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BOOK OF ABSTRACTS

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Oral presentations

First Evidence of Naturally-Occurring Electric Fields in Biofilms Documented in Anammox Granules

M. Tucci[1], M.M. Corneliussen[2], L.F.M. Rosa[3], C. Picioreanu[3], F. Aulenta[1,4], U. Marzocchi[2,5]

[1] *Water Research Institute (IRSA), National Research Council (CNR), 00010 Montelibretti (RM), Italy*
[2] *Center for Water Technology (WATEC), Department of Biology, Aarhus University, Aarhus, Denmark*
[3] *Environmental Science and Engineering (EnSE), Biological and Environmental Science and Engineering (BESE), King Abdullah University of Science and Technology (KAUST), Thuwal, Saudi Arabia National*
[4] *Biodiversity Future Center, Palermo 90133, Italy*
[5] *Center for Electromicrobiology (CEM), Department of Biology, Aarhus University, Aarhus, Denmark*

matteo.tucci@irsa.cnr.it

Beyond their environmental relevance, biofilms are central to numerous biotechnological applications, including bioremediation, biocatalysis, biosensing, and the production of probiotics and biomaterials. The effective design and operation of such biofilm-based processes require a detailed understanding of mass transport of substrates, nutrients, and metabolites within the biofilm matrix. Many substrates sustaining microbial metabolism exist in ionic form, therefore unequal diffusion rates of anions and cations can lead to local charge imbalances. Such imbalances give rise to electric fields (EFs), which in turn influence ion transport through ionic migration.

Herein, we report for the first time the occurrence of intrinsic electric fields within anammox granules collected from two different wastewater treatment plants in Aarhus (DK). Electric fields were quantified as depth-dependent increases in electric potential using custom-built electric potential microsensors. Freshly harvested granules incubated at 32°C exhibited electric field ranging from 145 to 319 V/m, representing unprecedented magnitudes for biological systems.

Mathematical modelling indicates that negatively charged bacterial cell walls and extracellular polymeric substances within the granules function as weak ion exchangers for soluble cations, thereby generating diffusion potentials. These electric fields enhance the inward migration of NH_4^+ toward the granule core while retarding the transport of negatively charged NO_2^-. Model results further suggest that at electric field of approximately 120 V/m, ionic migration contributes to total ion fluxes to the same extent as molecular diffusion.

The identification of strong, naturally occurring electric fields within biofilms has significant implications for the conceptualization and optimization of biofilm-based technologies, particularly where the interplay between mass transport and reaction kinetics governs system performance.

Enhancing In-Situ Biomethane Production: A Comparative Analysis of Electrochemical Configurations in AD-MEC Systems

Clara Marandola[1,2], Flavio Massera[1,2], Federico Scopetti[1,2], Costanza Arvigo[1,2], Giuseppe Lembo[2], Gaia Salvatori[1], Marianna Villano[1], Antonella Marone[2], Antonella Signorini[2]

[1] *Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy*
[2] *ENEA Casaccia Research Center, Via Anguillarese 301, 00123 Roma, Italy*

clara.marandola@uniroma1.it

The transition toward the circular bioeconomy requires the optimization of waste-to-energy technologies. Anaerobic Digestion (AD) is a consolidated process for treating organic wastes, with the resulting biogas typically consisting of the 50-70% (v/v) CH_4, limiting its direct application as a transport fuel or grid-quality natural gas substitute. Conventional biogas upgrading technologies rely on physical-chemical methods that are energy-intensive and often economically and environmentally unviable. Recently, the integration of biotechnology and electrochemistry has led to the development of Bioelectrochemical Systems (BESs), such as Microbial Electrolysis Cells (MECs), as a versatile platform to drive the biological reduction of CO_2 into CH_4. Indeed, the H2 generated electrochemically at the MEC cathode serves as an electron donor for hydrogenotrophic methanogens, converting CO_2 into CH4. Unlike water electrolysis, which requires a theoretical voltage of 1.23 V for H2 production, MECs utilize electrons derived from the oxidation of organic matter at the anode to allow H2 production at significantly lower voltages (e.g., theoretically 0.12 V with acetate oxidation)1. By integrating MECs directly into anaerobic digesters (AD-MEC), it could be possible to achieve in-situ biogas upgrading (CH4 ≥ 98%, v/v)2. This study provides a critical evaluation of reactor configurations and potential control modes to identify the optimal conditions for maximizing CH_4 production while minimizing energy input. The research explored two reactor architectures, single-chamber (SC) and dual-chamber (DC) under three electrochemical control strategies: Experiment I with the anode potentiostatic control at +0.0 V vs. Ag/AgCl (i.e., 0.20 V vs. SHE) in a single-chamber MEC; Experiment II with the application of fixed potential difference between the electrodes (ΔV = 0.9 V and 1.1 V); and Experiment III with the cathode potentiostatic control at −0.80 V vs. Ag/AgCl (i.e., −0.60 V vs. SHE) in a two-chamber MEC. In Experiment I, the CH_4 fraction in the headspace of the polarized reactor increased by up to 33% compared with the control, whereas volumetric CH_4 production increased by up to 66%. H2 was never detected in the reactor headspace and stoichiometric analysis confirmed its imminent consumption, and the current flowed in the system fully justified the surplus CH4 produced. Experiment II resulted in a 15% increase in volumetric CH_4 production compared to the control open circuit tests. However, this configuration did not significantly alter the composition of the biogas. No differences were observed between the two investigated ΔV values. Experiment III achieved the highest gas purity, with CH4 content reaching up to 90% (v/v) in the cathode chamber. However, this performance required high ΔV making the process economically unfavorable. The results demonstrate the feasibility of coupling MEC technology with AD for in-situ biomethanation. While the dual-chamber setup offers superior gas purity, the high energy cost and high reactor complexity challenges its scalability. Conversely, the single-chamber configuration with potentiostatic anode regulation emerges as the most promising solution minimizing operational complexity and reducing plan costs to biologically upgrade biogas to biomethane.

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Electrode potential as a metabolic switch: genomic insights into simultaneous hydrogen, PHB, and biomass production by Rhodopseudomonas palustris 42OL

Chiara Capelli[1], F. Muniesa-Merino[2], A. Esteve-Núñez[2], A. Adessi[1], Viti C.[1], Bellabarba A.[1], M. Daghio[1]

[1]*DAGRI, Univ. of Florence, Piazzale delle Cascine 18, Florence, Italy*
[2]*Dept. of Chemical Engineering, Univ. of Alcalá, Alcalá de Henares, Madrid, Spain*

chiara.capelli@unifi.it

The transition to a circular economy requires wastewater treatment technologies that go beyond simple remediation to achieve resource recovery. Brewery wastewater (BWW), characterized by high organic content, is an ideal substrate for purple phototrophic bacteria (PPBs) such as Rhodopseudomonas palustris. These versatile bacteria can potentially produce three value products simultaneously: bio-hydrogen (H2) via photo-fermentation, polyhydroxybutyrate (PHB) via carbon storage, and single-cell protein (biomass). However, achieving high yields for all three products is challenging due to the competition for reducing power (electrons) and carbon skeletons1. Bio-electrochemical systems (BESs) offer a novel solution to this problem by using a cathode to donate electrons, potentially relieving redox constraints2. This study explores the capacity of R. palustris 42OL to act as a multi-product cell factory in a BES. To our knowledge, this study represents the first experimental effort to couple BES performance with targeted genomic analysis in PPBs. By integrating electrochemical data with RT-qPCR, we aim to reveal the specific regulatory mechanisms that enable cathodic polarization to influence metabolic partitioning between H2, PHB, and biomass.

Single-chamber BESs were inoculated with R. palustris 42OL and fed with sterile BWW under anaerobic, illuminated conditions. Three cathodic potentials were applied: -0.3 V, -0.6 V, and -0.8 V (vs Ag/AgCl), alongside a non-polarized control. Performance was assessed via total organic carbon (TOC) removal, biomass growth (OD660), gas chromatography for H2 measurements, and HPLC for PHB quantification. To elucidate the metabolic shifts, quantitative RT-qPCR analyzed the expression of key functional genes: nifH (nitrogenase for H2 production), phaC (PHB synthase for bioplastic accumulation), and rbcL (RuBisCO for CO_2 fixation/biomass production).

The results showed that electrode potential is crucial for synchronizing the production of H2, PHB and biomass. −0.6 V emerged as the optimal potential, enabling the simultaneous maximization of all the target products. At this potential, H_2 production was at its highest (70.35 ± 1.67 mL H2/L), supported by significant upregulation of nifH. This indicates that cathodic electrons were effectively channelled towards nitrogenase to dissipate excess reducing power. −0.6 V also promoted the greatest PHB accumulation (115.3 ± 22.4 mg PHB/g VSS). The sustained expression of phaC suggests that PHB biosynthesis acts as an auxiliary electron sink alongside H_2 evolution, helping to maintain the intracellular redox balance. Unlike the most negative potential of −0.8 V, −0.6 V supported robust biomass accumulation (OD660 = 1.64 ± 0.07), as demonstrated by the maintenance of rbcL expression. In contrast, its downregulation at −0.8 V indicates that excessively reducing conditions compromise the
Calvin–Benson–Bassham cycle. Overall, this study provides the first evidence that cathodic polarization at −0.6 V can enhance the production of H_2, PHB and biomass from BWW synergistically, avoiding trade-offs between products. This enables *R. palustris* 42OL to exploit H_2 evolution and PHB storage as complementary strategies for balancing the redox state while sustaining growth. This work offers a rational framework for designing integrated, PNSB-based biorefineries that aim to maximise the recovery of value from industrial effluents.

Acknowledgements: Supported by Italian Ministry of University and Research, PRIN2022 PNRR project WHISPER (No. P2022W4MNM).

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Pyrolised Melamine Foam with adsorbed CNTs as 3D anodes for BES

Giorgio De Checchi (1,2), Paule Salvin (1), Giulia Errichiello (3), Andrea Bogo (3), Frederic Ferrer (2), Marcella Bonchio (3), Florent Robert (1)

(1) L3MA UR4_1, Université des Antilles, Schoelcher – Martinique,France;
(2) Société Anonyme de la Raffinerie des Antilles, Le Lamentin – Martinique, France;
(3) Università di Padova, NanoMolCat , Padova (PD) - Italy
Tel.: +596 0696691559 Mobile: +39 3347495584

dechecchi.giorgio@gmail.com

Addressing the urgent need for sustainable energy storage solutions, hydrogen emerges as a promising clean and efficient energy carrier. Microbial Electrolysis Cells (MECs), a subset of BioElectrochemical Systems (BEMs)1, offer an innovative alternative to the current production methods, water electrolysis, and steam methane reforming, which generate significant CO2 emissions. The anode is the central functional component of microbial electrolysis cells, as it hosts the electroactive biofilm responsible for coupling microbial metabolism to the external electrical circuit. Its properties directly control current generation, Coulombic efficiency, startup time, and long-term stability. Carbon-based materials are widely employed as anode materials in MECs due to their excellent electrical conductivity, biocompatibility, morphological adaptability, low overpotentials, and cost-effectiveness. Common options include carbon cloth, graphite brushes, carbon felt, and graphite granules. This study explores the potential of 3D carbon structures coupled with Carbon Nanotubes (CNTs) to enhance MEC performance.
For this purpose, we produced and characterised an anode composed of melamine foam with adsorbed CNTs and a successive pyrolysis step. Different types of CNT concentrations and functionalisation, anode geometry, and pyrolysis temperature have been tested, together with $\pi - \pi$ riboflavin stacking, to boost External Electron Transfer (EET) for exoelectrogenic bacteria, with a focus on Geobacter spp. and Shewanella spp. 2-3.
This study shows the potential in such a simple method of production for higher-efficiency anodes, with high potential for further functionalisation.

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Bioelectrochemical enhancement of anaerobic sludge digestion

B. Fomegong Kana, S. Ghezzi, A. Callegari, A. G. Capodaglio.

Department of Civil Engineering & Architecture, University of Pavia, 27100 Pavia, Italy

andrea.capodaglio@unipv.it

Excess biological sludge processing and disposal have significant impact on the energy balance and economics of wastewater treatment operations. Various methods are proposed to face it out including Anaerobic Digestion (AD) which consists in a biochemical process in which organic matter is converted to biogas helping to recover energy and stabilize the sludge. Biogas is composed by major fraction (usually 65-75%) of methane (CH4) a consistent fraction of CO2 (25-35%) and other minor gases: in order to use biogas efficiently, methanation (conversion of CO2 to CH4, or CO2 removal) is adopted to upgrade it to natural gas standards. This additional step, however, is economically and energetically expensive, reducing the overall recovery efficiency. To address this problem, Bio-Electrochemical Systems (BESs) could improve AD performance in terms of biogas quantity and quality yield by improving anaerobic biomass metabolic processes as a consequence of enhanced electronic exchange (Direct Interspecies Electron Transfer – DIET).

AD BES-augmentation tests were conducted in two 10 L reactors: a conventional one, as control, and a BES-assisted one. In the AD-BES system, an external voltage of 0.8 V is applied between a cathode made of a stainless-steel mesh rolled up into a 10 cm long cylinder (diameter 3 cm) and a carbon brush anode (10 cm long, diameter of 3 cm). Excess biological sludge from a neighboring wastewater treatment plant, is fed to both reactors, with Hydraulic Retention Time (HRT) of 30 days. The reactors' influent and effluent are tested for pH, Electro Conductivity, COD and TN. Biogas produced volume is measured, stored in aluminum foil bags and subsequently analyzed for composition. Results (after about 6 months) show that the BES-augmented reactor produces a much larger biogas volume (> 2.5 times) than the control reactor, with specific (L/g COD removed) production over 3 folds higher. Overall COD removal is not significantly different between reactors. Tests in different conditions were carried out to assess the augmentation effect of BES technology on AD, and to test its resiliency, showing that the AD-BES process is more resistant to upsets and unexpected stresses.

Bioelectrochemical sensing of cyanobacteria

Stefano Freguia

The University of Melbourne, Department of Chemical Engineering, Victoria 3010 Australia

stefano.freguia@unimelb.edu.au

Cyanobacteria are some of the earliest life forms on Earth, but after 3.5 billion years they have turned from life creators to opportunistic pollutants, causing widespread damage to water bodies as they bloom with increasing frequency due to nutrient pollution and a warming climate. We discovered that cyanobacteria and some microalgae are capable of generating anodic currents at high potentials (>0.6 V vs. SHE)) and this effect can be used to track their growth in the environment. We also expose the unique electrochemical features of several species.

