geobacter sulfurreducens*, multiheme *c*-type cytochromes form transmembrane electron transfer chains that connect intracellular quinone pools with extracellular interfaces (Choi & Sang, 2016; Shi et al., 2016; Zacharoff & El-Naggar, 2017). A representative example is the Mtr pathway of *S. oneidensis* MR-1, in which electrons are transferred from CymA through periplasmic *c*-type cytochromes to the outer-membrane MtrCAB/OmcA complex (Hirose et al., 2018). Conductive appendages, including outer-membrane extensions and nanowires, can further extend electron transfer networks beyond the cell surface and improve electrical contact with extracellular interfaces (Pirbadian et al., 2014; Reguera et al., 2005; Ueki, 2021). Gram-positive *Thermincola* sp. can also perform DET through cell membrane associated multiheme *c*-type cytochromes that transfer electrons across the thick peptidoglycan layer to extracellular acceptors (Carlson et al., 2012; Costa et al., 2019). These natural DET systems indicate that efficient artificial DET cannot be achieved by introducing a single redox component alone. Instead, it requires a continuous, host-compatible, and directionally aligned DET pathway that links intracellular electron donors or acceptors with extracellular interfaces. Therefore, when DET modules are transplanted into nonclassical-EAMs, the key question is not simply whether a *c*-type cytochrome or conductive structure can be

expressed, but whether the introduced components can form a functional electron conduit that is compatible with the cell membrane, endogenous redox metabolism, cofactor maturation capacity, and target electron-flow direction.

By contrast, IET relies on diffusible electron shuttles to transfer electrons between cells and extracellular electron acceptors or donors without direct contact (Fig. 1**B**). Natural electron shuttles include flavins, phenazines, quinones, and humic substances, whereas artificial systems may also use exogenous electron shuttles such as neutral red, methylene blue, or methyl viologen (Glasser et al., 2017; O'Loughlin, 2008). In *S. oneidensis* MR-1, flavins can accelerate electron transfer between outer-membrane *c*-type cytochromes and electrodes, while phenazines secreted by *Pseudomonas* can mediate IET without outer-membrane *c*-type cytochromes (Chen et al., 2021; Glasser et al., 2014; Lin et al., 2021; Marsili et al., 2008). Because soluble electron shuttles are diffusible, they can sustain electron transfer with redox interfaces located several centimeters away from microbial cells (Michelson et al., 2019). This feature is particularly important for nonclassical-EAMs that are unable to establish intimate cell-electrode contact or form electroactive biofilms. However, this spatial flexibility also makes shuttle-mediated EET vulnerable to electron shuttle loss, nonspecific redox reactions, and inefficient electron cycling. Effective artificial IET therefore requires appropriate electron shuttle redox potential, membrane permeability, intracellular reduction or oxidation routes, electron shuttle retention, low toxicity, and compatibility with redox metabolism (Zhang et al., 2024b). In nonclassical-EAMs, artificial IET should be designed as a controlled redox-cycling process that selectively links extracellular interfaces to relevant intracellular redox nodes while minimizing nonspecific electron leakage and metabolic disturbance.

2.2. Biofilm-level EET templates

Natural EET also shows that efficient EET is not only a cell-level process, but also an interfacial and community-level process (You et al., 2023). EAMs usually operate as electroactive biofilms rather than as isolated planktonic cells. In electroactive biofilms, cells directly contacting the electrode exchange electrons at the cell-electrode interface, whereas cells located in outer biofilm layers rely on extracellular conductive networks for long-range electron transport (Yu et al., 2020) (Fig. 1**C**). The extracellular polymeric substances matrix provides structural support and can retain redox-active components, including *c*-type cytochromes, flavins, and other electron shuttles (Wang et al., 2021; Xiao et al., 2017). Conductive filaments or nanowires further connect cell–cell and cell-electrode interfaces, thereby improving biofilm-level conductivity (Liu et al., 2019a). The biofilm template is especially important because engineered nonclassical-EAMs usually lack the naturally evolved electrode adaptation and biofilm conductivity found in model EAMs. Increasing biofilm thickness or biomass loading may improve cell retention and total electron flux, but excessive biofilm growth can restrict the diffusion of substrates, gases, light, electron shuttles, and products, thereby reducing functional electron transfer and cellular activity. Therefore, artificial electroactive biofilms should not be designed simply to maximize cell attachment or biomass accumulation. Instead, they should be engineered to balance biomass retention,

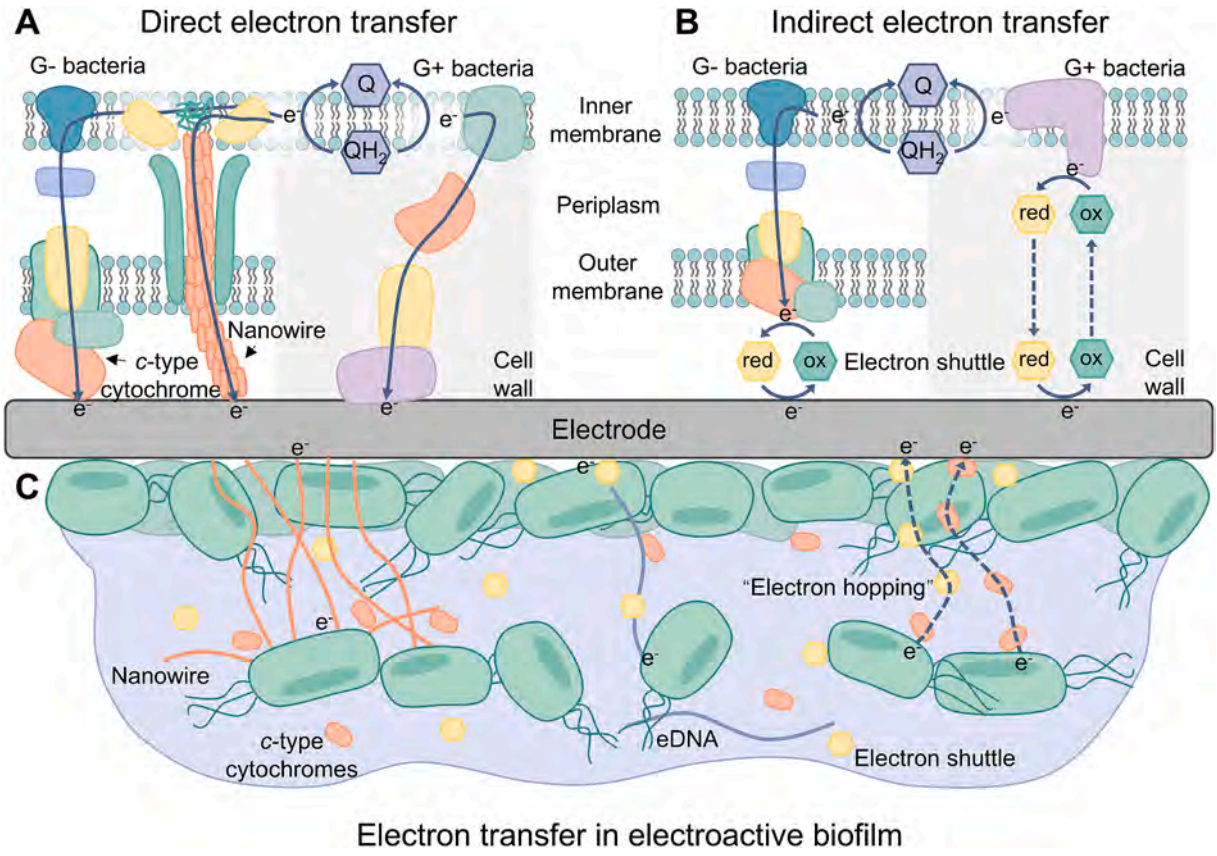

Fig. 1. Mechanisms of natural EET. (A) Direct electron transfer (DET). In Gram-negative bacteria, electrons are transferred from the inner-membrane quinone pool through periplasmic and outer-membrane *c*-type cytochromes (e.g., CymA-FccA-MtrCAB/OmcA from *Shewanella* sp.) to extracellular acceptors or electrodes, while conductive nanowires can support long-range electron transport. In Gram-positive bacteria, cell membrane associated multiheme *c*-type cytochromes mediate electron transfer across the thick peptidoglycan layer to extracellular acceptors. (B) Indirect electron transfer (IET). Diffusible electron shuttles, such as flavins, phenazines, and quinones, shuttle electrons between cells and the electrode, enabling electron exchange without direct contact. (C) Electron transport in electroactive biofilms. In biofilms, only surface cells directly contact the electrode, while inner cells rely on long-range electron transport through conductive networks formed by *c*-type cytochromes, electron shuttles, conductive nanowires, and eDNA, facilitating electron flow across the community.

electrical connectivity, and mass-transfer efficiency. This balance becomes particularly important in flowing or long-term systems, where cells, electron shuttles, and conductive materials may be washed out, diluted, detached, or spatially disconnected from interfaces.

