

Biochar cathodes for power-to-protein: a circular approach for anaerobic digestion side-product valorisation

Gabriele Soggia^a, Amin Narimani^a, Barbara Mecheri^b, Beatrice Ricciardi^b, Niccolò Forin^c, Giuseppe Concheri^c, Fabrizio Adani^{a,*}

^a Gruppo Ricicla Lab., Department of Agricultural and Environmental Sciences, University of Milan, Via Celoria 2, 20133, Italy

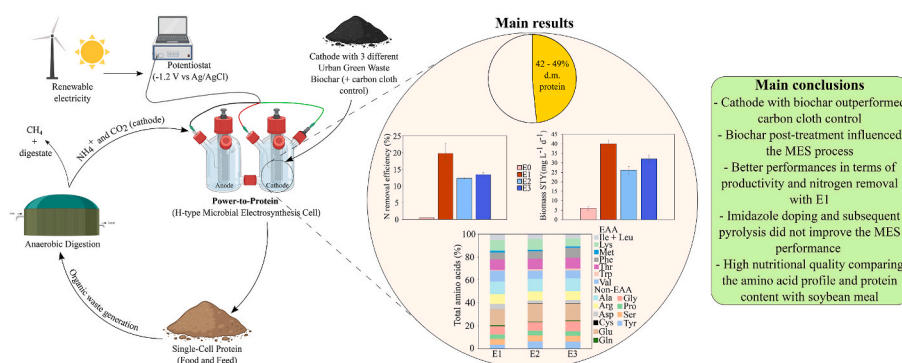
^b Department of Chemical Science and Technologies, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, Rome, 00133, Italy

^c Department of Agronomy, Food, Natural Resources, Animals and Environment, DAFNAE, University of Padova, Viale dell'Università 16, Legnaro, PD, 35020, Italy

HIGHLIGHTS

- Urban green waste biochar improved cathode performance compared to carbon cloth.
- CO₂-activated biochar functionalized cathode outperformed the post-treated version.
- Production of Single-Cell Protein with protein content of 42 - 49% on dry matter.
- Valuable amino acid profile with 39 - 41% of essential amino acids relative content.
- Improved results compared to previous study applying circular economy approach.

GRAPHICAL ABSTRACT



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ABSTRACT

Power-to-Protein is considered an innovative approach to store renewable energy by producing Single-Cell Protein as an alternative protein source to feed the growing global population, while lowering production impacts and recovering industrial side-streams such as CO₂ and NH₃. Nevertheless, a win-win approach should balance protein yield with cost reduction.

Three different biochar-functionalized cathodes were tested in Microbial Electrosynthesis Cells at a cathode potential of -1.2 V vs. Ag/AgCl for the recovery of CO₂ and NH₃ from anaerobic digestion. One biochar was activated with CO₂, while the other two underwent imidazole impregnation and subsequent pyrolysis.

The biochar application on the cathode led to improved performance compared to bare carbon cloth control in terms of protein productivity, protein content on dry matter, and nitrogen capture efficiency. However, the biochar post-treatments (i.e., nitrogen doping with imidazole and second-round pyrolysis) did not provide any additional benefit with respect to the key performance indicators considered. In particular, the untreated biochar

Abbreviations: BES, bioelectrochemical system; BET, Brunauer–Emmett–Teller; BJH, Barrett–Joyner–Halenda; CE, Coulombic Efficiency; DET, direct electron transfer; d.m., dry matter; GHG, greenhouse gas; HOB, hydrogen-oxidizing bacteria; IET, indirect electron transfer; MES, microbial electrosynthesis cell; PEM, proton exchange membrane; PM, particulate matter; SCP, single-cell protein; SDGs, Sustainable Development Goals; STY, space-time yield.

* Corresponding author.

E-mail address: fabrizio.adani@unimi.it (F. Adani).

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achieved 47% and 59% higher protein space-time yield compared to the two post-treated biochars. Regarding relative protein content and amino acid profile, no significant differences were observed among the biochar-functionalized cathodes, all of which exhibited a satisfactory proportion of essential amino acids (39 - 41% of total amino acids). The protein-rich biomass obtained in this study shows protein contents and essential amino acid proportions comparable to those reported for soybean, highlighting the potential of Power-to-Protein as a sustainable biotechnological route for future feed and food applications supporting energy transition and applying circular economy approach.

1. Introduction

Global population reached more than 8 billion in 2024, and it is projected to peak at around 10 billion by the mid-2080s (United Nations, 2024). Population growth is driving a substantial increase in global food demand (Sillman et al., 2019). The current food production system relies heavily on agriculture to supply proteins, essential biomolecules for human nutrition and health (European Parliamentary Research Service, 2024). However, the conventional protein production system is characterized by low nitrogen conversion efficiency, which results in excessive application of nitrogen fertilizers. A significant portion of this nitrogen is lost to the environment in the form of nitrous oxide (N_2O), a potent greenhouse gas, ammonium (NH_4^+), which contributes to PM 2.5 air pollution, and nitrate (NO_3^-), which leads to eutrophication when it reaches aquatic ecosystems (Pous et al., 2023). Only about 4% and 14% of the nitrogen supplied during animal- and plant-based protein production, respectively, is retained in the final consumable products (Pikaar et al., 2017). To worsen the situation, 50% of arable lands are already used (Sobhi et al., 2023) and global warming is expected to impact negatively agricultural yield in the future (Lumsden et al., 2024; Sillman et al., 2019). For those reasons, increasing the traditional agricultural production to support growing population demand for protein will be insufficient and unsustainable (Thornton et al., 2023).

Rethinking protein production systems toward more sustainable, circular, and environmentally friendly alternatives will be crucial to ensure sufficient protein supply and to simultaneously safeguard both ecosystem health and human well-being as required by the United Nations Sustainable Development Goals (SDGs), particularly Goal 2 (Zero Hunger), Goal 12 (Responsible Production and Consumption) and Goal 13 (Climate Action) (United Nations, 2015).

Alternative protein sources are emerging as a promising strategy to meet the above-mentioned requirements (Malila et al., 2024). Among these, single-cell proteins (SCPs), namely protein-rich microbial biomass, stand out due to their considerable protein content on dry weight (up to 80%), high productivity and resource utilization efficiency (Cedeno et al., 2025). SCPs offer numerous advantages over conventional animal and plant-based proteins, including lower water and land requirements, which could be further reduced by exploiting non-arable land. Furthermore, SCP production is independent of seasonal and climatic conditions (enabling year-round production) (Rovira-Alsina et al., 2025a). Additionally, SCPs are considered a high-quality protein source due to their considerable content of essential amino acids, and high digestibility, as demonstrated by a Protein Digestibility Corrected Amino Acid Score close to 1 (Cedeno et al., 2025).

Recently, the production of SCPs in bioelectrochemical systems (BES), specifically in microbial electrosynthesis (MES), has gained attention due to the capabilities of certain microorganisms to utilize electrons provided by a cathode either directly (via direct electron transfer, DET) or indirectly (through intermediates like H_2 or formate formed at the electrode surface, via indirect electron transfer, IET) to reduce CO_2 (Rominger et al., 2025). Furthermore, MES systems offer the possibility of CO_2 and NH_3 valorisation from industrial waste streams, contributing to carbon and nitrogen recovery within a circular economy and bioeconomy framework. For instance, CO_2 contained in off-gases or biogas can serve as a carbon source, while NH_3 present in the liquid fraction of the digestate can be used as a nitrogen source, thus aiding

nitrogen removal from wastewater (Soggia et al., 2024).

Despite their potential, MES systems for SCP production are still confined to the laboratory scale (maximum TRL around 4) due to challenges related to biomass productivity, high cost of components, particularly electrode and membrane materials, and Ohmic losses that reduce overall efficiency (Goglio et al., 2025; Roy et al., 2022; Schievano et al., 2019; Thulluru et al., 2025).

The study of alternative electrodes is therefore required to improve biocatalyst-cathode interactions, enhancing electron transfer, which is one of the main bottlenecks, and consequently increasing SCP productivity, while simultaneously reducing the cost and the environmental impact of traditional electrodes (Rovira-Alsina et al., 2025a; Sun et al., 2025).

Functionalization of carbon-based electrodes, such as carbon cloth, represents one of the most straightforward strategies to address the abovementioned challenges (Antic Gorrazzi et al., 2023; Raj et al., 2025; Thulluru et al., 2025). Biochar is among the most studied functionalizing agents for BES applications and has proven effective in increasing the electrode surface area, providing active sites that both promote interactions with microorganisms and facilitate redox reactions such as the reduction of protons to H_2 (Schievano et al., 2019). Furthermore, it is renewable and offers excellent biocompatibility, chemical and mechanical stability, good electrical conductivity and lower cost compared to graphite or metal-based cathodes (Antic Gorrazzi et al., 2023; Ghiara et al., 2023; Goglio et al., 2025).

The aim of this study was to investigate how different biochar activation and post-treatment strategies influence SCP production in MES. Biochars were produced from the same urban green waste feedstock and subjected to different post-treatments before being applied onto a carbon cloth support, resulting in biochar-functionalized cathodes with identical loadings. Carbon cloth without biochar was used as a control. Although biochar has been increasingly investigated as a functional material for bioelectrochemical applications, its use as a cathode material specifically for SCP production in MES remains limited, as most previous studies have relied on metal-based or noble-metal electrodes (Nyyssölä et al., 2021; Pous et al., 2022, 2023; Rovira-Alsina et al., 2025b; Tian et al., 2025; Yang et al., 2022a, 2022b). In this context, waste-derived biochar applied on carbon cloth represents a potentially more sustainable alternative, combining lower material costs and reduced environmental impacts with the reuse of urban biomass residues. By isolating the effect of biochar post-treatment while keeping feedstock, electrode loading and operating conditions constant, this study provides new insight into how cathode physicochemical tuning, rather than biochar presence per se, governs SCP productivity in MES. Combining the exploitation of CO_2 and NH_3 from anaerobic digestion as carbon and nitrogen sources, this study further provides insights into cathode design for SCP-oriented MES within a circular framework based on waste valorisation and nutrient reuse. To the best of the authors' knowledge, it is one of the first studies to explore how different biochars produced from the same feedstock affect SCP production in MES.

