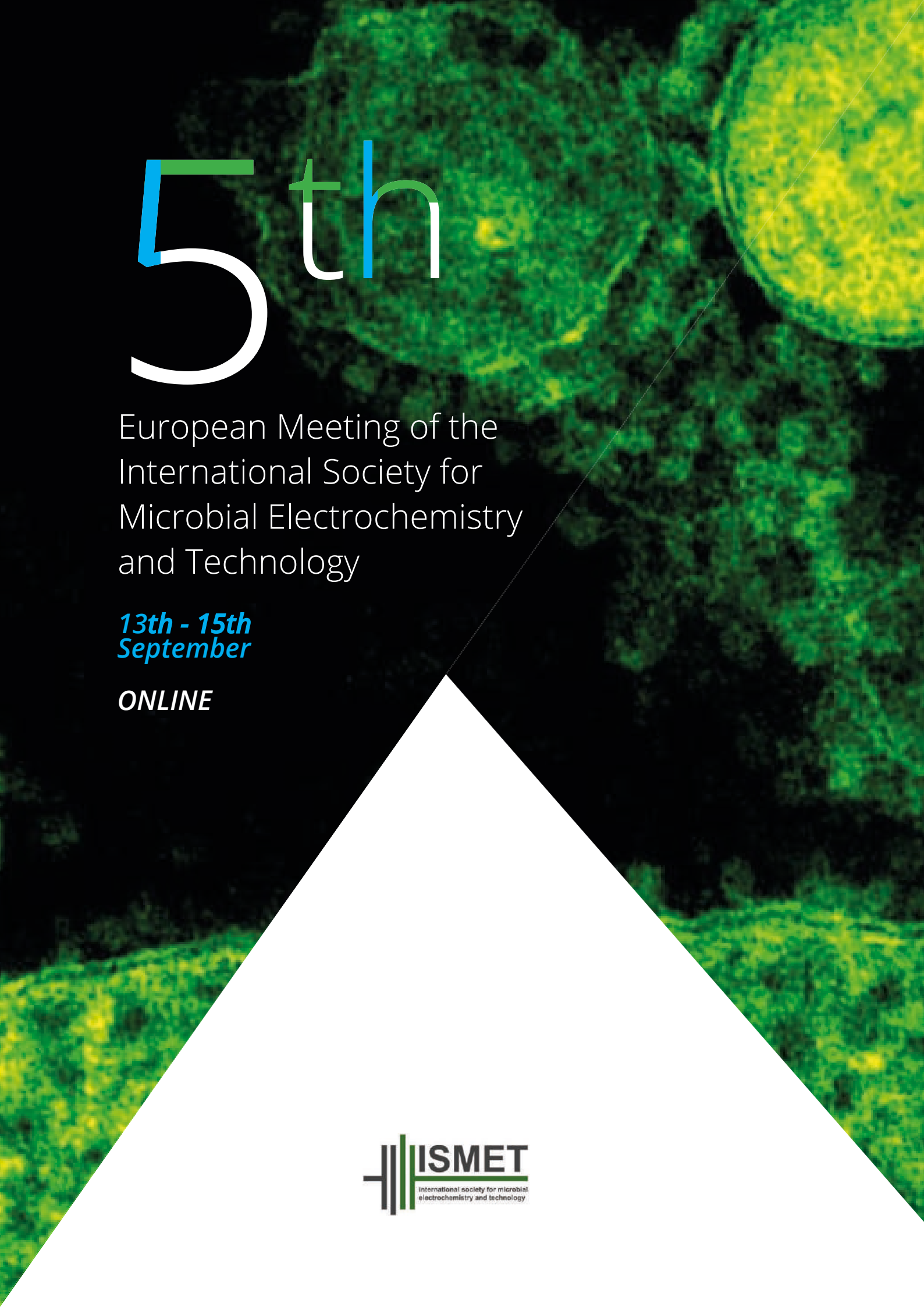
The “electro-fermentation effect” of Geobacter sulfurreducens co-cultured with Clostridium pasteurianum is not induced by electron transfer

María Fernanda Pérez-Bernal, Roland Berthomieu, Elie Desmond-Le Quéméner, Nicolas Bernet, Eric Trably



5th

European Meeting of the International Society for Microbial Electrochemistry and Technology

13th - 15th September

ONLINE

ISMET
international society for microbial electrochemistry and technology

Scientific & Organizing

COMMITTEES

ISMET
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5th EU-ISMET ONLINE
5th European Meeting of the International Society for Microbial Electrochemistry and Technology

