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Study on Electron Transfer Mechanisms of Electroactive Bacteria in Microbial Fuel Cells

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Abstract Electroactive bacteria (EAB) play a crucial role in microbial fuel cells (MFCs) by facilitating electron transfer processes that are essential for energy generation and environmental remediation. This review paper delves into the molecular mechanisms underlying electron transfer in EAB, highlighting recent advancements and key differences between Gram-positive and Gram-negative bacteria. The review also explores the diversity of electroactive microorganisms, including iron-reducing bacteria and electrotrophic microorganisms, and their applications in bioelectrochemical systems. Strategies to enhance electron transfer efficiency, such as the use of electron-conducting polymers and nanostructured materials, are discussed. Additionally, the role of cell-surface exposed conductive proteins and the impact of biofilm spatial structure on electron transfer efficiency are examined. This research aims to provide a deeper understanding of the electron transfer mechanisms in EAB, thereby contributing to the optimization and advancement of microbial fuel cell technologies.

Keywords Rapeseed oil; Biodiesel; Production process; Economic analysis; Sustainable development

1 Introduction

Microbial fuel cells (MFCs) have garnered significant attention as a sustainable technology for wastewater treatment and bioelectricity generation. MFCs utilize electroactive bacteria to convert chemical energy from organic substrates directly into electrical energy through electrochemical reactions at the anode and cathode (Logan et al., 2019; Aiyer, 2020). This dual functionality of MFCs not only addresses environmental pollution but also provides a renewable source of energy, making them a promising solution for sustainable development (Zhou et al., 2020). The efficiency and performance of MFCs are influenced by various factors, including electrode materials, microbial species, and the mechanisms of electron transfer from bacteria to the anode (Li et al., 2021).

Understanding the electron transfer mechanisms in electroactive bacteria is crucial for optimizing the performance of MFCs. Electroactive bacteria, such as Geobacter and Shewanella species, can transfer electrons to the anode either directly through conductive pili and nanowires or indirectly via redox mediators (Lan et al., 2018; Aiyer, 2020). The efficiency of these electron transfer processes determines the overall power output and stability of MFCs. Direct electron transfer (DET) involves the use of outer membrane cytochromes and conductive appendages, while mediated electron transfer (MET) relies on naturally secreted or externally added redox compounds (Pankratova et al., 2019; Aiyer, 2020). Enhancing our understanding of these mechanisms can lead to the development of more efficient MFCs with higher power densities and broader applications in bioelectrochemical systems (Zhao et al., 2020).

The primary objective of this study is to investigate the various electron transfer mechanisms employed by electroactive bacteria in MFCs and their impact on the performance of these systems. This includes examining both direct and mediated electron transfer processes, the role of biofilm formation, and the influence of different electrode materials and configurations. By elucidating these mechanisms, the study aims to provide insights into optimizing MFC design and operation for enhanced bioelectricity production and environmental remediation. The scope of the study encompasses a comprehensive review of current literature, experimental investigations, and the development of mathematical models to predict and improve electron transfer efficiency in MFCs.

2 Fundamentals of Electron Transfer in MFCs

2.1 Description of electron transfer processes in MFCs

Microbial fuel cells (MFCs) are innovative bioelectrochemical systems that convert chemical energy from organic substrates directly into electrical energy through the metabolic activities of microorganisms. The core process in MFCs involves the transfer of electrons from microbial cells to the anode, a process known as extracellular electron transfer (EET). This electron transfer is crucial for the generation of electricity in MFCs and is facilitated by electroactive bacteria (EAB) that can transfer electrons to solid-phase electron acceptors such as electrodes (Logan et al., 2019; Pankratova et al., 2019; Aiyer, 2020).

The EET process begins with the microbial oxidation of organic matter, which releases electrons and protons. The electrons are then transferred to the anode, while the protons migrate through a proton exchange membrane to the cathode, where they combine with oxygen to form water. The efficiency of this process is influenced by several factors, including the type of microorganisms, the nature of the electrode material, and the presence of electron mediators (Lan et al., 2018; Aiyer, 2020; Li et al., 2021).

2.2 Role of electroactive bacteria in electron transfer

Electroactive bacteria (EAB) play a pivotal role in the electron transfer processes within MFCs. These microorganisms possess unique metabolic pathways that enable them to transfer electrons to external electron acceptors. Among the most studied EAB are species from the genera Geobacter and Shewanella, which have been shown to produce high power densities in MFCs (Logan et al., 2019; Zhao et al., 2020; Zhou et al., 2020).