2.3. Design logic for engineering nonclassical-EAMs

Taken together, natural DET, IET, and electroactive biofilm mechanisms indicate that effective EET is a systems-level property rather than the result of a single electron-transfer component. For artificial EET engineering in nonclassical-EAMs, four interrelated design dimensions can be extracted from natural systems. First, nonclassical-EAMs compatibility determines whether an introduced EET module can be expressed, matured, localized, retained, and connected to endogenous redox metabolism in the selected nonclassical-EAMs. Second, electron-transfer directionality determines whether the redox-potential relationships among intracellular electron carriers, EET components, electron shuttles, electrodes, and target metabolism are suitable for outward or inward electron flow. Third, metabolic coupling determines whether artificial EET contributes to the intended function, such as signal generation, product synthesis, pollutant transformation, or waste conversion, rather than producing only apparent electroactivity. Fourth, interfacial stability determines whether engineered cells, electron shuttles, materials, and biofilms can maintain effective electron transfer during long-term operation.

This integrated view shifts artificial EET engineering from component-based construction to system-level design. DET engineering, shuttle-mediated IET, and electroactive biofilm engineering should therefore not be treated as isolated strategies, but as different routes for constructing nonclassical-EAMs compatible, directional, functionally coupled, and stable electron transfer in nonclassical-EAMs. This framework provides the basis for evaluating current artificial EET strategies and for designing application-oriented engineered nonclassical-EAMs in the following sections.

3. Engineering strategies for artificial EET in nonclassical-EAMs

Based on the design logic outlined above, this section compares the implementation, suitable scenarios, and major limitations of different artificial EET engineering strategies in nonclassical-EAMs. Artificial EET engineering aims to construct controllable EET pathways between intracellular metabolism and extracellular electronic interfaces. Guided by natural EET mechanisms, current strategies can be broadly grouped into three categories: engineering DET pathways, constructing electron shuttle-mediated IET pathways, and engineering electroactive biofilm interfaces. These approaches differ in nonclassical-EAMs type, electron transfer direction, target metabolic process, and operating environment. The following sections discuss their representative implementations, advantages, limitations, and future design priorities.

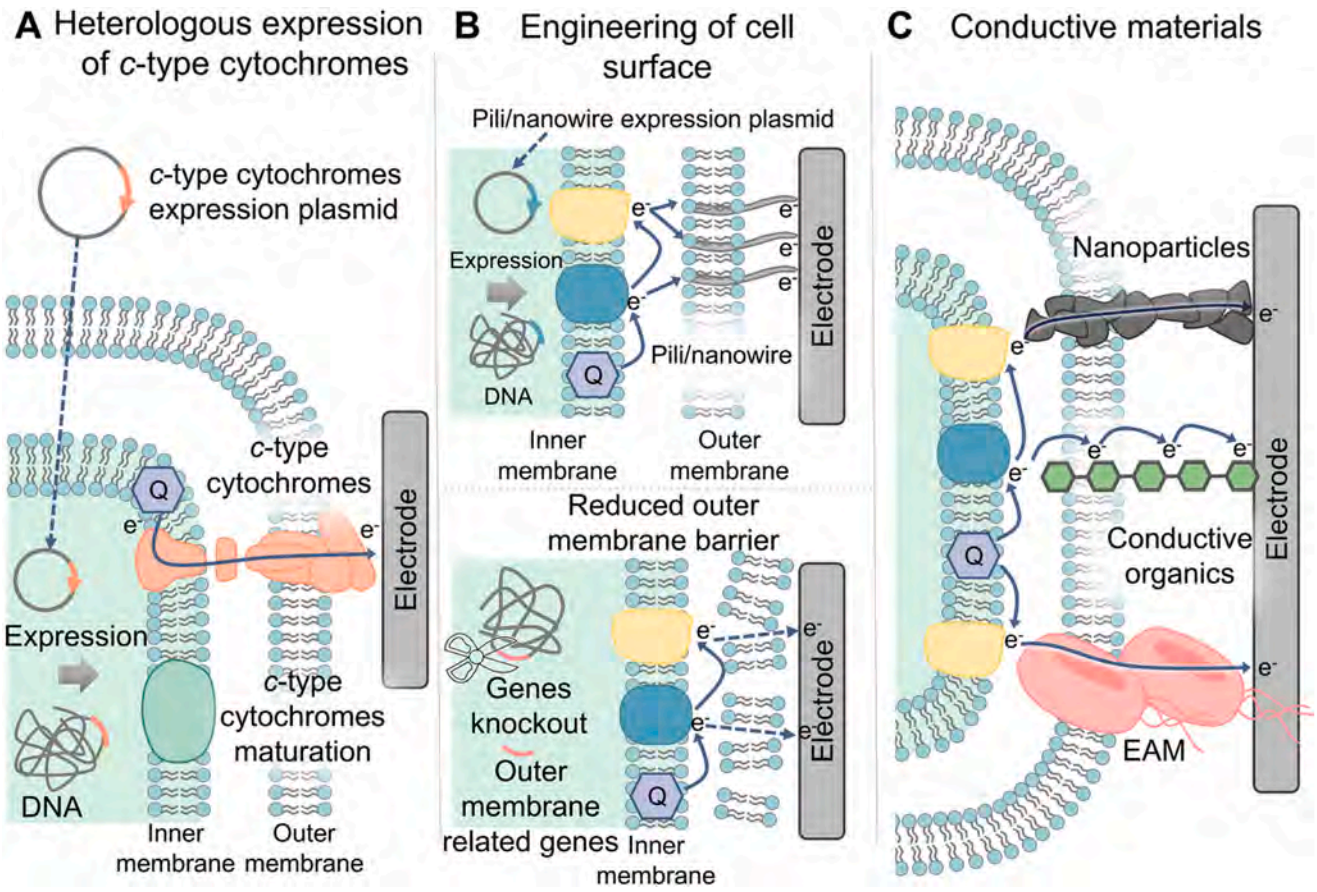

Fig. 2. Representative strategies for constructing DET pathways in nonclassical-EAMs. (A) Heterologous expression of *c*-type cytochromes and their maturation machinery to construct DET pathways. Q, quinone pool. (B) Engineering of the cell surface to enhance cell-electrode electron exchange, including heterologous expression of conductive pili or nanowires and the removal or reducing of the outer membrane barrier through knockout of outer membrane related genes. (C) Introduction of conductive materials, including nanoparticles, conductive organic molecules, and conductive biomaterials, into the periplasm or at the outer membrane/cell surface forms artificial conductive bridges for constructing DET pathways.

3.1. Engineering direct electron transfer pathways

3.1.1. Synthetic biology strategies for constructing direct electron transfer pathways

Synthetic biology-based DET engineering aims to reconstruct genetically encoded DET pathways in nonclassical-EAMs by introducing natural EAM-derived EET components. Its main advantage is that the introduced electron-transfer machinery can, in principle, be maintained and renewed during cell growth, making it more suitable for long-term and programmable bioelectrochemical systems than non-renewable external modifications. Heterologous expression of the *S. oneidensis* MR-1 Mtr pathway, together with the Ccm *c*-type cytochrome maturation system, in *Escherichia coli* provided an early proof of concept that nonclassical-EAMs can acquire detectable electroactivity through EET pathway transplantation (Donald et al., 2008; Jensen et al., 2010) (Fig. 2**A**). However, this proof-of-concept also shows that pathway transplantation alone is insufficient for efficient electron flux. A series of optimization studies in engineered *E. coli* further illustrates this limitation. Transcriptional tuning of Mtr/Ccm expression and directed evolution of the *c*-type cytochrome maturation component CcmH enhanced current output by increasing the maturation and expression levels of *c*-type cytochromes (Goldbeck et al., 2013; Su et al., 2020). Electron entry from intracellular metabolism is another critical constraint. Additional expression of inner-membrane or periplasmic *c*-type cytochrome proteins, including NapC/NapB, CymA, and STC, mainly reinforced transmembrane and periplasmic electron transfer steps, thereby further improving the artificial DET pathway and significantly increasing current output (Mouhib et al., 2023). These findings indicate that efficient DET also requires a continuous electron-transfer chain that connects intracellular redox metabolism with periplasmic and outer-membrane electron carriers. Heterologous expression of the Mtr pathway in the marine nonclassical-EAM *Marinobacter atlanticus* further suggests that the Mtr pathway has some degree of portability (Bird et al., 2023). Nevertheless, this portability should not be interpreted as universal transferability. Differences in cell-membrane architecture, membrane composition, redox metabolism, and protein maturation capacity can strongly influence whether a DET module functions in a new nonclassical-EAMs. Thus, future DET engineering should match EET modules with metabolism instead of assuming that a pathway optimized in one organism can be directly transferred to another.