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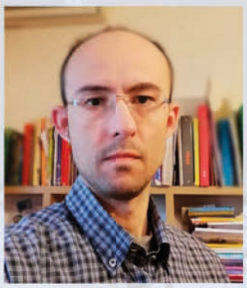
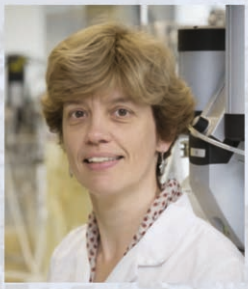
Lars Angenent


Federico Aulenta

Juan A Baeza


Belén Barroeta


Heleen De Wever


Abraham Esteve Núñez


Sarah Glaven

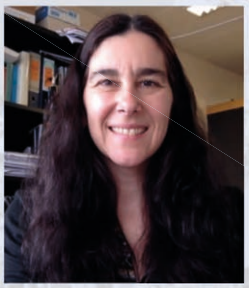

Ola Gomaa


Albert Guisasola


Falk Harnisch


Bruce Logan


Deepak Pant


Catarina Paquete


Korneel Rabaey


Miriam Rosenbaum


Pascal E. Saikaly


Annemiek ter Heijne


Marianna Villano


Bernardino Virdis


Eileen Yu

Keynotes

Synthetic biology for electroactive marine bacteria

Lina J. Bird[1], Daniel A. Phillips[2], Dasha Leary[1], Matthew D. Yates[1], Leonard M. Tender[1], Christopher A. Voigt[3], Sarah M. Glaven[1]

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Harnessing and controlling extracellular electron transfer pathways using bioengineering and synthetic biology promises to heighten the limits of established technologies and open doors to new possibilities. Synthetic biology is a powerful tool which nevertheless has limitations when applied to environmentally relevant organisms. Most synthetic biology parts are developed in E. coli, a model organism that grows well in the lab but poorly in the environment, especially in marine conditions. We have taken synthetic biology tools developed for E. coli and adapted them to Marinobacter atlanticus, a chassis that thrives under a wide range of conditions. Here, we present a three dimensional-printed flow cell for simultaneous electrochemistry and fluorescence imaging. Current-producing biofilms of Marinobacter atlanticus constitutively expressing green fluorescent protein were grown on the flow cell working electrode. Increasing current corresponded with increasing surface coverage and was comparable to biofilms grown in typical stirred-batch reactors. An isopropyl β-D-1-thiogalactopyranoside (IPTG) inducible system driving yellow fluorescent protein was used to assess the spatiotemporal activation of protein expression within the biofilm at different stages of growth and induction dynamics. We then used these tools to refactor a well-studied extracellular electron transfer system from Shewanella oneidensis and used it to program M. atlanticus for electronic communication with an electrode. In doing so, we gained a deeper understanding of the requirements of an inducible bioelectrochemical system, which could not have been determined from either homologous refactoring of the system, or expression of the system in traditional lab chassis.

Keynotes

Industrial biotechnology for CO_2 conversion

Heleen De Wever

Europe wants to become the first climate neutral continent by 2050 and aims at a 55% greenhouse gas emission reduction by 2030. To reach this challenging goal, decarbonization is a good strategy for energy and transport but not for chemicals and materials which are largely carbon based. A first part of the presentation will focus on renewable carbon as a means to cover future (increasing) carbon demand. The second part will zoom in on the renewable carbon source CO_2 and Carbon Capture and Utilization (CCU) strategies. The third part will discuss industrial biotechnology for CO_2 conversion, its claimed benefits and current challenges with indications on desired features. Examples will be included from research work and industrial scale experience.

Keynotes

Mediator-Based Electroactivity for Biotechnological Applications

Miriam A. Rosenbaum

Leibniz-Institute for Natural Product Research and Infection Biology - Hans-Knöll-Institute, Germany

Miriam.rosenbaum@leibniz-hki.de

Microbial extracellular electron transfer via self-produced diffusible redox-mediators has some significant advantages for biotechnology over direct electron transfer pathways. Most importantly, it is not dependent on a 2-dimensional electrode biofilm and it can enable also other microbial community partners to engage in extracellular electron transfer and. However, today, electron transfer via redox-mediator is much less efficient for the microorganisms than direct electron transfer and does not yield ATP for the cells.

One of the best known producers of natural redox-mediators is Pseudomonas aeruginosa, which produces phenazine-type mediators. Isolated from several wastewater treating microbial fuel cells it showed synergistic electroactivity relationships with the fermenter Enterobacter aerogenes. Thereby, Enterobacter stimulates phenazine synthesis in P. aeruginosa and in turn uses these mediators much more efficiently than Pseudomonas for anaerobic respiration with the anode. Our goal is to investigate the use of phenazines in both organisms with the longterm aim to reconstruct energy yielding mediator-based respiration in biotech production strains.