2. Materials and methods

2.1. Inoculum selection

The inoculum used in this work (OFMSW) was sampled from a

municipal solid waste landfill dating back to the 1980s and located in the Veneto region, which was subjected to a landfill mining operation in 2022. After sampling, it was acclimated in 500 mL glass serum bottles with a modified medium from Patil et al. (2015) with a NH_4Cl content of 1.25 g L^{-1} . The inoculum was fed every day with a gas mixture of H_2 : CO_2 (80:20% v/v) and agitated using an orbital shaker to ensure proper gas-liquid mixing. The methanogenesis inhibitor 2-bromoethanesulfonate (2-BES) was added at a concentration of 10 mM to the medium to reduce the rate of competing reactions that could withdraw reducing power from the desired product, i.e., protein (Pan et al., 2025; Serna-García et al., 2023).

2.2. Surface area characterization

Nitrogen adsorption-desorption isotherms were measured using a Micromeritics® TriStar II Plus surface area and porosity analyzer. Prior to analysis, all samples were degassed under vacuum at 350°C for 3 h to ensure the complete removal of physically adsorbed species and potential contaminants originating from air exposure. After degassing, the samples were transferred under inert conditions into the instrument's analysis ports. The specific surface area was calculated using the Brunauer-Emmett-Teller (BET) method applied to the adsorption data within the appropriate relative pressure range. The total pore volume was determined from the quantity of nitrogen adsorbed at a relative pressure (P/P_0) of 0.99, assuming pore saturation under these conditions.

Pore size distributions were derived from the desorption branch of the isotherms using the Barrett-Joyner-Halenda (BJH) model, allowing the evaluation of mesoporous structure characteristics. All measurements were performed at standard liquid-nitrogen temperature (77 K).

The electrochemically active surface area (ECSA) was determined from cyclic voltammetry (CV) measurements performed in N_2 -saturated 0.1 M PBS within a potential window of $0.5\text{--}0.0 \text{ V}$ vs. Ag/AgCl at scan rates ranging from 5 to 100 mV s^{-1} . The double-layer capacitance (Cdl) was first calculated from the slope of the linear relationship between the anodic current density measured at 0.3 V vs. Ag/AgCl and the scan rate. Subsequently, the ECSA was estimated using the obtained Cdl value, the mass of biochar deposited on the electrode, and a specific capacitance value of 0.3 F m^{-2} , according to a previously reported procedure (Nisa et al., 2026).

2.3. Cathode preparation

Carbon cloth ($3 \times 7 \text{ cm}$) was used as a conductive support. E0 consisted of bare carbon cloth air-sprayed with $20 \text{ wt\%}$ PTFE, followed by thermal treatment and hot pressing. This cathode was used as a control to assess the protein production without any electrode functionalization. The other cathodes were functionalized using air-sprayed biochar derived from urban green waste with a total loading of 6 mg cm^{-2} . Briefly, biochar and PTFE ($20 \text{ wt\%}$) were dispersed in an IPA/ H_2O mixture ($2:1 \text{ v/v}$) by ultrasonic treatment and the resulting ink was spray-coated onto preheated carbon cloth substrates (80°C). The coated electrodes were then hot-pressed (2.5 MPa , 90°C , 15 min) and subsequently heat-treated at 350°C for 30 min . A second biochar layer (catalyst layer) was then deposited using an ink containing $22 \text{ wt\%}$ Nafion as a binder, followed by a hot-pressing step (2.5 MPa , 80°C , 10 min). The biochar used for E1 was produced by pyrolyzing the biomass at 550°C with a heating rate of $10^\circ\text{C min}^{-1}$ and a residence time of 30 min under an N_2 atmosphere. The resulting char was then subjected to a consecutive CO_2 activation at 800°C (heating rate $20^\circ\text{C min}^{-1}$, no residence time). The biochar used for E2 was obtained through a two-step procedure. After the initial pyrolysis at 550°C , the cooled char was impregnated with imidazole and subsequently activated in CO_2 at 800°C for 1 h . The biochar used for E3 followed a three-step process: the precursor was first pyrolyzed at 550°C , then the resulting char was impregnated with imidazole, washed with HNO_3 , and finally

subjected to a second high-temperature pyrolysis at 900°C for 2 h under N_2 (Table 1).

2.4. Bioelectrochemical system configuration

A double-chamber H-type MES cell was used for SCP production. Each chamber had a working volume of 100 mL . The anolyte consisted of medium with conductivity and pH values of 6.189 mS cm^{-2} and 8.65 , respectively (Patil et al., 2015). The catholyte was prepared by mixing 50 mL of acclimated inoculum with 50 mL of modified medium as described in Section 2.1. Anodized titanium mesh ($1 \times 4 \text{ cm}$) was used as the anode. An Ag/AgCl (3 M KCl) electrode was used as the reference; henceforth in this work the potential will be referred to this reference electrode. A NEV 4 potentiostat (Nanoelectra S.L., Spain) was used in a three-electrode configuration. The two chambers were separated by a Nafion® 117 (DuPont™, US) proton exchange membrane (PEM).

The inoculum was acclimated for three weeks in the MES system at a cathode polarization of -1.0 V , which was identified as the best-performing polarization for protein production in a previous study with similar conditions (Soggia et al., 2024). After the acclimation phase, the cathode potential was shifted to -1.2 V , and this phase was considered the experimental period for performance evaluation (43 days). All key performance indicators described in the following sections were calculated considering the experimental period and thus excluding the acclimation phase.

The MES was fed by saturating every day the headspace (approximately 65 mL) with $99\% \text{ CO}_2$ derived from biogas upgrading (SIAD, Bergamo, Italy). All the experimental phases, including the acclimation period, were carried out at $20 \pm 2^\circ\text{C}$.

2.5. Biomass quantification

Biomass was harvested by means of centrifugation of the catholyte, i.e. at $16,000 \text{ rpm}$ for 20 min at 4°C (Optima L-100K Ultracentrifuge, Beckman Coulter, US) and resuspended in ultrapure (MilliQ) water. The harvested biomass and the cathode were lyophilized (Scanvac CoolSafe, LaboGene). The samples were weighed to assess the biomass production and were used for the following analyses.

2.5.1. Carbon and nitrogen quantification

Carbon, hydrogen and nitrogen biomass content were determined using a PerkinElmer CHN 2004 instrument (Waltham, MA, USA).

Biomass Space-Time Yield (STY) was calculated as described in Equation (1):

$$\text{STY} (\text{mg L}^{-1} \text{ d}^{-1}) = \frac{W}{V * t} \quad (1)$$

where W is the biomass weight recovered from the catholyte and the electrode, V is the cathodic chamber working volume (100 mL), and t is the experiment duration (43 days).

Nitrogen capture efficiency ($\%N_{\text{capt.}}$) was determined as indicated in Equation (2):

Table 1
Description of the cathodes used in the MES for SCP production.

Abbreviation	Support and biochar	Biochar treatments
E0	Carbon cloth (no biochar)	-
E1	Carbon cloth with urban green waste biochar	CO_2 activation (pyrolysis at 800°C , $20^\circ\text{C min}^{-1}$, CO_2 atmosphere)
E2		N impregnation (imidazole) and CO_2 activation (pyrolysis at 800°C for 1 h , $20^\circ\text{C min}^{-1}$, CO_2 atmosphere)
E3		N impregnation (imidazole), washing in HNO_3 and pyrolysis (900°C for 2 h , $20^\circ\text{C min}^{-1}$, N_2 atmosphere)

$$\%N_{\text{capt}} = \frac{W * N * MM_N^{-1}}{W_{\text{NH}_4^+} * MW_{\text{NH}_4^+}^{-1}} \quad (2)$$

where W is the biomass weight, N is the percentage of nitrogen in the biomass from CHN analysis, MM_N is the molar mass of nitrogen ($14.007 \text{ g mol}^{-1}$), $W_{\text{NH}_4^+}$ are the grams of supplied NH_4^+ , and $MW_{\text{NH}_4^+}$ is the molar mass of NH_4^+ (18.04 g mol^{-1}).

Apart from microbial NH_4^+ assimilation, nitrogen can be lost during the experiment due to other processes such as volatilization caused by high catholyte pH and other minor microbial activities such as nitrification and denitrification. The nitrogen fraction that was lost due to the abovementioned processes ($\%N_{\text{lost}}$), i.e., the nitrogen fraction that was not recovered in the biomass, over the total removed nitrogen in the medium was estimated as follows (Equation (3)):

$$\%N_{\text{lost}} = \frac{\Delta N - W_N}{\Delta N} \quad (3)$$

where ΔN represents the net nitrogen removed from the liquid phase, calculated from measured nitrogen concentrations at the beginning and the end of the experiment; therefore, nitrogen remaining in solution is explicitly accounted for and not included in the nitrogen lost fraction; W_N is the nitrogen content in the harvested biomass calculated from biomass weight and nitrogen content obtained from CHN. While nitrogen remaining in the liquid phase was explicitly quantified and excluded from the nitrogen lost fraction, the present study does not allow the relative contribution of individual nitrogen removal pathways, such as ammonia volatilization or biological nitrogen transformations, to be resolved. These processes are therefore discussed only in qualitative terms, based on operating conditions and previous MES literature.

2.5.2. Protein and aminoacidic content

The amino acid profile was determined using HPLC-DAD with the method detailed in Ceriani et al. (2024). Protein content (% d.m.) was determined by multiplying the biomass nitrogen content by the conversion factor of 6.25 (ISO, 2016; WHO and FAO, 2019). To verify the consistency of this approach and account for potential non-protein nitrogen, protein content was independently cross-checked by summing the quantified amino acids obtained from the amino acid profile analysis.

2.6. Electrochemical and chemical analysis

pH and conductivity of the anolyte and the catholyte were monitored throughout the whole experiment using a pH and conductivity meter (Eutech Instrument, PC 2700). Ammonium concentration was analysed using Ammonium 100 test kits ($4\text{--}80 \text{ mg L}^{-1} \text{ NH}_4^+\text{-N}$) by Nanocolor® (Macherey-Nagel).

Coulombic Efficiency (CE) was estimated according to Yang et al. (2025), with some modifications, as indicated in Equation (4):

$$CE = \frac{n * z * F}{\int_{t=0}^t I dt} \quad (4)$$

where n is the carbon moles in the biomass (derived from CHN analysis), z is the number of electrons required to reduce 1 mol of CO_2 to biomass (oxidation number of carbon in the biomass is assumed to be 0, thus z is equal to 4), F is the Faraday constant ($96,500 \text{ C mol}^{-1}$) and $\int_{t=0}^t I dt$ is the integral of current over time.

