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ABSTRACT

Microbial fuel cells (MFCs) are bioelectrochemical systems in which microorganisms convert organic compounds into electrical energy. To date, the scientific literature has predominantly focused on wastewater treatment and electricity generation. In contrast, the application of MFCs for the biodegradation of complex, hydrophobic pollutants and the biosynthesis of surface-active compounds remains relatively underexplored. Despite the growing interest in bioelectrochemical systems, few studies have focused on the relationship between hydrocarbon degradation, electrochemical activity, and biosurfactant synthesis in MFCs. This study aims to address this gap through a comprehensive, interdisciplinary analysis of the processes occurring in MFCs, integrating electrochemical, microbiological, metabolomic, and metagenomic approaches.

The objective of this dissertation was to characterize the structure and functionality of anodic microbial communities developing in MFCs, capable of hydrocarbon biodegradation, biosurfactant production, and electricity generation under diverse experimental conditions.

In the initial phase, the potential of MFC technology for the biodegradation of benzene, selected as a model aromatic compound, was evaluated. A strategy combining pure-strain inoculation followed by enrichment with activated sludge was employed. Additionally, the effect of glucose as a co-substrate was assessed, enabling the formation of functional anodic communities and achieving record power density values. Comprehensive electrochemical, microbiological (16S rRNA), and metabolomic (NMR) characterization demonstrated high bioelectrochemical efficiency, among the highest reported to date. Benzene acted as a selective factor, promoting the growth of microorganisms capable of its degradation and electron transfer, including *Citrobacter freundii*, *Comamonas testosteroni*, *Arcobacter faecis*, *Myroides odoratus*, and *Dysgonomonas sp*.

Subsequently, the influence of applied anode potential on the structure and biodiversity of anodic microbial communities in diesel-fed MFCs was investigated. It was shown that negative anode polarization (-0,3 V) promoted the formation of more complex and functionally specialized anodic biofilms, which correlated with the highest power density achieved. Communities developing under this potential exhibited higher biodiversity and an increased abundance of taxa capable of direct electron transfer and hydrocarbon degradation, including members of the genera *Comamonas*, *Gordonia*, *Pseudomonas*, *Geobacter*, and *Rhodococcus*. These results indicate that anode potential plays a key selective role in shaping the functionality of the anodic microbiome and the electrochemical performance of MFCs in hydrocarbon biodegradation processes.

The biodegradation potential of crude oil and the electrochemical activity of nine inocula from diverse ecological niches were then evaluated. Implementation of a co-substrate strategy using sodium acetate supplementation resulted in nearly a fourfold increase in average power density, reaching a record 18,3 W/m^3 for microbial communities enriched from a petroleum compound separator. High energy efficiency was associated with a decrease in the surface tension of the anolytes, indicating active biosurfactant synthesis that enhances hydrocarbon bioavailability. For the first time, this study identified the presence of functional genes involved in hydrocarbon

degradation in MFCs, including *nahB*, *tmoF*, *ladA*, *ahyA*. Their detection under bioelectrodegradation conditions provides new insight into the molecular mechanisms coupling hydrocarbon degradation with extracellular electron transfer.

Further experiments addressed MFC scale-up and long-term operational stability. Three of the most efficient microbial consortia, enriched in the previous stage, were selected. Increasing the anode surface area three times and the reactor working volume four times resulted in a 42 % increase in maximum power density, from 18,3 W/m^3 to 26 W/m^3. This performance exceeds by more than fourfold previously reported values for crude oil-fed systems integrated with biosurfactant production. LC-MS/MS analysis enabled in situ identification of biosurfactant profiles, while metagenomic analysis confirmed the synergistic coexistence of exoelectrogenic taxa (*Geobacter anodireducens*, *Arcobacter faecis*) with species responsible for hydrocarbon degradation and surface-active compound biosynthesis (*Pseudomonas stutzeri*, *Gordonia terrae*).

The final stage of the study focused on an experiment using waste frying oil as a substrate for biosurfactant synthesis in MFCs. First, an LC-MS/MS analytical protocol was developed for the identification of rhamnolipids in anolyte samples. Based on this protocol, analyses revealed the presence of eleven biosurfactants, including both mono- and dirhamnolipids. Taxonomic profiling showed that the anodic community was dominated by *Pseudomonas aeruginosa*, known for its ability to perform extracellular electron transfer, produce mediators (e.g., pyocyanin), and synthesize rhamnolipids. The observed diversity of rhamnolipids was higher than in MFCs supplied with petroleum-derived fractions. These results indicate the potential of using waste frying oil as a substrate for biosurfactant production in MFC systems.

Overall, the results demonstrate that microbial fuel cells represent an effective platform for the simultaneous biodegradation of hydrophobic pollutants, biosurfactant production, and electricity generation. Inoculum type, anode potential, and substrate characteristics determine both exoelectrogenic activity and microbial community structure. These findings provide significant insights into the functioning of microbial communities in the presence of complex hydrocarbons and confirm the high application potential of MFC technology in bioremediation processes and the development of sustainable energy technologies.