In past years, we investigated this phenazine-based synergistic electroactivity in detail. Interestingly, the co-culture stimulation is also found for other 2,3-butanediol fermenters. However, the synergism is only partly determined by the fermentation product 2,3-butanediol and also other molecular factors are involved. Further, environmental factors, especially oxygen, can be used to steer co-culture performance, phenazine production and electroactivity. On a molecular basis, phenazine production is highly controlled and we deciphered strong antagonistic regulation of the different phenazine synthesis genes. A big factor for a limited phenazine usage in P. aeruginosa could be the access of phenazines to electron donating metabolic steps. Our recent work, confirms a significant phenazine reduction already in the periplasm but also shows that oxidized phenazines can indeed reenter the cell.

We are now applying our molecular understanding of phenazine synthesis, regulation, and molecular physiology towards a heterologous utilization of phenazines for mediator-based microbial electrocatalysis.

Monday,

13th,

Tuesday,

14th

Wednesday,

15th

September 2021

PROGRAMME

ISMET
international society for microbial electrochemistry and technology

5th EU-ISMET ONLINE
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13th - 15th September 2021

Main Room

Monday, 13th
September 2021

9:00-12:30 EU-ISMET WORKSHOP

9:00-9:15 **Welcome session**
Chair: ***Narcís Pous***

9:00-09:50 W1: Introduction to microbialelectrochemistry: fundamentals and characterization tools. ***Bernardino Virdis*** (University of Queensland)

10:00-10:50 W2: On the thermodynamics of electroactive microorganisms

Benjamin Korth (Helmholtz Center for Environmental Research)

11:00-11:50 W3: How basic biology research can inspire us to innovate in microbial electrochemistry.
Sara Tejedor-Sanz (Rice University)

12:00-12:30 Separated meetings for screen-to-screen discussion with workshop speakers **(Networking area)**

14:00-14:30 Opening ceremony

Annemiek ter Heijnen & ***Sebastià Puig***

14:30-15:10 Plenary: "Mediator-based electroactivity for biotechnological applications"

Miriam Rosembaum

15:15-16:35 Parallel Session 1: Fundamentals of the electron transfer processes

Chair: ***Catarina Paquete***

In situ and non-destructive real time microscopic investigation of multi-species electroactive biofilms on transparent microfluidic BES.
Lucila Martinez Ostormujof

La persistència de l'entropia: Measuring the microbial electrochemical Peltier heat of different electrode materials.
Benjamin Korth

Role of phenazine-enzyme physiology for current generation in a bioelectrochemical system.
Anthony Chukwubuikem

Exploration of the reactivity of multiheme cytochromes and how it can be improved for (bio)electrocatalysis.
Ricardo Louro

Degustació de pernil a diferent voltatge de l'ànode: The influence of the anode potential on single cell yield coefficient of Geobacter sulfurreducens.
Francesco Scarabotti

16:35 – 16:45 Coffee Brea

16:45-18.05 Parallel Session 3: Fundamentals of the electron transfer processes

Chair: ***Lluís Bañeras***

Crossing the wall: extracellular electron transfer in Gram-positive bacteria.
Catarina Paquete

Extracellular electron transfer in a C. ljungdahlii-based platform for microbial electrosynthesis.
Santiago Treceño Boto

Bioelectrochemical characterization of a cytochrome belonging to the NapC/NirT family from Sideroxydans lithotrophicus ES-1.
Anaísa Coelho

Charge transfer parameters through biofilms of an enhanced current-producing Geobacter sulfurreducens strain reveal increased electron transfer diffusion rate.
Fernanda Jimenez Otero

Electron uptake in MES: How does Sporomusa ovata accept electrons at the cathode?.
Joana Madjarov

18:05-19:00 Social event. Girona guided tour

Note: The time zone is UTC+2.

Room 2

Monday, 13th
September 2021

15:15-16:35 Parallel Session 2: Commercialisation, Scale-up of METs

Chair: ***Esteve Núñez Abraham***

Scaling Up of Electro-Stimulated Anaerobic Reactor (ELSARTM) in the industrial wastewater treatment sector.
Antonio Gimenez Lorang

Competitive advantages of a circular bioeconomy based on microbial electrosynthesis and CO_2.
Jamin Wood

IoT biosensing: a microbial electrochemical sensor for monitoring water quality.
Antonio Berna

Wastewater treatment with a 1000 L microbial fuel cell: a 16-month experience.
Fabian Fischer

Role of the C/N ratio in the feeding solution of a micro pilot microbial electrolysis cell aimed at biogas upgrading.
Lorenzo Cristiani

16:30-16:45 Coffee Break

16:45-18.05 Parallel Session 4: Commercialisation, Scale-up of METs

Chair: ***Juan Antonio Baeza Labat***

Full scale operation of decentralized urban wastewater using METland® technology.
Abraham Esteve-Núñez

Development of Innovative Soil Microbial Fuel Cells for Energy Harvesting.
Jakub Dziegielowski.

