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USE OF MICROBIAL FUEL CELL SYSTEMS IN WASTEWATER TREATMENT IN CONNECTION WITH BIOENERGY GENERATION.

UTILIZAÇÃO DE SISTEMAS DE CÉLULAS COMBUSTÍVEIS MICROBIANAS NO TRATAMENTO DE ÁGUAS RESIDUAIS COADUNADO À GERAÇÃO DE BIOENERGIA.

USO DE SISTEMAS DE CELDAS DE COMBUSTIBLE MICROBIANAS EN EL TRATAMIENTO DE AGUAS RESIDUALES EN RELACIÓN CON LA GENERACIÓN DE BIOENERGÍA.

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ABSTRACT:

Microbial fuel cells (MFCs) provide a sustainable solution for wastewater treatment and renewable energy generation, with their efficiency being highly dependent on microbial community composition, which varies considerably across effluent types and operational conditions. In this study, metagenomic sequencing libraries of 16S rRNA amplicons were obtained from 227 samples across 30 published MFC studies. These libraries were processed using the QIIME2 v2025.5.1 amplicon data analysis pipeline to perform taxonomic classification, evaluate microbial diversity metrics, and infer functional pathway associations. Despite the variability observed, common electroactive genera such as *Geobacter* (1.10%) and organic matter degraders like *Proteiniphilum* (0.84%) were consistently identified, along with halotolerant *Halobacteriota* in high-salinity environments. At the phylum level, *Pseudomonadota* (13.45%) and *Bacteroidota* (9.88%) were predominant, with functional pathways linked to extracellular electron transfer, biofilm formation, and pollutant degradation. These findings underscore key microbial taxa and metabolic processes critical to MFC performance, providing a foundation for optimizing microbial consortia and refining operational strategies to enhance bioenergy production and wastewater treatment across diverse environmental contexts..

KEY WORDS: Bioelectricity, Amplicon, QIIME 2, Microbial fuel cells, Metagenomics, Biofilms, Electron transfer, Bioenergy.

RESUMO:

As células combustíveis microbianas (CCMs) oferecem uma solução sustentável para o tratamento de águas residuais e a geração de energia renovável, sendo sua eficiência altamente dependente da composição da comunidade microbiana, que varia consideravelmente entre diferentes tipos de efluentes e condições operacionais. Neste estudo, bibliotecas de sequenciamento metagenômico de amplicons de RNAr 16S foram obtidas a partir de 227 amostras de 30 estudos publicados sobre MFCs. As bibliotecas foram processadas usando a pipeline de análise de dados de amplicons do QIIME2 v2025.5.1 para realizar a classificação taxonômica, avaliar métricas de diversidade microbiana e inferir associações com vias funcionais. Apesar da variabilidade observada, gêneros eletroativos comuns, como Geobacter (1,10%), e degradadores de matéria orgânica, como Proteiniphilum (0,84%), foram consistentemente identificados, além de Halobacteriota, tolerantes à salinidade, em ambientes com alta salinidade. No nível de filo, Pseudomonadota (13,45%) e Bacteroidota (9,88%) foram predominantes, com vias funcionais associadas à transferência extracelular de elétrons, formação de biofilme e degradação de poluentes. Esses achados destacam os principais táxons microbianos e processos metabólicos críticos para o desempenho das MFCs, fornecendo uma base para a otimização de consórcios microbianos e o aprimoramento de estratégias operacionais para aumentar a produção de bioenergia e o tratamento de águas residuais em diversos contextos ambientais.

KEYWORDS: *bioeletricidade, amplicon, QIIME 2, células combustíveis microbianas, metagenômica, biofilmes, transferência de elétrons, bioenergia.*

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RESUMEN:

Las celdas de combustible microbianas (CCMs) ofrecen una solución sostenible para el tratamiento de aguas residuales y la generación de energía renovable, siendo su eficiencia altamente dependiente de la composición de la comunidad microbiana, la cual varía considerablemente entre diferentes tipos de efluentes y condiciones operacionales. En este estudio, se obtuvieron bibliotecas de secuenciación metagenómica de amplicones de ARNr 16S a partir de 227 muestras de 30 estudios publicados sobre MFCs. Las bibliotecas fueron procesadas utilizando la tubería de análisis de datos de amplicones de QIIME2 v2025.5.1 para realizar la clasificación taxonómica, evaluar métricas de diversidad microbiana e inferir asociaciones con rutas funcionales. A pesar de la variabilidad observada, se identificaron consistentemente géneros electroactivos comunes como Geobacter (1,10%) y degradadores de materia orgánica como Proteiniphilum (0,84%), además de Halobacteriota, tolerantes a la salinidad, en ambientes con alta salinidad. A nivel de filo, Pseudomonadota (13,45%) y Bacteroidota (9,88%) fueron predominantes, con rutas funcionales asociadas a la transferencia extracelular de electrones, la formación de biopelículas y la degradación de contaminantes. Estos hallazgos destacan los principales taxones microbianos y procesos metabólicos críticos para el rendimiento de las MFCs, proporcionando una base para la optimización de consorcios microbianos y la mejora de estrategias operacionales para aumentar la producción de bioenergía y el tratamiento de aguas residuales en diversos contextos ambientales.