Harnessing Geothermal Soils for CO_2 Bioelectrochemical Reduction

Gabriella Caucia[1], Elena Cazzulani[2], Ramya Veerubhotla[3], Ugo Marzocchi[3], Francesca Pittino[1], Andrea Franzetti[1], Pierangela Cristiani[4]

[1] *Università degli studi Milano-Bicocca, Piazza della Scienza U1, 20126, Milano (IT)*
[2] *Università degli Studi di Milano, Department of Chemistry, v. Golgi 19, 20133 Milano (IT)*
[3] *Center for Electromicrobiology, Department of Biology, Aarhus University, Aarhus 8000, Denmark.*
[4] *Ricerca sul Sistema Energetico – RSE v. Rubattino 54, Milano (IT)*

g.caucia@campus.unimib.it

Geothermal sites host microbial communities adapted to extreme environments, steep geochemical gradients, and high CO_2 fluxes. In this study, we exploit the unique potential of soil microbial communities from the Monte Amiata geothermal area (Italy), to drive CO_2 reduction into value-added compounds using bioelectrochemical systems (BESs). Under a fixed applied voltage of 6 V (Ecell) over 30 days, gas chromatography revealed maximum CH4 production rates of 6.2 $L \cdot m^{-2} \cdot day^{-1}$ in the cathodic chamber, while acetate accumulated to concentration of 5000 ppm as compared to 50 ppm in the unpolarized control. CO2 microsensor measurements revealed complete substrate consumption within 48 hours, suggesting a highly active microbial community driving CO2 reduction processes. O2 microsensor profiles confirmed anoxic conditions near the cathode. Microbial community analysis based on 16S rRNA gene sequencing shows the acetogen Sporomusa as the dominant member of the community followed by Methanobrevibacter, explaining both acetate accumulation and methane production. qPCR analysis of mcrA gene further confirms methanogens colonization on the cathode surface, reaching 106 copies per cm-2. Colonization by active microbial community has been further revealed by scanning electron microscopy (SEM). Cyclic voltammetry measures a progressive increase in the electrochemical active surface area (ECSA) of cathodes, reaching 90 cm2 compared to 5 cm2 in controls. As the investigated geothermal area harboured sites with varying levels of sulfate, we further tested the effect of initial sulfate concentration on the bioelectrochemical CO2 reduction process. Elevated sulfate levels negatively affected CH4 and acetate production processes, promoting enrichment of sulfate reducers (Desulfovibrio). Overall, our results demonstrate that geothermal soils represent a promising source of microorganisms for bioelectrochemical CO_2 reduction, provided that sulfate concentrations are low. This approach offers a sustainable strategy to valorize geothermal CO_2 fluxes while recovering high-energy and valuable products.

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Phenazines as Redox Mediators: How Determinant Are They in Microbiologically Influenced Corrosion of pure iron?

Giorgia Ghiara[1,2], Roberta Amendola[3], Amit Acharjee[3], Matthew Fields[4], Iwona Beech[4], Pierangela Cristiani[5]

1) Dipartimento di Scienza Applicata e Tecnologia (DISAT), Politecnico di Torino, Turin/ITALY
2) Laboratoire des Sciences de l'Ingénieur pour l'Environnement (LaSIE), La Rochelle Université, La Rochelle/FRANCE
3) Dept. of Mechanical and Industrial Engineering, Montana State University, Bozeman/USA
4) Center for Biofilm Engineering, Montana State University, Bozeman/USA
5) Ricerca sul Sistema Energetico S.p.A, Milan/ITALY

giorgia.ghiara@polito.it

Interactions between microorganisms and iron materials are widely recognized as a key factor in degradation processes, particularly in microbiologically influenced corrosion (MIC). Biofilm formation on metal surfaces alters local electrochemical conditions and promotes the onset of redox reactions involving both the metal and the microorganisms, which utilize electrons and Fe ions for their metabolism. In this context, redox mediators produced by bacteria play a crucial role in facilitating extracellular electron transfer between the metal surface and microbial cells. Among these compounds, phenazines—secondary metabolites and redox mediators of microbial activity—are known to influence biofilm development, electron transfer processes, and metal dissolution, in the case of stainless steel particularly [1]. Molecules such as pyocyanin (PYO), phenazine-1-carboxylic acid (PCA), and phenazine-1-carboxamide (PCN) exhibit redox properties that are highly relevant to corrosion processes; however, the distinct contribution of each compound, and their relevance in driving corrosion processes, especially for materials which experience high corrosion rate under anaerobic condition also, such as carbon steel, remain not fully understood.
In this work, the role of PCA—produced by the model microorganism *Pseudomonas fluorescens* and a biosynthetic precursor of PYO—was investigated in relation to the corrosion behavior of ARMCO® pure iron under anaerobic conditions. Electrochemical tests were performed using a three-electrode configuration, with the metal sample as the working electrode, a saturated Ag/AgCl (sat. KCl) electrode as the reference, and an anodized titanium mesh as the counter electrode. The results show that PCA significantly modifies the electrochemical response of the material, affecting charge transfer resistance and the properties of the oxide layer. Although this redox mediator contributes to an increase in corrosion kinetics, the observed corrosion process appears to be complex and may involve different types of metabolites and/or a direct influence of the biofilm. Overall, the study confirms that PCA does not play a dominant role in the process of iron dissolution under anaerobic conditions, and highlights the extremely complex nature of microbiologically influenced corrosion.

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Bacteria-based Semi-Artificial Photosynthesis for Solar Fuels, Current Generation, and Self-Powered Biosensors

Matteo Grattieri [1,2,3], Dario Lacalamita [1], Rosa Maria Matteucci[4], Umberto Mattia[4], Jefferson Honorio Franco[1], Pierluigi Lasala[1], Paolo Stufano[5], Savino Grieco[1], Danilo D'Agostino[1], Rossella Labarile[2], Massimo Trotta[2], Chiara Ingrosso[2], M. Lucia Curri[1,2,3], Elisabetta Fanizza[1,2,3], Gianluca M. Farinola[1]

[1]*Department of Chemistry, Università degli Studi di Bari "Aldo Moro", Bari, Italy*
[2]*CNR-IPCF, National Research Council of Italy, Bari, Italy*
[3]*National Interuniversity Consortium of Materials Science and Technology, INSTM, Bari Research Unit, via E. Orabona 4, 70125, Bari, Italy*
[4]*Dipartimento di Ingegneria Elettrica e dell'Informazione, Politecnico di Bari, Bari, Italy*
[2]*CNR-NANOTEC, National Research Council of Italy, Bari, Italy*

matteo.grattieri@uniba.it

Bioelectrochemical systems employing anoxygenic photosynthetic bacteria offer the unique feature to accomplish semi-artificial photosynthesis, where sunlight energy conversion is coupled to current generation, the removal of organic compounds, and solar fuel production.[1, 2] These processes are possible thanks to the complex enzymatic machinery of photosynthetic purple bacteria that combines light-induced reactions to their respiratory metabolism. However, to successfully obtain biohybrid electrochemical systems, the bacterial cells must be efficiently wired to an electrode surface, and the electron transfer process, both anodic and cathodic, should be tuned to avoid altering the fragile redox balance of the biological organisms.[3]
his work will present the approaches undertaken by our group to tackle this challenge, with the implementation of biocompatible polymers and inorganic nanomaterials towards facilitating the extracellular electron transfer process and drive a desired process while avoiding redox imbalance.[4, 5] The engineering approaches implemented allowed obtaining systems capable of H2 and syngas production, enhanced current generation, and self-powered monitoring of contaminants.6 Interestingly, the operation of such systems in complex matrices has shown promising results for the future implementation of these devices for distributed, low cost, environmental monitoring and waste reuse.

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Screening of Redox Mediators for Improved Electron Transfer in Rhodopseudomonas palustris 42OL-Driven Photobio-cathodes

Karima Guehaz

Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Piazzale delle Cascine,18 and Via Maragliano 77, Florence, Italy

karima.guehaz@unifi.it

The significant organic load and sheer scale of agri-food by-products present substantial logistical and environmental challenges. However, the high concentration of nutrients and organic compounds that complicates wastewater management also renders these streams ideal candidates for upcycling. To address these issues, WHISPER project introduced a circular "waste-to-energy" framework designed to close the loop in industrial processing. By integrating photosynthetic purple non-sulfur bacteria (PNSB) into bio-electrochemical systems (BES), the project established a multi-functional platform for the concurrent degradation of organic matter and the biosynthesis of green hydrogen and high-added-value metabolites (PHB).

The present study aimed to identify a potent redox mediator to enhance the growth of Rhodopseudomonas palustris 42OL within this bio-electrochemical setup. In an H-cell configuration with an applied potential of –600 mV (vs. Ag/AgCl), three distinct mediators were evaluated at a concentration of 500 µM: anthraquinone-2,6-disulfonate (AQDS), methyl viologen, and neutral red. To determine the most efficient redox intermediate, several parameters were monitored, including biomass yield, organic acid consumption, hydrogen evolution, and PHB accumulation. Electrochemical analyses were also evaluated via cyclic voltammetry and chronoamperometry.

Results revealed that the highest biomass production occurred in H-cells supplemented with AQDS, followed by methyl viologen and neutral red, respectively. This growth was positively correlated with increased malic acid consumption by R. palustris 42OL. Under these specific experimental conditions, however, no significant enhancement in hydrogen production or PHB accumulation was observed. This study represents a critical phase of the WHISPER project, identifying a key intermediate capable of shuttling electrons from the electrode to the bacterial inoculum, thereby improving electro-bacterial contact and stimulating metabolic growth.

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Why Make Acetate or methane When You Can Make Ectoine? A Value-Driven Bioelectrochemical Platform Using Halomonas elongata

Lorenzo Cristiani, Subal Parashar, Miriam A-Rosenbaum

Leibniz-HKI, Leibniz Institute for Natural Product Research and Infection Biology – Hans Knöll Institute (HKI), Jena, Germany

lorenzo.Cristiani@leibniz-hki.de

The sustainable production of high-value chemicals remains a critical bottleneck for the advancement of bioelectrochemical systems toward industrial relevance. While microbial electrosynthesis has demonstrated the possibility of coupling bioproduction to renewable electricity, the intrinsic complexity and cost of these systems raise a fundamental question: under which conditions can their use be justified compared to established fermentation technologies?
Halophilic microorganisms represent a compelling opportunity in this context, as they naturally synthesize compatible solutes such as ectoine, betaine, and trehalose to cope with osmotic stress. Among these, ectoine stands out as a high-value product with a market price of approximately 1000 \$ kg^{-1} and broad applications in cosmetics, pharmaceuticals, and biotechnology. However, conventional ectoine production relies on highly saline fermentations that are associated with high energy demand, severe gas–liquid mass transfer limitations, and complex downstream processing. Among halophiles, Halomonas elongata is the most widely used industrial ectoine producer, owing to its exceptional robustness, high productivity, and well-characterized physiology under high-salinity conditions. In this study, we use H. elongata as a benchmark organism to evaluate whether bioelectrochemical cultivation can provide tangible advantages when targeting the production of high-value molecules rather than commodity chemicals. Conventional ectoine production relies on highly saline fermentations, where gas–liquid mass transfer already represents a major limitation. Under hypersaline conditions, the solubility of key gases such as oxygen and hydrogen is further reduced to extremely low levels, exacerbating process constraints and energy demand. Bioelectrochemical operation offers an alternative strategy by decoupling microbial metabolism from external gas supply and enabling new redox control concepts in conductive saline media. We present our initial investigations on the bioelectrochemical cultivation of H. elongata, focusing on its performance under anodic conditions as an alternative to oxygen-dependent metabolism. The effects of salinity, electrode potential, and cultivation regime on growth, electrochemical behavior, and ectoine accumulation are systematically assessed. Under the investigated conditions, we currently achieve a daily ectoine production of 69 g per kg of glucose, demonstrating the feasibility of coupling bioelectrochemical cultivation with high-value product formation while preserving the industrial performance of H. elongata. Rather than positioning bioelectrochemical systems as universal replacements for conventional fermentations, this work advances the perspective that their higher complexity and cost can only be justified when they enable or intensify the production of high-value compounds. Building on this foundation, the next step will be the development of integrated bioelectrochemical routes for ectoine production from CO_2, establishing a value-driven pathway toward electricity-powered and carbon-efficient bioproduction.

Electrogenetics of Bacillus subtilis for tunable production of high-added value biochemicals

Enrico Marsili, Sunday Oguntomi, Sadia Nawab

University of Nottingham Ningbo, China

enrico.marsili1@gmail.com

More than 20% of the global greenhouse gas emissions are released from chemical industries that use non-renewable resources. To mitigate industry-related climate change emissions, China has highlighted specific bioeconomy enhancement goals in her 14th 5-year plan (2021-2025).
The major green platform that drives the availability of value-added chemicals is Industrial Biotechnology, which involves the use of microorganisms as biorefineries or biocatalysts to produce antibiotics, hormones, vitamins, and biopolymers, among others. However, Industrial Biotechnology is not yet competitive with conventional biochemical industry, due to the low efficiency, yield, and specificity of most industrial fermentation processes. These inefficiencies are partly caused by the redox bottleneck associated with the metabolic pathway in microbial fermentation.
We are currently working on electrofermentation, a biotechnology that addresses redox imbalances by controlled application of electrical potential to modulate the metabolism of microorganisms growing in microstructured communities known as biofilms. In our first electrofermentation case study, we focused on a biopolymer known as γ-polyglutamic acid (γ-PGA), using *Bacillus subtilis* as a biocatalyst.
B. subtilis is a platform microorganism used in numerous fermentation processes, owing to its generally regarded as safe (GRAS) status, its genetic tractability, and well-known metabolic pathways. However, little is known about its performance in electrofermentation.
γ-PGA is a water-soluble, biodegradable, and non-immunogenic biopolymer produced by fermentation and used in regenerative medicine, food, cosmetics, and pharmaceutical industries as hydrogels, drug carrier, and wound healing agent. The global γ-PGA market size is estimated to be worth USD 350 million in 2022 and projected to rise to USD 477.2 million by 2028. China currently ranks first in γ-PGA consumption with 75% of the market by value. The key biotechnological players in γ-PGA production include Vedan Biotechnology, Shandong Freda, and Guanghua Group.
The rising demand for γ-PGA and other biopolymers in various industries lies on the strict specificity of its molecular weight. To date, there is no industrial process leading to property-specific biopolymer production in electrofermentation.
Gene expression in electrofermentation, can be regulated through electrogenetic methods. Although differential gene expression patterns have been observed in biofilms grown on polarized electrodes, little is known about the molecular mechanisms that sense electrochemical potential and bring about the genetic regulation in response to this potential. We observed that the molecular weight of γ-PGA produced in *B. subtilis* PB5760 biofilms changed with the applied electrochemical potential. The application of 0.2 and 0.4 V vs. Ag/AgCl reduced the molecular weight of γ-PGA in the biofilm from 3800 to 4070 kDa at open circuit potential (OCP) to 1040–1590 kDa at 0.2 V and 2170–2730 kDa at 0.4 V, respectively. Under the same conditions, the relative levels of expression of pgdS, a gene that codes for a γ-PGA-specific endo-hydrolase in cells growing at OCP, 0.2 and 0.4 V vs. Ag/AgCl were 1.12 ± 0.57, 2.74 ± 0.54, 1.61 ± 0.57, respectively, which correlates with the molecular weight reduction. This is the first biotechnological application of electrogenetics for producing γ-PGA with a tunable molecular weight.