2.7. 16S rRNA sequencing and data analysis

Genomic DNA was extracted using the DNeasy® PowerSoil® Kit (Qiagen, Germany), following the manufacturer's protocol. DNA concentrations were quantified using a Qubit fluorometer (Thermo Fisher Scientific). DNAs samples were sent to Novogene Co. Ltd. (Cambridge,

UK) for PCR amplification and sequencing. The V3–V4 hypervariable region of the 16S rRNA gene was amplified using primers 341F (5'-CCTAYGGGRBGCASCAG-3') and 806R (5'-GGACTACNNGGTTATC-TAAT-3'). Amplicon libraries were prepared and sequenced on the Illumina NovaSeq 6000 platform, generating 250 bp paired-end reads.

Raw reads, already trimmed of adapters and primers, were analysed using the microbiome bioinformatics platform QIIME 2 (Bolyen et al., 2019). Denoising and quality control, including chimera removal, were performed with the DADA2 (Callahan et al., 2016) plugin using the *qiime dada2 denoise-paired* command, applying the “pooled” method for chimera detection. Taxonomic assignment of amplicon sequence variants (ASVs) was conducted using the Greengenes2 database (version 2024.09) (McDonald et al., 2024). ASV table, taxonomy annotations, and sample metadata were imported into RStudio (RStudio Team, 2023) and integrated with the *microeco* package (Liu et al., 2025). ASVs with total counts ≤ 5 were filtered out, along with those that did not belong to the Bacteria or Archaea kingdoms, and ASVs annotated as Mitochondria or Chloroplast. Count data were normalized using Total Sum Scaling (TSS) to convert raw counts into relative abundances. Taxonomic bar plots were produced with the *microeco* package, displaying the 20 most abundant taxa for each sample group. Functional potential was predicted using FAPROTAX v1.2.10 (Functional Annotation of Prokaryotic Taxa) (Louca et al., 2016) via the *transfunc* module in *microeco* with percentage-based functional profiles from TSS-normalized, group-aggregated data. Functional profiling limited to biofilm and suspended biomass samples was performed using PICRUST2 (Phylogenetic Investigation of Communities by Reconstruction of Unobserved States) with the recently updated PICRUST2-SC database (Wright and Langille, 2025). Following the PICRUST2 output, the dataset was subsequently filtered to retain pathways relevant to electroactive microbial metabolism, including extracellular electron transfer, acetogenesis, hydrogen oxidation, and translation-associated processes (Table S1). The dataset was normalized using Total Sum Scaling (TSS), and a heatmap was generated to visualize enrichment patterns across samples, enabling targeted comparison of functional profiles between biofilm and suspended biomass communities. Additionally, beta diversity analysis was conducted on the filtered and normalized dataset. Bray-Curtis dissimilarity was calculated and visualized using Principal Coordinates Analysis (PCoA) to assess clustering patterns. The effect of the experimental group on community composition was tested using permutational multivariate analysis of variance (PERMANOVA; adonis2).

2.8. Statistical analysis

All experiments and analysis were performed using three replicates. Mean and standard deviation (SD) values were determined following the standard procedure through R Studio (Posit team (2023). RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL <http://www.posit.co/>). Determination of significant differences among the parameters analysed at a level of significance of $p < 0.05$ was carried out by analysis of variance (ANOVA) and Tukey's post-hoc test using R considering biological replicates ($n = 3$).

3. Results

The biochar materials used in this study were previously fully characterized in terms of structure, porosity, and electrochemical behaviour (Nisa et al., 2026). All three biochars employed to modify the electrodes are highly porous and electrically conductive, providing a suitable platform for bioelectrochemical applications. However, the biochar used for E1 exhibits progressive changes in structure and surface chemistry as a consequence of nitrogen functionalization and post-pyrolysis treatments. Nitrogen functionalization (E2) introduces additional surface functionalities but also increases structural heterogeneity. In contrast, the post-pyrolysis treatment (E3) promotes graphitization and improves intrinsic electrochemical properties while

shifting the pore-size distribution toward a higher fraction of micropores at the expense of mesoporosity (see Table 1).

3.1. Biomass productivity and nitrogen capture efficiency

The biomass STY values recorded at the end of the experiment were respectively $40 \pm 2 \text{ mg L}^{-1} \text{ d}^{-1}$ ($1900 \pm 78 \text{ mg m}^{-2} \text{ d}^{-1}$) using E1, $32 \pm 2 \text{ mg L}^{-1} \text{ d}^{-1}$ ($1516 \pm 117 \text{ mg m}^{-2} \text{ d}^{-1}$) using E3, $26 \pm 2 \text{ mg L}^{-1} \text{ d}^{-1}$ ($1216 \pm 117 \text{ mg m}^{-2} \text{ d}^{-1}$) using E2, and $6 \pm 1 \text{ mg L}^{-1} \text{ d}^{-1}$ ($297 \pm 23 \text{ mg m}^{-2} \text{ d}^{-1}$) using E0. As indicated in Table 2, the biofilm grown on the cathodes contributed between 22 and 35% of the total STY without statistically significant differences between the electrodes indicating that the cathode functionalization did not significantly influence the balance between biofilm and planktonic cells in MES.

Nitrogen capture efficiency (Fig. 1a) followed the same trend as the produced biomass with higher nitrogen removal using E1, i.e., $19.8 \pm 3.0\%$ of total N supplied, followed by $13.4 \pm 0.7\%$ for E3, $12.3 \pm 0.2\%$ for E2 and $0.48 \pm 0.04\%$ of the control E0.

The difference between the total nitrogen removed from the medium and the nitrogen recovered in the harvested biomass was defined as the fraction of nitrogen lost through other processes, i.e., not assimilated in the biomass (Fig. 1b). This fraction accounted for approximately 99% of the removed nitrogen in E0 and for 73% and 69% in E2 and E3, respectively, while a lower fraction (55%) was observed for E1, indicating a greater fraction of nitrogen assimilated into biomass, consistent with the higher protein content and productivity using this electrode.

This nitrogen fraction not recovered in the biomass may originate from a combination of abiotic and biotic processes commonly reported in microbial electrosynthesis systems, including ammonia volatilization under alkaline catholyte conditions (Losantos et al., 2021; Yeruva and Angelidaki, 2025) and microbial nitrogen transformations (Pous et al., 2023; Sun et al., 2020). However, these pathways were not directly quantified in the present study and are therefore considered unaccounted nitrogen within the overall nitrogen balance.

3.2. Protein content and amino acid profile

From a protein content (% d.m.) point of view, no significant differences were observed among the biochar-functionalized cathodes: biomass harvested from MES with E1 contained $48.7 \pm 5.3\%$, similar to the $47.8 \pm 3.8\%$ observed in E2 biomass and $41.8 \pm 5.4\%$ in E3 biomass. It should be noted that protein content estimation based on nitrogen conversion factors may lead to overestimation due to the presence of non-protein nitrogen (e.g., nucleic acids) in microbial biomass (Chu et al., 2026; Nyssölä et al., 2021; Rovira-Alsina et al., 2025a). Although a general factor of 6.25 is widely used (Cui et al., 2026; Pous et al., 2022, 2023; Rovira-Alsina et al., 2025b; Soggia et al., 2024; Tian et al., 2025; Yang et al., 2022b), its applicability can vary depending on microbial composition. In the present study, this limitation was addressed by independently validating protein content through total amino acid quantification, which yielded comparable values. The protein content derived by summing the single amino acid content obtained from the amino acid profile analysis (Table 3) resulted in similar values compared with the one obtained from the N content, namely, $46 \pm 6\%$ d.

Table 2

Main key performance indicators of the SCP production: C/N ratio, Space-Time Yield, percentage of cathodic biofilm productivity on total STY to estimate the contribution of cathode biofilm and planktonic cells, protein content on dry matter, protein STY and average current density. Mean $\pm$ st. dev. (n = 3); values followed by the same letter are not statistically different ($p < 0.05$; ANOVA, Tukey test).

Electrode	C/N ratio	STY ($\text{mg L}^{-1} \text{ d}^{-1}$)	Cathodic biomass on STY (%)	Protein (%)	Protein STY ($\text{mg L}^{-1} \text{ d}^{-1}$)	Average current density (mA cm^{-2})	CE (%)
E0	$12.3 \pm 0.9\text{a}$	$6 \pm 1\text{c}$	$33 \pm 4\text{a}$	$7.6 \pm 0.1\text{b}$	$0.47 \pm 0.04\text{c}$	-0.16 ± 0.07	n.d. ^a
E1	$5.3 \pm 0.5\text{b}$	$40 \pm 2\text{a}$	$29 \pm 2\text{a}$	$48.7 \pm 5.3\text{a}$	$19.5 \pm 2.9\text{a}$	-0.20 ± 0.19	$14.7 \pm 0.2\text{b}$
E2	$5.7 \pm 0.4\text{b}$	$26 \pm 2\text{b}$	$22 \pm 7\text{a}$	$47.8 \pm 3.8\text{a}$	$12.2 \pm 0.2\text{b}$	-0.10 ± 0.04	$19.3 \pm 4.7\text{ab}$
E3	$6.3 \pm 1.0\text{b}$	$32 \pm 2\text{b}$	$35 \pm 4\text{a}$	$41.8 \pm 5.4\text{a}$	$13.2 \pm 0.7\text{b}$	-0.11 ± 0.05	$21.6 \pm 0.6\text{a}$

^a Not determined.

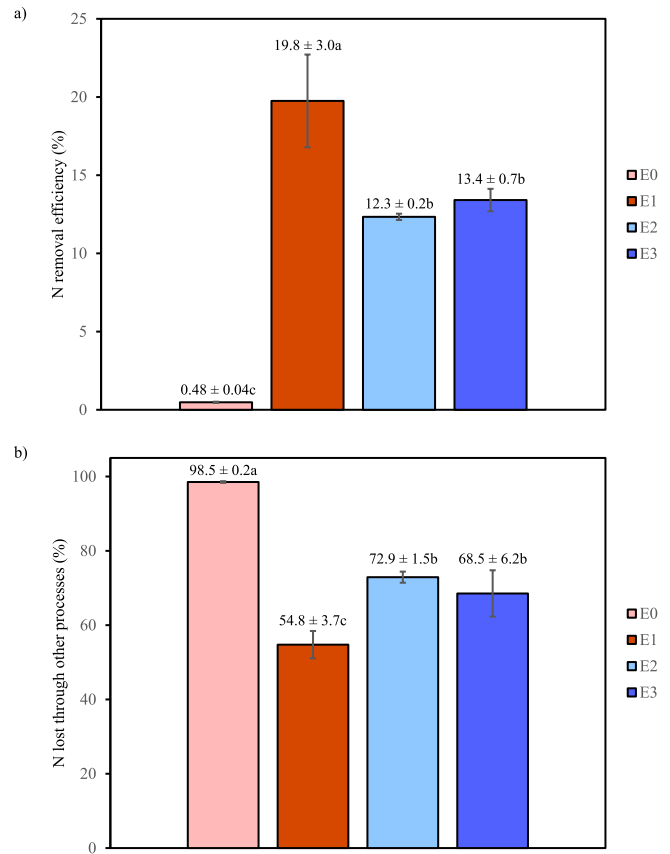


Fig. 1. a) Nitrogen capture efficiency calculated on the total nitrogen supplied and b) percentage of nitrogen lost through processes other than microbial assimilation (such as volatilization, nitrification, and denitrification) expressed as percentage of the total nitrogen removed from the medium at the end of the experimentation. Mean $\pm$ st. dev. (n = 3); values followed by the same letter are not statistically different ($p < 0.05$; ANOVA, Tukey test).

w., $51 \pm 5\%$ and $42 \pm 7\%$ for E1, E2 and E3.