Palabras clave: *Bioelectricidad, Amplicón, QIIME 2, Celdas de combustible microbianas, Metagenómica, Biofilms, Transferencia de electrones, Bioenergía.*

INTRODUCTION

The growing demand for sustainable wastewater treatment and renewable energy solutions has driven the development of microbial fuel cells (MFCs), bioelectrochemical devices that convert organic matter in wastewater into electrical energy. MFCs offer a dual benefit of effluent treatment and bioenergy production, aligning with global sustainability goals (Jadhav et al., 2021). These systems consist of an anode and a cathode separated by an ion-exchange membrane. In the anode chamber, microorganisms oxidize organic matter, releasing electrons and protons. Electrons are transferred to the anode, generating an electric current, while the cathode facilitates reduction reactions, typically involving oxygen. The anode chamber is particularly critical, as it hosts the microbial communities responsible for organic matter degradation and electron transfer (Li, 2018).

Recent advances have focused on understanding the microbial communities in MFC effluents, which play a pivotal role in system efficiency. Studies using 16S rRNA amplicon high throughput sequencing have revealed the diversity and metabolic capabilities of these prokaryotic microbiomes. However, variability in effluent sources and operational conditions remains a challenge for MFC standardization and optimization (Bolyen et al., 2019).

The efficiency of MFCs is closely tied to microbial composition and the ability of microorganisms to transfer electrons to the anode, a process often mediated by extracellular cytochromes and other electron transfer proteins. Biofilm formation provides a stable environment for microbial communities, enhancing organic compound degradation and electric current generation. The three-dimensional structure of biofilms facilitates nutrient retention and resistance to adverse conditions, ensuring the continuity of bioelectrochemical processes. Nevertheless, the complexity of microbial consortia and operational diversity make system optimization challenging (Bolyen et al., 2019).

This study aims to conduct a detailed survey of microbial abundance at different taxonomic levels using publicly available 16S rRNA amplicon sequencing data from MFC experiments, proposing an analysis combined with bioinformatics tools to explore microbial diversity and its functional implications. A comparative approach based on effluent source categorization helps to understand taxonomic sensitivity and shifts, providing insights for optimizing MFC operational conditions.

METHODS

Publicly available data were obtained from the NCBI Sequence Read Archive (https://www.ncbi.nlm.nih.gov/sra), focusing on 16S rRNA amplicon sequencing samples from MFC studies labeled as "bioanode" or "anode." Samples were included if they consisted of 16S rRNA amplicon sequences from any sequencing platform and specified the operational condition of effluent type. Metadata retrieved via the SRA Run Selector identified 227 samples from 30 studies. Fastq-dump tool v3.0.3 from NCBI SRA Toolkit was used to download the sequencing data. Effluent type served as the primary comparison criterion, and Table 1 summarizes the distribution of studies by effluent category, along with their respective authors/institutions and publication years.

Table 1 – Distribution of studies by condition groups distributed among types of effluents.

Anode Chamber Source (ID)	**Starter biofilms (BIOFILM)**	**Soil (SOIL)**	**Effluents from palm oil extraction plant (OIL)**	**Effluents from fish canning industry (FISH)**	**Activated sludge/anaerobic sludge from wastewater treatment (SLUDGE)**	**Freshwater sediments (SEDIMENT)**	**Pig slurry (SLURRY)**
Study identification (number of samples)	SCHMIDT_2018 (2)	ALAGAPPA_2017 (2)	ALBARRACIN-ARIAS_2021 (2)	CASTELLANO-HINOJOSA_2024 (48)	LIU_2019 (4), LI_2022 (3), PENN_2017 (3), PARK_2017 (6), WANG_2021 (5), SRINIVASAN_BUTLER_2017 (11), GUO_2020 (1), SHANDONG_2023 (10), DONGHUA_2023 (4), IRENA_2017 (5), CASR_2022 (4), ZHEJIANG_2020 (2), ZAKARIA_2018 (2), GUALTIERI_2023 (1), BEIHANG_2022 (4), LASCU_2022 (10), CUOG_2023 (20), FIHES_2023 (2), SCUT_2023 (2), SPIESS_2023 (1), DONGHUA_2022 (3), ZHANG_2020 (7), SPIESS_2021 (4), CAO_2019 (3)	ZHAO_2017 (32)	CERILLO_2016 (28)
Samples per category	2	2	2	48	113	32	28
Total samples	**227**						

Source: Authors (2024).