Development of gas diffusion electrode – microbial electrosynthesis (GDE-MES) cells for CO_2 reduction: computational fluid dynamics modelling and experimental verification

Santiago Martínez Sosa[1], Mingming Tong[2], Deepak Pant[3], Pau Farràs[1], Paolo Dessì[4]

[1]*School of Biological and Chemical Sciences, Ryan Institute, University of Galway, University Road, H91 CF50, Galway, Ireland.*

[2]*School of Engineering, University of Galway, University Road, H91 HX31, Galway, Ireland; Research Ireland I-Form Research Centre for Advanced Manufacturing, Ireland*

[3]*Deepak Pant: Electrochemistry Excellence Centre, Materials & Chemistry Unit, Flemish Institute for Technological Research (VITO), Boeretang 200, 2400, Mol, Belgium.*

[4]*Department of Agricultural Sciences, University of Naples Federico II, Piazza Carlo di Borbone 1, 80055 Portici, Italy.*

paolo.dessi@unina.it

Microbial electrosynthesis (MES) is a promising carbon capture and utilization (CCU) technology to convert carbon dioxide (CO_2) into added-value compounds (Dessì et al., 2021). However, low production rates and high energy consumption still hinder its application on a large scale. Gas diffusion electrodes (GDEs) can be applied to overcome gas transfer limitations, resulting in higher production rates, while saline electrolytes can help decreasing the ohmic resistance, resulting in energy savings. In our previous study (Dessì et al., 2023), we showed that three-chamber GDE-MES cells for CO_2 reduction to acetate can be operated under mild saline conditions (5 g/L NaCl) without negative impact on acetate production. The cells achieved an acetate production rate of 0.9 g/L/d, with over 80% coulombic efficiency. In this study, we further advance the technology by designing and realizing a GDE-MES cell with a serpentine pattern design, aiming at improving gas and electrolyte distribution in comparison to the original, squared design. Computational fluid dynamics (CFD) simulations confirmed that the serpentine-shaped cells avoid the appearance of "dead zones" with poor CO_2 and electrolyte flows on the cathode surface, establishing hydrodynamic parameters favourable to biofilm development. Biotic experiments in batch mode confirmed the superiority of the serpentine shaped cells, that achieved 40% higher acetate production rate than the squared cells. The serpentine-shaped cells were then operated in continuous mode (first time for GDE-based MES cells) for over 300 days at decreasing hydraulic retention time (HRT) from 7.03 to 0.81 days. Continuous operation resulted in a further 30% increase in acetate production, halving the electric energy consumption per kg of acetate produced in comparison to batch operation. The cells reached the highest acetate production rate of 2.2 g L^{-1} d^{-1} at 1.08 days HRT and were able to withstand HRTs as low as 0.81 days with minimum performance losses. This work highlights the importance of cell design and modelling for advancing MES, and the benefits that continuous operation can bring. Further improvements, especially on the electrical efficiency of the system, are key to the development of an industrially viable process.

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Exciting perspectives in Soil Microbial Fuel Cell research and routes to practical implementations

Mirella Di Lorenzo

Centre for Bioengineering and Biomedical Technologies (CBio) and Department of Chemical Engineering, University of Bath, Claverton Down, Bath, United Kingdom, BA2 7AY

mdl28@bath.ac.uk

We here present our recent achievements in the field of soil microbial fuel cells (SMFC) for energy harvesting and sensing. SMFCs stand as a promising source of renewable energy, leveraging electroactive microorganisms in soil to produce clean electricity. Despite their potential, limited understanding of operational fundamentals and thermodynamic constrains hinders their power density, and market viability. With our work we seek to tackle these challenges to optimise and scale the SMFC technology, striving for performance levels suitable for low-power applications. To do so, the life cycle of conventional flat-plate SMFCs was closely monitored and assessed, revealing four critical stages in their evolution. To increase the power output, we have tested a variety of SMFC reactor designs and developed arrays of SMFCs electrically connected to scale the power output. This approach was applied in field trials in the North-East of Brazil, where an array of SMFCs powered an electrochemical water treatment reactor. Field generated data matched the laboratory results, proving the concept and treating 3L of water per day. To address performance instability issues highlighted during the field studies, a custom power management system (PMS) was developed. The PMS utilised a model-based power estimation strategy, tailored to the voltage dynamics of the SMFC. Overall, these results underscore the importance of comprehensive optimisation, emphasizing the critical role of fine-tuning all components of the SMFC system and PMS to achieve optimal performance. This insight is crucial for future research endeavours, to bring the SMFC technology closer to commercial readiness. Finally, we are exploring the opportunities that SMFCs open up in the field of self-powered remote soil diagnostics. While this is a very early days, preliminary results are very encouraging, paving the way for new perspectives in agriculture.

Accelerating anaerobic digestion through bioelectrochemical systems integration: the AD-BES technology

Daniele Molognoni,[1] Marian Garcia-Montolio,[1] Pau Bosch-Jimenez,[1] Gaspard Bouteau,[2] Geoffrey Karakachian,[2] Eduard Borràs[1]

[1] *LEITAT Technological Center, Terrassa, Barcelona/Spain*
[2] *ENGIE Lab CRIGEN, Stains/France*

d.molognoni@leitat.org

The integration of bioelectrochemical systems (BES) into anaerobic digestion (AD) has emerged as a promising strategy to enhance biogas production and AD process stability. This study evaluates the performance of a **continuously fed AD-BES reactor operated with real liquid digestate** from a full-scale biogas plant, under mesophilic conditions, and compared it to a conventional AD control. The liquid digestate was characterized by high conductivity (>20 mS/cm), elevated ammoniacal nitrogen (5.0 ± 0.1 g-N/L), recalcitrant organic matter and low C/N ratio (≈7), posing significant inhibitory challenges for methanogenesis. To mitigate these effects, both reactors were inoculated with acclimated digestate, enabling stable operation despite ammonia stress. The AD-BES reactor (10 L) incorporated a custom polyamide structure supporting five carbon brush electrodes (three cathodes, two anodes), with a cathode surface-to-vessel volume ratio of 13 m^2/m^3. An applied voltage of 0.7 V was maintained throughout the experiment. **Hydraulic retention time (HRT) was progressively reduced from 40 to 20 days**, to assess system resilience under increasing organic loading rates. Electrochemical monitoring revealed stable current generation during the initial phase (0.42 ± 0.07 mA/cm^2 at HRT 40 days), with variations linked to substrate availability and operational conditions. Cyclic voltammetry confirmed electrode-associated redox activity, suggesting involvement of exoelectrogenic and syntrophic acetate-oxidizing consortia, as well as electromethanogenic pathways via direct electron transfer. Biogas production and methane content were monitored over time. While methane yield of both reactors decreased slightly at the shortest HRT of 20 days, **AD-BES maintained a higher process stability** compared to the AD control, with 22-25% higher methane production. Correlation analysis indicated a monotonic relationship between current density and methane production at lower HRTs, highlighting the potential of BES to accelerate digestion kinetics and improve methane recovery from challenging substrates. Anodic Coulombic efficiencies remained low (<16%), consistent with previous reports on electrofermentation processes. This work demonstrates the feasibility of integrating BES into semi-industrial AD systems treating real digestate, bridging the gap between lab-scale trials and practical applications. The findings support BES as a viable strategy to enhance biogas recovery, reduce digester's HRT and valorize residual methane potential in digestate streams, contributing to circular economy and decarbonization goals.

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Monitoring Bioelectrolysis systems by Distribution of Relaxation Times elaboration of Electrochemical Impedance Spectroscopy data

Daniele Moraschini[a], Stefano Ravasi[b], Silvia Franz[a], Pierangela Cristiani[b]

[a] *Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Via Mancinelli 7, 20131 Milano (Italy)*
[b] *Ricerca sul Sistema Energetico - RSE Via Rubattino 54 20134 Milano (Italy)*

daniele.moraschini@polimi.it

Bioelectrolysis, as a new technology, can produce high-added-value products, recover wastewaters, and offer lower costs compared to conventional systems based on Platinum Group Metals (PGM) catalysts. Like all electrochemical systems , bio electrolyzers undergo degradation over time, with the biofilm evolution being a crucial factor for the system efficiency. One possible technique to monitor anode/cathode degradation and biofilm establishment is Electrochemical Impedance Spectroscopy. In this study, a system composed of six independent bioelectrochemical cells, constituted of a double-chamber configuration, was tested, with anode and cathode being separated by a terracotta separator, and an anaerobic bacteria pool being established on the cathode. For all the studied cells, the anodic materials consisted of a commercially available Ti-Ir electrode, while three different cathodes were tested: (i) Ti-Ir, (ii) Ti-Ir with graphite, (iii) Ti-Ir with graphite, Cu and Hydroxyapatite, and the study aimed at defining what cathode could provide the best surface for biofilm formation and the best conditions for the power-to-gas conversion. Preliminary results indicate the cell configuration maintains strict anaerobic conditions at the cathode, with negligible oxygen crossover. The cells evolution was monitored using electrochemical impedance spectroscopy, analysed via the distribution of relaxation times (DRT), demonstrating strong capability to track resistive and capacitive changes associated with cell phenomena. Two-electrode EIS performed under working conditions quantified the combined contributions of anode, biocathode and terracotta separator, while three-electrode measurements allowed separate assessment of anode and biocathode performance evolution, confirming EIS/DRT as an effective diagnostic tool for this system.

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Real-time, label-free electrochemical technique for detection and quantification of early biofilm

Opeyemi Temitope Otemoye

University of Nottingham Ningbo China

opeyemitemitope.OTEMOYE@nottingham.edu.cn

Biofilms are one of the most resilient and adaptive strategies that microorganisms use to survive under difficult conditions. Their ability to form protective matrices makes them a critical threat in healthcare, as they often cause persistent infections. While established techniques exist to characterize biofilms, they face several challenges, primarily due to labor intensity, time consumption, complex sample preparation, and the high cost of imaging equipment. In developing countries, costs are particularly high due to limited detection infrastructure, highlighting the need for a complementary characterization technique that enables standardized, cheap, and rapid characterization of biofilm composition and metabolic activity.

In this study, we developed and optimized a short-term potentiometric method to investigate early biofilm formation by measuring in real-time the charge and discharge of bacterial membrane potentials. When grown on a polarized surface, the bacterial attachment generates microscale currents that can be correlated to cell concentration, nutrients, and environmental conditions, among other factors. By analyzing the bacterial current output, we were able to detect early biofilm formation within 4–6 hours, a significant improvement over conventional methods, which typically require 18–24 hours and at a lower cost (~1 USD per sample). The results obtained were validated against a control experiment (open-circuit potentiometry) with established biofilm characterization methods to verify the non-destructive capability of the developed technique.

The proposed technique can be effectively used to detect early biofilms without the need for redox mediators, thereby extending its application to the bioelectrochemical analysis of microorganisms of clinical importance, which are often weak electricigens.

Adaptive Laboratory Evolution (ALE) for the bioelectrosynthesis of Multicarbon Organic Compounds from HCO3- by exploiting Cupriavidus necator metabolism.

Serena Narducci, Vincenzo Pasquale, Rosa Anna Nastro

Laboratory of Microbiology and Biochemistry, Department of Science and Technology, University of Naples, Parthenope, Centro Direzionale Isola C4, Naples (Italy)

rosa.nastro@uniparthenope.it

Chemiolitothrophic bacteria are known for their ability to synthesize organic compounds for biosynthesis of molecules for biomass production, intracellular carbon storage, and fermentation products. However, the conversion of inorganic carbon such as CO_2 or HCO_3^- can be achieved in species able to utilize electrons supplied exogenously. Microbial electrosynthesis (MES) or Electrofermentation (EF) can overcome some of the issues associated to conventional C1 compound fermentation. In MES, the extracellular redox potential is modified by supplying external electrons through the cathode, therefore displacing the intracellular redox balance required to obtain the desired fermentation products [1] The ability of microorganisms to give or receive electrons can take advantage of both direct and indirect electron exchange mechanisms such as nanowires and H_2 / shuttles respectively. In this work,
we demonstrated the efficiency of an Adaptive Laboratory Approach (ALE) for the improvement of CO_2/HCO_3^- conversion into added-value compounds in bioelectrochemical systems by exploiting *Cupriavidus necator* metabolic pathways. At the same time, we tested the ability of activated sludge microflora (growing using sodium acetate 0.2M as sole source of carbon in mineral medium) to provide the electrons needed to sustain chemiolitothrophic metabolism in *C.necator* [2]
In this work, we thoroughly investigated the metabolome obtained from both *C.necator* at the cathode and from the microbial community at the anode when grown in Microbial Fuel Cells (MFCs), in Microbial Electrosinthesis Cells (MECs) and in control cultures, i.e without any electrodes available to foster microbial electrogenesys. Our results showed that, by applying ALE, we were able to induce the selection of strains with better phenotypes by long-term culture under a specific selection pressure or growth environment. Among the aim is to perform, in future, a metabolic modelling of *C.necator* grew. We also investigated the metabolic profile in terms of carbon source utilization (ECOLOG plates – BIOLOG) of C.necator when used for several thousands of generations in Microbial Fuel Cells (MFCs) a. The obtained profile was compared with the original strain purchased at DSMZ, which had never grown in BESs.
Our results showed that inorganic carbon utilization rate and metabolites production change under the applied redox potential, with significantly improved CO_2 capture in BESs than in traditional microbial cultures. Further steps will include the metabolic modelling of *C.necator* grown under different redox potentials.