Synthetic biology can also improve DET by engineering cell-surface structures. The objective of this strategy is to reduce electronic barriers between the cell and the electrode by creating new conductive appendages or modifying insulating surface layers (Fig. 2**B**). For example, *E. coli* pili were engineered by incorporating aromatic amino acids, particularly tryptophan, thereby generating programmable self-assembling conductive protein nanowires and increasing conductivity by more than 80-fold (Shapiro et al., 2022). To address the low outer-membrane permeability of autotrophic cyanobacteria and their limited coupling to external electron interfaces, the nanowire protein OmcS from *Geobacter* was heterologously expressed in an engineered *Synechococcus elongatus* PCC 7942 strain, thereby constructing DET and enhancing NH_3 production by approximately 13-fold (Dong et al., 2021). Conversely, repression of slr0688 in *Synechocystis* sp. PCC 6803 weakened the anchoring of the outer membrane to the peptidoglycan layer, leading to substantial outer-membrane detachment in the *slr0688i* mutant. This modification produced an order-of-magnitude enhancement in photocurrent generation by reducing the outer membrane permeability barrier and facilitating electron transfer (Kusama et al., 2022). These examples show that surface engineering can reduce interfacial electronic barriers, but they also highlight a trade-off between improved electron transfer and cell-membrane integrity. Excessive modification of cell-surface structures may compromise cell viability, stress tolerance, or long-term stability.

Therefore, genetically encoded DET should be developed as a modular, nonclassical-EAMs compatible electron transfer system rather than as simple pathway transplantation. An effective EET pathway must continuously connect intracellular reducing equivalents with periplasmic and outer-membrane electron carriers while remaining compatible with host metabolism, cofactor maturation, membrane localization, and cellular growth. The central challenge is to convert enhanced DET into functional electron flow, in which electrons derived from intracellular redox metabolism are transferred through the engineered DET pathway and quantitatively coupled to the intended metabolic process. Otherwise, increased expression of EET components may merely divert reducing equivalents from central metabolism, resulting in electron leakage. Current evidence supports genetically encoded DET primarily as an outward electron transfer strategy, whereas efficient inward transfer requires dedicated electron inward components that couple cathodic electrons to intracellular reductive metabolism. Future designs should therefore coordinate intracellular electron supply, DET pathways expression, and target metabolism. Their performance should be evaluated by examining the relationship between current generation and target metabolic processes, the extent of the intended reaction or product formation, and the long-term stability of the engineered DET pathways.

3.1.2. Material-assisted construction of direct electron transfer pathways

Material-assisted strategies aim to construct artificial conductive bridges between microbial metabolism and electrodes, thereby reducing interfacial resistance and facilitating DET (Cao et al., 2021; Yu et al., 2020). Compared with genetic reconstruction of native EET pathways, this approach provides a more rapid and chassis-flexible route for conferring electroactivity on nonclassical-EAMs. However, material-enabled DET should not be viewed as a simple substitute for synthetic biological EET machinery, because its performance depends on whether the introduced materials can form electronically continuous, biocompatible, and metabolically connected interfaces. Conductive materials differ substantially in electrical conductivity, particle size or molecular dimensions, morphology, synthesis and assembly methods, cellular localization, and biocompatibility, all of which can strongly influence material-microbe interface formation and DET efficiency(Li et al., 2026; Zhang et al., 2018).

Inorganic conductive materials, characterized by small particle sizes, relatively high electrical conductivity, and compatibility with in situ biomineralization or intracellular deposition, have been widely used as particulate electron relays to construct DET pathways in nonclassical-EAMs. In situ deposition of palladium nanoparticles within the periplasm and on the cell surface of the sulfate-reducing bacterium *Desulfovibrio desulfuricans* markedly enhanced DET capability (Wu et al., 2011), while biosynthesized FeS nanoparticles enabled *Desulfovibrio vulgaris* to use electrode-derived electrons for sulfate reduction (Deng et al., 2020). Similarly, FeS nanoparticle synthesis in *Citrobacter freundii* JH increased current output by 54-fold and shifted sulfamethoxazole degradation from an intracellular process to the extracellular interface, leading to a 10-fold improvement in degradation efficiency (Liu et al., 2023). These examples indicate that inorganic materials can act as artificial electron relays, but they also show that material localization is critical. Materials must be positioned close to relevant redox-active cellular structures; otherwise, increased conductivity at the cell surface may not translate into effective coupling with intracellular metabolism. More recent studies have moved toward hybrid strategies that combine biological targeting with conductive materials. Displaying a gold-binding peptide on type IV pili of *Synechocystis* sp. PCC 6803 enabled targeted AuNP assembly and increased photocurrent density by approximately 4.3-fold (Wang et al., 2026). Compared with nonspecific material deposition, targeted assembly provides better spatial control over material localization and may improve electronic contact with defined cellular structures. This suggests that material-assisted EET should move from passive material loading toward spatially programmed bioelectronic interface construction.

Compared with particulate inorganic materials, conductive organic

materials generally possess tunable molecular structures, greater conformational flexibility, and better compatibility with lipid membranes, thereby providing a relatively mild means of constructing membrane-associated artificial DET pathways through molecular insertion, adsorption, or self-assembly. Carbon dots restored current generation in the outer-membrane *c*-type cytochrome mutant *S. oneidensis* MR-1 Δ*omcA/mtrC* from approximately 1.5 μA to 28.1 μA (Yang et al., 2020), and were further used to build artificial transmembrane electron conduits in *E. coli* and *S. aureus*, increasing microbial fuel cell power output by 172-fold (Zhang et al., 2023b). Amphiphilic conjugated oligoelectrolytes such as $DSSN^+$ can spontaneously insert into phospholipid bilayers and enhance electricity generation in *E. coli* K-12 microbial fuel cells to 300 % of the original level (Garner et al., 2010; Wang et al., 2013). These studies show that organic conductive materials can reduce membrane-associated electron-transfer barriers, but they also highlight the need to balance conductivity with membrane compatibility. Excessive membrane insertion or high material loading may disrupt membrane integrity, substrate transport, or cellular growth.

Naturally derived conductive biomaterials represent a potentially promising class of materials for constructing artificial DET pathways because of their favorable biocompatibility and environmental compatibility; however, relevant studies remain limited, their EET efficiency is relatively modest, and their modes of fusion with host cells and spatial localization remain difficult to resolve. *c*-type cytochrome-enriched vesicles secreted by *G. sulfurreducens* were used to modify *E. coli*, thereby conferring DET capability, which was proposed to result from vesicle fusion with the *E. coli* outer membrane to form electron conduits enabling electron transport from the inner membrane through the periplasm to the outer membrane (Liu et al., 2020). Besides, *G. sulfurreducens* could serve as an electron conduit for the nonclassical-EAM *Clostridium intestinale*; after co-cultivation and cell fusion, *C. intestinale* was able to transfer electrons to the electrode through the *c*-type cytochromes network on the *G. sulfurreducens* membrane (Liu et al., 2024). These bio-derived strategies are attractive because they use naturally evolved conductive components, but their mechanisms, reproducibility, and long-term stability require further clarification before they can be broadly applied.

The main advantage of material-assisted strategies is their broad chassis range. Because they do not fully depend on nonclassical-EAMs genetic tractability or precise expression of multi-component cytochrome pathways, they can be applied to diverse nonclassical-EAMs, including organisms that are difficult to engineer genetically **(**Fig. 2**C)**. However, enhanced conductivity does not necessarily generate functional electron flow. In most of the studies discussed above, conductive materials were deposited or assembled in a largely nonspecific manner, making it difficult to control their connection with the target metabolic pathway. Establishing functional electron flow therefore requires the spatially controlled localization of conductive materials near key redox enzymes, electron carriers, or other electron transfer nodes associated with the intended metabolism, thereby directing the transferred electrons toward target processes such as pollutant degradation or product formation. Although conductive materials are intrinsically compatible with bidirectional electron flow, material-assisted DET has been demonstrated more extensively for electron outward than for electron inward, which requires more precise material localization and redox matching. Future material-assisted DET should therefore optimize material localization, redox properties, retention, and biocompatibility while strengthening its integration with endogenous electron transfer chains and target metabolic processes.