Increasing the voltage in series-stacked bioelectrochemical systems, a road towards technology up-scaling.
Pau Bosch-Jimenez

Life cycle assessment of Microbial Electrosynthesis.
Siddharth Gadkari

MIDES H2020 Project: Microbial Desalination for Low Energy Drinking Water. From lab-scale concept to Pilot Pant validation.
Juan Manuel Ortiz

Main Room

Tuesday, 14th
September 2021

8:30-9:10 Plenary: "Industrial biotechnology for CO2 conversion"

Heleen De Wever

Chair: ***Sebastià Puig***

9:15-10:35 Parallel Session 5: Microbial electrosynthesis and electro-fermentation

Chair: ***Eileen Yu***

Insights in the electro-fermentation process with 13C-labelled experiments supported by NMR spectroscopy analysis.
Gaia Salvatori

Experimental optimization of long chain fatty acids synthesis from CO2 using statistical design.
Narnepati Krishna Chaitanya.

Enhancing butanol production by Clostridium beijerinckii through cathodic electro-fermentation approach.
Daniele Molognoni

Statistical analysis on factors affecting microbial electrosynthesis (MES).
Lakshmi Pathi Thulluru

Voleu crear models per fer una drecera? Development of a model for microbial electrosynthesis with planktonic cells and mediator based electron transfer.
Johannes Nelles

10:35 – 10:45 Coffee Break

10:45-12:20 Parallel Session 8: Microbial electrosynthesis and electro-fermentation

Chair: ***Marianna Villano***

Bioinorganic electrosynthesis of single-cell protein from CO2 and green electricity.
Mingyi Xu

Alliance of microbial electrochemical technologies and fermentation for the conversion of carbon dioxide into elongated chemical building blocks.
Meritxell Romans Casas

3D bioprinted MES biofilms enhancing the acetate production rate of Sporomusa ovata.
Adolf Krige

Towards bioelectrochemical degradation of hydrophobic wastes coupled with synthesis of biosurfactants.
Grzegorz Pasternak

In-situ production of microbial protein with reclaimed ammonium in a microbial electrochemical recovery conversion cell.
Xiaoyong Yang

Biogas upgradation through CO2 conversion into acetic acid via microbial electrosynthesis.
Moumita Roy

12:25-13:00 Plenary session: Horizon Europe

Lydia González

Chair: ***Sebastià Puig***

13:00-14:00 Lunch

Main Room

Tuesday, 14th
September 2021

14:00-14.40 Plenary session: Synthetic biology for electroactive marine bacteria

Sarah Glaven

Chair: ***Sebastià Puig***

14:45-16:05 Parallel Session 11: Electro-microbiology

Chair: ***Ricardo O Louro***

Competition of two highly specialized and efficient acetoclastic electroactiv bacteria for acetate in biofilm anode of microbial electrolysis cell.
Krishna Katuri

Enhancing power generation and biocompatibility exo-electrogenic bacteria in microbial fuel cell using NiWO4/rGO as anode electro-catalyst and optimized process parameter.
Geetanjali

Haloalkaliphilic nitrate-reducing electroactive microbial biofilm enriched from the Lonar Lake sediments.
Srishti Chaudhary

The “electro-fermentation effect” of Geobacter sulfurreducens co-cultured with Clostridium pasteurianum is not induced by electron transfer.
Elie Desmond-Le Quéméner

Design of electroactive bacteria by coupling genetic networks to redox environments.
Angel Goñi-Moreno

16:05-16:15 Coffee Break

16:15-17:30 Parallel Session 13: Electro-microbiology

Chair: ***Luis Bañeras Vives***

La última batalla: Interaction between Geobacter spp. dominated biofilms and anaerobic digestion effluents.
Daniel Dzofou Ngoumelah

Bacteria coated cathodes as an in-situ hydrogen evolving platform for Microbial Electrosynthesis.
Elisabet Perona-Vico

Selection and enrichment of electroactive microbial communities for removal of pharmaceuticals in microbial electrochemical systems.
Razieh Rafieenia

How can microbial heat help in optimising the energy conversion of Microbial Electrochemical Systems (MES)?.
Pavlina Theodosiou

17:30-18:00 ISMET event - News

Room 2

Tuesday, 14th
September 2021

9:15-10:35 Parallel Session 6: Microbial electrosynthesis and electro-fermentation