Data processing and analysis Data processing was conducted using the QIIME2 2024.5.1 pipeline (Amplicon 2024.5 distribution). Single-end and paired-end high-throughput sequence data runs were imported separately, and preprocessing was performed using the DADA2 denoising extension. This step

involved error correction, identification of unique sequence variants (ASVs), and removal of chimeric sequences. Feature tables were generated, detailing counts of each unique sequence variant per sample, representative sequences, and a statistical summary of filtered, corrected, and retained reads. To ensure equitable comparisons, rarefaction was performed with qiime2 feature-table rarefy, applied at a depth of 2,849 features per sample considering the minimum necessary in order to maintain all samples, retaining 7,332 features in total among all samples.

Taxonomic classification and data visualization

Taxonomy was assigned considering the rarefied ASVs table and representative sequences. Naive Bayes classifier trained on the Greengenes2 2024.09 database (confidence threshold: 0.8) assigned taxa. Bray-Curtis dissimilarity metrics were calculated and visualized through Principal Coordinates Analysis (PCoA) plots. Shannon alpha diversity indices were computed, and violin plots were generated to compare diversity across the condition groups. All metrics were calculated with QIIME2 2024.5.1 native distance matrix tools.

Abundance data for phyla and genera were transformed using the Hellinger method to reduce compositional bias. Pie charts and stacked bar plots were generated using Python libraries BIOM-format v2.1.15, matplotlib v3.8.4, seaborn v0.12.2 and pandas v2.2.2 to visualize the taxonomic composition at these levels. Co-occurrence networks were constructed using SCNIC v0.6.6 based on the SparCC correlation metric to highlight ecological interactions among phyla, with minimum p-value of 0.5.

Functional enrichment inference

PICRUSt2 v2.5.3 was used to predict differential community functions based on ASV representative sequences, with the MetaCyc database identifying PWY metabolic pathways associated with extracellular electron transfer, pollutant degradation, biofilm formation, energy metabolism, and other processes reported in microbial fuel cell studies. Metabolic pathway counts underwent Hellinger transformation, and Jaccard distances were computed to construct a dissimilarity matrix. The transformed data were analyzed to identify abundant predicted pathways across condition groups and those exclusive to specific groups.

All scripts, visualizations, and supplementary data are available at https://github.com/jvtarss/ccm-2024.

RESULTS AND DICUSSION

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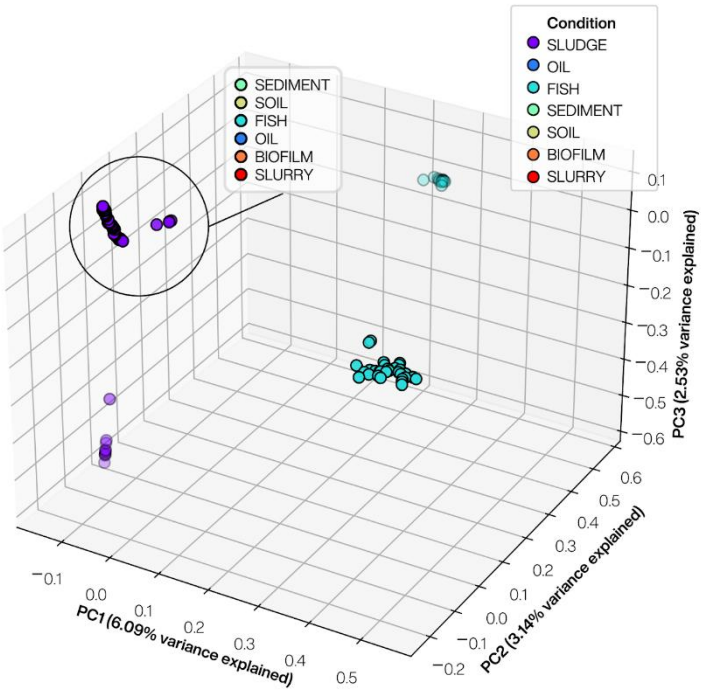
Taxonomic analysis

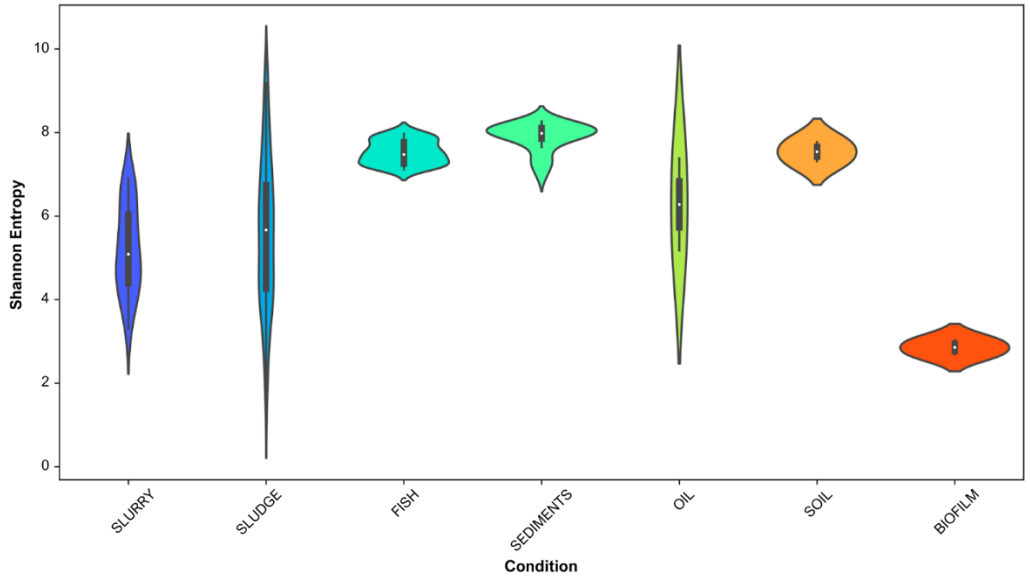
Figure 1 – PCoA plot with three principal components for the