3.2. Engineering electron shuttle mediated indirect electron transfer pathways

3.2.1. Endogenous biosynthesis of electron shuttles

Engineering nonclassical-EAMs to biosynthesize electron shuttles represents an important strategy for constructing artificial IET pathways. By continuously biosynthesizing diffusible redox-active molecules, engineered cells can mediate bidirectional electron exchange with extracellular electron donors or acceptors, thereby partially bypassing the insulating barrier of the cell membrane. Phenazines are among the most widely engineered endogenous electron shuttles because they can function as diffusible redox electron shuttles and are genetically encoded by relatively well-defined biosynthetic pathways. Heterologous reconstruction of pyocyanin biosynthesis in nonclassical-EAMs such as *Pseudomonas putida* KT2440 and *E. coli* has demonstrated that engineered phenazine production can construct artificial IET pathways and increase current output (Feng et al., 2018; Schmitz et al., 2015). These studies show that heterologous construction of pyocyanin biosynthesis is sufficient to enable nonclassical-EAMs to endogenously produce phenazine electron shuttles and construct artificial IET pathways. But higher electron shuttle production should not automatically be regarded as better, its effect must be evaluated together with growth, redox balance, and target metabolic performance (Fig. 3**A**).

Flavin-mediated IET further indicates that the redox activity of electron shuttles alone is insufficient to confer electroactivity on nonclassical-EAMs, as their function also depends on their ability to cross the cell membrane. In *E. coli*, flavin-mediated IET can be established by expressing the flavin-permeable porin OprF, which increases cell membrane permeability and enables flavin transport across the cell membrane (Fig. 3**B**) (Yong et al., 2013). In engineered *E. coli* equipped with Mtr pathway, enhancement of flavin biosynthesis increased extracellular FMN and riboflavin concentrations, thereby coupling flavin-mediated IET with the pre-existing DET pathway and further improving current output (Mouhib et al., 2025). This contrast indicates that flavins often function less as stand-alone IET modules than as auxiliary redox components whose performance depends on transportability and integration with existing DET pathways.

The major advantage of endogenous electron shuttle biosynthesis is that it enables nonclassical-EAMs to mediate IET through diffusible redox-active molecules. Because these electron shuttles can diffuse between cells and electrodes, they may support electron transfer over longer distances and are particularly useful for connecting cells located away from the electrode surface. In addition, endogenous biosynthesis allows engineered cells to continuously produce electron shuttles, reducing the need for repeated external electron shuttle supplementation. However, increased shuttle production does not necessarily lead to functional electron flow. Electron shuttles biosynthesis and secretion consume intracellular reducing equivalents, while the resulting redox-active molecules may exchange electrons with multiple intracellular redox pools, leading to futile redox cycling and unnecessary energy dissipation. Functional electron flow can therefore be established only when electron shuttle cycling remains effectively coupled to the target metabolism, allowing the transferred electrons to contribute to the intended reaction rather than being dissipated through nonspecific redox exchange. Endogenously synthesized electron shuttles generally favor outward electron transfer because they are naturally compatible with specific intracellular redox sites and can readily accept reducing equivalents from cellular metabolism. Beyond genetic engineering, future studies could explore material-based or other external stimuli to promote electron shuttle secretion and thereby enhance IET (Li et al., 2026; Wang et al., 2025; Zhang et al., 2026). Future designs should balance shuttle production with host fitness, coordinate shuttle redox properties and transport with intracellular metabolism, and evaluate current generation together with target substrate conversion, product formation, and electron recovery.

3.2.2. Exogenous supplementation of transmembrane electron shuttles

Exogenous supplementation with transmembrane electron shuttles provides a simple and rapid route for constructing IET in nonclassical-EAMs. Early studies showed that neutral red can act as a transmembrane electron shuttle to mediate IET in *E. coli*, significantly

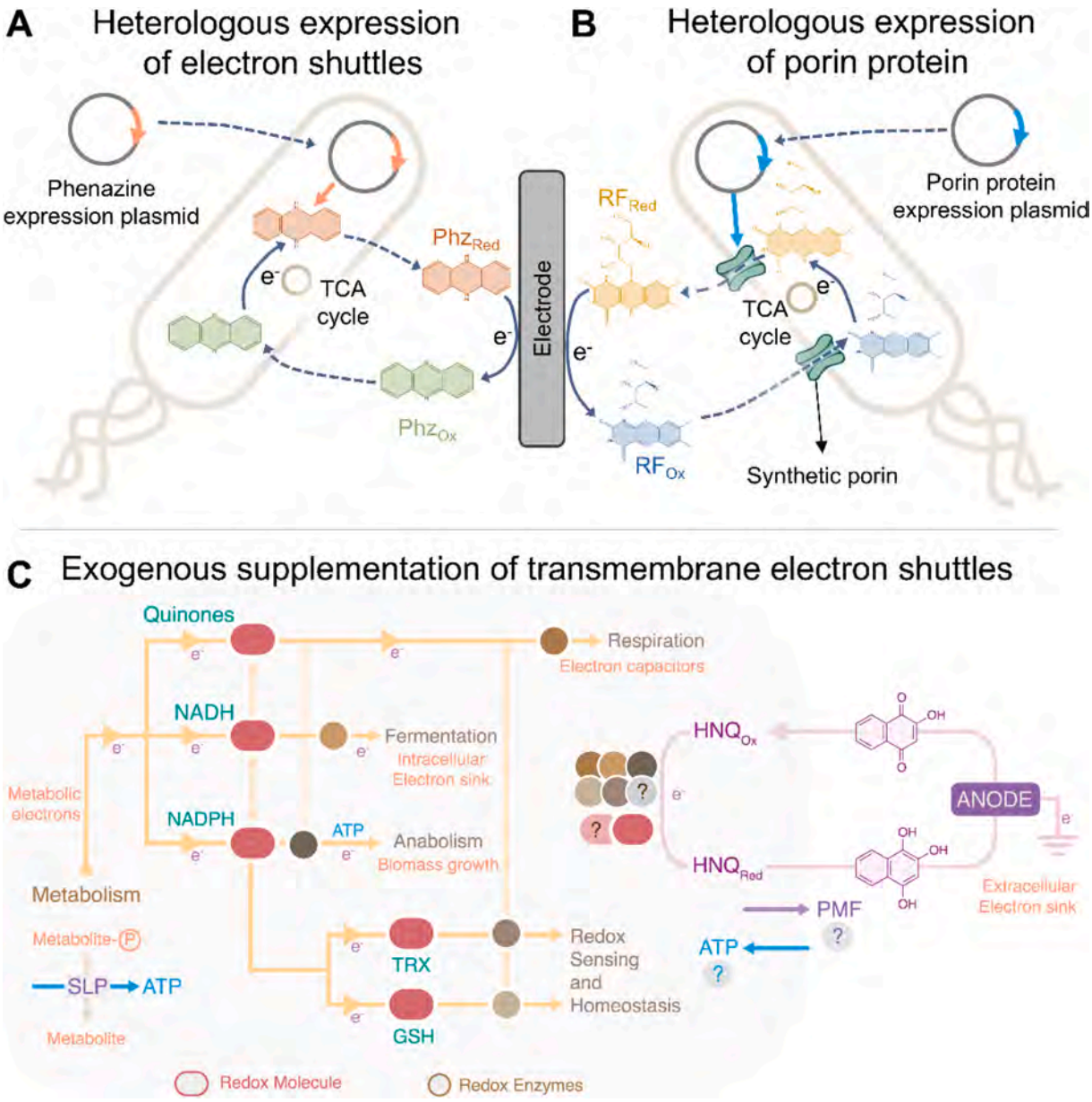

Fig. 3. Representative strategies for constructing electron shuttle mediated IET pathways in nonclassical-EAMs. (A) Heterologous expression of electron shuttle biosynthetic pathways engineers the endogenous production of electron shuttles. (B) Heterologous expression of porin proteins facilitates shuttle transport across the cell membrane. (C) Exogenous addition of transmembrane electron shuttles establishes artificial electron transfer routes between intracellular redox metabolism and extracellular electron sinks such as anodes, thereby engineering electron shuttle mediated IET pathways. C reprinted from reference (Kundu et al., 2025), with permission from Elsevier.

enhancing current generation by exchanging electrons with the inner-membrane menaquinone pool and thereby transferring intracellular electrons to the extracellular interface (Harrington et al., 2015; McKinlay & Zeikus, 2004; Park & Zeikus, 2000). Subsequently, a variety of other dye-like small molecules, including methylene blue, resazurin, and methyl viologen, were also shown to mediate IET in diverse nonclassical-EAMs (Montoya-Vallejo et al., 2023; Ramanavicius et al., 2016; Rhodes et al., 2021). These studies established the feasibility of using exogenous transmembrane electron shuttles to construct artificial IET pathways in nonclassical-EAMs. This limitation has motivated the development of more biocompatible and structurally tunable electron shuttles (Liu et al., 2018). For example, the transmembrane redox-active copolymer poly(2-methacryloyloxyethyl phosphorylcholine-*co*-vinylferrocene) promoted IET in *Ralstonia eutropha* and increased polyhydroxybutyrate production by 60 % (Nishio et al., 2014), while a modified low-potential pyocyanin was used to extract electrons from the photosynthetic electron transport chain of *Synechocystis* sp. PCC 6803, increasing photocurrent by fourfold (Clifford et al., 2021). Therefore, exogenous supplementation electron shuttle engineering should move beyond the screening of usable dyes toward rational electron shuttle design guided by nonclassical-EAMs compatibility and electron flux control.