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Engineering Reactors for Microbial Electrocatalysis

Rossi, R. [1], Kim, D.[1], Du, H.[1]

[1]*Johns Hopkins University, Baltimore, MD, USA*

rrossi4@jhu.edu

Bioelectrochemical technologies merge the efficiency and environmentally friendly nature of living microorganisms with renewable electricity to produce valuable energy, fuels, and chemicals from waste resources. Despite their usefulness in a circular economy, bioelectrochemical systems have rarely exited academic laboratories and have not yet been widely adopted for commercial applications on a large scale. While great strides on the microbiological aspect of these technologies allowed to understand the evolution and operation of microbial communities on the electrodes, little progress has been made on the electrochemical engineering side of bioelectrochemical systems, resulting in the widespread adoption of reactor configurations with poor design, high internal resistances, and consequently low current density and poor productivity. This lack of fundamental understanding of how the reactor design and operational parameters dictate the electrochemical characteristics of the cell has hampered efforts in scale-up and deployment of bioelectrochemical systems by the private sector. Here, I will show the impact of reactor engineering on microbial and electrochemical performance and how improving the cell design can boost microbial metabolism and accelerate the production of valuable products. I will show how reactor engineering and the development of asymmetric and vapor-fed reactor designs unlocked unprecedented performance in bioelectrochemical systems. For example, an asymmetric-fed microbial fuel cells produced the largest power and current densities to date (8.8±0.5 W/m2, 42±1 A/m2), while vapor-fed microbial electrolysis and electrosynthesis cells resulted in record current (44.4±0.9 A/m2), hydrogen (81±3 LH2/Lreactor-d), methane (748 mmol/m2-d), and volatile fatty acid (1300±280 mmol/m2-d) production rates. This advancement was enabled by a reduction of local acidification of the bioanode that, in turn, boosted microbial activity, leading to a large increase in efficiency and production rates. COMSOL flow dynamics simulations were used to provide a hydrodynamic framework to guide the scale up the reactor configuration without negatively affecting electrolyte velocity and distribution in the cell. Larger productivity and efficiencies were then confirmed at scale, as the internal resistance of the bioelectrochemical cell was maintained in check following scale-up of the geometric electrode area from 9 cm2 (11.7 ± 0.5 mΩm2) to 100 cm2 (19.7 ± 1.3 mΩm2). Finally, the performance of asymmetric and vapor-fed bioelectrochemical systems was assessed with real waste streams, from unamended anaerobic digestor effluents (8.8 ± 0.3 A/m2, 32 ± 6 LH2/Lreactor-d) to lignocellulosic fermentation effluent (24 ± 6 A/m2, 75 ± 19 LH2/Lreactor-d). Advancing microbial electrochemical technologies requires moving forward with both the microbial and the electrochemical aspects of this technology. New reactor designs and architecture, such as asymmetric and vapor-fed bioelectrochemical systems, provide an efficient route to fully exploit the activity of electroactive biofilms.

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Electrochemical Characterization of a Digestate-Fed Bioanode in a Continuous-Flow Microbial Electrolysis Cell for Hydrogen Production

Gaia Salvatori[a], Laura Infante[a], Clara Marandola[a], Bernardino Virdis[b], Marianna Villano[a]

[a]*Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy*
[b]*Australian Centre for Water and Environmental Biotechnology (ACWEB), The University of Queensland, Brisbane, QLD 4072, Australia*

gaia.salvatori@uniroma1.it

Microbial Electrolysis Cells (MECs) have been extensively investigated as a technology that couples waste-water treatment at a bioanode, with the production of reduced bio-products (e.g., bio-hydrogen or bio-methane) at a cathode. So far, MECs have been tested for their capacity to treat organic wastes, including effluents from dark fermentation [1][2]. However, limited evidence is available on the use of digestate, resulting from the anaerobic conversion of organic waste [3].
In this study, digestate derived from anaerobic valorization of urban and agricultural organic waste was used as substrate of a MEC-bioanode. In particular, the digestate was characterized by high protein content (27.7 ± 0.8 g COD/L, Chemical Oxygen Demand), and carboxylic acids accounting for approximately 40% of the soluble COD.
The MEC was operated with the anode potential controlled at +0.20 *vs*. SHE (Standard Hydrogen Electrode) to promote the growth of electroactive microorganisms on the graphite surface. The organic loading rate was fixed at 0.5 gCOD/L/d (based on soluble COD) and the catalytic current generated by the bioanode was continuously monitored to evaluate the bio-H_2 yield and the overall MEC performance.
The bioanode achieved a COD removal efficiency of 48±3 %, with a COD removal rate of 108±8 mgCOD/d, which corresponded to a complete conversion of the removed COD into current (i.e., Coulombic Efficiency accounted for 114±20 %). As for the cathode performance, H_2 production rate accounted for 10.5±0.5 mmol/d with a corresponding cathode capture efficiency (CCE) of 82±4 %. The overall H_2 yield, based on the biologically driven oxidation of organic substrates at the bioanode, accounted for 3.5±0.5 mLH_2/mgCOD.
Furthermore, electrochemical characterization was performed using polarization curves to evaluate the internal resistances of both the bioanode and the cathode in the linear region dominated by ohmic losses over a range of current densities. Based on the individual electrode data, the cathode was the largest source of resistance in the system (Rcathode= 4.2 Ω m^2), with the bioanode contributing 74% less resistance (Ranode=1.1 Ω m^2). This approach based on polarization curve has also been performed feeding the MEC-bioanode with a model substrate such as acetate. As expected, the anode resistance remained the same in the ohmic region. In contrast, the power density curve showed an increase of 33% in terms of current density when the system was fed with acetate. These findings suggest that the observed differences in current density are likely related to the availability of readily biodegradable COD and, more generally, to the composition of the substrate. However, digestate oxidation at the bioanode showed a high conversion of COD into catalytic current, highlighting that the low value of current density (σ= 0.05 A m^{-2}) was associated with the nature of the feedstock.
Overall, these findings suggest that digestate valorization into H_2 generation in MEC is strongly dependent on the fraction of the feedstock organic content that is accessible to the bioelectrochemical process.

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Development and validation of Bioelectrochemical processes for the Remediation of groundwater contaminated by chlorinated aliphatic hydrocarbons: from proof of concept to pilot-scale application

Geremia Sassetto, Laura Lorini, Marco Petrangeli Papini, Marco Zeppilli

Department of Chemistry University of Rome Sapienza, Piazzale Aldo Moro 5 00185 Rome

marco.zeppilli@uniroma1.it

The remediation of contaminated sites represents one of the most complex environmental challenges of recent decades, particularly concerning the management of groundwater polluted by chlorinated aliphatic hydrocarbons (CAHs). In this scenario, Bioelectrochemical Systems (BES) have offered an innovative approach to provide reducing power (i.e. electrons) to stimulate and sustain the biological reductive dechlorination process. Different from other BES applications, such as hydrogen production from wastewater treatment, groundwater remediation needs different technological and operational paradigms. Indeed, CAHs and other contaminants are usually present at low concentrations, while aquifer velocity significantly decreases contaminant load rates. Moreover, remediation actions are usually temporary installations with the main aim of removing toxic and persistent contaminants from the environmental matrix. This contribution aims to illustrate the research and technological development actions conducted by the Department of Chemistry at Sapienza University of Rome, focusing on the engineering of bioelectrochemical solutions for the treatment of contaminated aquifers. Specifically, the present study describes the fundamental milestones of the research actions directed toward simplifying reactor design and operating parameter control from proof-of-concept to the scale- up of the technology. The contribution describes both promising results and difficulties and bottlenecks related to technology development and validation. The present contribution will conduct a rigorous comparison of the different technological approaches through mass and energy balances, giving a comprehensive overview of the research efforts conducted on the bioelectrochemical CAHs remediation. Future and current research actions directed towards technology field scale validation and technology implementation will analyse possible solutions to overcome present limitations.

Microbial extracellular electron transfer in the environment: Potential role in arsenic dynamics in rice field soil

Sarah Zecchin[a], Stefania Marzorati[b], Lucia Cavalca[a]

[a] *Dipartimento di Scienze per gli Alimenti, la Nutrizione e l'Ambiente (DeFENS)*
[b] *Dipartimento di Scienze e Politiche Ambientali (ESP)*
Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy

sarah.zecchin@unimi.it

Microbial Extracellular Electron Transfer (MEET) is hypothesized to be widespread in nature and to play a crucial role in biogeochemical cycles of anoxic environments. Flooded rice field soils are considered natural hotspots of electroactive processes, where the cycling of redox-active elements such as iron, sulfur and arsenic has major implications for food safety. Despite increasing interest, the identity, metabolic pathways, spatial distribution and ecological relevance of electroactive microorganisms remain poorly understood. This knowledge gap is largely due to the lack of robust functional biomarkers for monitoring MEET in the environment and to the difficulty of linking natural systems with engineered bioelectrochemical platforms. This study aims to develop an integrated framework to investigate environmental MEET by combining: (i) the identification of universal markers for monitoring MEET in natural systems, (ii) the enrichment of electroactive anodic biofilms from rice field soils, and (iii) the investigation of the role of MEET in arsenic biogeochemistry. The potential occurrence of electroactive microorganisms was first assessed based on Illumina sequencing-based taxonomic profiling of microbial communities retrieved from different rhizosphere compartments of rice plants cultivated in fields managed under various agronomic regimes. Metadata analyses indicated that putative electroactive bacterial population were more abundant in close proximity to plant roots.
Different experimental set ups were evaluated to enrich electroactive microorganisms from soil, allowing the analysis of their metabolic pathways through downstream molecular and physiological characterization. The enrichment of soil-derived anodic biofilms in single chamber Microbial Fuel Cell (MFC) systems was compared with enrichment in soil bioelectrochemical systems (soil BES). MFC were filled with minimal mineral culture medium added with small organic molecules (acetate, formate, lactate; 0.6% v/v) as electron donors and inoculated either with 5 g of freshly sampled soil or with anodes pre-incubated in soil BES. In the soil BES, anodes were buried in the soil and connected to floating cathodse. The hypothesis that increased soil conductivity promotes electroactive microbial populations was tested by mixing soil with electroactive biochar. Soil BES were periodically amended with the same small organic molecules used in the MFCs and some of the BES were exposed to 1.5 mM arsenite.
Biochar-amended soil BES exhibited significantly lower chemical oxygen demand and bacterial 16S rRNA gene copy number compared to non-amended controls (250 vs 40 mg/L and 10^8 vs 10^3 gene copies/g soil). However, microbial communities enriched in biochar-amended microcosms showed a significantly higher current transfer efficiency compared to controls (100 vs 30 µA/gene copies).
In MFC systems, while both fresh soil-derived and non-biochar-mixed soil BES-derived anodic biofilms reached average currents of 10 µA, biofilms derived from biochar-amended soil BES exhibited average currents above 50 µA. This suggests that pre-incubation of soil with enhanced conductivity promotes the enrichment of electroactive microbial populations. Preliminary results indicated that exposure to 1.5 mM arsenite in the culture medium increased current transfer compared to non-exposed controls.
Overall, these results support the hypothesis that modulation of soil conductivity selectively enriches highly electroactive microbial consortia as well as the existence of a relevant link between MEET and arsenic redox transformations in rice paddy soils. This integrated approach provides a basis for defining functional MEET markers and for linking environmental electroactivity with controlled bioelectrochemical systems, with implications for arsenic mitigation in rice-based agroecosystems.

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Resilience and Adaptation of a Bioelectrochemical System to Cr(VI) during TCE Dechlorination

Geremia Sassetto[1], Maria Presutti[1], Bruna Matturro[2], Marco Petrangeli Papini[1] & Marco Zeppilli[1]

[1] *Department of Chemistry, University of La Sapienza, Piazzale Aldo Moro 5, 00185, Rome, Italy*
[2] *Water Research Institute, IRSA-CNR, Via Salaria km 29, Rome, Monterotondo, 300, 00015, Italy*

geremia.sassetto@uniroma1.it

Groundwater contamination by chlorinated solvents, like trichloroethylene (TCE), and heavy metals, such as hexavalent chromium (Cr(VI)), represents a major environmental and public-health concern. Conventional remediation strategies, including pump-and-treat, are frequently expensive and exhibit limited efficiency. Bioelectrochemical systems (BESs) provide a more sustainable alternative by leveraging electroactive microbial communities to promote both TCE reductive dechlorination and Cr(VI) reduction. Nonetheless, the co-occurrence of these pollutants may cause inhibitory interactions, especially affecting dehalorespiring microorganisms.

This study investigates a bioelectrochemical approach for the reductive dechlorination of TCE coupled with Cr(VI) removal from contaminated water. The process was implemented in a membrane-less tubular microbial electrolysis cell (MEC) equipped with a granular graphite cathode and an internal counter electrode composed of graphite granules. This architecture supports a simple, adaptable, and cost-effective design. The MEC was operated in continuous flow and fed with a solution containing TCE (100μM) and Cr(VI) (50μM), while the system was polarized under galvanostatic control with the cathode serving as the working electrode. Performance was assessed across a range of operating conditions, including different applied currents (galvanostatic mode) and hydraulic retention times (HRTs).

The BES showed strong tolerance to Cr(VI), consistently achieving near-complete TCE removal (>99%) and quantitative Cr(VI) reduction (100%). Importantly, the distribution of dechlorination byproducts shifted with operating conditions, indicating a dynamic microbial response to process settings. Although Cr(VI) initially inhibited dechlorinating bacteria, the system displayed substantial adaptive capacity, with dechlorination efficiency recovering over time. Overall, these results highlight BES technology as a robust and flexible platform for the simultaneous remediation of chlorinated solvents and heavy metals in contaminated groundwater.