Geobacter sulfurreducens, for instance, is known for its ability to form conductive biofilms and transfer electrons directly to the anode via conductive pili or nanowires. These biofilms enhance the surface area for electron transfer and improve the overall efficiency of the MFC (Logan et al., 2019; Li et al., 2021). Similarly, Shewanella oneidensis can transfer electrons through outer membrane cytochromes and soluble redox mediators, facilitating both direct and mediated electron transfer mechanisms (Pinto et al., 2018; Zhao et al., 2020).

The diversity of EAB extends beyond these model organisms, with many other bacteria, fungi, and archaea also capable of EET. This diversity offers opportunities for optimizing MFC performance by selecting or engineering microorganisms with enhanced electron transfer capabilities (Logan et al., 2019; Zhao et al., 2020).

2.3 Types of electron transfer mechanisms

The electron transfer mechanisms in MFCs can be broadly categorized into direct electron transfer (DET) and mediated electron transfer (MET).

Direct Electron Transfer (DET): In DET, electrons are transferred directly from the microbial cells to the anode without the involvement of soluble mediators. This process is facilitated by conductive structures such as pili, nanowires, and outer membrane cytochromes. For example, Geobacter species utilize conductive pili and cytochromes to transfer electrons directly to the anode, forming a highly efficient electron transfer pathway (Lan et al., 2018; Aiyer, 2020; Li et al., 2021). The spatial structure of the biofilm and the interaction between the microbial cells and the electrode surface are critical factors influencing DET efficiency (Pinto et al., 2018; Li et al., 2021).

Mediated Electron Transfer (MET): In MET, soluble redox mediators facilitate the transfer of electrons from the microbial cells to the anode. These mediators can be naturally produced by the microorganisms, such as flavins and pyocyanins, or artificially added to the system, such as methylene blue and neutral red (Kalathil et al., 2016; Liu et al., 2018). The mediators shuttle electrons between the microbial cells and the anode, enabling electron transfer even when direct contact is not possible. The efficiency of MET depends on the redox potential of the mediators and their ability to diffuse between the cells and the electrode (Kalathil et al., 2016; Liu et al., 2018).

Both DET and MET are essential for the operation of MFCs, and understanding these mechanisms is crucial for optimizing the design and performance of these systems. Advances in nanotechnology and bioengineering hold promise for enhancing both DET and MET, thereby improving the overall efficiency and applicability of MFCs in sustainable energy production and environmental remediation (Kalathil et al., 2016; Li et al., 2021).

3 Key Electroactive Bacteria in MFCs

3.1 Identification of major electroactive bacterial species

Microbial fuel cells (MFCs) rely on the unique capabilities of electroactive bacteria to transfer electrons to the anode, facilitating electricity generation. Among the diverse array of electroactive microorganisms, three key species have been extensively studied: *Geobacter sulfurreducens*, Shewanella oneidensis, and Pseudomonas species.

Geobacter sulfurreducens is a well-known iron-reducing bacterium that produces high power densities in MFCs. This species is particularly effective in transferring electrons directly to the anode via conductive pili or nanowires, making it a model organism for studying extracellular electron transfer (EET) mechanisms (Logan et al., 2019; Li et al., 2021). Shewanella oneidensis is another prominent electroactive bacterium that utilizes both direct and mediated electron transfer mechanisms. It can produce redox-active compounds such as flavins, which facilitate electron transfer to the anode (Pinto et al., 2018; Aiyer, 2020). Pseudomonas species, although less studied, have shown significant electrochemical activity in MFCs, contributing to both electricity generation and bioremediation processes (Zhou et al., 2020).

3.2 Metabolic pathways facilitating electron transfer

The metabolic pathways of electroactive bacteria are crucial for their ability to transfer electrons to the anode in MFCs. These pathways involve complex biochemical processes that enable the bacteria to extract energy from organic substrates and transfer the resulting electrons to external electron acceptors.