More recent studies indicate that transmembrane electron shuttles-mediated IET is not merely a passive diffusion-driven process, but depends strongly on the compatibility between the transmembrane electron shuttles and nonclassical-EAMs physiology. A representative example is HNQ, whose redox potential is well matched to the menaquinone pool of *E. coli*, thereby enabling electrode-respiring EET and supporting cell growth with the electrode as the sole electron acceptor (Fig. 3**C**) (Kundu et al., 2025). More importantly, the same study showed

that the efficiency of HNQ-mediated electron transfer was strongly influenced by membrane transport processes in *E. coli* as well as intracellular redox cycling involving the nitroreductases NfsA and NfsB. These results suggest that the performance of exogenous electron shuttles is determined not only by their electrochemical properties, but also by their compatibility with physiological processes in *E. coli*. More broadly, this work indicates that transmembrane electron shuttles may be developed not merely as externally supplied redox materials, but also as tunable functional modules for constructing artificial IET in nonclassical-EAMs, whose selection and optimization should consider both electrochemical behaviour and physiological compatibility. In addition to promoting outward electron release, exogenous transmembrane electron shuttles can also function as electron input modules in nonclassical-EAMs to drive reductive metabolism. For example, neutral red was used in a polymer dots-*Ralstonia eutropha* H16 biohybrid system to mediate transmembrane injection of photoelectrons, thereby increasing intracellular NADPH availability and promoting CO_2 conversion to PHB (Yu et al., 2023). Likewise, in an engineered *E. coli* photocatalyst-mineralized biofilm, methyl viologen transferred photoelectrons from extracellular CdS nanoparticles to formate dehydrogenase, enabling CO_2 reduction to formate (Wang et al., 2022). Collectively, these studies demonstrate that transmembrane electron shuttles can mediate both outward and inward electron transfer, thereby coupling intracellular metabolism with extracellular electron acceptors or donors.

The main advantages of exogenous transmembrane electron shuttle supplementation are its simplicity, rapid implementation, and broad nonclassical-EAMs range. This strategy can be applied without extensive genome engineering and can be tuned by changing the molecular structure, redox potential, or concentration. However, the ability of an exogenous electron shuttle to cross the cell membrane does not by itself ensure functional electron flow. Its redox potential and intracellular accessibility must also allow it to exchange electrons with the metabolic node that supplies or receives electrons in the target pathway. Otherwise, the electron shuttle may enhance electron exchange with the electrode without effectively directing those electrons toward the intended metabolic process. The key challenge is therefore to coordinate membrane transport, and redox-potential matching so that the direction and destination of electron transfer are consistent with the target metabolic processes. Exogenous transmembrane electron shuttles allow flexible control over electron transfer direction, because selecting shuttles with different membrane permeabilities and redox potentials can direct electron flow either from intracellular redox sites to the extracellular interface or from extracellular electron donors to intracellular electron accepting sites. Future designs should focus on structurally tunable electron shuttles whose redox potential can be adapted to different metabolic processes. Immobilization, and recycling should also be incorporated to improve shuttle retention and stability while reducing toxicity and environmental release.

3.3. Engineering electroactive biofilms

Electroactive biofilm engineering aims to translate artificial EET from individual cells to electrode-associated community. Although DET and IET strategies construct EET pathways at the cellular level, high-performance microbial electrochemical systems require engineered nonclassical-EAMs to attach to electrode surfaces (Steidl et al., 2016). This requirement is particularly important because the EPS of nonclassical-EAM biofilms generally differ from those of natural EAM biofilms in both composition and electrical function. Natural EAM biofilms often contain redox-active components, such as *c*-type cytochromes, nanowires, and retained flavins, which support electron transport beyond cells directly contacting the electrode (Wang et al., 2021; Xiao et al., 2017). By contrast, the EPS of many nonclassical-EAMs mainly consists of polysaccharides and structural fibers that primarily support adhesion and mechanical stability but provide limited intrinsic conductivity. Consequently, introducing an intracellular or cell-surface EET pathway into a nonclassical-EAMs does not necessarily establish a stable, long-range electron transfer network throughout the biofilm. Therefore, electroactive biofilm engineering generally involves three interrelated strategies: improving cell attachment to electrode surfaces, constructing conductive-material hybrid biofilms to enhance community-level electron transfer, and optimizing electrode architecture to coordinate electron transfer, mass transport, and cellular activity.

First, enhancing cell attachment to electrode surfaces is an important prerequisite for effective cell-electrode coupling in engineered nonclassical-EAMs (Fig. 4**A**). Stable attachment increases the proportion of cells electrically connected to the electrode and reduces the loss of engineered cells during prolonged operation. In engineered *E. coli*, electrocatalytic oxygen reduction activity was first introduced through genetic engineering and then further coupled to electrodes by depositing polypyrrole nanoparticles on the cell surface. This surface polymerization increased interfacial roughness and strengthened cell–electrode contact, thereby promoting the formation of a more efficient electrode-associated electroactive interface (Niu et al., 2024). Similarly, self-assembly of the conjugated polyelectrolyte PEDOT-S with cyanobacteria generated a three-dimensional biocomposite interface with mixed ionic-electronic conductivity and increased single-cell photocurrent output by 10-fold (Chen et al., 2025). Second, conductive materials and composite matrices can be used to construct hybrid electroactive biofilms that facilitate electron transfer at the community scale (Fig. 4**B**). These materials may compensate for the limited conductivity of nonclassical-EAM EPS by forming conductive bridges between cells and between cells and the electrode. Encapsulation of Mtr pathway expressing engineered *E. coli* in a sodium alginate-agarose hydrogel immobilized cells on electrodes and increased biosensor signal intensity by 30 % relative to planktonic cells, while TiO_2@TiN nanocomposites further enhanced interfacial electron transfer (Atkinson et al., 2022). Dopamine-derived conductive coatings have also been used to immobilize *Azotobacter vinelandii* and construct electroactive biofilms for electricity-driven ammonia synthesis (Zhang et al., 2024c). Third, electrode architecture also strongly influences artificial EET performance (Fig. 4**C**). Engineered nonclassical-EAMs are generally less well adapted to long-range electron transport and to local environmental fluctuations within thick biofilms. As cell loading increases and biofilms thicken, system performance may be increasingly influenced by light attenuation, limited substrate and gas diffusion, local accumulation of metabolic products and uneven electron collection. Under these conditions, electrode structure may affect cell distribution, pore architecture and interfacial mass transport, and thus influence overall electron-transfer efficiency and cellular activity. For example, hierarchical indium tin oxide electrodes with micropillar arrays improved light delivery, gas diffusion, and electron collection in cyanobacterial biofilms, thereby enhancing current output (Chen et al., 2022).