Posters

Microbial Fuel Cells with Biochar–Hydroxyapatite Cathodes: Implications for Organic Matter and Heavy Metals Removal

Niccolò Bassani[1], Mirko Magni[1], Sebastiano Campisi[2], Sarah Zecchin[3], Marco Parolini[1], Lucia Cavalca[3], Antonella Gervasini[2], Stefano Pierpaolo Marcello Trasatti[1], Stefania Marzorati[1]

[1]*Dipartimento di Scienze e Politiche Ambientali, Università degli Studi di Milano, v.Celoria 2, Milano*
[2] *Dipartimento di Chimica, Università degli Studi di Milano, via Golgi 19, 20133 Milano*
[3] *Dipartimento di Scienze per gli Alimenti, la Nutrizione e , Università degli Studi di Milano, via Celoria 2, 20133 Milanol'Ambiente*

stefania.marzorati@unimi.it

This study investigates the combined use of biochar and hydroxyapatite (HAP) as electrode materials in single chamber microbial fuel cells (MFCs), within the context of not energivorous wastewater treatment. MFCs represent a promising bioelectrochemical technology for the sustainable management of civil and industrial effluents. The main objective was to assess whether biochar–hydroxyapatite composite cathodes could enhance organic matter degradation and heavy metal removal while maintaining electrochemical performance comparable to more conventional carbon-based active material used in the preparation of positive electrodes in MFCs.
Three biochars were produced via pyrolysis of food waste materials—pumpkin seeds, tomato peels, and coffee silverskin. Hydroxyapatite was incorporated at 10 wt% relative to biochar to form homogeneous composites immobilized on carbon cloth and employed in air-exposed cathodes. Eight MFCs were operated in duplicate, with cathodes consisting of: pumpkin seed biochar, pumpkin seed biochar with HAP, tomato peel biochar, tomato peel biochar with HAP, silverskin biochar, silverskin biochar with HAP, commercial carbon, and commercial carbon with HAP. An additional MFC was maintained under open-circuit conditions as a control. All systems were fed with brewery wastewater. Key physicochemical and electrochemical parameters, including pH, conductivity, redox potential, chemical oxygen demand (COD), and current generation, were monitored throughout the experiment. Bacterial and archaeal communities in the biofilm colonizing the surface
of biochar granules were characterized by Illumina sequencing of 16S rRNA genes using domain-specific primers.
The results showed that MFCs with cathodes made of biochar (with or without HAP) achieved similar performance - in terms of current generation and organic load reduction - to those using commercial carbon. Although ex situ adsorption studies on simulated solutions containing single metal ions (Pb2+, Cd2+, Zn2+) demonstrated that the addition of hydroxyapatite to biochar significantly enhances removal capacity under dynamic conditions, leading to residual metal concentrations up to ten times lower than those observed for pristine biochar, this improvement is not reflected when the biochar–hydroxyapatite composite is immobilized on the cathode. In this case, the addition of HAP composites did not result in significant differences in the speed or efficiency of lead, zinc, and cadmium cation removal. This suggests that the hydroxyapatite in the composite loses much of its adsorptive capacity once immobilized on the cathode surface. Additionally, the introduction of heavy metals inhibited the bacterial community's activity, drastically reducing electricity production and slowing the degradation of organic matter in the treated water. The composition of electroactive bacterial community colonizing the surface of biochar granules was strongly affected by the starting food waste material, while the archaeal community was stable and dominated by the hydrogenotrophic methanogen Methanothermobacter.
While these results highlight challenges for MFC robustness, they also suggest potential applications in developing biosensors capable of real-time detection of heavy metals contamination in water. Overall, the findings indicate that further optimization of hydroxyapatite integration—such as its direct application in the aqueous phase—and the selection of metal-tolerant microbial communities could enhance the multifunctionality and resilience of MFCs.

Microbiologically Influenced Corrosion (MIC): Integrating Metagenomics and Machine Learning for Predictive Risk Assessment and Mitigation

Francesca Pittino[1], Andrea Franzetti[1], Carlo Santoro[1], Stefano Trasatti[2], Roberto Ambrosini[2], Pierangela Cristiani[3]

[1] *Università degli studi Milano-Bicocca, Piazza della Scienza U1, 20126, Milano (IT)*
[2] *Università degli Studi di Milano, Department of Chemistry, v. Golgi 19, 20133 Milano (IT)*
[3] *Ricerca sul Sistema Energetico – RSE v. Rubattino 54, Milano (IT)*

francesca.pittino@unimib.it

Microbiologically influenced corrosion (MIC) is a complex phenomenon in which microbial communities contribute to the deterioration of metals through direct and indirect mechanisms. Key microbial players include sulfate-reducing bacteria producing corrosive hydrogen sulfide, iron-oxidizing bacteria promoting iron oxide deposition, acid-producing bacteria, and methanogens facilitating metal anodic dissolution. MIC is observed across multiple environments, including marine settings, oil and gas facilities, and wastewater treatment plants, resulting in substantial economic and safety impacts [1].
Current research often focuses on single microbial species under controlled laboratory conditions, overlooking the complexity of natural microbial consortia and their interactions with environmental factors and metal surfaces. Conventional inspection and electrochemical methods fail to detect MIC at early stages, highlighting the need for predictive tools and preventive strategies.
This contribution presents a research project that aims to advance understanding and management of MIC by integrating metagenomic sequencing and machine learning to characterize microbial communities on metal surfaces, including unculturable strains, and to predict their corrosive potential. The objectives include: mapping microbial diversity and associated metabolic pathways, analyzing environmental and material influences on MIC, and developing predictive models to distinguish high- and low-risk microbiomes.
The methodological approach integrates a meta-analysis of existing MIC literature with targeted laboratory and field investigations to complement available data, enabling the development of robust and predictive models. The ultimate goal is to provide practical guidelines, material selection strategies, and maintenance protocols for the prevention and management of MIC.

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Impact of the integrated application of conductive nanoparticles and bioelectrochemical stimulation on the anaerobic digestion of organic waste matrices

Alessandra Scotti, Matteo Tucci, Carolina Cruz Viggi, Federico Aulenta

CNR-IRSA, Istituto di Ricerca sulle Acque, Consiglio Nazionale delle Ricerche, Strada Provinciale 35d, km 0,7, 00010 Montelibretti (RM), IT

alessandra.scotti@uniroma1.it

Background and objective: Anaerobic digestion (AD) is a biological process that converts organic matter into biogas through the degradation of sludge and other organic substrates. Biogas is rich in methane and therefore represents a renewable energy source. However, conventional AD technologies still suffer from several limitations, including low rate methane production rates. Recent studies have shown that bioelectrochemical system (BES) can enhance AD performance by promoting hydrogenotrophic methanogenesis over the slower acetoclastic pathway. In addition, BESs can improve the degradation of recalcitrant compounds and prevent volatile fatty acids (VFAs) accumulation. Other studies have demonstrated that electrically conductive materials (ECMs) can accelerate syntrophic conversions, thereby enhancing AD efficiency and process stability. The aim of this study is to demonstrate that the integration of BESs and ECMs into AD can significantly improve methane production rates.

Methods: Two reactors (working volume: 1 L), each containing 0.5 L of synthetic organic matrix designed to mimic anaerobic sludge (COD: 25 g/L) and inoculated with 10 mL of activated sludge, were operated at 35°C with continuous stirring. One reactor was supplemented with magnetite nanoparticles (1 g/L), while the other served as an unamended control. Both reactors were equipped with a carbon brush anode and a stainless-steel cathode and were operated at an applied voltage of 0.8 V during the first two cycles, followed by open-circuit operation during the third cycle. Methane production and VFAs concentrations were monitored throughout the experiment. Methane was quantified using gas chromatography with a thermal conductivity detector (GC–TCD), while VFAs were analyzed by gas chromatography with a flame ionization detector (GC–FID).

Results: The results showed a clear synergistic effect between the bioelectrochemical system (BES) and magnetite nanoparticles, with an increase in methane production rates ranging from 34% to 51% compared to the BES alone. In contrast, the addition of magnetite nanoparticles alone had a marginal impact on process kinetics. Chemical analyses revealed that the performance improvement observed in the BES+NP system was associated with a reduced accumulation of VFAs, particularly acetic acid, suggesting a more efficient conversion of metabolic intermediates. This behavior is likely attributable to the rapid oxidation of acetate at the anode, resulting in electrical current generation and subsequent hydrogen production at the cathode, which was readily converted into methane by hydrogenotrophic methanogenic microorganisms. The presence of magnetite nanoparticles may further enhance extracellular electron transfer processes between microorganisms and electrodes.

Conclusion: Overall, this study highlighted, for the first time, a positive synergistic effect arising from the combination of bioelectrochemical systems and conductive magnetite nanoparticles, capable of accelerating the methanogenic conversion of complex organic substrates and enhancing the degradation of volatile fatty acids. Future studies will be required to assess the technical and economic feasibility of this approach at full scale.

Phototaming of Bacterial Bioelectricity

Giuseppe Maria Paternò

Dipartimento di Fisica, Politecnico di Milano

Giuseppemaria.paterno@polimi.it

Engineering living matter aims to confer organisms with controllable functions by coupling biological specificity with designed inputs. A powerful, yet underexploited, axis for such control is cellular bioelectricity: the membrane potential (MP) that links redox chemistry, transport and metabolism to signalling and behaviour. In bacteria this link is particularly intimate because ATP synthesis and many ion fluxes occur at the membrane; recent work shows that MP is dynamic and participates in cell-to-cell communication, shaping metabolism, motility, dormancy towards antibiotics and community behaviours such as biofilm formation.

Here, we demonstrate that membrane-localized photoactive molecules can produce reliable optical control of bacterial MP.[1] Membrane isomerization at the site of action drives either hyperpolarisation or depolarisation depending on the excited-state relaxation pathway (optomechanical versus dipolar), a biomimetic mechanism reminiscent of retinal photochemistry. Such control can evoke neuron-like bioelectric responses in bacteria and reveal the functional involvement of ion channels previously uncharacterised in microbial electrophysiology. Finally, we show how light-driven MP modulation can be used to influence antibiotic uptake and efficacy,[2] and to steer motility — opening routes to both fundamental insight and translational applications in diagnostics and antimicrobial strategies.

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Bioelectrochemically-assisted degradation of 1,1,2-trichloroethane by a co-culture of *Dehalobacter* and *Dehalococcoides*

Camilla Valli[1], Ernest Marco-Urrea[2], Paqui Blánquez[2], Sarah Zecchin[1], Lucia Cavalca[1], David Fernández-Verdejo[2]

1 Department of Food, Environmental and Nutrition Sciences (DeFENS), University of Milan, Milan, Italy
2 Biorem UAB, Department of Chemical, Biological and Environmental Engineering, School of Engineering, Universitat Autònoma de Barcelona, 08193, Bellaterra, Barcelona, Spain

lucia.cavalca@unimi.it

Chlorinated aliphatic hydrocarbons (CAHs) represent a challenge for groundwater remediation. While the anaerobic biodegradation of chlorinated ethenes has been extensively investigated, the biodegradation of chlorinated ethanes remains largely unexplored. Among these, 1,1,2-trichloroethane (1,1,2-TCA), widely used as an industrial degreasing agent, is a widespread groundwater contaminant due to improper storage and accidental releases.

Under anoxic conditions, reductive dechlorination is expected to be the dominant transformation mechanism and can proceed via two main pathways: hydrogenolysis and dichloroelimination. During dichloroelimination, two vicinal C–Cl bonds of 1,1,2-TCA are cleaved to form 1,2-dicloroethane (1,2-DCA) and vinyl chloride (VC), a compound more toxic than its parent, whereas hydrogenolysis involves the sequential cleavage of single C–Cl bonds, producing 1,2-dichloroethane (1,2-DCA) and monochloroethane. These reactions are catalyzed by organohalide-respiring bacteria (OHRB), which can use 1,1,2-TCA as a terminal electron acceptor.

The growth of OHRB is constrained by the limited availability of suitable electron donors and by the difficulty of maintaining strictly reducing conditions in groundwater. Bioelectrochemical systems (BESs) offer a promising strategy to overcome these limitations by enabling the controlled delivery of reducing equivalents at the cathode through electrochemical hydrogen evolution.

In this study, we aim to evaluate the feasibility of coupling two syntrophic OHRB cultures of *Dehalobacter* and *Dehalococcoides* in the cathodic chamber of a BES to promote the sequential transformation of 1,1,2-TCA to ethene. *Dehalobacter* is expected to catalyze the transformation of 1,1,2-TCA to VC and 1,2-DCA, while *Dehalococcoides* to further reduce them to ethene. The *Dehalobacter* culture (strain 8M) was originally enriched from chloroform-contaminated groundwater, while the *Dehalococcoides mccartyi* strain derived from a CAH-contaminated site.

The system will be operated under several controlled cathodic potentials to regulate hydrogen production and to distinguish between biotic from abiotic transformation pathways. Moreover, cathodic potentials not capable of driving hydrogen evolution will be applied to assess the feasibility of direct electron transfer for both, *Dehalobacter* and *Dehalococcoides* reductive reactions. Several electrode materials will be evaluated to determine their performance as electron donors under both configurations.

The experimental approach will initially focus on optimizing a growth medium compatible with both OHRB cultures and on investigating the potential inhibitory effects of 1,1,2-TCA on *Dehalococcoides*, in order to determine the maximum pollutant concentrations that the mixed consortium can effectively treat. Subsequently, abiotic cathodic conditions will be assessed to minimize non-biological dichloroelimination, together with the optimization of the BES design. The growth of both *Dehalobacter* and *Dehalococcoides* in the cathode will me monitored by qPCR.

Overall, this work aims to explore the potential of BES as a versatile and controllable strategy to support syntrophic dechlorinating communities and to enhance the complete degradation of chlorinated ethanes and their byproducts in anaerobic environments.

Thermal Selection of Microbial Consortia Controls Corrosion Behavior of Copper and Copper Alloys

Elena Cazzulani[1], Giorgia Ghiara[2], Andrea Franzetti[3], Marcella Balordi[4], Paolo Fedeli[4], Francesco Pini[4], Pierangela Cristiani[4*], Gian Luca Chiarello[1*]

[1]*Department of Chemistry, Università degli Studi di Milano, Milano, Italy*
[2]*Department of Applied Science and Technology (DISAT), Politecnico di Torino, Italy*
[3]*Department of Earth and Environmental Sciences, University of Milan Bicocca, Italy*
[4]*RSE-Ricerca sul Sistema Energetico S.p.A., Milano, Italy*

Elena.cazzulani@unimi.it

Microbiologically influenced corrosion (MIC) poses significant challenges to copper and copper-based alloys, particularly in thermophilic environments such as methanation reactors and biogas plants. This study investigates how thermal treatments selectively enrich microbial communities [1] and consequently modulate the corrosion behavior of high-purity copper (≥99.9 wt.%) and duplex brass OT60 (40 wt.% Zn). Batch experiments were conducted at 45°C for 15 days using thermophilic microbial consortia from a methanation pool subjected to different thermal treatments: (i) untreated (biotic control), (ii) boiling (120 min), (iii) double autoclaving (121°C, 20 min), and (iv) extended autoclaving with filtration and UV exposure (sterile control). Microbial communities were characterized by 16S rRNA gene sequencing, while corrosion processes were monitored using electrochemical impedance spectroscopy (EIS), scanning electron microscopy (SEM), and micro-Raman spectroscopy.