Chair: ***Lars Angenent***

Theory of transport and recovery in microbial electrosynthesis of acetate from CO2.
Jouke Dykstra

Power-to-algae: carbon dioxide to bio-oil in a BES-supported microalgae biorefinery.
Silvia Bolognesi

Screening for hyperthermophilic electroautotrophs for the microbial electrosynthesis of organic compounds.
Guillaume Pillot

Characterization of fluidized vs. fixed granular activated carbon beds as cathodes for microbial electrosynthesis of carboxylates from CO_2.
Igor Vassilev

Insights into electro-fermentation of caproate from CO_2 and ethanol.
Laura Rovira-Alsina

10:35-10:45 Coffee Brea

10:45-12:20 Parallel Session 9: Microbial electrosynthesis and electro-fermentation

Chair: ***Marika Kokko***

CO_2 conversion by combining a Cu electrocatalyst and microorganisms.
Konstantina-Roxani Chatzipanagiotou

Biocathode and photoanode development for solar energy-driven microbial electrosynthesis.
Paolo Dessi

A general model for biofilm-driven microbial electrosynthesis of carboxylates from CO_2.
Oriol Cabau Peinado

The impact of cathode acclimation methods on electrotrophic biofilm formation and performance of electromethanogenic cells.
Amin Ghaderikia

Bioelectrosynthesis of organic acids from CO_2 using fluidized bed electrodes in a 3 phase reactor.
María Llorente Remartínez

Strategies for increasing production of bio-products from CO_2 by Microbial electrosynthesis (MES).
Eileen Yu

13:00-14:00 Lunch

Room 2

Tuesday, 14th *September 2021*

14:45-16:05 Parallel Session 12: Bioremediation, water treatment and resource recovery from waste

Chair: ***Marco Zeppilli***

Large-scale literature meta-analysis: the impact of the bioelectrochemical process configurations on the electrochemical and wastewater treatment performances.
Roman Moscoviz

Filtrating Microbial Fuel Cells for biofertiliser and energy production.
Iwona Gajda

Exploring avoided environmental impacts and resource recovery from desalination brine through Microbial Desalination Cell treatment.
Rosa Anna Nastro

Optimization of filtering anodes for the integration of bioelectrochemical systems into anaerobic membrane bioreactors.
Pavari Viwatthanasittiphong

Comprehensive and long-term assessment of critical operational parameters of a pilot MEC for hydrogen production from urban wastewater.
Oscar Guerrero

16:05-16:15 Coffee Break

16:15-17:30 Parallel Session 14: Bioremediation, water treatment and resource recovery from waste

Chair: ***Albert Guissaola***

Low energy consuming bioelectrochemical system for ammonium recovery from wastewater as liquid fertilizer.
Eduard Borràs

Ammonia recovery from digestates in a three-chambered bioelectrochemical system through hydrophobic membranes.
Míriam Cerrillo Moreno

Effect of hydraulic conditions in PFR reactors using bio-electrochemical processes to influence greenhouse gas emission.
Annegret Budach

Simulation of Pilot Scale Microbial Electrochemical Technologies.
Jordan Day

Note: The time zone is UTC+2.

Room 3

Tuesday, 14th
September 2021

9:15-10:35 Parallel Session 7: Novel applications of METs

Chair: ***Tom Sleutels***

Biophotovoltaics for hydrogen production using cyanobacteria.
Bin Lai

Hyperthermophilic hydrogen production by iron reducing Archaea in microbial electrolysis cells (MECs).
Yasemin Dilsad Yilmazel

Electrifying biotrickling filters for the treatment of aquaponics wastewater.
Narcis Pous

Metabolic Pathway Involved in CO2 Fixation under Photo-Bioelectrochemical conditions by a Purple Phototrophic Bacteria Biofilm.
Sara Díaz-Rullo Edreira

10:35-10:45 Coffee Break

10:45-12:20 Parallel Session 10: Novel applications of METs & MET-based sensor technology

Chair: ***Annemiek Ter Heijne***

Bidirectional microbial biofilms with sulfur based energy storage.
Paniz Izadi

Bioenergetic and biotechnological implications of extracellular electron transfer in lactic acid bacteria.
Sara Tejedor Sanz

Microbial activity and biomass monitoring in freshwater ecosystems using a MEC-based biosensor.
Marta Fernandez-Gatell

Microbial Electrochemical Systems: Principles, Construction and Biosensing Applications.
Rabeay Hassan

Biocathodes with graphene oxide coatings enhance methane production
Daniela Carrillo

Note: The time zone is UTC+2.