In *Geobacter sulfurreducens*, the primary pathway involves the oxidation of organic compounds, such as acetate, coupled with the reduction of iron or other metal oxides. The electrons generated during this process are transferred to the anode via conductive pili or nanowires, which act as biological wires (Logan et al., 2019; Li et al., 2021). Shewanella oneidensis employs a similar strategy but also produces soluble redox mediators like flavins and quinones that shuttle electrons from the cell to the anode, enhancing the efficiency of electron transfer (Pinto et al., 2018; Aiyer, 2020). Pseudomonas species utilize a variety of metabolic pathways, including the production of pyocyanin, a redox-active compound that facilitates electron transfer to the anode (Liu et al., 2018; Zhou et al., 2020).

3.3 Genetic and physiological characteristics contributing to electroactivity

The genetic and physiological characteristics of electroactive bacteria play a significant role in their ability to transfer electrons to the anode in MFCs. These characteristics include the presence of specific genes encoding for electron transfer proteins, the structure of the cell envelope, and the formation of biofilms.

Geobacter sulfurreducens possesses genes encoding for outer membrane c-type cytochromes, which are essential for direct electron transfer to the anode. These cytochromes are located on the outer membrane and interact with conductive pili or nanowires to facilitate electron transfer (Logan et al., 2019; Li et al., 2021). The thick cell wall of Shewanella oneidensis contains multiple layers of cytochromes and other redox-active proteins that enable both direct and mediated electron transfer. The ability to produce redox mediators like flavins further enhances its electroactivity. Pseudomonas species exhibit a high degree of genetic diversity, with genes encoding for various redox-active compounds and electron transfer proteins. The production of biofilms by these bacteria also contributes to their electroactivity by providing a stable environment for electron transfer processes (Zhou et al., 2020; Liu et al., 2018).

In conclusion, the study of electroactive bacteria in MFCs reveals the intricate mechanisms by which these microorganisms transfer electrons to the anode. Understanding the identification, metabolic pathways, and genetic and physiological characteristics of key electroactive bacteria such as *Geobacter sulfurreducens*, Shewanella oneidensis, and Pseudomonas species is essential for optimizing MFC performance and developing practical applications for sustainable energy generation and environmental remediation.

4 Direct Electron Transfer (DET) Mechanisms

4.1 Mechanisms of direct electron transfer between bacteria and electrodes

Direct electron transfer (DET) is a crucial process in microbial fuel cells (MFCs) where electroactive bacteria transfer electrons directly to the electrode without the need for soluble electron shuttles. This process is facilitated by specific structural and functional adaptations in bacteria, such as the presence of outer membrane c-type cytochromes (OM c-Cyts) and conductive pili or nanowires. These components form a conductive pathway that allows electrons generated from cellular metabolism to be transferred across the cell surface to the electrode (Figure 1) (Aiyer, 2019; Li et al., 2021; Paquete et al., 2022).The spatial structure of electroactive biofilms (EABs) plays a significant role in enhancing DET. For instance, modifying the anode with materials like iron phthalocyanine (FePc) can improve the affinity between the anode and OM c-Cyts, leading to a more active EAB and higher power density in MFCs (Li et al., 2021). This modification decreases the charge transfer resistance and accelerates the interfacial reaction rate, thereby promoting DET (Li et al., 2021).

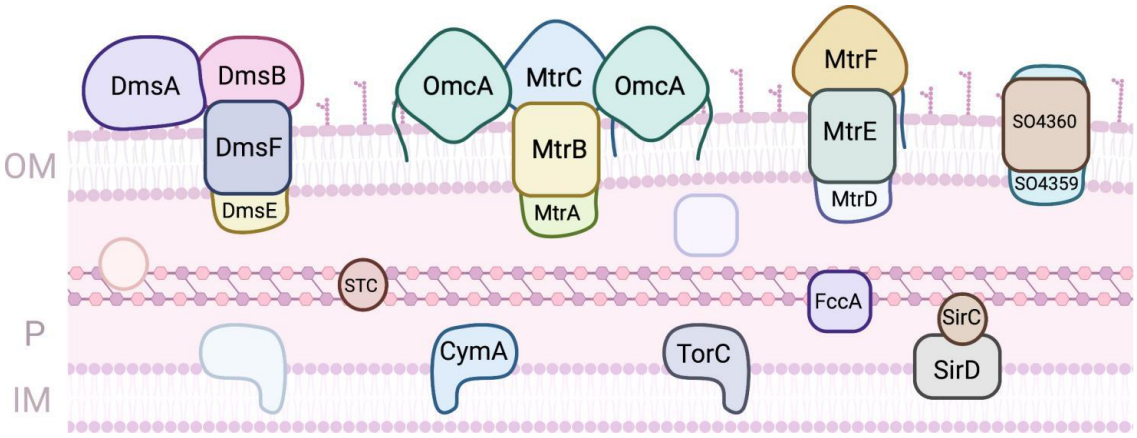

Figure 1 Proteins involved in EET processes of S. oneidensis MR-1 (Adopted from Paquete et al., 2022)