Results revealed that thermal treatments induced marked shifts in microbial community structure without achieving complete sterilization. For pure copper, abundant bacterial populations accelerated early-stage copper dissolution and delayed surface passivation, while archaeal-dominated biofilms [2] promoted copper immobilization through precipitation of mixed copper phosphate phases, leading to diffusion-controlled corrosion and mitigation of localized attack. In contrast, sterile conditions favored chloride-driven pitting corrosion with the highest copper release.

For brass, autoclaving enriched spore-forming Bacilli (*Caldalkalibacillus, Brevibacillus, Effusibacillus*) [3], which promoted formation of a protective Cu_2O/CuO-$CaCO_3$ bilayer and achieved the highest total resistance (42 kΩ·cm² after 15 days). The biotic and boiled conditions, dominated by thermophilic *Tepidiphilus*, developed intermediate Cu-Zn phosphate layers with lower protective efficiency (3.8-6.5 kΩ·cm²). The sterile condition exhibited the most severe corrosion with direct alloy dissolution (Cu/Zn ratio: 7.25) and formation of fragile, non-protective deposits (14 kΩ·cm²).

These findings demonstrate that the balance between bacterial and archaeal activity governs the evolution from corrosion acceleration to mineral-mediated stabilization [4]. Selective microbial enrichment through controlled thermal treatments represents a promising strategy for mitigating MIC in copper and copper alloys operating in thermophilic biotechnological systems.

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Using soil microflora to powering low-power systems for environmental monitoring and remediation: the Bio-Renewable Intelligent Data & Green Energy project.

Raffaele D'Apuzzo[1], Serena Narducci[2], Valeria Di Onofrio[2], Vincenzo Pasquale[2], Alfredo Autorino[3], Claudio Autorino[1], Rosa Anna Nastro[2]

[1] *PROTOM ROBOTICS srl,- Centro Polifunzionale di Napoli Ed.6, 80143 Naples – Italy*
[2] *Department of Science and Technology, University of Naples "Parthenope"- Centro Direzionale Isola C4, 80143 Naples, Italy*
[3] *PROTOM GROUP spa - Via Vittoria Colonna, 14 80121 Napoli*

serena.narducci@unina.it

Microbial Fuel Cells (MFCs) and, more generally, bioelectrochemical systems have the potential to provide energy from renewable sources (microbial metabolism) to areas not served by electricity distribution networks, in a process that is CO_2 neutral. Experimental data available in international literature clearly show the ability of MFCs to exploit microbial metabolism, while using organic and inorganic compounds (pollutants included) from wastewater, groundwater, sediments, soil (1). If successfully implemented, they could be used in a process that does not require the use of solvents, surfactants or soil oxygenation, which are usually applied in "*in situ*" remediation processes. Beyond their use for remediation purposes, MFCs applied to soils can also bring benefits in the agronomic field, both through the provision of energy for use in rural areas and because the biocompatibility of MFCs with plant growth, as shown by the creation of plant-MFCs in various parts of the world, besides of treating wastewater that can be used for irrigation purposed (2). Few or no data, instead, are available about the potential effects of electrogenesys on nitrogen fixing bacteria, which play a pivotal role in soil ecosystems and fertility.
The Bio-Renewable Intelligent Data & Green Energy (BRIDGE) project's objective places within the topic of "Smart and Green Environment for the telecommunications networks of the future". because the research envisages the integration of MFCs with an electronic device provided with a low-power technologies such as Bluetooth Low Energy (BLE) and a sensor for monitoring environmental temperature and humidity. BRIDGE was essentially aimed at setting up a lab-scale prototype for demonstrating the potentialities of MFCs as energy devices for decentralized telecommunication networks for agriculture/remediation purposes. For this proof-of-concept proposal, we used MudWatts as already well proven systems for energy production from mudTogether with microflora from gardening soil, we tested the effect of compost, kerosene and $CdSO_4$ on MudWatts elect4rochemical behaviour and energy outputs. We also investigated the quali-quantitative variation in microbial tests on plant MFCs including young bonsai to evaluate the potential effects of electrogenesis on plants growth. Two modules made up by three MudWatts connected in parallel were able to continuously power the electronic system for one week and more, Mudwatts, after a first month of activity where great part of pollutants are removed from soil, can be integrated into the modules. Last, but not the least, electrogenesys seems to have not negative effects on plant growth. On the contrary, the production of compounds from roots when the plant is stressed, can cause voltage reversal. The results about the effects on nitrogen fixing bacteria and, more in general on soil microflora, are discussed.

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VisioNing: valorization of agro-industrial wastewater. From research bench to business

Mirko Magni[a,c], D. La Corte[a], P. Scavone[a], K. Rajagopalan[a], S. Marzorati[c], D. Meroni[b], F. Adani[d], C.L. Bianchi[b], S. Guzzetti[a], E. Falletta[a,b]

[a] *VisioNing srl, via F. Olgiati 26, 20143, Milan, Italy*
[b] *Department of Chemistry, Università degli Studi di Milano, via C. Goli, 19, 20133 Milan, Italy*
[c] *Department of Environmental Science and Policy, Università degli Studi di Milano, via G. Celoria, 2, 20133 Milan, Italy d Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, Università degli Studi di Milano, via G. Celoria, 2, 20133 Milan, Italy*

mirko.magni@unimi.it

The escalating pollution caused by agro-industrial wastewater is an alarming environmental threat. Apart from the obvious water wastage, valuable nutrients within are also lost [1]. Besides that, valuable strategies to treat fluids waste coming from anaerobic digestion of biomass and biowaste are still under scouting by many industrial actors. The wastewater treatment sector and that of fertilizers' production account for over 3% and 1.2% of world's energy [2]. Moreover, fertilizer costs are continually growing as they are related to the energy cost and population growth. This represents a significant economic damage to companies in the sector. The agro-industrial wastewater treatment market is witnessing a surge in interest towards efficient and sustainable technologies. In fact, technological innovation and the embrace of eco-friendly solutions are driving industry transformation. In this context, the mission of VisioNing is simple but ambitious: transforming wastewater from a problem to a resource with 100% circularity and sustainability.
VisioNing is a deeptech startup conceived and founded after a meticulous examination of the challenges in wastewater treatment. Combining synergistically spontaneous and low-energy demanding treatments, our process not only purifies water but also recovers and reuses the nutrients it contains. While numerous wastewater treatment solutions exist, our combined technology offers dual benefits: energy efficiency and recyclability. This empowers us to position ourselves as frontrunners in sustainability and innovation, ensuring a 30% reduction in operational costs compared to traditional systems. Having conducted in-depth research and developed prototypes at the University of Milan, we have now refined some operational and highly efficient pilot plants (500 L capacity). Three pilot plants have been conducting with several companies, spread along the peninsula, financed by the "Farming Future" investment fund of CDP Venture Capital SGR and ToSeed & Partners, confirming the preliminary results in terms of nutrients recovery and water purification.

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Utilisation of Sludge derived Ligno-Humic-Like Compounds as Electrocatalytic Booster for Bioelectrochemical applications

Angela Marchetti[a], Marco Agostini[b], Maria Lacalamita[c], Luigi di Bitonto[d], Marco Zeppilli[a]

[a] *Department of Chemistry University of Rome Sapienza, Piazzale Aldo Moro 5 00185 Rome*
[b] *Department of Chemistry and Drug Technologies, University of Rome Sapienza, Piazzale Aldo Moro 5 00185 Rome, Italy*
[c] *Department of Earth and Geoenvironmental Sciences, University of Bari, Bari, Italy.*
[d] *Water Research Institute, National Research Council-C.N.R, Bari, Italy*

angela.marchetti@uniroma1.it

Sewage sludge management is a global challenge, yet it contains valuable components for biofuel and material recovery. While lipids, cellulose, sugars, and proteins contained in sewage sludge are widely exploited, lignohumic-like (LHL) compounds, representing 4–20% of sludge solids, remain underutilized. LHL compounds are a complex mixture of molecules with a structure that is a hybrid of lignin and humic substances. This study investigates the direct use of LHLs extracted from different waste organic streams as “boosters” of electrocatalytic activity in microbial electrolysis cells (MECs). LHLs, extracted from anaerobic digestate (AUD), primary sludge (AUP) and digested sludge (AUDIGE), were combined with graphite-based electrodes and tested in single chamber MEC reactors to assess their potential use as electrocatalytic booster. The LHLs residual matrix from the different sludges were dispersed on the surface of carbon felt electrodes using the coating techniques. The produced electrodes were tested in a single chamber reactor using cyclic voltammetry to identify redox centres and capacitive charge. Preliminary results indicated that the AUD electrode showed the most significative effect on electrocatalytic activity showing a consistent increase in electrocatalytic capacity. Preliminary results showed the effective possibility to use LHLs derived compounds to increase electrocatalytic activity of carbon-based electrodes. These preliminary results are promising with respect the possibility to valorise a by-product material from sewage sludge valorisation to promote circularity in wastewater treatment by coupling organic carbon removal and hydrogen generation.

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Rapid screening and performance optimization of a dual-cathode microbial electrolysis cell for sustainable hydrogen production from synthetic and real waste

Angela Marchetti[a], Syrine Berhouma[a,b], Habib Chouchane[b], Marco Zeppilli[a]

[a]*Department of Chemistry University of Rome Sapienza, Piazzale Aldo Moro 5 00185 Rome*
[b]*University Manouba, ISBST, BVBGR-LR11ES31, Biotechpole Sidi Thabet, Ariana, Tunisia*

angela.marchetti@uniroma1.it

Currently, about 96% of global hydrogen is still produced via fossil-based steam methane reforming, a highly energy-intensive process associated with significant CO_2 emissions. In this context, microbial electrolysis cells (MECs) represent a promising alternative to conventional alkaline water electrolysis, as they enable hydrogen production at substantially lower applied voltages by exploiting electroactive microorganisms to oxidize organic substrates. When acetate is used as electron donor, the required voltage can be reduced from 1.23 V to approximately 0.187 V, corresponding to an energy input reduction of about 85%. However, the practical implementation of MEC technology is still limited by challenges related to reactor configuration, process optimization and system stability, especially when complex real substrates are employed.

The present study proposes a dual-cathode MEC configuration designed to enhance energy conversion efficiency and hydrogen recovery by improving current distribution, stabilizing anodic overpotential and minimizing cathodic overvoltage. In addition, for the first time, a rapid screening approach is introduced to systematically evaluate the performance of dual-cathode MECs under different operating conditions, with the aim of accelerating system optimization according to predefined process objectives. The screening methodology is based on the progressive variation of hydraulic operating parameters, influent flow rate and hydraulic retention time, while maintaining each condition for a sufficiently long but limited period, allowing the system outputs to reach stable values without requiring prolonged operation.

This approach enables the fast identification of optimal operational regimes depending on the target performance, such as maximization of hydrogen production, enhancement of organic matter removal, or achievement of a balanced anodic and cathodic outputs. The system was operated under six conditions by varying hydraulic retention time and organic loading rate, using both a synthetic medium and a real fermented effluent derived from dark fermentation of food waste and sewage sludge. The real substrate was characterized by a high organic acid content (82% OA/COD_sol) and a low redox potential (≈ −0.278 V vs SHE), indicating strong electron-donating capacity and high suitability for bioelectrochemical conversion. System performance was evaluated in terms of current generation, COD removal, hydrogen recovery, and coulombic and energy efficiencies, supported by potentiostatic polarization analysis to assess the electrochemical response of the reactor.

The dual-cathode MEC achieved stable operation across all tested conditions, with a cathodic coulombic efficiency (CCE) values approaching or exceeding 100% for both substrates, indicating complete or over-stoichiometric electron recovery into hydrogen. At an organic loading rate of 4 g COD L^{-1} d^{-1}, average currents of 98.5 mA and 73.2 mA were obtained for the synthetic and real feeding solutions, respectively. Compared to conventional single-cathode architectures reported in literature, the proposed configuration consistently enhanced electrochemical performance, improving productivity and maintaining high hydrogen yields even when treating complex real waste streams. Overall, the combined dual-cathode design and rapid screening methodology provide an effective platform for accelerating MEC management and optimization, demonstrating that appropriate reactor configuration and operational tuning are key factors to unlock the full potential of MECs for real-waste valorisation and sustainable biohydrogen production within circular economy and low-carbon energy frameworks.

An experimental approach to geothermal electromethanogenesis

Stefano Ravasi[a], Daniele Moraschini[b], Silvia Franz[b], Pierangela Cristiani[a]

[a]*Ricerca sul sistema Energetico – RSE SpA, v. Rubattino 54, Milano (Italia)*
[b]*Politecnico di Milano, Department of Chemistry, Materials and Chemical Engineering "Giulio Natta", Via Mancinelli 7, 20131 Milano (Italy)*

stefano.ravasi@rse-web.it

Electromethanogenesis is an emerging technology that offers a sustainable pathway for converting CO_2 emissions into green CH_4, hydrogen, and other value-added products using renewable electricity. However, the large-scale implementation of this process requires the development of sustainable, low-cost materials for electrodes and chamber separators to improve its economic and environmental feasibility.
Among the potential CO_2 sources, geothermal emissions from natural gas vents represent a particularly promising opportunity for a process that can be defined as geothermal electromethanogenesis, as these streams are primarily composed of CO_2 and contain only trace amounts of oxygen [1]. Moreover, the anaerobic microorganisms involved in electromethanogenesis exhibit a high tolerance to impurities, enabling the direct utilization of CO_2 from natural sources such as geological soil emissions.
Despite these advantages, several challenges still limit the performance, development, and scale-up of bioelectrochemical systems. This work presents a pilot-scale setup designed to investigate a simple, low-cost electromethanogenesis system suitable for both laboratory and field applications. The system includes an automated unit for CO_2 dosing and flow control, as well as for the polarization of electrodes in six bioelectrochemical cells. In addition, an innovative double-chamber configuration is proposed, in which the anode and cathode are separated by a terracotta cylinder, allowing continuous flow or recirculation of CO_2 and produced gases.
Preliminary results demonstrate that the proposed cell configuration ensures strict anaerobic conditions at the cathode, with negligible oxygen crossover. Furthermore, the terracotta separator exhibits a low internal resistance, below 10 Ω, confirming its suitability as a cost-effective alternative for electromethanogenesis applications.