Electroactive biofilm engineering is a necessary step for applying engineered nonclassical-EAMs in practical bioelectrochemical systems. By enhancing cell attachment, constructing hybrid conductive interfaces, and optimizing electrode architecture, these strategies can improve electron-transfer efficiency, biomass retention, and operational stability. However, a central challenge is to balance biofilm thickness with mass transport. Increasing biofilm thickness can enhance electron flux and system stability, but it also increases diffusion barriers for substrates, gases, electron shuttles, and metabolic products, ultimately leading to reduced electron-transfer efficiency and impaired cellular activity. Therefore, future electroactive biofilm engineering should focus on designing biofilm-electrode interfaces that coordinate biomass retention, electron transfer, and mass transport. The key design principle is not simply to maximize biofilm formation, but to construct biofilms with appropriate thickness, controlled porosity, stable conductivity, and efficient diffusion pathways. Future studies should also consider mixed-species biofilms and explore how conductive matrices, spatial organization, and engineered interspecies electron

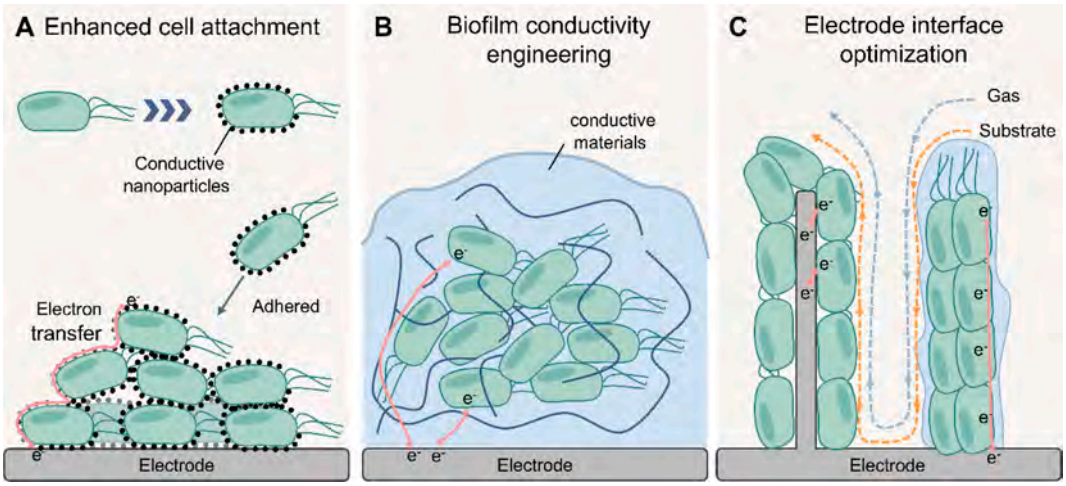

Fig. 4. Representative strategies for engineering conductive biofilms. (A) Introduction of conductive nanoparticles onto the cell surface enhances cell attachment to the electrode, thereby improving cell-electrode coupling and conductive biofilm formation. (B) Incorporation of conductive materials into the biofilm engineers conductive networks within the biofilm, thereby enhancing long-range electron transfer. (C) Optimization of electrode architectures and interfacial microenvironments improves gas and substrate transport, electron collection, and cell distribution, thereby engineering biofilm-electrode interfaces for more efficient EET.

transfer pathways can electrically integrate microbial subpopulations performing complementary functions.

4. Application-oriented artificial EET in engineered nonclassical-EAMs

Artificial EET engineering expands METs from systems based on a limited number of naturally electroactive microorganisms to platforms built with function-oriented microbial chassis. Once electron-input or electron-output modules can be installed in nonclassical-EAMs, microorganisms can be selected according to the requirements of the target application, including molecular recognition, metabolic conversion, substrate utilization, environmental tolerance, and industrial relevance. Although most engineered nonclassical-EAM-based METs are still at the proof-of-concept stage, these studies have demonstrated the application potential of artificial EET by linking task-specific microbial functions with extracellular electronic interfaces. This changes the design logic of METs from adapting applications to available EAMs toward constructing

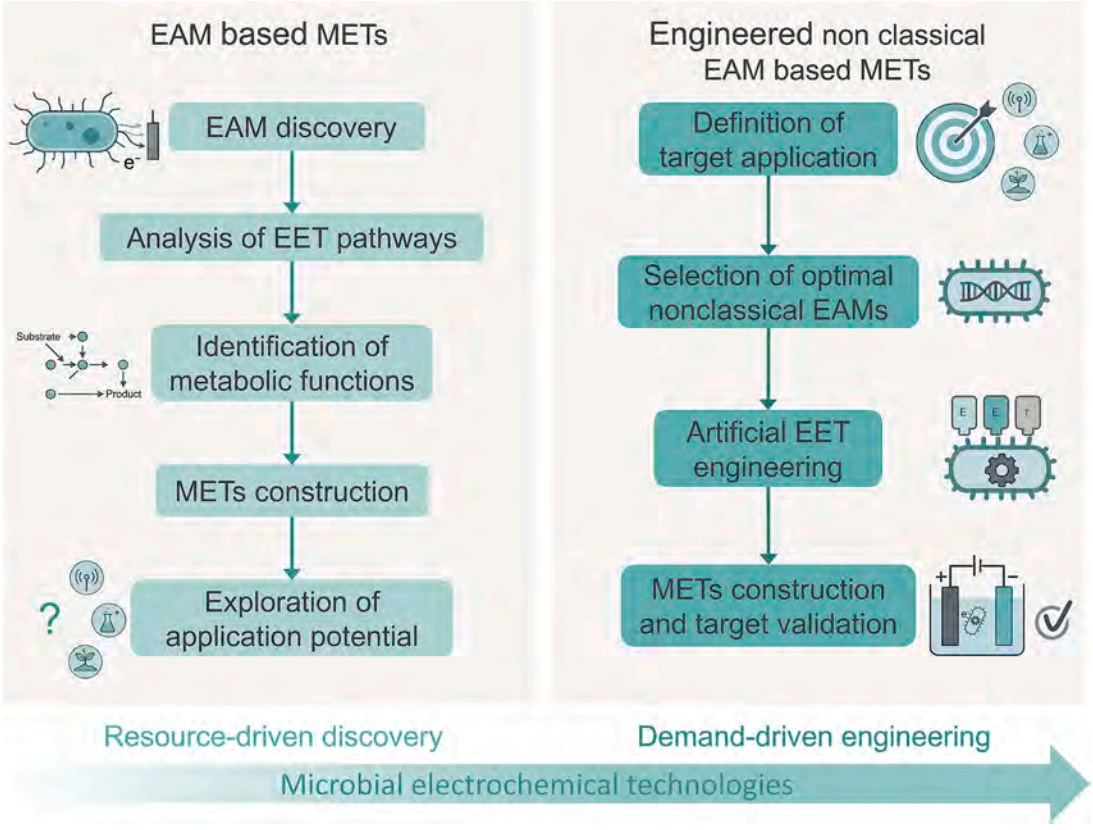

Fig. 5. Conceptual comparison of application development workflows between conventional EAM-based METs and engineered nonclassical-EAM-based METs, highlighting the shift from resource-driven discovery to demand-driven engineering.

electroactive microorganisms for specific tasks (Fig. 5). Within this framework, engineered nonclassical-EAMs show emerging promise in programmable biosensing, microbial electrosynthesis, CO_2 conversion, waste valorization, and environmental remediation.

4.1. Programmable biosensing

Biosensing is one of the most promising near-term applications of engineered nonclassical-EAMs because artificial EET can connect molecular recognition with electrical signal output. In native EAM-based sensors, current generation usually reflects global metabolic activity and can be affected by substrate availability, oxygen exposure, biofilm state, and environmental stress (Li et al., 2016; Liao et al., 2023). Engineered nonclassical-EAMs provide a more modular design strategy because recognition modules and electrochemical output modules can be separated and recombined. Recent engineered E. coli electrochemical sensing platforms have demonstrated that synthetic input, coupling, and output modules can be assembled to convert specific molecular recognition events, such as thiosulfate detection, into current generation. Further incorporation of protein switches and conductive nanomaterial encapsulation has enabled engineered E. coli electrochemical sensors to detect endocrine-disrupting compounds in real water samples (Atkinson et al., 2022). These studies indicate that the value of engineered nonclassical-EAMs in biosensing lies in the coordinated design of three modules: molecular recognition, EET mediated signal output, and stable cell-electrode interfacing. Genetic circuits provide specificity, artificial EET pathways convert biological recognition into electrical output, and electroactive biofilm or encapsulation strategies improve cell retention, interfacial stability, and signal robustness under realistic sample conditions. Artificial EET also enables biosensor architectures with higher information capacity. By constructing multiple EET pathways in E. coli, different analytes such as Cd^{2+} and As^{2+} can regulate distinct electron-transfer pathways and generate distinguishable electrochemical outputs (Zhang et al., 2025). This multi-route strategy shows that engineered nonclassical-EAMs can support multiplexed sensing by assigning different molecular inputs to different EET outputs. Thus, artificial EET expands microbial electrochemical sensing from single-target current response toward programmable multi-input and multi-output bioelectronic detection. Overall, the value of engineered nonclassical-EAMs in biosensing lies in programmable input–output control rather than simply stronger current generation. Future biosensor design should focus on reducing background electron transfer, improving genetic circuit insulation, strengthening the coupling between recognition modules and EET output modules, constructing stable electroactive biofilm interfaces for real-sample applications, and developing multiplexed sensing capabilities. Key evaluation criteria should include specificity, signal-to-noise ratio, response time, detection limit, matrix tolerance, operational stability, and the ability to distinguish multiple analytes.