Image caption: This diagram illustrates the key proteins in the bacterial electron transport chain and their locations within the cell membrane. The image includes various electron transport proteins located in the outer membrane (OM), periplasm (P), and inner membrane (IM). DmsA, DmsB, DmsE, and DmsF are responsible for electron transfer from the outer membrane to the inner membrane. OmcA, MtrC, MtrA, and MtrB form a transmembrane complex that assists in electron transfer across the outer membrane. CymA, TorC, and FccA are located in the inner membrane and play roles in electron transport. SO4360 and SO4359 indicate proteins associated with metal reduction. The coordinated action of these proteins facilitates the electron transfer process within the cell, which is significant for the study of microbial fuel cells and other bioelectrochemical systems (Adopted from Paquete et al., 2022)

4.2 Role of cytochromes and conductive pili in DET

Cytochromes, particularly multiheme c-type cytochromes, are essential for DET as they facilitate electron transfer across the bacterial cell envelope to the electrode. These proteins are embedded in the outer membrane and form a conductive pathway that bridges the intracellular electron donors and the extracellular electron acceptors (Kracke et al., 2015; Paquete et al., 2022). The presence of these cytochromes is a common feature among electroactive bacteria, including Geobacter and Shewanella species, which are well-known for their efficient DET capabilities (Zhao et al., 2020; Paquete et al., 2022).

Conductive pili, also known as microbial nanowires, are another critical component in DET. These pili are filamentous structures that extend from the bacterial cell surface and can conduct electrons over micrometer distances. They enable bacteria to establish electrical connections with distant electrodes or other cells, thereby enhancing the overall electron transfer efficiency in bioelectrochemical systems (Aiyer, 2019; Lovley et al., 2021). The combination of cytochromes and conductive pili allows bacteria to effectively transfer electrons directly to the electrode, bypassing the need for soluble mediators (Lovley et al., 2021; Paquete et al., 2022).

4.3 Case studies highlighting DET in specific bacteria (e.g., Geobacter spp.)

Geobacter species are among the most studied electroactive bacteria due to their remarkable ability to perform DET. These bacteria possess a high abundance of OM c-Cyts and conductive pili, which are crucial for their

electron transfer processes. In one study, the introduction of FePc into a carbon cloth electrode significantly enriched the Geobacter population in the EAB, increasing their abundance from 6.97% to 44.83% (Li et al., 2021). This enrichment led to a substantial increase in power density and biomass loading, demonstrating the effectiveness of Geobacter in DET (Li et al., 2021).

Another study highlighted the role of Geobacter in a neutral red-mediated MFC, where the addition of neutral red enhanced the electron transfer and induced the growth of exoelectrogens, including Geobacter (Chen et al., 2021). This resulted in improved MFC performance, showcasing the potential of Geobacter in bioelectrochemical applications (Chen et al., 2021).

Overall, the ability of Geobacter species to efficiently transfer electrons directly to electrodes makes them ideal candidates for enhancing the performance of MFCs and other bioelectrochemical systems. Their well-characterized DET mechanisms provide valuable insights into the design and optimization of these systems for sustainable energy production and environmental remediation (Zhao et al., 2020; Chen et al., 2021; Li et al., 2021; Paquete et al., 2022).

5 Mediated Electron Transfer (MET) Mechanisms

5.1 Use of redox mediators in electron transfer

Mediated Electron Transfer (MET) in microbial fuel cells (MFCs) involves the use of redox mediators to facilitate the transfer of electrons from electroactive bacteria to the anode. Redox mediators are compounds that can shuttle electrons between the microbial cells and the electrode, thereby enhancing the efficiency of electron transfer. These mediators can be either naturally secreted by the bacteria or artificially added to the system. Natural redox mediators include compounds like flavins and pyocyanins, which are secreted by certain bacteria to facilitate electron transfer (Aiyer, 2019). Artificial mediators, such as methylene blue and neutral red, are often added to MFCs to improve electron transfer rates and overall system performance (Kalathil et al., 2016; Aiyer, 2019).