The protein content measured in the biomass produced using E0 ($7.6 \pm 0.1\%$ d.m.) was markedly lower than that obtained with biochar-functionalized cathodes. This value is atypically low for microbial biomass and was associated with an elevated C/N ratio (12.3 ± 0.9), suggesting that the measured carbon content may have been influenced by the presence of inorganic carbon fractions (Soggia et al., 2024). Although the possible contribution of carbonate precipitation cannot be excluded under the operating conditions of the cathode chamber, inorganic carbon was not directly quantified. Therefore, the interpretation of E0 as a benchmark condition is affected by an unresolved uncertainty related to potential inorganic carbon contributions and thus protein content values obtained for E0 should be interpreted with caution and were not considered representative of microbial protein composition. For this reason, amino acid composition was not determined for E0, and

Table 3

Amino acid profile of the biomasses produced using the three biochar-functionalized cathodes expressed in mg 100 g⁻¹ d.w. Mean $\pm$ st. dev. (n = 3); values followed by the same letter are not statistically different (p < 0.05; ANOVA, Tukey test).

Amino acids	E1	E2	E3
Essential aa	(mg 100 g ⁻¹ d.w.)		
Isoleucine + Leucine	2422 $\pm$ 944a	2166 $\pm$ 193a	1593 $\pm$ 275a
Lysine	4289 $\pm$ 202a	4768 $\pm$ 510a	2813 $\pm$ 252b
Methionine	797 $\pm$ 7a	705 $\pm$ 43a	461 $\pm$ 84b
Phenylalanine	2655 $\pm$ 91a	3227 $\pm$ 287a	3567 $\pm$ 627a
Threonine	4310 $\pm$ 521a	4691 $\pm$ 212a	3985 $\pm$ 795a
Tryptophan	259 $\pm$ 334a	613 $\pm$ 149a	662 $\pm$ 272a
Valine	4255 $\pm$ 235a	3540 $\pm$ 167a	2873 $\pm$ 1,166a
Non-essential aa			
Alanine	5114 $\pm$ 546a	5601 $\pm$ 351a	4719 $\pm$ 964a
Arginine	3977 $\pm$ 1,020a	4002 $\pm$ 442a	3193 $\pm$ 1,148a
Aspartate	2259 $\pm$ 2,055a	1287 $\pm$ 51a	1330 $\pm$ 814a
Cysteine	32 $\pm$ 21a	69 $\pm$ 19a	56 $\pm$ 31a
Glutamate	6449 $\pm$ 289a	7958 $\pm$ 784a	6145 $\pm$ 1,117a
Glutamine	428 $\pm$ 382a	162 $\pm$ 5a	241 $\pm$ 187a
Glycine	3320 $\pm$ 97a	3832 $\pm$ 294a	3801 $\pm$ 1,470a
Proline	1794 $\pm$ 183a	2050 $\pm$ 181a	1613 $\pm$ 315a
Serine	2312 $\pm$ 453a	2692 $\pm$ 214a	2100 $\pm$ 210a
Tyrosine	1625 $\pm$ 560a	3272 $\pm$ 1,255a	2441 $\pm$ 1,039a

quantitative comparisons of protein quality were focused on biochar-functionalized cathodes.

The highest protein STY was obtained with E1 (19.5 ± 2.9 mg L⁻¹ d⁻¹ or 928 ± 139 mg m⁻² d⁻¹), followed by E3 (13.2 ± 0.7 mg L⁻¹ d⁻¹ or 630 ± 34 mg m⁻² d⁻¹) and E2 (12.2 ± 0.2 mg L⁻¹ d⁻¹ or 580 ± 9 mg m⁻² d⁻¹).

Fig. 2 illustrates that the amino acid profile of the biomasses obtained using the three different cathodes was similar from a qualitative point of view with no statistically significant differences apart from Lysine and Methionine, which were slightly lower in the E3 biomass. With all the cathodes tested, Glutamate was the dominant amino acid with relative amounts ranging from 14 to 16%. Alanine and Threonine

followed with values of 11% and in the range of 9–10%, respectively. The least abundant amino acids were Cysteine (0.1%), Glutamine (0.3–0.9%), Tryptophan and Methionine both with values ranging from 1 to 2%.

The essential amino acids in the produced biomass were $41 \pm 4\%$, $39 \pm 1\%$ and $39 \pm 2\%$ for E1, E2 and E3 respectively. The most abundant essential amino acids in the SCP were Lysine, Threonine and Valine with values ranging from 7 to 10%. As shown in Table 3, some amino acids exhibited relatively high standard deviations. This variability reflects the biological nature of the system, as values are derived from independent biological replicates, and may also be influenced by biomass heterogeneity and analytical variability.

3.3. Electrochemical and chemical characterization

The highest average cathodic current density (Table 2) was observed in E1, with a value of -0.20 ± 0.19 mA cm⁻². Fig. S1 shows that E1 reached higher values compared to the other MES system, especially between day 10 and 15, when values ranged from -0.75 to -1.00 mA cm⁻². The MES equipped with the other cathodes had lower current densities, i.e., -0.16 ± 0.07 mA cm⁻² for E0, -0.11 ± 0.05 mA cm⁻² for E3 and -0.10 ± 0.04 mA cm⁻² for E2.

The Coulombic Efficiency (CE) of the biomass production resulted was $14.7 \pm 0.2\%$, $19 \pm 4.7\%$ and $21.6 \pm 0.6\%$ for E1, E2 and E3, respectively. The CE value for E0 was negligible due to the low production of biomass during the experiment compared to the Coulomb supplied through electric current.

The catholyte pH decreased at the end of the experiment reaching values around neutrality regardless of the cathode used: 6.8 ± 0.0 for E0, 7.1 ± 0.6 for E1, 7.1 ± 0.6 for E2 and 7.0 ± 0.1 for E3. Nevertheless, throughout the experiment, the pH reached higher values (ranging between 8 and 9) as expected due to pH splitting caused by the PEM between the MES chambers. The conductivity of the medium increased throughout the experiment reaching values between 12 and 13 mS cm⁻².

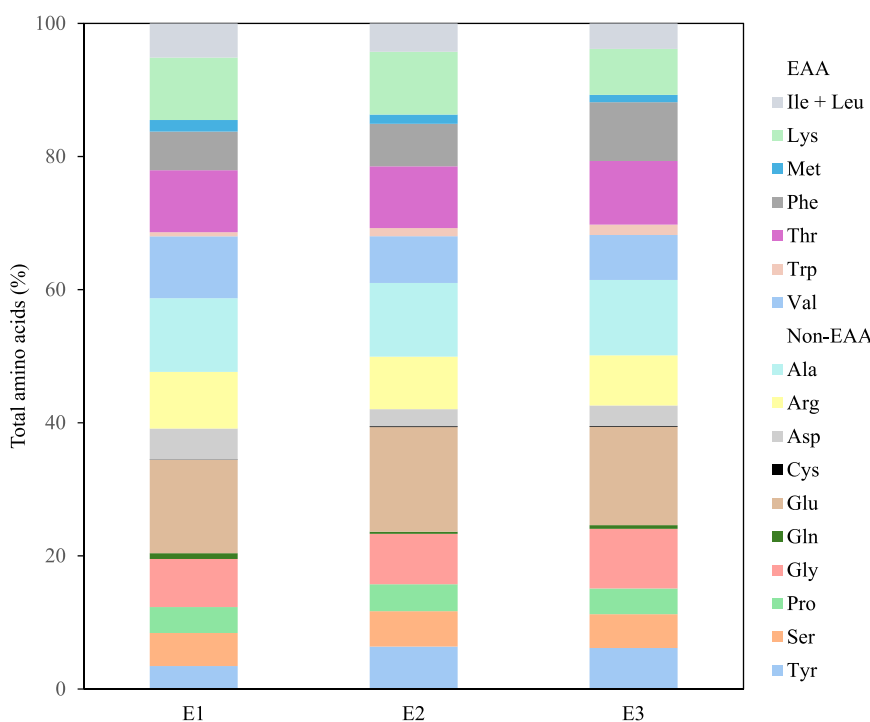


Fig. 2. Amino acid profile of the biomass recovered from catholyte of MES equipped with the three biochar-functionalized cathodes.

3.4. Microbial community analysis

To assess microbial community dynamics (bacteria and archaea), V3–V4 16S rRNA gene sequencing was performed on the original OFMSW inoculum, the OFMSW acclimated and 2-BES-treated cultures, and on biofilm and suspended biomass samples collected from MES reactors E0, E1, E2, and E3 (Fig. 3). The comparison revealed clear shifts in community composition between the pre- and post-MES samples,

particularly between MES E0 (the electrode without biochar) and the biochar-amended systems (MES E1–E3).