4.2. Electrosynthesis and CO_2 conversion

Electrosynthesis and CO_2 conversion require extracellular electrons to be delivered into intracellular reductive metabolism and ultimately directed toward target products. Natural EAMs possess efficient EET pathways, but they are not always ideal production chassis because their product spectra, metabolic programmability, genetic tools, and industrial robustness are often limited. By contrast, many nonclassical-EAMs, including *E. coli*, *Bacillus subtilis*, yeasts, cyanobacteria, and other industrial or photosynthetic microorganisms (Kavscek et al., 2015; Liu et al., 2019b; Matsumoto et al., 2017; Vavitsas et al., 2019), have strong metabolic engineering potential but lack efficient bioelectronic interfaces. Artificial EET therefore provides a strategy to combine extracellular electron supply with programmable production metabolism.

For artificial EET to support electrosynthesis, imported electrons must pass through three functional steps: entry into the cell, conversion into intracellular reducing power, and allocation to product-forming pathways. Engineered *E. coli* systems that use artificial electron-input routes to support cathode-derived electron uptake and succinate formation demonstrate the first step, namely enabling a nonclassical-EAM production chassis to access extracellular electrons (Wu et al., 2019). However, electron entry alone does not guarantee efficient electrosynthesis, because imported electrons may still be lost through competing electron sinks, or other metabolism processes. The second step is to connect electron input with intracellular reducing-power metabolism. This is particularly important for CO_2 conversion and electrosynthesis, because most product-forming pathways use NADH or NADPH rather than extracellular electrons directly. In polymer dots-*Ralstonia eutropha* H16 biohybrids, neutral red-mediated photoelectron injection increased intracellular NADPH availability and promoted CO_2 conversion to polyhydroxybutyrate (Nishio et al., 2014). This indicates that artificial EET becomes more productive when electron transfer is coupled to intracellular reducing process and energy metabolism, rather than remaining an isolated electron-entry process. The third step is to direct reducing equivalents toward specific enzymes or metabolic pathways. In engineered *E. coli* photocatalyst-mineralized biofilms, methyl viologen transferred photoelectrons from extracellular CdS nanoparticles to formate dehydrogenase, enabling CO_2 reduction to formate (Guo et al., 2018). This pathway-level targeting is essential because electrons that enter the cell but are not linked to a defined reductive enzyme or biosynthetic route may increase apparent electrochemical activity without improving product selectivity or electron recovery.

Therefore, the central design issue in electrosynthesis oriented artificial EET is control over the intracellular fate of imported electrons. Efficient systems must coordinate electron entry, cofactor regeneration, carbon-flux distribution, and product-pathway activity. Future designs should match electron-input modules with nonclassical-EAMs redox metabolism and product-specific cofactor demands, while also controlling electron shuttle toxicity, photocatalyst stability, enzyme specificity, competing reactions, and energy losses. Evaluation should move beyond current density and product titer to include Faradaic efficiency, electron recovery into products, product selectivity, carbon conversion efficiency, cofactor balance, energy efficiency, and long-term production stability.

4.3. Waste valorization and environmental remediation

Waste valorization and environmental remediation are important application areas of METs, but systems based only on natural EAMs are often constrained by limited substrate range, pollutant-transforming capacity, and environmental adaptability. Although natural EAMs possess efficient EET, they are not always suitable for complex wastewater or contaminated environments that require broad substrate utilization, specific pollutant-degrading capacity, and tolerance to toxic or fluctuating conditions. Artificial EET provides a route to incorporate functional nonclassical-EAMs into METs. Many nonclassical-EAMs already possess the metabolic ability to degrade specific pollutants or transform complex organic substrates, but their weak electrical communication with electrodes limits their direct use in bioelectrochemical systems. By constructing artificial electron-transfer interfaces, these native degradation or substrate-conversion functions can be coupled with extracellular electron recovery. Thus, the value of artificial EET in environmental applications is not simply to make cells more electroactive, but to electronically connect pollutant-degrading or waste-transforming microorganisms with electrodes, enabling pollutant removal and energy or resource recovery to occur in the same system.

Several nonclassical-EAM-based environmental systems have shown this potential. Constructing artificial EET pathways in nonclassical-EAMs has improved wastewater treatment, organic substrate removal, and electricity recovery (Whisonant et al., 2024; Zhao et al., 2026), suggesting that effective electron-transfer interfaces can help couple native substrate-conversion metabolism with electrode-associated electron recovery. FeS-based artificial EET pathways further enhanced

tetracycline degradation by *Bacillus cereus* and sulfamethoxazole degradation by *Citrobacter freundii* (Lan et al., 2025; Liu et al., 2023). These results indicate that artificial EET can provide a feasible route for incorporating microorganisms with native waste-conversion or pollutant-degradation capacities into METs, thereby linking pollutant removal with electron recovery. Stable operation is essential for translating this advantage into practical environmental applications. Because many nonclassical-EAMs possess pollutant-degrading capacity and greater environmental tolerance than typical EAMs, artificial EET provides an opportunity to build more robust environmental METs. The key challenge is to ensure that the constructed artificial EET pathways remain compatible with this robustness during long-term operation. Conductive biofilms, immobilization matrices, and structured electrodes should therefore be optimized to maintain stable coupling between pollutant-degrading metabolism and electron recovery. Future studies should evaluate engineered systems under real wastewater, mixed pollutants, fluctuating pH and salinity, toxic loading, and continuous or semi-continuous operation. Key evaluation criteria should include pollutant removal efficiency, electron recovery efficiency, Coulombic efficiency, and long-term operational robustness.

5. Research needs and future directions

Artificial EET pathway engineering has demonstrated that nonclassical-EAMs can be electronically connected to electrodes through genetically encoded pathways, conductive materials, electron shuttles, and engineered electroactive biofilms (Kundu et al., 2025; Mouhib et al., 2023; Mouhib et al., 2025). However, most current studies remain at the proof-of-concept stage and primarily focus on generating or enhancing electrochemical activity. Future research should shift from maximizing electrochemical signals toward the function-oriented design of artificial electron flow. Accordingly, we propose a design framework that integrates four key aspects: demand-driven selection of nonclassical-EAM chassis and artificial EET strategies, quantitative analysis of functional electron flow, directional and dynamic control of electron transfer, and application-oriented evaluation of operational stability, ecological robustness, and biosafety. This framework aims to guide the construction of artificial EET pathways in which electron transfer is thermodynamically feasible, physiologically compatible, controllable, functionally effective, and safe under application-relevant conditions.

5.1. Demand-driven selection of nonclassical-EAM chassis and artificial EET strategies

Artificial EET pathways design should begin with a clear definition of the target application, including the intended metabolic function, operating environment, and required operational duration. An appropriate microbial chassis should then be selected according to its substrate utilization capacity, target product formation, environmental tolerance, and physiological robustness. The intracellular redox node that supplies or receives electrons should also be identified, because it determines where the artificial EET pathways must connect with endogenous metabolism. The artificial EET strategy should subsequently be matched to the physiological and engineering characteristics of the selected chassis. Key considerations include membrane permeability, genetic tractability, stress tolerance, biofilm forming capacity, system openness, and tolerance to conductive materials or electron shuttles. Genetically encoded DET pathways may be suitable for genetically tractable chassis when a heritable and programmable electron-transfer route is required. Material-assisted DET may provide a more practical option for genetically recalcitrant hosts, whereas electron shuttle mediated IET may be preferable when transmembrane or long-range electron transfer is needed. Hybrid strategies may be considered when no single approach can satisfy all application requirements.

Future research should move beyond empirical strategy selection and establish transferable principles for matching nonclassical-EAM chassis with artificial EET strategies. Comparative studies across hosts with different membrane properties, genetic accessibility, and electron-transfer requirements should clarify how chassis characteristics affect strategy suitability. Such studies could support the development of rational chassis-strategy matching criteria and enable artificial EET engineering to progress from case-specific testing toward application-oriented design.