The use of redox mediators is crucial in overcoming the limitations posed by the cell envelope and the low redox potential difference between the intracellular and extracellular environments. By acting as electron carriers or bridges, these mediators enable efficient extracellular electron transfer (EET), which is essential for the operation of MFCs. The efficiency of MET is influenced by the redox potentials of the mediators and the microbial oxidative metabolism that liberates electrons (Aiyer, 2019).

5.2 Identification of natural and synthetic mediators

Natural redox mediators are typically produced by electroactive bacteria as part of their metabolic processes. For instance, *Geobacter sulfurreducens* and Shewanella oneidensis are known to produce flavins and other redox-active compounds that facilitate electron transfer to the anode (Logan et al., 2019; Zhao et al., 2020). These natural mediators are advantageous because they are inherently biocompatible and can be continuously produced by the bacteria, reducing the need for external additions.

Synthetic mediators, on the other hand, are externally added compounds that can enhance the electron transfer capabilities of MFCs. Common synthetic mediators include methylene blue, neutral red, and various quinones (Kalathil et al., 2016; Aiyer, 2019). These compounds are selected based on their redox potentials and their ability to interact with the microbial cells and the electrode surface. The use of synthetic mediators allows for greater control over the electron transfer process and can significantly improve the performance of MFCs.

5.3 Case Studies illustrating MET in Various MFC configurations

Several case studies have demonstrated the effectiveness of MET in different MFC configurations. For example, a study on the use of iron phthalocyanine (FePc) modified carbon cloth (CC) electrodes showed that the modification significantly improved the viability of electroactive biofilms and enhanced electron transfer rates (Li et al., 2021). The FePc-CC anode achieved a much higher power density and biomass loading compared to unmodified CC, highlighting the potential of using synthetic mediators to boost MFC performance.

Another study investigated the effect of electrode position on the performance of MFCs designed for simultaneous Cr(VI) reduction and bioelectricity production. The results indicated that optimal electrode spacing could enhance electron transfer and electrochemical activity, leading to higher power densities and more efficient contaminant removal. This study underscores the importance of system configuration in maximizing the benefits of MET.

Furthermore, research on the application of specific potentials at the bioanode has shown that different potentials can stimulate the formation of electroactive biofilms and influence the mode of electron transfer (Pinto et al., 2018). For instance, a negative applied potential favored mediated electron transfer and biofilm formation, while a positive potential promoted direct electron transfer. These findings provide valuable insights into the complex interactions between bacteria and electrodes in MFCs and the role of redox mediators in these processes.

In summary, the use of redox mediators, both natural and synthetic, plays a critical role in enhancing electron transfer in MFCs. By understanding and optimizing the use of these mediators, researchers can improve the efficiency and performance of MFCs for various applications, including wastewater treatment, bioenergy production, and environmental remediation.

6 Factors Influencing Electron Transfer Efficiency

6.1 Impact of electrode materials and surface properties

The choice of electrode materials and their surface properties significantly influence the efficiency of electron transfer in microbial fuel cells (MFCs). For instance, the modification of carbon cloth (CC) electrodes with iron phthalocyanine (FePc) has been shown to enhance the affinity between the anode and outer membrane c-type cytochromes (OM c-Cyts), leading to a highly active electroactive biofilm (EAB). This modification resulted in a substantial increase in power density and biomass loading, as well as a significant reduction in charge transfer resistance, thereby promoting direct electron transfer (Li et al., 2021). Additionally, the use of electron-conducting polymers as mediators has been suggested as a promising strategy to enhance electron transfer efficiency, particularly in Gram-positive bacteria, which typically exhibit weak extracellular electron shuttling activity due to their thick, non-conductive cell walls (Yang et al., 2019).