The starting inoculum was dominated by Rhizobiaceae, Flavobacteriaceae, Cellulomonadaceae, and Mycobacteriaceae. During acclimation, Flavobacteriaceae and Methanobacteriaceae became dominant, while the addition of 2-BES suppressed methanogenesis, leading to the disappearance of Flavobacteriaceae and a marked decline in Methanobacteriaceae, favouring non-methanogenic fermentative

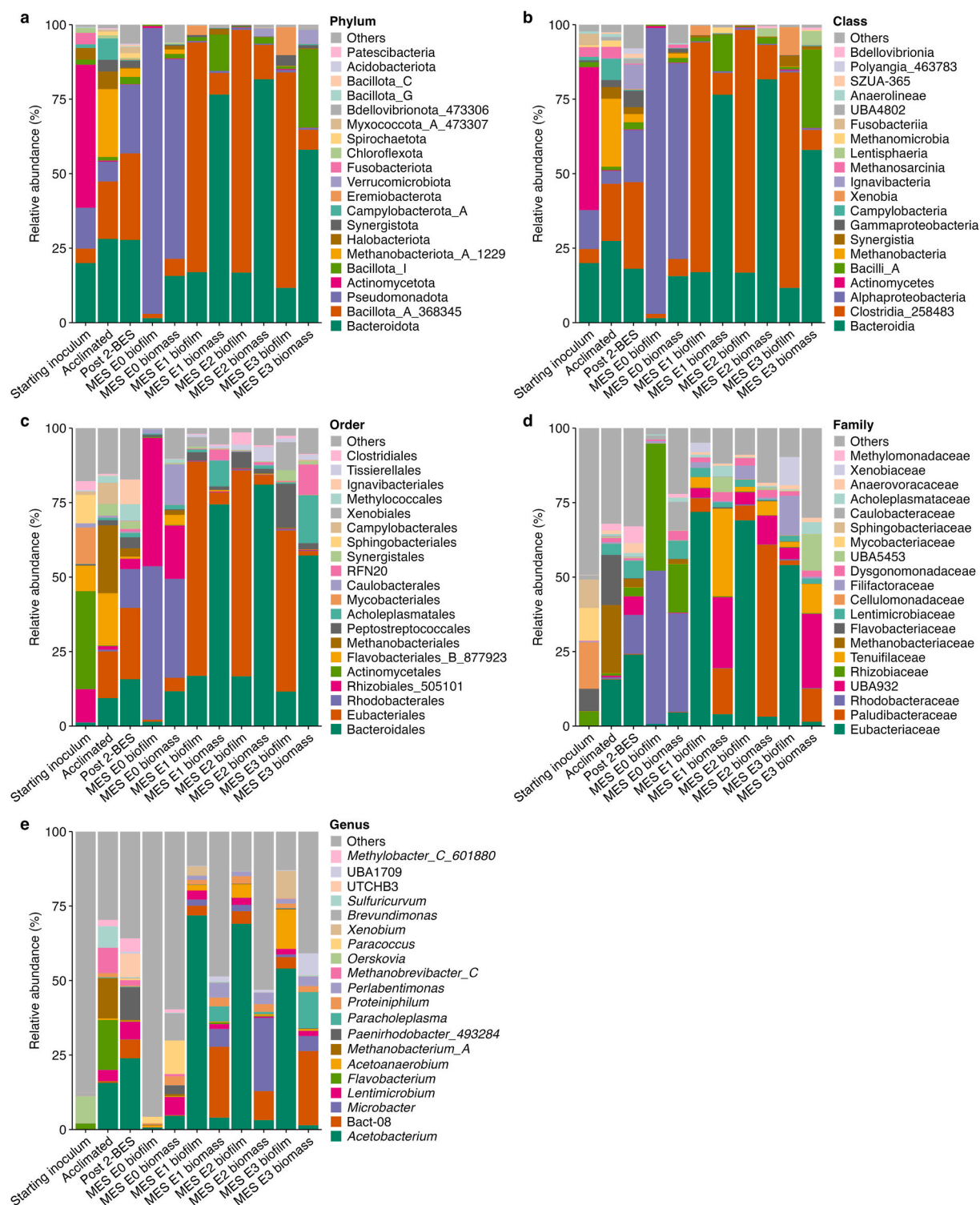


Fig. 3. Mean relative abundances of the 20 most prevalent microbial taxa at the phylum (a), class (b), order (c), family (d), and genus (e) levels. Remaining taxa are grouped as 'Others'.

populations.

MES reactors equipped with biochar electrodes were colonized by acetogenic Clostridia, including *Acetobacterium* (family Eubacteriaceae) and *Acetoanaerobium* (family Filifactoraceae), along with members of Bacteroidota (e.g., *Bact-08* and *Microbacter*). *Acetobacterium* and *Acetoanaerobium*, genera that include homoacetogenic species capable of producing acetate from CO₂ and H₂, showed higher relative abundance in the electrode-associated biofilm compared to the suspended biomass. In particular, *Acetobacterium* represented approximately 72%, 69%, and 54% of the biofilm communities associated with E1, E2, and E3, respectively, whereas it accounted for less than 1% in the E0 biofilm and 1–5% in suspended biomass samples. This distribution may reflect adaptation to the environmental conditions established at the cathode. *Acetobacterium* is frequently reported as a dominant electrotrophy in microbial electrosynthesis systems and typically prevails within biofilm communities rather than suspended fractions (Jourdin et al., 2015; Song et al., 2017). Its enrichment is in line with FAPROTAX predictions indicating a higher potential for reductive acetogenesis, supporting the hypothesis that biochar-functionalized cathodes may provide favourable conditions for microbial functions associated with CO₂ conversion and protein synthesis (Fig. 4). Despite this, these functional assignments are based on taxonomic inference and should therefore be interpreted as functional potential rather than direct evidence of metabolic activity.

Amplicon sequence variants (ASVs) affiliated with Paludibacteraceae (e.g. *Microbacter*) and Bacteroidales bacterium *Bact-08* were also enriched in biochar-based MES, particularly in biomass samples (Fig. 3). These anaerobic fermenters, together with *Lentimicrobium*, a strict anaerobic genus known for acetate production under certain conditions (Sun et al., 2016), may contribute to the degradation of residual organic matter from the OFMSW inoculum. These processes are known to produce intermediates such as acetate and formate, which could support

downstream syntrophic interactions within the MES community (Deng et al., 2018; Satinover et al., 2020).

In addition, *Proteiniphilum* was detected in both suspended and biofilm communities, albeit in low relative abundance. This genus exhibits a broad range of metabolic capabilities, including hydrolysis, fermentation, and acetate oxidation, suggesting a versatile role in organic matter turnover and syntrophic interactions within the MES microbiome (Feng et al., 2023).

Biochar-based cathodes were associated with changes in microbial community composition, including a higher relative abundance of fermentative and acetogenic taxa compared to the control. Functional annotation based on FAPROTAX suggested a potential enrichment of metabolic functions related to CO₂ reduction, H₂ oxidation and anaerobic chemoheterotrophy (Fig. 4); yet these predictions reflect functional potential rather than directly measured metabolic activity. Therefore, conclusions regarding acetogenesis, hydrogen oxidation and protein synthesis should be interpreted as indicative and require validation through direct metabolic, transcriptomic or proteomic analyses. Such metabolic functions, typical of syntrophic and acetogenic consortia operating under reducing conditions, are consistent with a microbial community structure potentially supporting redox-driven processes and the degradation of organic compounds from the OFMSW inoculum. The porous and conductive structure of biochar may provide suitable microhabitats and efficient electron-transfer interfaces that promoted biofilm development and possibly enabled direct interspecies electron transfer (Chen et al., 2014). In contrast, MES without biochar were dominated by heterotrophic Alphaproteobacteria such as Rhizobiaceae and Rhodobacteraceae, exhibiting lower predicted capacities for acetogenesis and hydrogen-based metabolism.

The PICRUST2 analysis showed a functional differences between suspended communities and electrode-associated biofilms, as well as

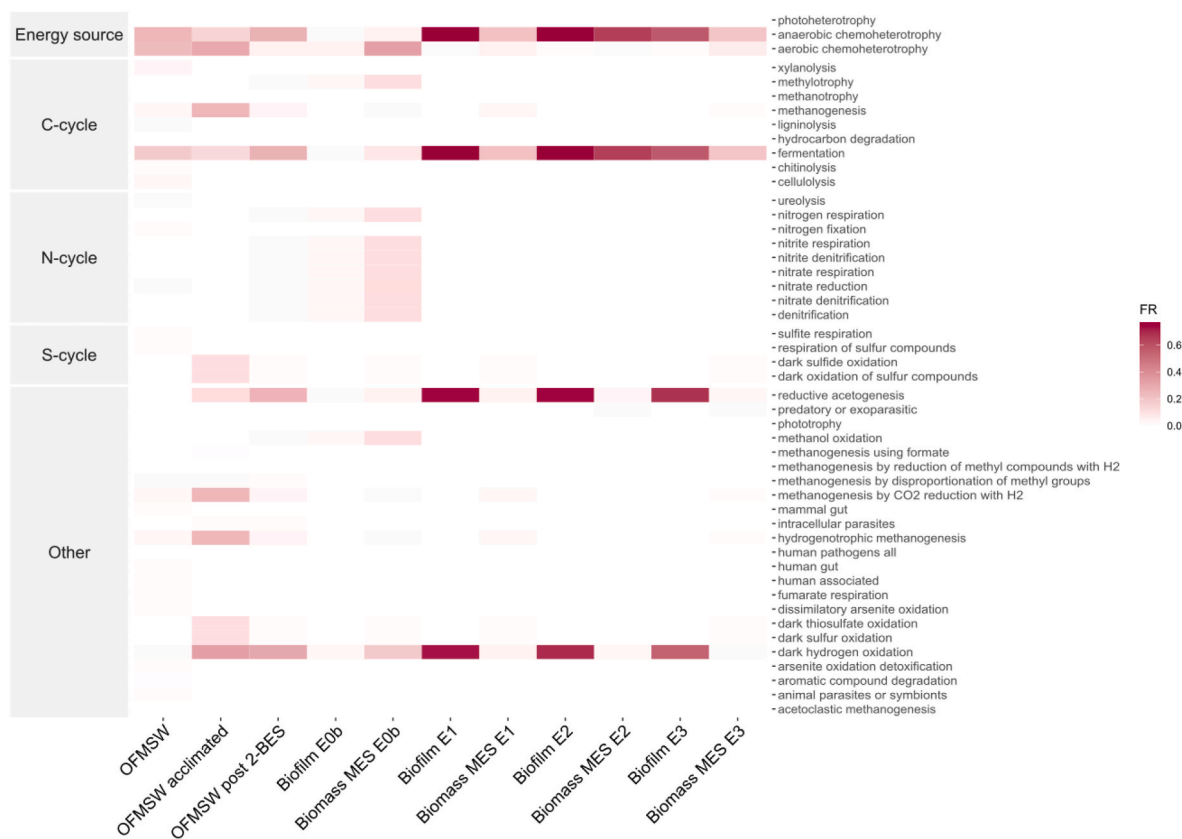


Fig. 4. Heatmap illustrating the predicted functional potentials of microbial communities based on FAPROTAX annotations. Functions are organized by category, and colour gradients represent the relative abundance of each function across the analysed samples. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