Source: Authors (2024).

Figure 1 displays the PCoA plot of Bray-Curtis dissimilarities, with a distinct clustering of samples from fish canning industry effluents (CASTELLANO-HINOJOSA_2024) is attributed to the shifts in salinity levels for each group of samples, which influences microbial composition. Nonetheless, the samples marked in the lowest part of the graph are associated with collections made directly in the anode chamber, while the others were collected from the influent (Castellano-Hinojosa et al., 2023). All 10 activated sludge/anaerobic sludge from wastewater treatment samples from SHANDONG_2023, exposed to 0.2V - 0.6V voltages, also showed distinct clustering, highlighting the impact of these operational conditions on microbial communities (Shi et al., 2023).

Figure 2 – Violin plot from Shannon diversity alpha diversity significance for each condition.

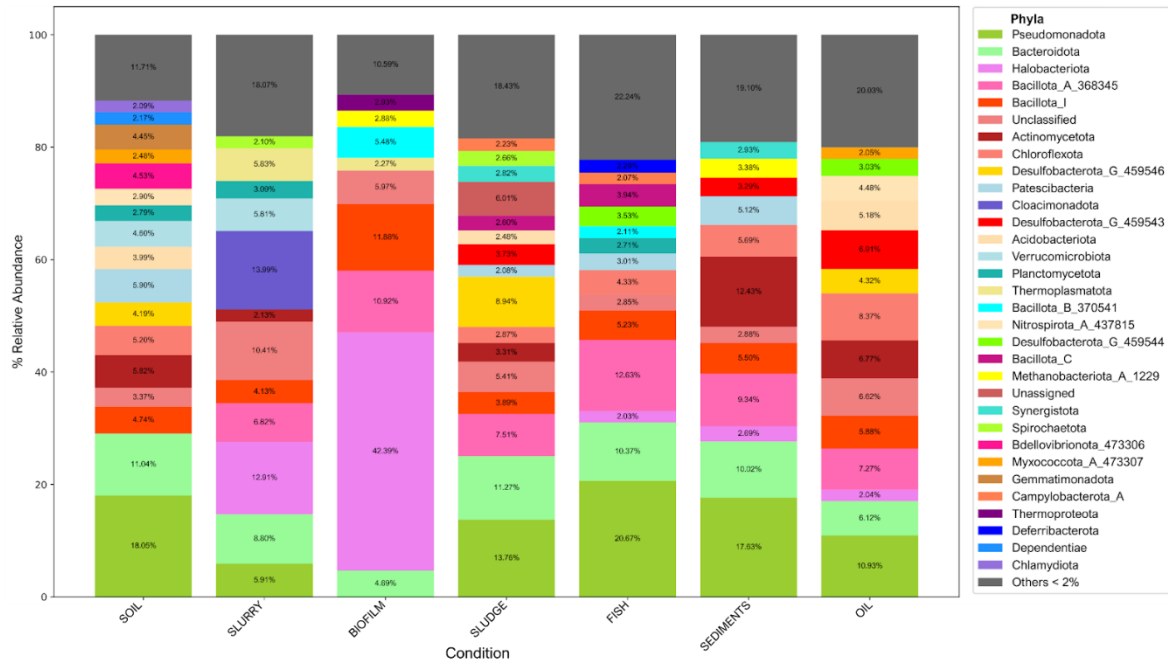


Source: Authors (2024).

Figure 2 illustrates the alpha diversity (Shannon entropy) across samples grouped by experimental conditions. The Kruskal-Wallis test revealed significant differences between the groups (H = 29.59, p = 5.45), indicating substantial variations in microbial diversity. Activated/anaerobic sludge from wastewater treatments and effluents from palm oil industry samples displayed the highest diversity, likely reflecting the greater number of samples and rich and heterogeneous microbial communities. In contrast, starter biofilms, freshwater sediments, effluents from fish canning industry and soil samples showed the lowest diversity, suggesting a more specialized or less diverse microbiota.

Phyla level analysis

Figure 3 – Stacked bar plot for mean relative abundances of phyla for pooled samples from samples for each condition.



Source: Authors (2024).