6.2 Environmental Conditions (pH, temperature, substrate availability)

Environmental conditions such as pH, temperature, and substrate availability play crucial roles in the efficiency of electron transfer in MFCs. The extracellular electron transfer (EET) mechanisms are highly dependent on the redox potentials of the species involved and the microbial oxidative metabolism that liberates electrons (Aiyer, 2019). For example, the presence of specific substrates can influence the metabolic pathways of electroactive bacteria, thereby affecting their electron transfer capabilities. In a study where an electroactive biofilm was switched from acetate oxidation to nitrate reduction conditions, it was observed that the same bacterial consortium could catalyze both anodic and cathodic reactions using the same electron conduit, highlighting the adaptability of electroactive bacteria to different environmental conditions (Pous et al., 2016).

6.3 Biofilm Formation and Its Influence on Electron Transfer

Biofilm formation is a critical factor that influences electron transfer in MFCs. The spatial structure and composition of the biofilm can significantly impact its electrochemical activity. For instance, the introduction of magnetite into the biofilm, facilitated by a magnetic field, has been shown to improve electron delivery within the biofilm, leading to increased power density and enhanced extracellular electron transfer (Figure 2) (Liu et al., 2018b). Moreover, the regulation of biofilm formation through engineering strategies, such as enhancing transmembrane electron transport via cytochrome protein channels and promoting microbe-electrode interface reactions, can further improve the EET capabilities of electroactive microorganisms (Zhao et al., 2020). The distribution and penetration of conductive materials within the biofilm are essential for facilitating efficient electron transfer across the biofilm, thereby improving the overall performance of MFCs.

In summary, the efficiency of electron transfer in MFCs is influenced by a combination of factors, including the properties of electrode materials, environmental conditions, and the formation and structure of biofilms. By optimizing these factors, it is possible to enhance the performance and practical applications of MFCs in

sustainable energy production and environmental remediation.

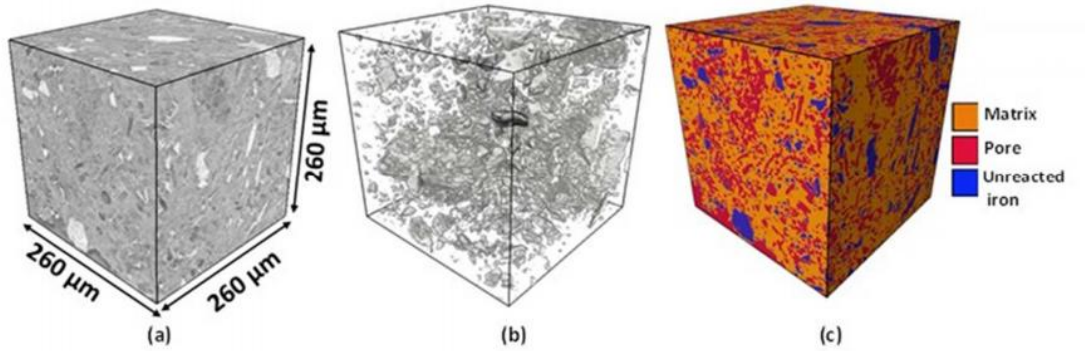

Figure 2 3D Reconstruction and Classification of Microstructure (Adopted from Liu et al., 2018b)

Image caption: (a) Original XRT image of the iron carbonate binder, (b) 3D image after segmentation of unreacted iron particles; and (c) 3D image after pore and unreacted iron segmentation (three-phase; pore, unreacted iron and solid). Please refer to the online version of the paper for color images (Adopted from Liu et al., 2018b)

7 Case Study: Electron Transfer in *Geobacter sulfurreducens* MFCs

7.1 Description of the case study setup and conditions

In this case study, we investigate the electron transfer mechanisms of *Geobacter sulfurreducens* in microbial fuel cells (MFCs). The setup involves cultivating G. sulfurreducens on graphite-based electrodes polarized to +400 mV versus Ag/AgCl for a period of 8 days. During this period, the biofilm formation and electron transfer processes are monitored and analyzed. The maximum current density achieved was (172±29) µA cm^{-2} after 7 days, indicating active electron transfer from the biofilm to the electrode (Fernandes et al., 2021). Additionally, the spatial structure of the electroactive biofilm (EAB) is tailored using iron phthalocyanine (FePc) modified carbon cloth (CC) electrodes to enhance the affinity between the anode and outer membrane c-type cytochromes (OM c-Cyts), resulting in a higher power density and biomass loading (Stöckl et al., 2019).