Main Room

Wednesday, 15th September 2021

8:30-9:10 Plenary: Microbial electrochemistry for bioremediation: the game changer

Korneel Rabaey

Chair: ***Sebastià Puig***

9:15-10:35 Parallel Session 15: Bioremediation, water treatment and resource recovery from waste

Chair: ***Uwe Schröder***

Desalination of Brackish Water sing à Microbial Desalination Cell: analysis of the electrochemical behaviour. ***Marina Ramirez***

Electrobioremediation by METland® solution differentiates among enantiomers in mixtures of pharmaceuticals and herbicides. ***Álvaro Pun García***

Development of a four-chamber microbial desalination cell for simultaneous treatment of Cr (VI) containing wastewater and by-products recovery. ***MD Tabish Noori***

Electrobioremediation of Pharma pollutants is boosted in a Microbial Electrochemical Fluidized Bed Reactor. ***Yeray Asensio***

Bioelectrochemical dechlorination of 1,2-dichloropropane by a Dehalogenimonas-enriched culture. David Juan Fernández Verdejo

10:30-10:45 Coffee Break

10:45-12.20 Parallel Session 17: Bioremediation, water treatment and resource recovery from waste

Chair: ***Pascal Saikaly***

Groundwater denitrification by expanded bioelectrochemcial systems. ***Xiaofei Wang***

Combined electrochemical water softening and nitrate electro bioremediation. ***Alba Ceballos-Escalera***

Electro-bioremediation of nitrate from saline groundwater and concomitant chlorine production. ***Giulia Puggioni***

Soil Microbial Fuel Cell for Bioremediation of Recalcitrant Hydrocarbons. Arpita Nandy

Simultaneous removal of oxidable and reducible contaminants from groundwater with the “bioelectric well”. ***Matteo Tucci***

Power Generation from Constructed Wetland Microbial Fuel Cells under Batch and Continuous Flow. ***María G. Salinas-Juárez***

12:20-13:00 Round Table on “electro-bioremedation”

Chair: ***Korneel Rabaey***

13:00-14:00 Lunch

Main Room

Wednesday, 15th September 2021

14:00-14.40 Eu-ISMET goes to high schools

Maria Teresa Bosch Vilardell, Narcís Pous and Miquel Àngel Ruiz

Chair: ***Belén Barroeta***

14:45-16:00 Parallel Session 19: Materials science and design

Chair: ***Falk Harnisch***

Magnetite anodes to inhibit oxygen production and to circumvent the use of membranes in microbial electrosynthesis.
Nils Rohbohm

Procedure for the screening of anode materials for microbial electrolysis cells operated with brewery wastewater.
Isaac Vázquez

Promising affordable separators for microbial electrolysis cells.
Oscar Santiago

Towards improving the performance of microbial fuel cells: Evaluation of inoculation strategies and anode materials.
Zainab Ul Kausar

Metal-free activated biochar as an oxygen reduction reaction catalyst in single chamber microbial fuel cells.
Tommy Pepè Sciarria