The stacked bar chart reveals distinctive patterns in microbial community composition across the different MFC conditions. Starter biofilms demonstrate a striking dominance of *Halobacteriota* (42.39%), suggesting adaptation to high salinity conditions that may influence biofilm stability and efficiency in saline effluents. Despite initial inoculation with electroactive species like *Geobacter sulfurreducens* and *Shewanella oneidensis*, the community shift indicates complex succession dynamics during biofilm development (Jiang et al., 2018).

Pseudomonadota exhibits highest abundances in effluents from palm oil extraction (22.24%) and freshwater sediments (20.03%), potentially reflecting their metabolic versatility in processing complex organic compounds. Activated sludge and anaerobic sludge from wastewater treatments show a more balanced distribution of phyla, with significant contributions from both *Pseudomonadota* and various *Desulfobacterota* groups, indicating active sulfate reduction processes. Soil samples exhibit considerable diversity with *Pseudomonadota* (18.05%) dominance, while effluents from fish canning industry show relatively high proportions of *Chloroflexota*, suggesting adaptation to the specific organic matter composition in these industrial wastewaters (Jiang et al., 2024).

Table 2 – Ranking of 15 most abundant phyla from all samples.

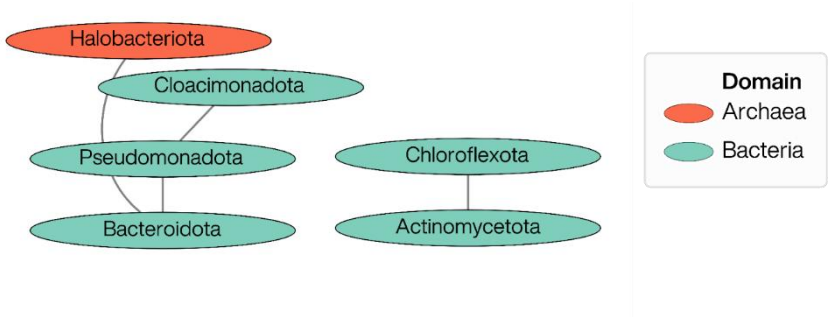
No.	Phylum	Normalized abundance of ASVs	Percentage (%)
1	*Pseudomonadota*	92.81	13.45
2	*Bacteroidota*	68.21	9.88
3	*Bacillota A 368345*	53.92	7.81
4	*Actinomycetota*	40.06	5.80
5	*Unclassified Desulfobacterota G*	37.86	5.49
6	*459546*	36.22	5.25
7	*Bacillota I*	32.20	4.66
8	*Chloroflexota*	29.28	4.24
9	*Desulfobacterota G 459543*	25.56	3.70
10	*Halobacteriota*	22.81	3.30
11	*Unassigned*	21.57	3.12
12	*Patescibacteria*	18.70	2.71
13	*Acidobacteriota*	16.11	2.33
14	*Cloacimonadota*	15.68	2.27
15	*Synergistota*	14.74	2.14

Source: Authors (2024).

The overall taxonomic analysis described in Table 2 reveals *Pseudomonadota* as the dominant phylum (13.45%), followed by *Bacteroidota* (9.88%) and *Bacillota A 368345* (7.81%), collectively accounting for approximately 31% of the total microbial community. This prevalence of *Pseudomonadota* aligns with their known roles in bioelectrochemical systems, particularly their diverse metabolic capabilities and potential for extracellular electron transfer. The significant presence of multiple *Desulfobacterota* groups (*G 459546*: 5.25% and *G 459543*: 3.70%) suggests active sulfate reduction processes that could compete with electrode reduction, potentially affecting MFC performance (Jiang et al., 2018) (Rumora et al., 2024).

Notably, while *Halobacteriota* shows dramatic dominance in biofilm samples (as seen in the condition-specific analysis), it represents only 3.30% of the overall community across all samples, indicating its specialized niche adaptation. The substantial presence of unclassified (5.49%) and unassigned (3.12%) taxa highlights the potential for novel microorganisms in these systems, suggesting unexplored metabolic capabilities that could be relevant for MFC optimization (Jiang et al., 2024).

Figure 4 – Co-occurrence network of significantly abundant phyla generated with SparCC (Sparse Correlations for Compositional data) correlation method (p-min-val of 0.5).