7.2 Analysis of electron transfer pathways in *Geobacter sulfurreducens*

The electron transfer (ET) pathways in *G. sulfurreducens* involve a complex network of multiheme c-type cytochromes that facilitate the transfer of electrons from the cell interior to the exterior. Key cytochromes identified include inner-membrane associated MacA, periplasmic cytochromes (PpcA, PpcB, PpcC, PpcD, PpcE, and GSU1996), and outer membrane-associated cytochromes (OmcF, OmcS, and OmcZ) (Santos et al., 2015). The redox properties of these cytochromes are critical for efficient ET, with OmcF playing a pivotal role in the reduction of metal oxides and electricity production in MFCs (Füeg et al., 2021). The ET process is further influenced by the extracellular polymeric substances (EPS) secreted by the biofilm, which enhance the biofilm's electroactivity and stability (Fernandes et al., 2021).

7.3 Results and implications for MFC performance

The results of this case study highlight several key findings regarding the ET mechanisms of G. sulfurreducens and their implications for MFC performance. Firstly, the rate-limiting steps in ET transition during biofilm growth were identified. In early to mid-stage biofilms, the rate-limiting step transitions from irreversible acetate turnover to electron transfer from inside the exoelectrogen to extracellular redox cofactors (ERCs) within the biofilm. Fully-grown biofilms exhibit a current density of more than 3.1 Am^{-2}, with the rate-limiting step being the electron transfer from ERCs within the biofilm to ERCs at the anode (Ren et al., 2020).

The introduction of FePc-modified CC anodes significantly improved the viability and activity of the EAB, resulting in a higher power density of 2 419 mW m^{-2} compared to 560 mW m^{-2} for unmodified CC anodes. This enhancement is attributed to the decreased charge transfer resistance and accelerated interfacial reaction rates, promoting direct electron transfer via OM c-Cyts (Stöckl et al., 2019). Additionally, the EPS secreted by *G. sulfurreducens* under electroactive conditions were found to be rich in proteins, which dominate all EPS fractions

and contribute to the biofilm's electroactivity (Fernandes et al., 2021).

These findings underscore the importance of optimizing the biofilm's spatial structure and the redox properties of cytochromes to enhance MFC performance. The detailed understanding of ET pathways and the role of EPS in biofilm stability and activity provide valuable insights for the development of more efficient microbial electrochemical technologies.

8 Applications of Understanding Electron Transfer Mechanisms

8.1 Enhancements in MFC design and performance

Understanding the electron transfer mechanisms in microbial fuel cells (MFCs) is crucial for enhancing their design and performance. The efficiency of electron transfer directly impacts the power output and overall efficiency of MFCs. For instance, the use of nanowire electron transfer mechanisms has been shown to significantly increase power generation, as demonstrated by mathematical models predicting enhanced current densities with optimized biofilm thicknesses (Lan et al., 2018). Additionally, the spatial structure of electroactive biofilms can be tailored to improve direct electron transfer, as seen with iron phthalocyanine-modified anodes, which resulted in a substantial increase in power density and biomass loading (Li et al., 2021). The position of electrodes also plays a critical role; optimal electrode spacing can enhance electrochemical performance and bioelectricity production, as evidenced by the superior performance of MFCs with 2 cm electrode spacing (Zhou et al., 2020). These insights into electron transfer mechanisms enable the development of more efficient and effective MFC designs, ultimately leading to better performance and scalability.

8.2 Potential for bioenergy production and wastewater treatment

The understanding of electron transfer mechanisms in MFCs opens up significant potential for bioenergy production and wastewater treatment. MFCs utilize electroactive bacteria to convert organic waste into electricity, making them a promising technology for sustainable energy generation and environmental remediation. The diversity of electroactive microorganisms, including exoelectrogens and electrotrophs, allows for the utilization of various organic substrates and terminal electron acceptors, thereby enhancing the versatility and efficiency of MFCs in different applications (Logan et al., 2019). For example, the reduction of Cr(VI) and simultaneous bioelectricity production in MFCs highlight their dual functionality in treating contaminated water while generating energy (Zhou et al., 2020). Moreover, the use of advanced nanostructured materials to improve bidirectional extracellular electron transfer (EET) processes can further enhance the efficiency of microbial electrosynthesis, enabling the production of high-value chemicals such as ethanol (Kalathil et al., 2016). These advancements underscore the potential of MFCs as a sustainable solution for both energy production and wastewater treatment.