16:00-16:10 Coffee Break

16:10 - 17:30 Parallel Session 21: Materials science and design

Chair: ***Eduard Borras***

Capacitive bioanodes in fixed and moving bed reactors.
Tom Sleutels

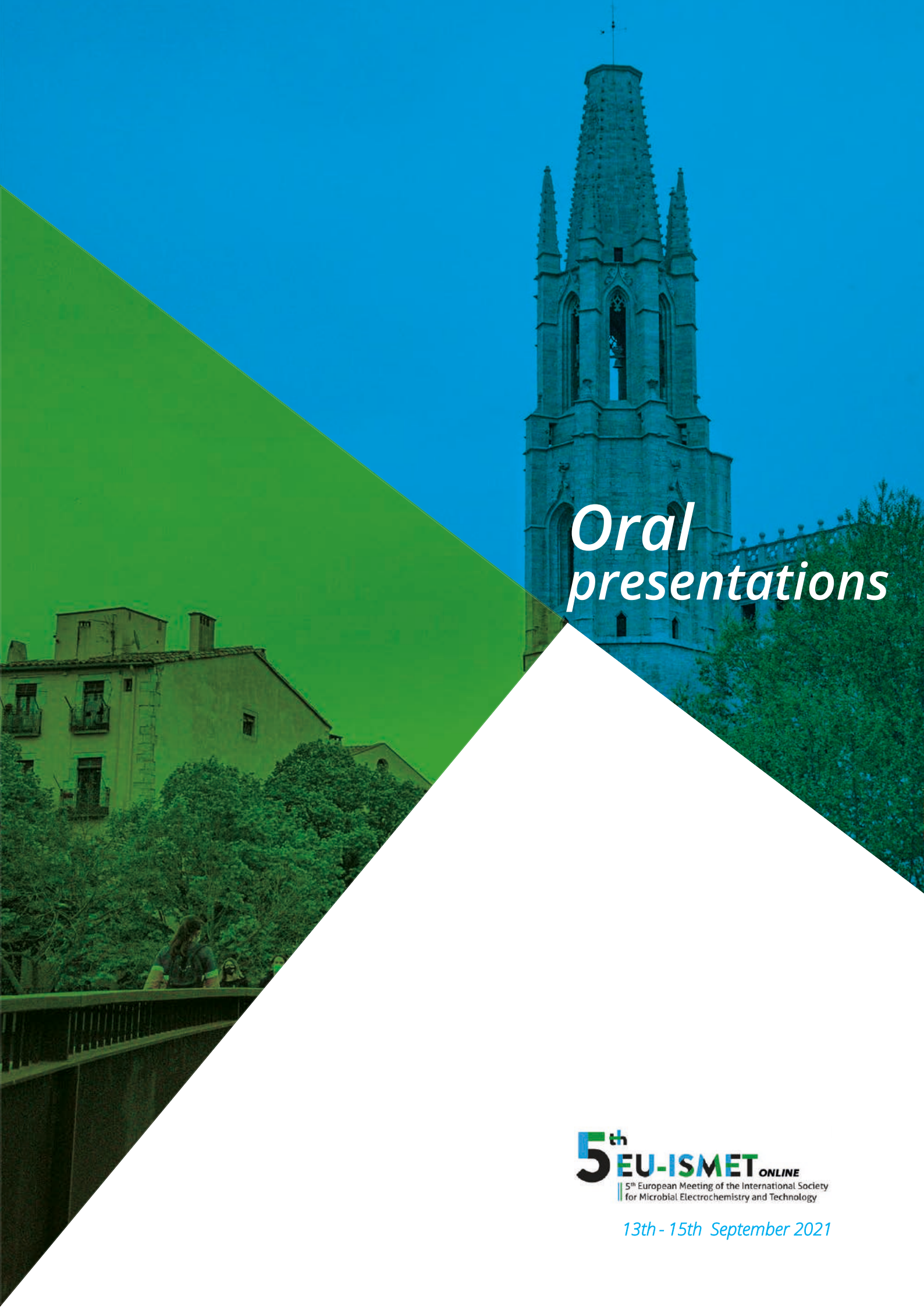
Graphene functionalization with metallic Pt nanoparticles: envisaging future cathodes for bioelectrochemical H2 production.
Pilar Sánchez Peña

Development and upscaling of gas diffusion electrodes for wastewater treatment and electrosynthesis of chemicals.
Deepak Pant

Estructures superficials com les d'Antoni Gaudi: Platinized titanium as cost-effective anode material for Kolbe electrolysis.
Katharina Neubert

17:35 - 18:00 Closing ceremony

Note: The time zone is UTC+2.



Oral presentations

5th EU-ISMET ONLINE

5th European Meeting of the International Society for Microbial Electrochemistry and Technology

13th - 15th September 2021

Biocathodes with graphene oxide coatings enhance methane production

D. Carrillo[1], R. Mateos[1], A. Morán[1], A. Escapa[1,2,*]

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[2] Department of Electrical Engineering and Automatic Systems, Campus de Vegazana s/n, Universidad de León, E-24071 León, Spain

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Microbial electrosynthesis (MES) uses certain types of electroactive microorganisms to obtain commercially valuable products (such as methane, CH_4) from carbon dioxide (CO_2). The aim of this work is to quantify the impact that the electrodeposition of graphene oxide on a cathode has on methane production in a MES system. To that end, an H-type electrochemical cell was used, with four identical carbon felt electrodes placed in the cathodic chamber (Fig. 1). Two of these cathodes were electrodeposited with graphene oxide (goCF) and the other two electrodes were used as control (without modifications). In terms of productivity and bioelectrochemical performance, the current density produced by the goCF was 30% higher than the control electrode. Furthermore, with the help of cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) it was confirmed that goCF electrode had lower charge transfer resistances and up to twice as much electrical capacitance compared to the unmodified electrode. Finally, at a CO_2 feed rate of 15-30 g CO_2 per m^2 of electrode and per day, methane was produced at concentrations above 95%.