across MES configurations with and without biochar (Fig. S2). In particular, electrode-associated communities in biochar-amended systems were characterized by an enrichment of pathways related to carbon fixation and energy metabolism. Notably, the reductive acetyl-CoA pathway (CODH-PWY) was more represented in biofilm samples from biochar-containing electrodes compared to both suspended communities and the biochar-free condition, indicating an association between biochar presence and the predicted enrichment of electroactive metabolic functions. More broadly, several biosynthetic pathways linked to microbial growth and metabolic activity, including branched-chain amino acid biosynthesis (BRANCHED-CHAIN-AA-SYN-PWY), lysine biosynthesis (PWY-2942; PWY-5097), and nucleotide biosynthesis (PYRIDNUCSYN-PWY), showed a tendency to be more structured and consistently distributed within electrode-associated communities, particularly in the presence of biochar. In contrast, suspended-phase communities exhibited greater variability in their functional profiles, pointing to a less specialized and more heterogeneous metabolic organization. However, not all pathways followed the same trend. For example, coenzyme A biosynthesis (COA-PWY) showed comparable distributions between suspended and attached communities, indicating that this function appears conserved and not specifically enriched by biochar or electrode attachment. This highlights that the influence of biochar seems to be pathway-specific rather than universal. Beta diversity analysis further supported the functional differences observed in the PICRUST2 output. Principal Coordinates Analysis (PCoA) based on Bray-Curtis dissimilarity revealed a clear separation between MES E0 samples (including both suspended biomass and biofilm) and all other conditions. In addition, a distinct clustering was observed between suspended and electrode-associated communities within biochar-amended MES systems (Fig. S3). These differences were statistically supported by PERMANOVA, which indicated a significant effect of the experimental grouping on functional composition ($R^2 = 0.563$, $F = 4.73$, $p = 0.001$).

4. Discussion

In this study, the role of integrating biochar into the cathode structure was highlighted, demonstrating its effectiveness in improving MES performance for SCP production. The bare carbon cloth cathode (E0) was included as a reference condition to evaluate MES performance in the absence of cathode functionalization. The unusually low protein content and elevated C/N ratio measured for E0 suggested that the biomass composition may have been influenced by non-biological carbon contributions, which could not be experimentally verified in the present study.

Importantly, regardless of the origin of this compositional uncertainty, the performance of biochar-functionalized cathodes was consistently superior to E0 across all key performance indicators, including biomass space-time yield, protein productivity and nitrogen capture efficiency. Therefore, the observed enhancement associated with biochar-functionalized cathodes did not rely on quantitative comparisons of protein composition with E0, but rather on the clear functional improvement of the MES system upon biochar integration.

Multiple reasons could have accounted for the diverse productivity levels. First, the modification of carbon cloth with biochar of high surface area led to an increase in the electrochemically active surface area of the electrode. Surface area is a key parameter influencing the adhesion and interaction between the cathode and microorganisms, as a larger surface area generally provides a greater number of available sites for microbial attachment and interaction. In addition, modification of the electrode with biochar enhanced its electrical properties, facilitating electron transport toward the biofilm and improving charge exchange processes (Antic Gorrazzi et al., 2023; Soggia et al., 2025). This is supported by the cyclic voltammetry comparison between E0 and E1 (Fig. 5). As previously observed in N_2 -saturated electrolyte, biochar-based electrodes exhibited a higher current response than bare

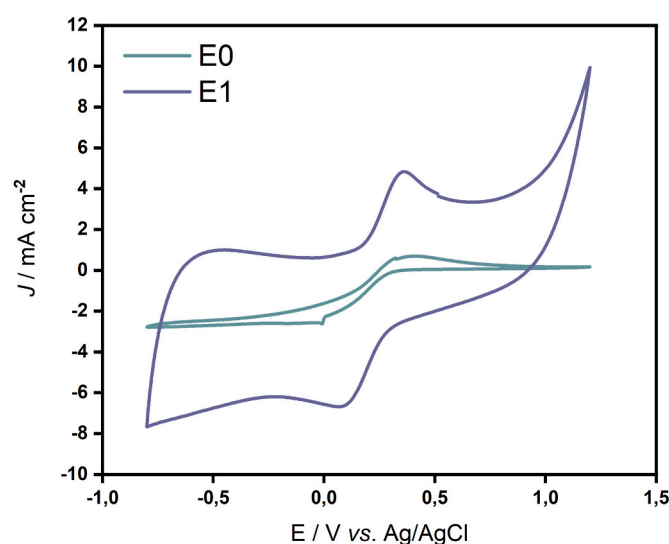


Fig. 5. Cyclic voltammogram for E0 and E1 recorded in 100 mM PBS buffer (pH 7.4) containing 10 mM potassium ferricyanide at a potential scan rate of 5 mV s^{-1} .

carbon cloth, indicating increased capacitive behaviour and electrochemically active surface area. In the presence of a ferricyanide redox probe, E1 showed both higher capacitive current and an increase in the faradaic peak current associated with the $\text{Fe}(\text{CN})_6^{3-}/\text{Fe}(\text{CN})_6^{4-}$ redox couple, indicating a larger electroactive surface area and improved charge transfer properties compared to E0 (Schievano et al., 2019; Wang et al., 2017).

The surface chemical properties of the biochar used for E1, E2, and E3 were extensively characterized in our previous study (Nisa et al., 2026) by means of FT-IR and XPS analyses. That work demonstrated the presence of oxygen- and nitrogen-containing functional groups, as well as clear differences in surface composition and nitrogen speciation arising from the different activation and post-treatment conditions. FT-IR analysis qualitatively evidenced the presence of $-\text{OH}$, $\text{C}=\text{O}$, and $\text{C}-\text{N}$ functionalities, confirming surface enrichment in oxygen- and nitrogen-containing groups, particularly for E1 and E2. In contrast, E3, due to its more extensively pyrolyzed and graphitized structure, exhibited a lower density of FT-IR-active functional groups. XPS analysis provided further insight into surface chemistry, revealing both the relative abundance of heteroatom-containing species and the nature of nitrogen functionalities. In particular, deconvolution of the $\text{N}1\text{s}$ spectra indicated the presence of pyridinic, pyrrolic, amine, and graphitic nitrogen species, whose distribution depends on the applied treatments. Among the materials, E2 showed the highest overall nitrogen content, while E1 also presented a relatively high abundance of oxygen- and nitrogen-containing functionalities; conversely, E3 was characterized by a more graphitized surface with a lower degree of functionalization. These differences in surface chemistry were directly relevant in bioelectrochemical systems, as oxygen- and nitrogen-containing functional groups are known to influence surface wettability, microbial adhesion, and electron transfer processes, potentially enhancing electrocatalytic activity compared to bare carbon-based electrodes (Artyushkova et al., 2015; Schievano et al., 2019; Wang et al., 2017).

Finally, as a consequence of the superior characteristics of the biochar-functionalized cathode compared to E0 described above, the enhanced cathodic performance was reflected in the hydrogen evolution reaction (HER) onset potential, which was more negative for E0 (-1.6 V vs Ag/AgCl) than for the biochar-functionalized electrodes (-1.2 V vs Ag/AgCl). This indicated that the presence of biochar reduced the overpotential required for hydrogen generation, thereby enhancing the HER kinetics and possibly favouring indirect electron transfer (IET) through H_2 production at the electrode interface. While direct electron

transfer (DET) cannot be excluded (Lekshmi et al., 2023; Pous et al., 2022), no direct experimental evidence supporting DET was obtained in the present study. Moreover, operating at less negative cathode potentials offers the additional advantage of lowering the system's energy demand, thus reducing overall operating costs.

Beyond the comparison between the control and biochar-functionalized cathodes, it is also essential to evaluate how the bioelectrochemical characteristics of the biochar influenced the MES performance. As reported by Li et al. (2025), the physical and chemical characteristics, and consequently the electrical properties, of biochar are strongly affected by the feedstock type, pyrolysis conditions, and any subsequent treatments.

In this study, the effect of different biochar post-treatments on cathode performance for single-cell protein (SCP) production via MES was thoroughly investigated. The post-treatments included a nitrogen-enrichment strategy through functionalization with a nitrogen-rich precursor (imidazole), followed by a high-temperature post-pyrolysis to increase the degree of graphitization of the biochar.

As described in Section 3, the best-performing electrode in terms of biomass STY, protein productivity, and nitrogen capture efficiency was E1. This result could be related to the morphology and porosity of the biochar applied to the cathodes. The total pore volume of the three biochar-functionalized cathodes was similar (approx. $0.3 \text{ cm}^3 \text{ g}^{-1}$, Table 4); in contrast, their pore-size distributions showed some differences. As widely reported in the literature (Mohamed et al., 2010), physical activation under CO_2 preferentially promotes the development of microporosity. Accordingly, micropores represented the dominant pore fraction in all three samples, although variations in their relative distribution and contribution could be observed among the materials. The biochar characterization (Fig. S4) showed that the material used for E1 exhibited a highly amorphous carbon structure with a rough, porous surface, characterized by irregular cavities (Nisa et al., 2026). N_2 -adsorption-desorption analysis indicated that E1 had a lower micropore fraction (86%) and a higher mesopore contribution (7%) compared to E3 (94% and 2%, respectively), while E2 showed intermediate values (88% micropores, 6% mesopores). Despite its slightly lower specific surface area and ECSA (Table 4), the relatively higher mesoporosity of E1 likely enhanced mass transport and the accessibility of surface functional groups, explaining its improved performance in the MES system (Li et al., 2024). This more favourable texture likely enhanced microbial adhesion and electron transfer from the cathode to bacteria, providing abundant interaction sites and resulting in 47% and 59% higher protein STY compared to E3 and E2, respectively (Chiliveru et al., 2025; Fiset and Puig, 2015; Wang et al., 2017).