8.3 Future research directions and technological advancements

Future research in the field of MFCs should focus on further elucidating the molecular mechanisms of electron transfer and exploring new materials and configurations to enhance performance. One promising area is the investigation of the electron transfer properties of less-studied electroactive microorganisms, such as Gram-positive bacteria, which could provide new insights and strategies for improving MFC efficiency (Pankratova et al., 2019). Additionally, the development of novel electrode materials, such as 2D nanomaterials, holds great potential for increasing power outputs and achieving industrial-scale applications (Slate et al., 2019). Research should also aim to optimize the conditions for biofilm formation and electron transfer, as different applied potentials can significantly influence the electrochemical behavior and architecture of biofilms. Furthermore, the integration of advanced nanostructured materials to improve the electrical connection between bacteria and electrodes could enhance both EET and microbial electrosynthesis processes (Kalathil et al., 2016). By addressing these research directions, the technological advancements in MFCs can be accelerated, paving the way for their widespread adoption in bioenergy production and environmental remediation.

In conclusion, a comprehensive understanding of electron transfer mechanisms in MFCs is essential for enhancing their design, performance, and applications. Continued research and technological advancements in this field will contribute to the development of more efficient and scalable MFCs, ultimately supporting sustainable energy

production and environmental protection.

9 Concluding Remarks

The study of electron transfer mechanisms in electroactive bacteria within microbial fuel cells (MFCs) has revealed several critical insights. Extracellular electron transfer (EET) is a pivotal process in MFCs, enabling microbes to convert chemical energy from biomass into electrical energy. This process can occur via direct transfer through conductive pili or nanowires, or mediated transfer involving redox mediators such as flavins and pyocyanins. The efficiency of these mechanisms is influenced by the redox potentials of the species involved and the microbial oxidative metabolism.

Research has highlighted the diversity of electroactive microorganisms, including iron-reducing bacteria like *Geobacter sulfurreducens*, which produce high power densities, and other microorganisms such as yeasts and hyperthermophilic archaea. The spatial structure of electroactive biofilms and the use of modified anodes, such as those incorporating iron phthalocyanine, have been shown to significantly enhance electron transfer and power density in MFCs. Additionally, mathematical models have been developed to predict the nanowire electron transfer mechanism, demonstrating that biofilm thickness has a minimal impact on MFC performance.

The findings from these studies have several implications for the development of more efficient MFCs. Understanding the mechanisms of EET can lead to the optimization of microbial and electrode interactions, thereby enhancing the overall efficiency of MFCs. For instance, the use of electron-conducting polymers and the regulation of biofilm spatial structure can improve electron transfer rates and power output. Additionally, the strategic positioning of electrodes can optimize electrochemical performance and pollutant reduction, as demonstrated by the enhanced Cr(VI) reduction and bioelectricity production in MFCs with optimal electrode spacing.

Furthermore, engineering strategies such as enhancing transmembrane electron transport, accelerating electron shuttle synthesis, and promoting microbe-electrode interface reactions can significantly improve the EET capabilities of electroactive microorganisms. The integration of advanced nanostructured materials, such as carbon nanotubes and graphene, has also been shown to enhance bidirectional EET processes, potentially expanding the practical applications of MFCs.

The future of research on electroactive bacteria in MFCs is promising, with several avenues for further exploration. Continued investigation into the molecular aspects of EET mechanisms, particularly in less-studied microorganisms, will provide deeper insights into optimizing MFC performance. The application of systems biology and synthetic biology approaches can lead to the development of high-performance electroactive microbial systems, potentially revolutionizing bioelectrochemical technologies.

Moreover, the use of redox mediators in microbial electrocatalysis offers opportunities to enhance charge transport and electrochemical reactions at the microorganism-electrode interface, promoting the widespread application of MFCs in various environmental and industrial processes. As research progresses, the integration of novel materials and engineering strategies will likely lead to more efficient and sustainable MFCs, contributing to the advancement of renewable energy technologies and environmental remediation efforts.

In conclusion, the study of electron transfer mechanisms in electroactive bacteria within MFCs has provided valuable insights that can drive the development of more efficient and versatile bioelectrochemical systems. Continued interdisciplinary research and technological innovation will be key to unlocking the full potential of MFCs in the future.

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Conflict of Interest Disclosure

The author affirms that this research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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