5.2. Toward functional electron flow analysis

Future artificial EET pathway construction should establish quantitative analytical frameworks that resolve the source, electron transfer pathway, and final functional destination of electrons. Current output, power density, and electrochemical signal intensity only indicate electron exchange between cells and electrodes and cannot determine whether the transferred electrons enter the target metabolic process. Future studies should therefore integrate electrochemical measurements with electron balances, mass balances, and application-specific functional metrics. Comparing the charge transferred to or from the electrode with the electron equivalents associated with substrate consumption, target-product formation, biomass synthesis, and by-product generation could quantify the allocation of electrons toward the intended function. Stable-isotope tracing could further resolve the metabolic material flows associated with electron transfer. Carbon-isotope-labeled substrates could be used to determine whether substrate oxidation is coupled to outward electron transfer, whereas labeled carbon dioxide could reveal carbon incorporation into biomass and target products during artificial EET pathways.

Future studies should also develop real-time and single-cell approaches to reveal the dynamics and cellular heterogeneity of artificial EET pathways. Fluorescent probes for intracellular redox states could monitor changes in key metabolic nodes, whereas single-cell fluorescence and electrochemical imaging could visualize electron transfer activity in individual cells and at cell-electrode interfaces. Integrating electron balances, isotope tracing, intracellular redox monitoring, and single-cell visualization with simultaneous measurements of current, substrate conversion, and product formation could ultimately establish a multiscale analytical framework linking artificial EET pathways to intracellular metabolism and the intended function, thereby distinguishing genuine functional electron flow from nonspecific electrochemical enhancement.

5.3. Directional and dynamic control of electron transfer

Artificial EET pathways should be designed according to both the direction and magnitude of electron flow required by the target application. Outward and inward EET involve different electron-transfer routes and energetic requirements. In outward EET, electrons must be transferred from intracellular electron donors to extracellular acceptors through an energetically favorable redox cascade. In inward EET, extracellular electrons must enter the cell and be delivered to intracellular cofactors or target enzymes. Therefore, the redox potentials of intracellular electron carriers, EET components, electron shuttles, conductive materials, and terminal electron donors or acceptors should be coordinated to establish thermodynamically feasible electron-transfer pathways in the intended direction. Electron flux should also be regulated rather than simply maximized. Excessive electron transfer may disturb intracellular redox homeostasis, increase metabolic burden and cellular stress, divert reducing equivalents from native metabolism, or reduce cell viability. In contrast, insufficient electron transfer may limit biosynthesis, pollutant conversion, current generation, or electrochemical signal output. Artificial EET systems should therefore maintain electron flux within a range that is compatible with host physiology and matched to the electron demand of the target process.

Future artificial EET pathways could achieve dynamic control by

integrating intracellular physiological states, application-specific process signals, and online electrochemical feedback. The balance between reduced nicotinamide adenine dinucleotide and oxidized nicotinamide adenine dinucleotide, together with the intracellular level of adenosine triphosphate, could reflect the coordination among intracellular electron availability, cellular energy status, and metabolic demand. These intracellular states, together with external and process-level variables such as substrate availability, target-compound concentration, product formation, and electrical output, could be used to regulate electron flux according to the requirements of the intended microbial electrochemical function. In parallel, online measurements of current, electrode potential, impedance, substrate consumption, or product formation could be used to adjust the applied potential or other operating parameters. Future studies should establish quantitative relationships among intracellular physiological states, electron flux, and functional performance. Such relationships could guide dynamic regulation to maintain electron flux within an appropriate operating range, preserve intracellular redox and energy homeostasis, minimize nonspecific electron leakage, and increase the proportion of electrons effectively coupled to the intended function, thereby enabling efficient and stable functional electron flow.

5.4. Toward stable, ecologically robust, and biosafe application-oriented systems

Long-term stability remains a major bottleneck for artificial EET systems. Different artificial EET strategies may lose function through different mechanisms. Genetically encoded pathways can impose metabolic burden, suffer from unstable expression, or reduce host fitness during extended cultivation. Electron shuttles may be lost from the system, degraded, or become toxic at effective concentrations. Conductive materials may detach from cells or electrodes, aggregate, become diluted during cell growth, or lose interfacial contact over time. Engineered biofilm interfaces can improve cell retention and electron-transfer continuity, but excessive biofilm formation may introduce mass-transfer limitations. Therefore, stability should not be evaluated only by current retention, but also by EET module persistence, cell viability, interface integrity, and maintenance of the target function.

Under application-relevant conditions, whether engineered nonclassical-EAMs can maintain stable population abundance and functional activity remain an important challenge. Most engineered nonclassical-EAMs are currently evaluated as pure cultures under controlled conditions, whereas practical applications commonly involve mixed microbial communities and complex environmental matrices. Microbial competition, environmental fluctuations, and interference from abiotic components may reduce the survival and colonization of engineered nonclassical-EAMs, ultimately weakening or disrupting functional electron flow. In open or semi-open systems, the escape and persistence of engineered nonclassical-EAMs outside the intended system may also raise biosafety concerns, including unintended ecological interactions and horizontal transfer of engineered genetic elements.

A key future direction is therefore to develop spatially confined electrode architectures in which engineered nonclassical-EAMs are immobilized or encapsulated within electroactive biofilms constructed using conductive materials and retained at the electrode interface. Such conductive biofilms could maintain a high local abundance of engineered nonclassical-EAMs and stable cell-electrode contact, thereby reducing direct competition with surrounding microorganisms for attachment sites and electron transfer interfaces. Spatial confinement could also reduce cell loss and environmental dispersal, thereby lowering the risk of unintended release. However, the design of confined electrodes must balance cell retention, electrical conductivity, mechanical stability, and mass-transfer performance. Excessively dense encapsulation may restrict the transport of substrates, products, gases, and electron shuttles, whereas insufficient confinement may lead to cell leakage and unstable EET performance. Future studies should therefore optimize the material composition, pore structure, conductivity, and long-term durability of confined electrode interfaces and determine whether they can maintain engineered nonclassical-EAMs abundance and functional electron flow under prolonged and complex operating conditions.

To support the translation and comparison of engineered nonclassical-EAMs future studies should establish standardized application-oriented evaluation frameworks. Current differences in nonclassical-EAMs chassis, EET modules, electrode materials, reactor configurations, electron shuttle concentrations, applied potentials, operating conditions, and normalization methods make it difficult to determine whether performance improvements originate from the artificial EET pathways constructing strategy itself. Therefore, studies should report both electron transfer metrics and application-specific functional outcomes, together with long-term functional retention and biosafety-related indicators. Such standardized evaluation will enable more reliable comparison among different systems and facilitate the identification of transferable design principles.

Overall, future development of artificial EET pathways in nonclassical-EAMs should integrate demand-driven selection of microbial chassis and EET strategies, quantitative analysis of functional electron flow, directional and dynamic control of electron transfer, and application-oriented validation of long-term stability and biosafety. Artificial EET pathways should be designed by first defining the target application, host requirements, intracellular redox node, electron-transfer direction, and operating conditions, and then selecting appropriate EET modules, conductive interfaces, regulatory strategies, and validation metrics. In practical applications, spatially confined electroactive biofilms should be further developed to maintain the local abundance and functional activity of engineered nonclassical-EAMs, reduce competition with surrounding microorganisms, and limit unintended environmental release. Such an integrated design framework will help move artificial EET pathways engineering beyond isolated demonstrations of electroactivity toward stable, controllable, functionally effective, and biosafe METs. Ultimately, engineered nonclassical-EAMs could provide programmable platforms for expanding METs in electricity generation, microbial electrosynthesis, biosensing, waste valorization, and environmental remediation.

6. Conclusions

Artificial EET engineering provides a route to expand METs beyond the limited set of naturally EAMs. By installing DET conduits, electron shuttle mediated redox cycles, or engineered electroactive biofilm, nonclassical-EAMs can be electronically connected to electrodes while retaining their native advantages in metabolism, genetic tractability, and environmental tolerance. This review highlights that successful engineering should not be judged by current output alone. The decisive criterion is whether electron flow is compatible with the host, aligned with the desired direction, coupled to target metabolism, and stable under application-relevant conditions. Future studies should therefore shift from empirical enhancement of electroactivity to demand driven design, quantitative electron flow tracing, dynamic flux regulation, and standardized performance evaluation. Such advances will enable engineered non-electroactive microorganisms to support programmable biosensing, microbial electrosynthesis, carbon conversion, waste valorization, and pollutant remediation, thereby broadening METs as practical tools for sustainable bioresource conversion and environmental management.

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used ChatGPT, developed by OpenAI, solely to improve the language, readability, and clarity of the manuscript. The tool was not used to generate scientific content, data, results, interpretations, conclusions, or references. After

using this tool, the authors carefully reviewed and edited the manuscript and take full responsibility for the content of the published article.

CRediT authorship contribution statement

Wei Chen: Writing – original draft, Conceptualization. **Yong Xiao:** Writing – review & editing, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Data availability

No data was used for the research described in the article.

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