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Microbial Fuel Cell cross-laboratory study

*Carlo Santoro[1], Plamen Atanassov[2], Pierangela Cristiani[3]

[1] *Electrocatalysis and Bioelectrocatalysis Laboratory, Department of Material Science, University of Milano-Bicocca Via Cozzi 55, 20125 Milan, Italy*
[2] *Department of Chemical & Biomolecular Engineering, National Fuel Cell Research Center (NFCRC), University of California, Irvine, CA 92697, USA*
[3] *Department of Sustainable Development and Energy Resources. Ricerca sul Sistema Energetico S.p.A. Milan, Italy*

carlo.santoro@unimib.it

The feasibility of achieving comparable performance in MFCs fed with wastewater-derived microbial consortia from different locations was demonstrated through a cross-laboratory study involving five institutions: (1) University of New Mexico located in Albuquerque, New Mexico, USA; (2) Ricerca sul Sistema Energetico and University of Milan, both located in Milan, Italy; (3) CNRS, Université de Toulouse, located in Toulouse, France; (4) Technische Universität Braunschweig, located in Braunschweig, Germany; (5) Helmholtz-Centre for Environmental Research – UFZ, located in Leipzig, Germany.
All the involved laboratories operated identical Microbial Fuel Cells (MFC) using domestic wastewater as inoculum source and i) the performance was assessed, and ii) the initial bacterial pools and the biofilm formed on the electrodes were identified.
The identical single chamber MFC used in this study had an air cathode and an empty volume of 125 mL. Graphite plates were used as anode having a surface area of 6.25 cm^2. The air breathing cathode had a surface area of 2.9 cm^2 geometric surface area. The cathode was fabricated by pressing a mixture of PTFE/AC (2:8 ratio) over a stainless-steel mesh. The MFCs were inoculated with local domestic wastewater, hence from plant sites in worldwide different locations in Europe and North America.
The overall study run for 6 weeks. The initial two weeks (acclimation stage), the MFCs were run by applying an external resistance of 1000 Ω. The stabilization phase was run for additional two weeks decreasing the external resistance to 100 Ω followed by the steady state phase. The inoculum affected importantly the startup phase. Notably, all the MFCs reached similar maximum power outputs and COD removal efficiencies, despite the large diversity found in their bacterial pool. Only in one case, MFCs run by CNR had very low power output along the experimentation due to the dilution in the initial inoculum. [1]. In a second case, MFCs run by RSE, the low performance induced by a dilute inoculum where just Pseudomonadales enriched, was remediated by using a new, more concentrated, inoculum coming from the same wastewater plant. In all performing MFCs, the syntrophic interaction of fermentative and strict anaerobic electroactive bacteria at anode and cathode lead to similar performance of the MFCs. Different bacterial groups compose the microbial consortia. However, they share similar functions both on anode and cathode of the different MFCs. Particularly, Clostridiales, Bacteroidales, Synergistales, Lactobacillales and Desulfovibrionales are the key bacteria orders found in MFCs of each institution. Faster energy recovery was positively associated with the abundance of Desulfovibrionales or Lactobacillales at the anode, while higher COD removal rate was clustered with fermentative bacteria such as Clostridiales, Bacteriodales and Synergistrales.[1]

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Impacts of long-term biochar application on greenhouse gas emissions and soil microbial community structure in vineyard soils

Elisabetta Toni[a], Matteo Daghio[a], Filippo Rocchi[b], Alessandra Lagomarsino[b], Roberta Pastorelli[b], Francesco Primo Vaccari[c], Marco Fusi[d], Carlo Viti[a]

[a] *Dipartimento di Scienze e Tecnologie Alimentari, Agrarie, Ambientali e Forestali, Università di Firenze, Piazzale delle Cascine 18, 50144, Firenze, Italy.*
[b] *Research Centre for Agriculture and Environment, Consiglio per la Ricerca in Agricoltura e l'Analisi dell'Economia Agraria (CREA-AA), Via di Lanciola 12/A, 50125 Firenze, Italy;*
[c] *Institute of BioEconomy (IBE), National Research Council (CNR), Via Madonna del Piano 10, 50019 Sesto Fiorentino, Firenze, Italy.*
[d] *Dove Marine Laboratory, School of Natural and Environmental Sciences, Newcastle University, Newcastle, NE1 7RU, UK.*

elisabetta.toni@unifi.it

Soil health, in particular the maintenance of its microbial diversity, is recognised as essential for the functioning of ecosystems, agricultural sustainability and human well-being. Unsustainable agricultural practices, such as excessive use of fertilisers, intensive monocultures and excessive ploughing, lead to progressive soil degradation, increased greenhouse gas (GHG) emissions and groundwater pollution. Among the strategies proposed to counter these trends, biochar, a carbon-rich material obtained through the pyrolysis of biomass, has attracted considerable attention for its ability to persistently modify soil environments.

Biochar incorporation into soils induces substantial changes in physical and chemical properties, including enhanced porosity, increased surface area, improved water-holding capacity, pH buffering, and greater nutrient retention. In addition, biochar exhibits distinctive electrochemical properties, such as a high density of surface charges, redox-active functional groups, and the ability to participate in electron transfer reactions. These electrochemical characteristics enable biochar to influence microbial metabolism and soil redox processes, acting as an electron shuttle or sink that can regulate key pathways involved in carbon and nitrogen cycling. Through these mechanisms, biochar has the potential to modulate microbial community structure and activity while simultaneously reducing CO_2 and N_2O emissions. However, the effects of biochar on soil over long time scales remain poorly understood. To address this limitation, we examined the long-term consequences of biochar addition using a mesocosm experiment based on a vineyard soil that had received biochar approximately 15 years earlier at two application rates (22 and 44 t ha^{-1}). Nitrogen was supplied as urea or digestate at a dose equivalent to 150 kg N ha^{-1}, reflecting common agronomic practices. We assessed bacterial community composition, enzymatic activities, functional gene potential, and greenhouse gas fluxes, and explored causal links between soil biochemical properties, bacterial community, and gas emissions.

Our findings indicate that biochar-amended soils, regardless of the nitrogen source applied, exhibited marked shifts in microbial community composition and functional potential, which were consistently associated with lower CO_2 and N_2O emissions. Furthermore, cumulative GHG fluxes were strongly shaped by the interaction between nitrogen fertilization strategy and both the presence and dose of biochar, highlighting their combined role in controlling soil GHG dynamics. This study demonstrates that biochar exerts long-lasting effects on soil biogeochemical processes, partly driven by its electrochemical properties, which contribute to the regulation of microbial activity and the mitigation of greenhouse gas emissions, thereby supporting more sustainable soil management strategies.

Magnetite Nanoparticles Promote Conductive Self-Assembled Bacterial Networks to Enhance Long-Distance Electron Transfer in Soil

Matteo Tucci[1], Marco Resitano[1], Carolina Cruz Viggi[1], Ugo Marzocchi[4,5], Federico Aulenta[1,3]

[1] *Water Research Institute (IRSA), National Research Council (CNR), 00010 Montelibretti (RM), Italy*
[3] *National Biodiversity Future Center, Palermo 90133, Italy*
[4] *Center for Electromicrobiology (CEM), Department of Biology, Aarhus University, Aarhus, Denmark*
[5] *Center for Water Technology (WATEC), Department of Biology, Aarhus University, Aarhus, Denmark*

matteo.tucci@irsa.cnr.it

Extracellular electron transfer (EET) is a mechanism that allows microorganisms to respire insoluble redox-active substrates, such as iron and manganese oxides, or solid electrodes, thereby sustaining their metabolism. Certain electroactive bacteria are capable of long-distance electron transfer (LDET) over tens of micrometers via conductive outer-membrane proteins, pili, or both, forming electroactive biofilms up to approximately 50 µm thick. Nevertheless, the limited extension of these biofilms restricts their effectiveness in industrial and environmental applications, especially in electrobioremediation, where the electrode's radius of influence is a key limiting factor.

In this study, we investigated whether magnetite nanoparticles could extend LDET in a microbial electrochemical system (MET) operated in a model soil environment. Two identical tubular bioelectrochemical reactors were packed with either sand alone or sand amended with 5% (w/w) magnetite nanoparticles. Graphite anodes were polarized at +0.2 V vs. SHE, and acetate (1 g/L) was provided as the sole electron donor to prevent direct interspecies electron transfer (DIET). The addition of magnetite nearly doubled the current generated from acetate oxidation (~5.5 mA compared to ~2.7 mA in the sand-only reactor), indicating a significantly enhanced acetate biodegradation rate. Cyclic voltammetry (CV) revealed progressive colonization of the anodes by electroactive microorganisms, with substantially higher oxidative currents in the magnetite-amended system.

To evaluate the spatial extension of microbial conductivity, CV measurements were performed using a titanium electrode positioned at increasing distances from the anode. No redox activity was detected in the sand-only reactor or in abiotic controls. In contrast, distinct electrocatalytic signals were observed up to 1.8 cm from the anode surface in the presence of magnetite, demonstrating the formation of an extended conductive microbial network. Overall, these results show that magnetite nanoparticles enhance LDET by increasing both the effective electrocatalytic surface and the spatial reach of microbial electron transfer. This strategy paves the way for field-scale environmental applications of MET, such as soil and groundwater electrobioremediation.

Rational design of gold nanoparticles-bacteria interfaces for optimized biophotocurrent generation

Umberto Mattia

Politecnico di Bari

u.mattia@phd.poliba.it

The development of biohybrid architectures integrating intact photosynthetic bacteria with metal nanoparticles (NPs) represents a transformative approach toward sustainable energy conversion[1]. These systems leverage the high electrical conductivity of metallic nanostructures to enhance extracellular electron tran sfer (EET) between biological photosynthetic apparatuses and external electrodes[2].
In this work, cells of Rhodobacter capsulatus, purple non-sulfur anoxygenic photosynthetic bacterium, are modified with Gold (Au) NPs tailored in size and surface chemistry to disclose the role of these two parameters in enhancing charge transport at the biotic-abiotic interface in biohybrid electrodes. Purposely selected solution-phase synthetic protocols are used to achieve control over NPs size, and optical density measurements are carried out to assess the viability of bacterial cells exposed to Au NPs in the 66-132 nM concentration range. TEM analysis provides critical insights into the spatial localization and assembly of the NPs on the bacterial scaffold. The photoelectrochemical performance is evaluated via cyclic voltammetry and chronoamperometry. Furthermore, electrochemical impedance spectroscopy under dark and visible light conditions was utilized to gain insights into electron transfer mechanism. By comparing different biohybrid configurations, this study aims at identifying the optimal nanobiointerface design for maximizing photocurrent generation, highlighting the crucial role played by Au NPs, towards the green energy transition.

Photo-driven syngas generation through microbial biocathodes

Dario Lacalamita [1], Jefferson Honorio Franco [1], Rosa Maria Matteucci [2], Paolo Stufano [3], Pietro Cotugno [1], Rossella Labarile [4], Massimo Trotta [4], Alessandra Tacca [5], Gianluca Maria Farinola [1], Matteo Grattieri [1,4]

[1]*Department of Chemistry, Università degli Studi di Bari "Aldo Moro", Bari, Italy*
[2]*Dipartimento di Ingegneria Elettrica e dell'Informazione, Politecnico di Bari, Bari, Italy*
[3]*CNR-NANOTEC, National Research Council, Bari, Italy*
[4]*CNR-IPCF, National Research Council, Bari, Italy*
[5]*ENI Novara Laboratories (NOLAB), Novara, Italy*

dario.lacalamita@uniba.it

Photosynthetic purple non-sulfur bacteria (PNSB) possess a highly versatile metabolism, making them valuable biocatalysts in biohybrid electrochemical systems for applications ranging from biosensing to wastewater remediation and sustainable chemical production [1]. While several artificial strategies have been developed to enhance extracellular photo-induced electron transfer, research has predominantly focused on anodic configurations, leaving cathodic processes comparatively underexplored [2]. In this work, we introduce a sustainable biohybrid photocathode based on a homemade polyhydroxybutyrate–carbon nanofiber electrode coated with a polydopamine–PNSB matrix [3]. Electrochemical characterization reveals that PNSB significantly contribute to the observed cathodic photocurrent, and their use with the homemade electrode allowed a 140-fold enhancement compared to glassy carbon biocathodes. Building on this platform, we demonstrate photo-driven hydrogen evolution and syngas production, achieving an H2:CO ratio of 1.7 ± 0.1 and a Faradaic efficiency of 22% at –0.19 V vs RHE after 3 hours, alongside formate, methanol, and trace ethanol. These results highlight the potential of PNSB-based biohybrid photocathodes for sustainable syngas and green hydrogen generation during wastewater treatment.