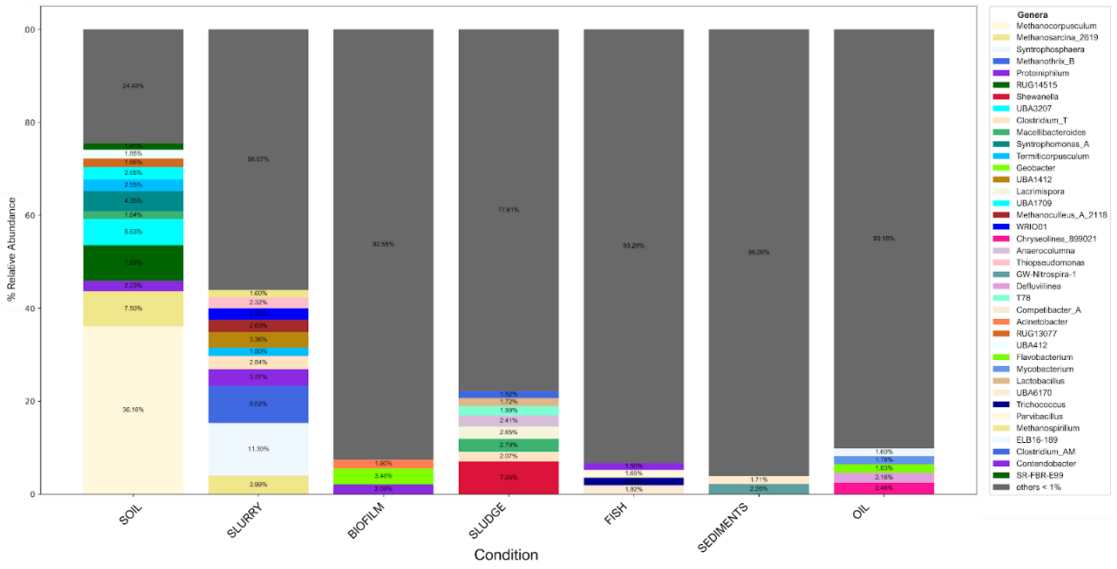
Source: Authors (2024).

The co-occurrence network in Figure 4 reveals key potential ecological relationships in MFC systems, highlighting significant correlations between *Halobacteriota* (*Archaea*) and bacterial phyla. Despite representing only 3.30% of the overall community, *Halobacteriota's* strong correlation with *Cloacimonadota* (2.27%) and its dramatic dominance in biofilm samples (42.39%) suggests important syntrophic relationships in biofilm development, particularly under high-salt conditions (Jiang et al., 2024).

The network also demonstrates robust interactions between the two most abundant phyla, *Pseudomonadota* (13.45%) and *Bacteroidota* (9.88%), suggesting synergistic organic matter degradation and electron transfer processes. A separate cluster linking *Chloroflexota* (4.24%) and *Actinomycetota* (5.80%) indicates potential complementary roles in metabolizing complex substrates, particularly relevant in industrial wastewater treatment applications (Jiang et al., 2024).

Genera level analysis

Figure 5 – Stacked bar plot for mean relative abundances of genera for pooled samples from samples for each condition.



Source: Authors (2024).

Starter biofilm samples showed a dominance of *Methanocorpusculum* (27.45%), a methanogen contributing to anaerobic degradation processes, with *Methanosarcina* (5.69%) and *Syntrophomonas* (3.31%) also present, highlighting the importance of syntrophic relationships for electron transfer. Soil samples displayed relatively low microbial abundance, with genera like *Chryseolinea* (1.55%) and *Defluviilinea* (1.36%) playing roles in organic matter degradation and nutrient cycling (Nath et al., 2021) (Jiang et al., 2024).

Effluents from palm oil extraction plants samples were enriched with *Shewanella* (5.43%), known for its electroactive properties, along with *Macellibacteroides* (2.14%) and *Clostridium T* (1.59%), which are likely involved in hydrocarbon degradation and fermentation processes (Nath et al., 2021). Effluents from the fish canning industry samples exhibited microbial diversity with *Competibacter* (1.33%) and *Trichococcus* (1.15%) contributing to organic matter breakdown, alongside potential pathogens such as Mycobacterium (0.94%) (Liu et al., 2023).

Activated sludge/anaerobic sludge from wastewater treatment samples were dominated by *Geobacter* (2.41%), a key electroactive bacterium essential for electron transfer in microbial fuel cells (MFCs), as well as *Proteiniphilum* (1.46%) and *Dysgonomonas* (0.93%), emphasizing their suitability for bioelectrochemical applications. According to the MiDAS 5 database, as of July 2024, these genera have been linked to processes such as carbon removal,

anaerobic ammonia oxidation, denitrification, nitrification, and biological phosphorus removal in activated sludge samples from different countries (Dahl et al., 2024) (Dueholm et al., 2022). Freshwater sediment samples were enriched with *Thiobacillus* (0.93%), a sulfur-oxidizing bacterium potentially enhancing cathodic reactions, and *Desulfobulbus* (0.83%), a sulfate-reducing bacterium indicative of anaerobic conditions (Ge et al., 2020).

Pig slurry samples showed a prominence of *Syntrophosphaera* (7.32%) and *Methanothrix* (5.19%), which are known for acetate oxidation and methane production, with additional contributions from *Proteiniphilum* (2.31%) and Clostridium T (1.84%) in organic matter degradation (Ge et al., 2020) (Rumora et al., 2024).

Table 3 – Ranking of 15 most abundant genera from all samples.