In contrast, the biochar used in E3 showed a more graphitic structure with a smooth, compact, and layered morphology. Although a higher degree of graphitization is generally associated with greater electrical conductivity (Antic Gorrizzi et al., 2023; Chiliveru et al., 2025; Gabhi et al., 2020; Li et al., 2025; Prado et al., 2019; Wang et al., 2025), the predominance of micropores, combined with the ordered graphitic structure and reduced availability of surface functional groups, may have hindered bacterial colonization, biofilm development and electron transfer, thereby reducing system productivity (Prado et al., 2019; Schievano et al., 2019; Vassilev et al., 2022). E2 biochar exhibited a heterogeneous structure, with disruptions in the graphitic framework

caused by imidazole treatment, which interacted with both organic and inorganic phases, leaving agglomerated nanoparticles. Previous studies have reported that nitrogen-based post-treatments, such as imidazole impregnation, can improve bioelectrochemical performance by introducing positively charged nitrogen groups (Li et al., 2025; Schievano et al., 2019; Wang et al., 2025). Under the investigated conditions, although cathodes incorporating imidazole-treated biochars (E2 and E3) performed better than E0, the overall benefit of nitrogen doping and post-treatments in general was not evident when compared with the biochar subjected only to CO_2 post-activation, as E1 achieved superior performance. Along with attributing this outcome to specific nitrogen doping parameters, the results suggested that the intrinsic electrochemical advantages observed for functionalized biochars may be attenuated when these materials are integrated into thicker electrode structures (Nisa et al., 2026). Indeed, differences in electrochemical behaviour are more clearly detectable in thin, well-dispersed films (e.g., rotating disk electrode experiments on glassy carbon), whereas they tend to diminish in practical configurations such as carbon cloth electrodes, where higher catalyst loading, film thickness, and the presence of binders (e.g., Nafion and PTFE) can influence charge transport and mask material-specific effects (Ehelebe et al., 2022; Imhof et al., 2023). These findings indicated that translating material properties from model electrochemical systems to bioelectrochemical devices remains a critical challenge.

The nitrogen balance indicated that a substantial fraction of the nitrogen removed from the medium was not recovered in the harvested biomass. It is important to emphasize that this fraction does not represent a quantified loss through specific pathways, but rather an unaccounted nitrogen fraction resulting from the comparison between supplied nitrogen and nitrogen assimilated into biomass. Under MES operating conditions, nitrogen not recovered in biomass may be associated with processes such as ammonia volatilization, particularly during periods of elevated catholyte pH (Section 3.3), as well as with biological nitrogen transformations occurring within complex microbial communities (Section 3.4). However, under the predominantly reducing conditions of the cathodic environment and in the absence of detectable classical nitrifying and denitrifying taxa (Section 3.4), canonical nitrification–denitrification pathways appeared less favoured and were therefore likely to represent a secondary contribution relative to ammonia volatilization driven by alkaline pH. Similar nitrogen gaps have been reported in previous MES studies focusing on nitrogen recovery and microbial protein production, where volatile losses were identified as a potentially relevant factor (Losantos et al., 2021; Yeruva and Angelidaki, 2025). In conclusion, as classic nitrifiers and denitrifiers were not detected, ammonia volatilization under alkaline catholyte pH conditions (8.0 - 9.0) represented a plausible pathway, but its actual contribution could not be resolved based on the available data and thus remained one of multiple possible explanations for the observed nitrogen not recovered in biomass.

The protein content obtained in this study with the biochar-functionalized cathodes (42–49% d.w.) was comparable to values reported in previous MES studies. As summarized in Table 5, these literature values ranged from 33% to 74% d.w. with an average of $57 \pm 13\%$ d.w. (Lee et al., 2025; Nyssölä et al., 2021; Pous et al., 2022; Rovira-Alsina et al., 2025b; Soggia et al., 2024; Tian et al., 2025; Yang et al., 2022a, 2022b). As can be inferred from the above-mentioned table, a standard configuration for SCP production via MES has not yet been established, with both protein content and productivity spanning a wide range of values (33–74% and $1\text{--}2700 \text{ mg L}^{-1} \text{ d}^{-1}$, respectively), highlighting the strong influence of reactor design and operational conditions. Several studies, mainly based on single-chamber configurations and metal electrodes, achieved higher productivities than those reported here; however, they rely on materials such as stainless steel, IrO_2 -based or Ti-based electrodes, which may limit large-scale implementation due to high costs and environmental burdens associated with raw material extraction and processing (Carrara et al., 2025;

Table 4

Specific surface area (SSA), total pore volume, and pore size distribution (meso- and micropore fractions) of the biochars used for E1, E2, and E3.

Biochar	BET SSA ($\text{m}^2 \text{ g}^{-1}$)	ECSA ($\text{m}^2 \text{ g}^{-1}$)	Tot. Pore volume ($\text{cm}^3 \text{ g}^{-1}$)	Mesopore volume	Micropore volume
E1	546	103	0.33	7%	86%
E2	450	190	0.26	6%	88%
E3	613	266	0.33	2%	94%

Table 5

Summary of studies investigating SCP production through MES, highlighting inoculum, MES configuration and equipment, operational conditions, protein content (%) and reported productivities (or yields where indicated).

Inoculum	MES	Applied conditions	Anode	Cathode	Catholyte	Gas feed	Protein (%)	Protein STY (mg L ⁻¹ d ⁻¹)	Reference
<i>Cupriavidus necator</i> H16	Single chamber	−6 mA cm ⁻²	Pt wire	Glassy carbon	Synthetic medium	CO ₂	56	-	Lee et al. (2025)
<i>Xanthobacter viscosus</i> 7d							72	-	
HOB ^a -enriched culture	Double chamber	150 mA	IrO ₂ -coated titanium mesh	Stainless-steel mesh	Synthetic medium	CO ₂ :O ₂	64	77 ± 1 ^b	Tian et al. (2025)
HOB-enriched culture	Single chamber	5 – 48 A m ⁻²	Ti-MMO ^c mesh	Stainless-steel mesh	Synthetic medium	CO ₂	74	2,700 ^b	(Rovira-Alsina et al., 2025)
Wastewater treatment plant pre-acclimated culture	Double chamber	−0.6 V vs Ag/AgCl −1.0 V vs Ag/AgCl Unpolarized control	Carbon cloth	Wood-chip biochar functionalized carbon cloth	Pig-manure derived wastewater medium	CO ₂	43 ± 1 69 ± 1 33 ± 1	2.2 ± 0.1 0.8 ± 0.0 0.5 ± 0.0	Soggia et al. (2024)
HOB-enriched culture	Single chamber	0.0 – 1.0 mA cm ⁻²	Ti-MMO mesh	Stainless-steel mesh	Synthetic medium	CO ₂	87 ± 8 ^d	411 ± 143 ^b	Pous et al. (2023)
HOB-enriched culture	Single chamber	−0.997 V vs Ag/AgCl	Ti-MMO mesh	Stainless-steel mesh	Synthetic medium	CO ₂	53 ± 8 64 ± 4	6.1 ^b 8.0 ^b	Pous et al. (2022)
<i>Cupriavidus necator</i> 335	Single chamber (HBI ^e)	3 V	IrO ₂ -coated alloy mesh	Titanium mesh	Influent from municipal WWTP ^f	CO ₂	65 ± 5	247 ± 1 ^g	Yang et al. (2022a); Yang et al. (2022b); (Yang et al. (2022a); Yang et al. (2022b))
<i>Cupriavidus necator</i> 335	Double chamber	3 V 4 V 5 V	IrO ₂ -coated alloy mesh	Titanium mesh	N-free synthetic wastewater	CO ₂ :H ₂ :O ₂	49 ± 7 57 ± 8 63 ± 10	200 ^g 466 ^g 513 ^g	(Yang et al. (2022a); Yang et al. (2022b))
<i>Nocardioideis nitrophenolicus</i> KGS-27	Single chamber	18 mA	IrO ₂ -coated titanium wire	Stainless-steel capillary	Synthetic medium	CO ₂ : N ₂ (20:80)	33 ± 2	87 ± 18 ^b	Nyyssölä et al. (2021)
<i>Rhodococcus opacus</i> DSM 43205							37 ± 1	20 ± 6 ^b	

^a Hydrogen Oxidizing Bacteria.

^b Value calculated by multiplying the biomass productivity by the average protein content.

^c Titanium covered with Mixed Metal Oxide.

^d High value probably due to biomass underestimation.

^e Hybrid biological inorganic system.

^f Wastewater treatment plant.

^g Value expressed in g L⁻¹ calculated by multiplying the harvested cell dry weight by the protein content.

Jung et al., 2020; Soggia et al., 2026). By focusing on waste-derived, metal-free cathode materials, this study addressed a key unresolved challenge in MES-based SCP production, namely the strong dependence of high-performance systems on metal or noble-metal electrodes. In this context, the work critically evaluated whether competitive protein contents can be achieved under comparable operating conditions. The results demonstrated that competitive protein contents can be achieved using waste-derived biochar, providing a relevant starting point for more sustainable system design, although further improvements are required to reach state-of-the-art productivity levels achieved with metal electrodes. At the same time, the environmental and economic implications of biochar production must be carefully considered, as its sustainability depends on feedstock, pyrolysis conditions, and post-treatments, which can significantly affect energy demand and environmental footprint (Huggins et al., 2014). Therefore, comprehensive techno-economic and life cycle assessments are required to fully evaluate the benefits of biochar-based electrodes.

Similarly, the values were in line with SCP produced through fermentation, which ranged from 29% to 83% with an average of 57 ± 16% d.w. (Soggia et al., 2024). With this protein content, SCPs are in the running as an alternative to conventional protein sources such as soybean meal with reported protein content of 44% to 49% d.w (Ceriani et al., 2024). with an average of 49.7 ± 2.6% d.w. (Soggia et al., 2024).

The amino acid profile highlighted that the biochar applied on the cathodes did not significantly influence the production of amino acids from a qualitative standpoint. Nonetheless, the amino acids distribution differed from that of conventional protein sources, such as soybean meal. Glutamine was the most abundant amino acid both in soybean

meal and SCP produced in this study, with relative abundance of 17.8 ± 1.4% in soybean meal and 14 - 15.7% in SCP. Considering the SCP from E1, lower amounts of Asparagine, Leucine, Isoleucine and Cysteine were detected compared to soybean meal, in which these amino acids account for 8.9 ± 4.1%, 7.6 ± 0.4%, 4.7 ± 0.4% and 1.4 ± 0.3%, respectively. Conversely, the microbial biomass contained higher quantities of Lysine (9.4 ± 1.7% vs 6.3 ± 0.2%), Valine (9.3 ± 1.7% vs 4.9 ± 0.3%), Alanine (11.1 ± 0.3% vs 4.4 ± 0.3), Glycine (7.2 ± 0.8% vs 4.3 ± 0.2%) and Threonine (9.3 ± 0.1% vs 3.8 ± 0.2%). Glutamine, Arginine, Phenylalanine, Proline, Serine, Tyrosine, Methionine and Tryptophan were present at levels comparable to those reported for soybean meal (n = 15) (Donadelli et al., 2019; García-Rebollar et al., 2016; Kumar et al., 2022; Lagos and Stein, 2017; Lammens et al., 2012; Ramachandran et al., 2007; Rodrigues et al., 2012; Su et al., 2022; Wang et al., 2016).