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Boosting microbial Power-to-Protein via waste-derived biochar cathodes in microbial electrosynthesis

Gabriele Soggia[a], Amin Narimani[a], Barbara Mecheri[b], Niccolò Forin[c], Andrea Squartini[c], Fabrizio Adani[a]

[a] *Gruppo Ricicla, Dipartimento di Scienze Agrarie e Ambientali: Produzione, Territorio, Agroenergia, Via Celoria 2, 20133, Milan, Italy*
[b] *Department of Chemical Science and Technologies, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, 00133, Rome, Italy*
[c] *Department of Agronomy, Food, Natural Resources, Animals and Environment, DAFNAE, University of Padova, Viale Dell'Università 16, 35020, Legnaro, Italy*

gabriele.soggia@rse-web.it

Power -to-Protein (PtP) technologies are emerging as a viable solution to store surplus renewable electricity in the form of microbial single-cell protein (SCP), providing a sustainable alternative to conventional plant- and animal-based protein sources. SCP production is characterized by high protein yields per unit of biomass, reduced land and water requirements, and the potential to exploit residual carbon and nitrogen streams.
When implemented in microbial electrosynthesis (MES) systems, PtP processes can be fully integrated into a circular bioeconomy framework, enabling the simultaneous valorization of CO_2 and nitrogen-rich waste streams derived from anaerobic digestion or other biowaste treatment processes.
In this study, the effect of biochar-based cathode functionalization on microbial activity, biomass productivity, SCP yield, and nitrogen capture efficiency was investigated in MES reactors operated under potentiostatic control at −1.2 V vs Ag/AgCl and supplied with 1.25 g L^{-1} NH_4Cl as the sole nitrogen source. Carbon cloth cathodes were either left unmodified (E0, control) or spray-coated with biochar produced from urban green waste and subjected to different post-treatment strategies (E1, E2, and E3). All reactors were equipped with a titanium mesh anode and separated by a Nafion® 117 cation exchange membrane. The cathodic chamber was inoculated with acclimated microbial biomass originating from the organic fraction of municipal solid waste.
Cathode functionalization resulted in a substantial improvement of MES performance compared to the control system. Biomass space–time yield (STY) increased from 6 ± 1 mg L^{-1} d^{-1} for the unmodified cathode (E0) to 40 ± 2 mg L^{-1} d^{-1} for E1, corresponding to an increase of more than 500%. Intermediate but still significantly enhanced biomass productivities were observed for E3 (32 ± 2 mg L^{-1} d^{-1}) and E2 (26 ± 2 mg L^{-1} d^{-1}). Nitrogen capture efficiency showed a similar trend, increasing from 0.48 ± 0.04% N for E0 to 19.8 ± 3.0% N for E1, while E2 and E3 reached 12.3 ± 0.2% N and 13.4 ± 0.7% N, respectively.
Cathode functionalization also strongly affected the biochemical composition of the produced biomass. The protein content of SCP obtained with E0 was limited to 7.6 ± 0.1% on a dry matter basis, whereas all biochar-functionalized cathodes achieved markedly higher protein contents, namely 48.7 ± 5.3% for E1, 47.8 ± 3.8% for E2, and 41.8 ± 5.4% for E3. As a consequence, protein-specific STY increased from 0.47 ± 0.04 mg L^{-1} d^{-1} for E0 to 19.5 ± 2.9 mg L^{-1} d^{-1} for E1, with values of 12.2 ± 0.2 mg L^{-1} d^{-1} and 13.2 ± 0.7 mg L^{-1} d^{-1} measured for E2 and E3, respectively. Amino acid analysis further revealed that essential amino acids accounted for up to 40% of the total protein fraction in SCP produced using biochar functionalized cathodes, highlighting its high nutritional value.
The superior performance of E1 compared to E2 and E3 was attributed to differences in biochar morphology induced by the applied post-treatments, which likely enhanced microbial adhesion, biofilm development, and electron transfer processes at the cathode –microorganism interface.
Overall, these results demonstrate that the functionalization of cathodes with low-cost, waste -derived biochar materials can significantly enhance microbial PtP performance in MES systems. This approach improves nitrogen recovery, increases protein yield and quality, and supports the production of value-added SCP from residual resources, reinforcing the role of bioelectrochemical technologies in sustainable and circular protein production.

Maximizing Hydrogen Production from organic waste: Bioelectrochemical Valorization of Dark Fermentation Effluents

Costanza Arvigo[1,2], Gaia Salvatori[1], Clara Marandola[1,2], Federico Scopetti[1,2], Eleonora De Santis[2], Silvia Rosa[2], Roberto Ciccoli[2], Luca Di Palma[3], Marianna Villano[1], Antonella Marone[2]

[1] *Department of Chemistry, Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy*
[2] *Department of Energy Technologies and Renewable Sources, ENEA, Italy*
[3] *Department of Chemical Engineering, Materials and Environment, Sapienza University of Rome, via Eudossiana 18, 00185 Rome, Italy*

costanza.arvigo@uniroma1.it

Dark fermentation (DF) is a biotechnological process in which organic wastes are degraded by microorganisms in the absence of oxygen to produce bio-H2 as gaseous effluent. However, fermentative H2 production typically achieves only partial oxidation of the organic substrate due to thermodynamic limitations. Indeed, two-thirds of the carbon and hydrogen content of the substrate are converted into microbial metabolic byproducts which, if unutilized, represent both a disposal burden and a waste of energy [1]. A promising approach to maximize H2 recovery from organic waste is the integration of DF with Microbial Electrolysis Cells (MEC) (Figure 1), wherein electroactive microorganisms (EAMs) oxidize the organic compounds present in the DF effluent at the anode, generating electric current that allows further H2 generation at the MEC cathode [1].
The aim of this work is the development of a cascade two-step H2 production process utilizing waste substrate. The MEC employed in this study features two 0.77 L chambers with a planar filter-press geometry and the anode chamber continuously fed. The anode and cathode compartments are separated by a proton exchange membrane (PEM). The anodic electrode consists of a granular graphite bed, designed to provide an high surface area for the development of an EAM biofilm, while a stainless-steel mesh is employed as the cathode to facilitate the hydrogen evolution reaction (HER). The experimental investigation was organized into two phases aimed at evaluating the performance of the bioelectrochemical system. The first phase (run I) was conducted using a synthetic substrate composed of a mixture of carboxylic acids (CA) as the MEC feedstock, specifically formulated to simulate the metabolic profile of the effluent from cheese whey dark fermentation; this approach allowed for the establishment of an electroactive biofilm with the required ability of DF metabolites oxidation and to set up a baseline reference under controlled conditions. Subsequently, during the second phase (run II), the experimentation proceeded with the use of the real effluent obtained from the dark fermentation of cheese whey to verify the influence of the complex real matrix and the possible presence of other residual organic compounds on the activity of the electroactive biofilm. To ensure the consistency and comparability of data collected from the two runs, all bioelectrochemical tests were operated controlling the anode potential at +0.20 V vs SHE (Standard Hydrogen Electrode) and with an applied organic loading rate (OLR) at the MEC anode of 2 gCOD/L·d Run II (conducted using the real effluent as substrate for the MEC anode), resulted in a COD removal efficiency of 86±2 %, with a near-complete conversion of the oxidized COD into current (i.e., the Coulombic Efficiency, CE, was equal to 98±4 %, meqi/meqCOD). H2 production accounted for 46.4±1.4 meqH2/d with a Cathodic Capture Efficiency (CCE) resulting of 87±5 (%, meqH2/meqi). Overall, the results demonstrate that DF metabolite-rich effluents can be effectively valorized toward hydrogen generation through integration with the MEC technology. As this system aims to maximize organic waste conversion into H2, future studies will focus on optimizing the anodic process to maximize substrate oxidation.

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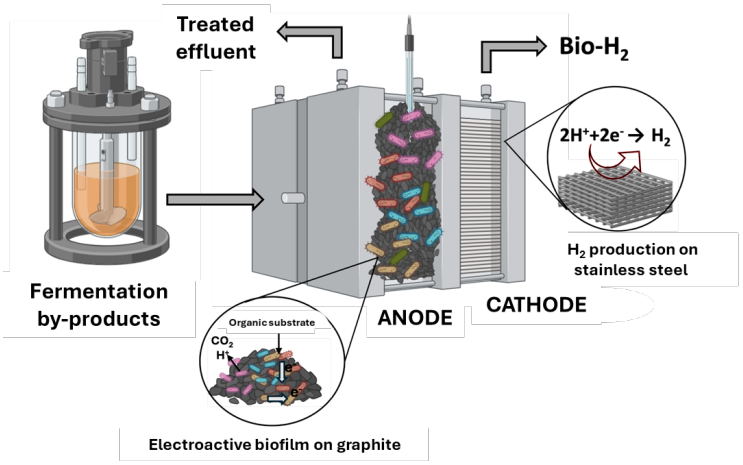

Figure 1: Scheme of the integrated DF-MEC process

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PARTECIPANTS LIST

Fabrizio	**Adani**	Università degli Studi di Milano	fabrizio.adani@unimi.it
Alessadra	**Adessi**	Univeristà degli Studi di Firenze	alessandra.adessi@unifi.it
Costanza	**Arvigo**	Università di Roma La Sapienza	costanza.arvigo@uniroma1.it
Niccolo	**Bassani**	Università degli studi di Milano	bassaniniccolo02@gmail.com
Arianna	**Callegari**	Università di Pavia	arianna.callegari@unipv.it
Chiara	**Capelli**	Università degli studi di Firenze	chiara.capelli@unifi.it
Andrea	**Capodaglio**	Università di Pavia	andrea.capodaglio@unipv.it
Gabriella	**Caucia**	Università degli Studi di Milano-Bicocca	g.caucia@campus.unimib.it
Elena	**Cazzulani**	Università degli Studi di Milano	elena.cazzulani@unimi.it
Lucia	**Cavalca**	Università degli Studi di Milano	lucia.cavalca@unimi.it
Lorenzo	**Cristiani**	Leibniz-HKI	lorenzo.cristiani@leibniz-hki.de
Pierangela	**Cristiani**	Ricerca sul Sistema Energetico - RSE SpA	pierangela.cristiani@rse-web.it
Carolina	**Cruz Viggi**	Istituto di Ricerca sulle Acque - CNR	carolina.cruzviggi@irsa.cnr.it
Matteo	**Daghio**	Università degli Studi di Firenze	matteo.daghio@unifi.it
Giorgio	**De Checchi**	Universite des Antilles	gio.giodk8@gmail.com
Paolo	**Dessi**	Università degli studi di Napoli Federico II	paolo.dessi@unina.it
Giuseppe	**Dies**		giuseppe.dies58@gmail.com
Mirella	**Di Lorenzo**	University of Bath	m.di.lorenzo@bath.ac.uk
Ermelinda	**Falletta**	VisioNing srl	ermelinda.falletta@visioning-tech.com
Andrea	**Franzetti**	Università degli Studi di Milano Bicocca	andrea.franzetti@unimib.it
Stefano	**Freguia**	University of Melbourne	stefano.freguia@unimelb.edu.au

Simone	**Ghezzi**	Università di Pavia	simone.ghezzi02@unipv.it
Giorgia	**Ghiara**	Politecnico di Torino	giorgia.ghiara@polito.it
Matteo	**Grattieri**	Università degli Studi di Bari Aldo Moro	matteo.grattieri@uniba.it
Seyedeheraheleh	**Yousefikafshgari**	University of Milano-Bicocca	seyedehraheleh.yousefikafshgari@unimib.it
Guehaz	**Karima**	Università di Firenze	gagakarima@gmail.com
Dario	**Lacalamita**	Università degli Studi di Bari	dario.lacalamita@uniba.it
Pierluigi	**Lasala**	Università di Bari	pierluigi.lasala@uniba.it
Giuseppe	**Lembo**	ENEA	giuseppe.lembo@enea.it
Mirko	**Magni**	Università degli Studi di Milano	mirko.magni@unimi.it
Clara	**Marandola**	Sapienza Università di Roma	clara.marandola@uniroma1.it
Angela	**Marchetti**	Università di Roma La Sapienza	angela.marchetti@uniroma1.it
Mario	**Marchisio**	Northeastern University of Shenyang	marchisio@mail.neu.edu.cn
Antonella	**Marone**	ENEA	antonella.marone@enea.it
Enrico	**Marsili**	University of Nottingham Ningbo China	enrico.marsili@nottingham.edu.cn
Stefania	**Marzorati**	Università degli Studi di Milano	stefania.marzorati@unimi.it
Umberto	**Mattia**	Politecnico di Bari	u.mattia@phd.poliba.it
Daniele	**Molognoni**	Leitat Technological Center	dmolognoni@leitat.org
Daniele	**Moraschini**	Politecnico di Milano	daniele.moraschini@mail.polimi.it
Elmira	**Mousavifard**	Univeristà degli Studi di Milano	elmira.mousavifard@studenti.unimi.it
Serena	**Narducci**	Università degli Studi di Napoli Parthenope	serenanarducci.pro@gmail.com
Rosa	**Nastro**	Università degli Studi di Napoli Parthenope	rosa.nastro@uniparthenope.it
Opeyemi Temitope	**Otemoye**	University of Nottingham Ningbo China	Opeyemitemitope.OTEMOYE@nottingham.edu.cn
Giuseppe Maria	**Paternò**	Politecnico di Milano	giuseppemaria.paterno@polimi.it
Pamela	**Principi**	Institute of Microbiology DACD	pamela.principi@supsi.ch

Francesca	**Pittino**	Università degli Studi di Milano Bicocca	francesca.pittino@unimib.it
Stefano	**Ravasi**	RSE SpA	s.ravasi17@campus.unimib.it
Melania	**Reggente**	SUPSI	melania.reggente@gmail.com
Ruggero	**Rossi**	Johns Hopkins University	rrossi4@jh.edu
Gaia	**Salvatori**	Sapienza University of Rome	gaia.salvatori@uniroma1.it
CARLO	**SANTORO**	University of Milano-Bicocca	carlo.santoro@unimib.it
Geremia	**Sassetto**	Università La Sapienza	geremia.sassetto@uniroma1.it
Alberto	**Scaccabarozzi**	Politecnico di Milano	alberto.scaccabarozzi@polimi.it
Gabriele	**Soggia**	RSE SpA	gabriele.soggia@rse-web.it
Stefano	**Trasatti**	Università degli Studi di Milano	stefano.trasatti@unimi.it
Matteo	**Tucci**	CNR - IRSA	matteo.tucci@irsa.cnr.it
Elisabetta	**Toni**	Università degli Studi di Firenze	elisabetta.toni@unifi.it
Carlo	**Viti**	Univeristà degli Studi di Firenze	carlo.viti@unifi.it
Carolina	**Viggi**	CNR - IRSA	carolina.cruzviggi@irsa.cnr.it
Marianna	**Villano**	Sapienza - Università di Roma	marianna.villano@uniroma1.it
Bernardino	**Viridis**	The University of Queensland	b.virdis@uq.edu.au
Sarah	**Zecchin**	Università degli Studi di Milano	sarah.zecchin@unimi.it
Marco	**Zeppilli**	Sapienza - Università di Roma	marco.zeppilli@uniroma1.it

“ATTIVITA’ DI RICERCA REALIZZATA NELL’AMBITO DEL PIANO NAZIONALE DI RIPRESA E RESILIENZA (PNRR) - MISSIONE 2 – COMPONENTE 2– INVESTIMENTO 3.5 “RICERCA E SVILUPPO SULL'IDROGENO”, FINANZIATO DALL’UNIONE EUROPEA – NEXT GENERATION EU, E SVOLTA IN ATTUAZIONE DEL PROGRAMMA OPERATIVO DI RICERCA (POR) APPROVATO DAL MINISTERO DELLA TRANSIZIONE ECOLOGICA IL 27.06.2022”