No.	Genus	Normalized abundance of ASVs	Percentage (%)
1	Unassigned	576.39	32.87
2	*Geobacter*	19.32	1.10
3	*Proteiniphilum*	14.78	0.84
4	*Acinetobacter*	12.91	0.74
5	*Methanothrix B*	11.89	0.68
6	*Bact-08*	11.36	0.65
7	*Syntrophosphaera*	11.25	0.64
8	*Clostridium T*	10.37	0.59
9	*Trichococcus*	9.34	0.53
10	*Competibacter A*	8.66	0.49
11	*Novosphingobium*	8.08	0.46
12	*Mycobacterium*	7.51	0.43
13	*Ignavibacterium*	7.46	0.43
14	*Desulfovibrio 446353*	7.34	0.42
15	*Comamonas F 589250*	6.83	0.39

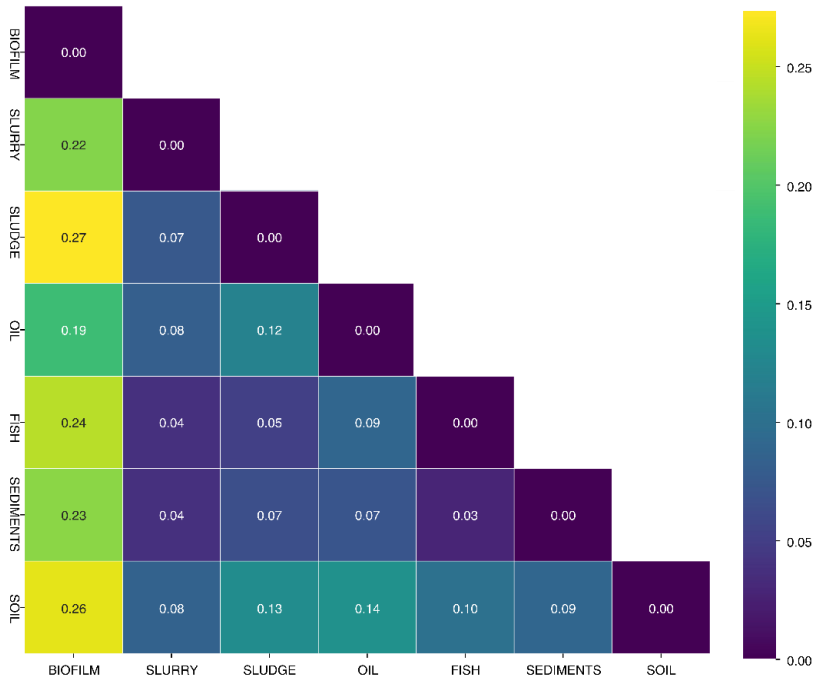
Source: Authors (2024).

Among the classified genera described in Table 3, *Geobacter* (1.10%), as stated before, is noteworthy considering its well-established role in extracellular electron transfer, which is crucial for the efficient operation of MFCs. Thus, other genera, such as *Proteiniphilum* (0.84%) and *Syntrophosphaera* (0.64%), are known for their contributions to the anaerobic degradation of organic matter, providing substrates for electroactive bacteria. *Acinetobacter* (0.74%) and *Novosphingobium* (0.46%) play roles in biofilm formation and pollutant degradation, enhancing microbial stability and diversity within the system. Additionally, methanogenic genera like *Methanothrix B* (0.68%) may indirectly influence MFC performance through syntrophic interactions, while *Desulfovibrio* (0.42%) supports sulfur cycling and electron transfer (Ge et al. , 2020).

Functional pathways prediction analysis

The dissimilarity among Hellinger-transformed metabolic pathway abundances, calculated using Jaccard distances between grouped conditions, is presented in Figure 6.

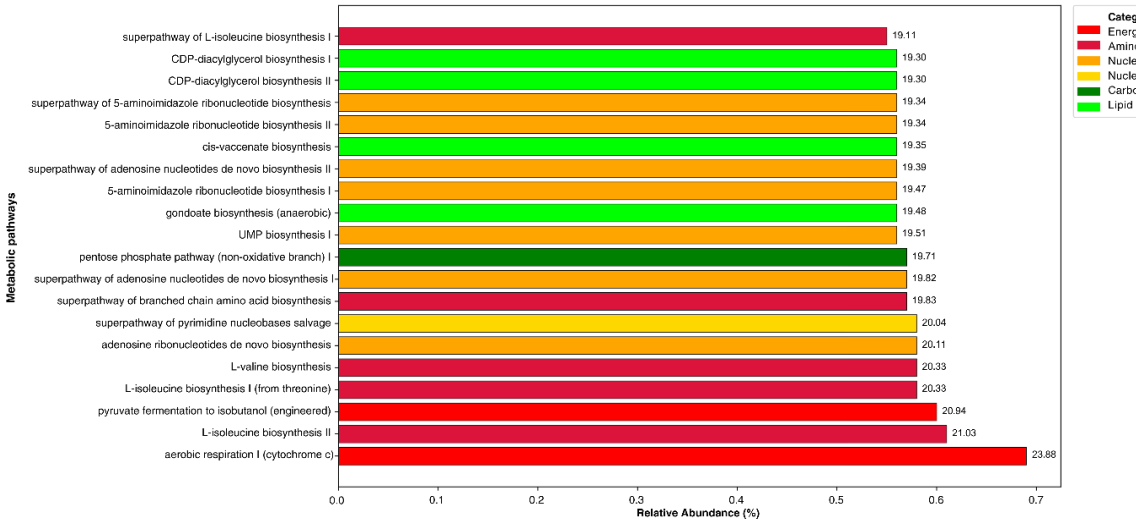
Figure 6 – Dissimilarity heatmap of Jaccard Hellinger transformed abundances of metabolic pathways among grouped conditions.