The essential amino acids content of the produced SCP (39 - 41%) was similar comparing E2 and E3, and higher comparing E1 to soybean meal essential amino acid content that is on average 37.6 ± 1.4% on total amino acid content (n = 15) (Donadelli et al., 2019; García-Rebollar et al., 2016; Kumar et al., 2022; Lagos and Stein, 2017; Lammens et al., 2012; Ramachandran et al., 2007; Rodrigues et al., 2012; Su et al., 2022; Wang et al., 2016).

In any case, the comparison with soybean meal was based on protein content and amino acid composition derived from literature data. While these compositional parameters indicated a promising nutritional potential, additional aspects are required to fully assess the suitability of SCP for feed and food applications. Future studies should therefore address protein digestibility, amino acid bioavailability, nucleic acid

content and safety aspects, which are known to play a key role in determining the nutritional performance and regulatory acceptance of microbial proteins. Integrating these analyses with compositional data will be essential to place MES-derived SCP within a unified nutritional assessment framework and to enable a robust comparison with established protein sources such as soybean meal. The observed variability in individual amino acid concentrations in this study (Table 3) did not alter the overall nutritional interpretation of the obtained SCP. Despite differences in specific amino acids, the relative proportion of essential amino acids remained consistent across biochar-functionalized cathodes, and values were within ranges reported for microbial proteins. From a nutritional perspective, the assessment of SCP quality is primarily based on overall protein content and essential amino acid balance rather than on the absolute concentration of individual amino acids. Nevertheless, future studies with increased sample size and extended biological replication would be beneficial to further reduce uncertainty and to refine the evaluation of amino acid variability in MES-derived SCP.

Soggia et al. (2024), under similar conditions but using biochar derived from wood chips, reported higher relative protein content compared to this study. Specifically, E1 exhibited approximately 29% lower protein content than the SCP produced with wood chip biochar cathodes. Nonetheless, both biomass and protein STY in this study increased by 11- and 8-fold with respect to the best performing MES reported by Soggia et al. (2024), respectively.

The average current density values in the two studies were of the same order of magnitude, indicating comparable electrochemical activity and, consequently, similar energy consumption. When comparing the best-performing MES from both studies, the essential amino acid content showed an 18% improvement. Furthermore, nitrogen capture efficiency increased by 195% compared to the previous work. Overall, these results revealed a significant enhancement in the performance of the MES for SCP production relative to the earlier investigations.

The biomass STY was compatible with previous studies, which reported values in the order of milligrams per liter per day, ranging from 12 to 473 mg TSS L⁻¹ d⁻¹ (Nyyssölä et al., 2021; Pous et al., 2022, 2023; Soggia et al., 2024; Tian et al., 2025).

Considering the Coulombic Efficiency (CE) of the different biochar-functionalized cathodes (Table 2), an inverse trend with biomass and protein productivity was observed, with E1 exhibiting the highest biomass and protein STYs but the lowest CE, and E3 showing lower productivity but higher CE. Higher current densities, as observed for E1, indicate increased electron availability at the cathode surface (Soggia et al., 2024), which can promote faster biomass growth and protein production. However, CE accounts only for the fraction of electrons recovered in biomass and does not capture the overall rate of electron transfer. Under conditions of high electron flux, a larger share of electrons may therefore be diverted to parallel processes not directly coupled to biomass formation, such as hydrogen evolution not fully assimilated by microorganisms and metabolic maintenance requirements, resulting in lower CE despite higher productivity. Conversely, the lower current densities observed for E3 may have favoured a more selective utilization of a smaller electron flux, leading to higher CE values but reduced biomass and protein production rates. Although minor contributions from other microbial processes such as residual methanogenic activity, acetogenesis or other side reactions (Dessi et al., 2021; Rovira-Alsina et al., 2025a) could not be completely ruled out, the observed trade-off between productivity and CE was consistent with a shift from electron-limited to electron-rich operating conditions rather than with a loss of system performance.

Finally, the power consumption associated with biomass and protein production was calculated to estimate the energy requirements for SCP production. No significant differences were observed among the functionalized cathodes, with values of 73 ± 45 Wh g⁻¹ d.w., 72 ± 14 Wh g⁻¹ d.w., 67 ± 16 Wh g⁻¹ d.w. for E1, E2 and E3, respectively. In contrast, the control E0 exhibited substantially higher energy requirements, reaching

508 ± 128 Wh g⁻¹. The values obtained with biochar-based cathodes were advantageous when compared with those reported by (Pous et al., 2022) for SCP production under potentiostatic control (303 Wh g TSS⁻¹), comparable to those achieved in galvanostatic HOB cultivation by (Pous et al., 2023) (37 ± 14 Wh g TSS⁻¹) and by (Soggia et al., 2024) in double-chamber MES for SCP production (49 ± 13 Wh g⁻¹ d.w.). Based on the SCP produced, the specific energy requirements per unit of protein were calculated as 145 ± 76 Wh g⁻¹ protein for E1, 150 ± 18 Wh g⁻¹ protein for E2, and 165 ± 59 Wh g⁻¹ protein for E3. It should be noted that the energy analysis presented in this study refers only to the MES operational phase and does not include upstream processes such as biochar production. Therefore, comprehensive life cycle assessment and techno-economic analysis are required to fully evaluate the overall sustainability of the proposed system.

Lastly, microbial community analysis indicated that biochar-based cathodes were associated with distinct shifts in microbial community composition compared to the control. In particular, these systems showed a higher relative abundance of fermentative and acetogenic taxa, suggesting a microbial structure compatible with Power-to-Protein processes. Functional predictions based on FAPROTAX indicated the potential presence of metabolic capabilities related to acetogenesis, CO₂ reduction and hydrogen oxidation; yet, these assignments reflected predicted functional potential rather than directly measured metabolic activity. Within these limitations, biochar-based MES may support microbial consortia compatible with the conversion of waste-derived carbon and nitrogen sources into microbial biomass, reinforcing their potential role in circular power- and waste-to-resource biorefinery concepts. PICRUST2 functional predictions supported a clear differentiation between suspended and electrode-associated communities, as well as between biochar-amended and control systems. Biochar-supported biofilms showed enrichment of carbon fixation and energy metabolism pathways, including a higher abundance of the reductive acetyl-CoA pathway (CODH-PWY), implying a metabolic potential compatible with CO₂ fixation via the Wood-Ljungdahl pathway. These observations, together with the lower HER onset potential measured for biochar-functionalized cathodes, indicated enhanced hydrogen evolution at the electrode surface. This suggests that hydrogen-mediated pathways may have contributed to electron transfer processes in the system. The enrichment of *Acetobacterium* and *Acetoanaerobium* in cathodic biofilms corroborated this hypothesis, as members of these genera include homoacetogenic organisms capable of utilizing H₂ and CO₂. However, their actual metabolic activity was not directly assessed in this study, and the proposed role in indirect electron transfer re-mains inferential.

At the process level, these community shifts were associated with improved MES performance, with biochar-functionalized cathodes achieving higher SCP productivity and a reduced fraction of nitrogen not recovered in biomass compared to controls. Overall, biochar functionalization promotes a selective taxonomic and functional organization of electrode-associated biofilms, supporting metabolic features compatible with enhanced Power-to-Protein performance in MES.

5. Conclusion

Power-to-Protein represents a promising approach to simultaneously store renewable energy and capture CO₂ and NH₃ from industrial side-streams within a circular economy framework. This technology enables the production of low-cost, low-impact protein for sustainable feed and food applications, thereby addressing the growing global demand for protein. Overall, Power-to-Protein stands out as a key enabler in the ongoing energy transition and the pursuit of a zero-waste, carbon-neutral economy.

This study showed how cathode functionalization with low-cost carbon-based conductive materials such as biochar from urban green waste considerably improved SCP production in MES cells in terms of biomass and protein productivity, protein relative content and nitrogen capture efficiency compared to bare carbon cloth cathode. Furthermore,

beyond the intrinsic conductivity of the biochar, the bioelectrochemical performance is governed by a combination of factors, including electrode architecture and mass transport, which can modulate the accessibility of active sites and electron transfer processes. While nitrogen functionalization and post-treatments have been shown to enhance electrochemical properties in model systems such as thin films on glassy carbon electrodes, their positive effects are not directly translated into improved performance in the present bioelectrochemical configuration. In this context, the results indicate that nitrogen doping and subsequent post-treatment did not lead to a measurable improvement in protein production, either quantitatively or qualitatively, likely due to the attenuation of material-specific properties within the electrode structure.

Nevertheless, several challenges remain to be addressed in future studies to reach the productivities obtained with other technologies such as fermentation and to scale up this technology beyond its current TRL of approximately 4. These include: a) optimizing the microbial community to enrich protein-accumulating genera capable of producing high biomass yields with elevated protein content and a balanced amino acid profile; b) investigating the impact of NH_4^+ concentration on nitrogen assimilation within the biomass, with the goal of maximizing nitrogen recovery from the medium while minimizing nitrogen losses through processes such as NH_3 volatilization by tuning ammonium concentrations, implementing capture or stripping strategies, adjusting catholyte pH, and managing nitrification/denitrification inhibition; and c) improving electron transfer from the cathode to microorganisms to achieve higher coulombic efficiencies.

Furthermore, the SCP produced in this study exhibited protein contents and essential amino acid proportions comparable to those reported for conventional protein sources. However, this comparison is limited to compositional parameters. Additional nutritional and safety assessments will be required in future studies to fully evaluate the suitability of MES-derived SCP for feed and food applications.

CRediT authorship contribution statement

Gabriele Soggia: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Amin Narimani:** Writing – original draft, Investigation, Formal analysis, Data curation. **Barbara Mecheri:** Writing – original draft, Formal analysis, Conceptualization. **Beatrice Ricciardi:** Formal analysis. **Niccolò Forin:** Formal analysis. **Giuseppe Concheri:** Formal analysis, Conceptualization. **Fabrizio Adani:** Writing – review & editing, Writing – original draft, Resources, Project administration, Methodology, Investigation, Funding acquisition, Data curation, Conceptualization.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Fabrizio Adani reports financial support was provided by European Union. Fabrizio Adani reports a relationship with University of Milan that includes: funding grants. NO has patent NO pending to NO. NO If there are other authors, they 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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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.jclepro.2026.149479>.

Data availability

Data will be made available on request.

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