Source: Authors (2024).

The dissimilarity matrix revealed the most distinct metabolic profiles among samples associated with starter biofilms, likely due to their technical limitations, while samples from palm oil industry effluents, effluents from fish canning industry and freshwater sediments showed lower divergence numbers (Schmidt et al., 2018).

Figure 7 – Dissimilarity heatmap of Jaccard Hellinger transformed abundances of metabolic pathways among grouped conditions.



Source: Authors (2024).

Figure 7 displays the 20 most relatively abundant predicted metabolic pathways across all samples. The analysis highlighted fundamental microbial metabolic elements, such as aerobic respiration (cytochrome C) (23.88%) and pyruvate fermentation to isobutanol (20.94%), alongside amino acid, carbohydrate, and nucleotide biosynthesis pathways, including L-isoleucine biosynthesis I and II (20.33% and 21.03%, respectively) and de novo biosynthesis of adenosine ribonucleotides (20.11%). This broad metabolic landscape reflects the general microbiome diversity studied, without significant deviations to identify relevant patterns (Caspi et al., 2014) (Ijoma et al., 2021).

The analysis of exclusive pathways associated with specific effluent conditions revealed 16 unique pathways in activated sludge and anaerobic sludge samples from wastewater treatments. These included pathways for toluene degradation (PWY-5179, PWY-5184) and naphthalene degradation (PWY-5427, PWY-6956), highlighting the capacity of these systems to break down persistent pollutants such as hydrocarbons and polycyclic aromatic compounds. Pathways like the 3-hydroxypropanoate/4-hydroxybutanate cycle (PWY-5789) and gluconeogenesis II (PWY-6142) are involved in energy metabolism, while mycolate biosynthesis (PWY-6113) and O-antigen building blocks biosynthesis (PWY-5823) indicate adaptations related to biofilm formation, which is essential for stable extracellular electron transfer (EET) and current production (Caspi et al., 2014) (Whitfield et al., 2020) (Thimmaryan et al., 2023) (Ijoma et al., 2021).

Effluents from fish canning industry samples exhibited the Entner-Doudoroff pathway III (PWY-2221), which optimizes glucose metabolism under high

salinity, and coumarins biosynthesis (PWY-7398), potentially aiding in stress response, which may be associated with microbial survival and EET efficiency in saline environments. Freshwater sediments samples showed dTDP-N-acetylviosamine biosynthesis (PWY-7316), likely supporting cell wall integrity in nutrient-limited conditions, while soil samples featured the L-methionine salvage cycle I (PWY-7528), important for sulfur metabolism and stress tolerance, both of which could indirectly support pollutant degradation and biofilm stability (Caspi et al., 2014) (Ijoma et al., 2021) (Duan et al., 2021) (Tokunou et al., 2022).

CONCLUSION

This analysis emphasizes the critical roles of key microbial taxa, such as *Geobacter* (electron transfer), *Proteiniphilum* (organic degradation), and *Methanothrix* (methanogenesis), as well as dominant phyla like *Pseudomonadota* and *Halobacteriota*, in driving the performance of microbial fuel cells (MFCs). Functional profiling uncovered effluent-specific metabolic adaptations, including hydrocarbon degradation pathways in activated sludge and salinity-resilient glucose metabolism in fish canning effluents, offering actionable insights for optimizing MFCs under diverse operational conditions. These findings highlight the potential of leveraging microbial community composition to enhance MFC efficiency, particularly through the enrichment of electroactive and stress-adapted taxa.

It is important to acknowledge methodological limitations, including differences in sequencing depth, the potential loss of features during rarefaction, and uneven sample grouping, which may have influenced the results. Despite these constraints, the study provides valuable insights into the microbial dynamics of MFCs.

Future research should build on these findings by integrating multi-omics approaches, such as metagenomics and metatranscriptomics, to further elucidate strain-level contributions and metabolic interactions. Additionally, the data presented here could serve as a foundation for developing models of MFC systems tailored to specific microbial compositions, advancing their scalability and application in sustainable wastewater treatment and bioenergy production. By combining these insights with controlled experimental designs, researchers can refine strategies for optimizing MFC performance and fully harnessing the potential of microbial communities in bioelectrochemical systems.

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