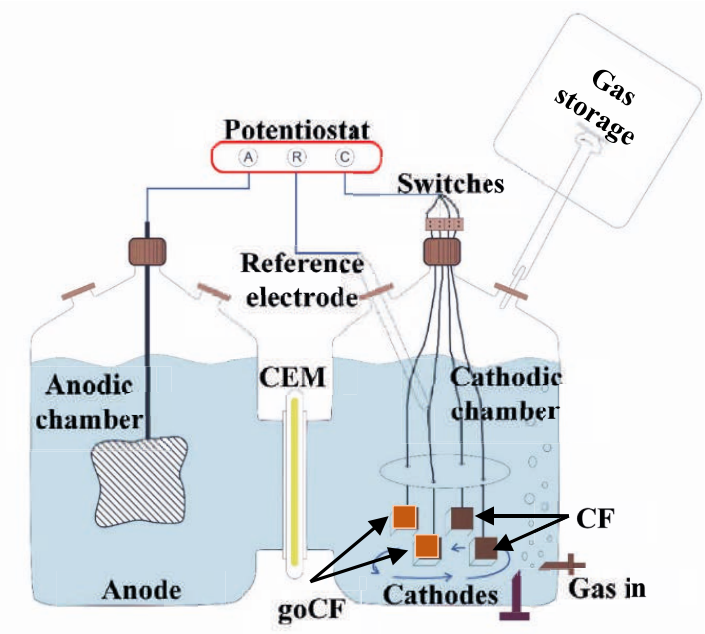

Fig. 1 Microbial electrosynthesis reactor (H-Cell MES) configuration.

Keywords: Biocathode, CO_2, graphene oxide, microbial electrosynthesis.

Funding: This research was possible thanks to the financial support of "Ente Regional de la Energía de Castilla y Leon" (No. EREN_2019_L3_ULE) and Project supported by 'Consejería de Educación de la Junta de Castilla y León' (No. LE320P18), a project co-financed by FEDER funds.

Microbial activity and biomass monitoring in freshwater ecosystems using a MEC-based biosensor

Marta Fernandez-Gatell[1, 2], Xavier Sanchez-Vila[2, 3], Jaume Puigagut[1,*]

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[2] Dept. of Civil and Environmental Engineering, Universitat Politècnica de Catalunya, c/ Jordi Girona 1-3, 08034 Barcelona, Spain

[3] Associated Unit: Hydrogeology Group (UPC-CSIC), Spain

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Climate change is one of the most important and probable aspects influencing the stability of human societies [1]. Greenhouse gas (GHG) emissions from natural or human-made freshwater ecosystems are linked to the anaerobic microbial activity (MA) and influence the global GHG emissions and dynamics [2]. Thus, better tools regarding the characterization of both the MA and biomass within ecosystems is of capital importance to deal with climate change. However, MA and biomass assessment methodologies are complex and cannot be applied continuously. Bioelectrochemical systems (BES) produce a current from the anaerobic metabolic activity of electroactive bacteria that naturally inhabit water bodies. This study addresses the use of the bio-electric signal of BES to estimate the MA and the biomass of its surrounding area. To this purpose, four Microbial Electrolysis Cell (MEC) reactors were operated for 29 weeks under different MA and biomass conditions. Bio-electric signal was recorded continuously, while biomass content and MA (estimated through ATP and enzymatic activity) were weekly evaluated (n = 6/reactor). Results (Fig. 1) showed that the MEC bio-electric signal correlates with statistical significance with both the MA and biomass, demonstrating that MEC-based biosensors could be applied for continuous monitoring of MA and biomass in freshwater ecosystems.

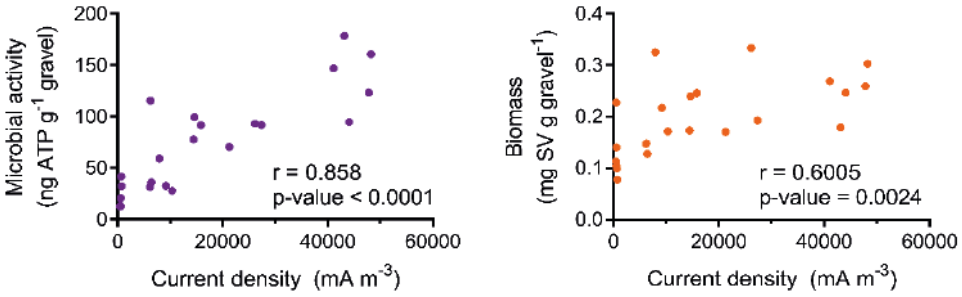

Fig. 1. Pearson's correlation results between current density from the MEC and biomass (left) and microbial activity (right).

Keywords: microbial activity, biomass, climate change, microbial electrolysis cell, biosensor

Funding: Ayudas para la promoción de empleo joven e implantación de la Garantía Juvenil en I+D+i (PEJ2018-005586-A). Generalitat de Catalunya (Consolidated Research Group 2017 SGR 1029

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[2] P. Bousquet *et al.*, "Contribution of anthropogenic and natural sources to atmospheric methane variability," *Nature*, vol. 443, no. 7110, pp. 439–443, Sep. 2006.

Bioenergetic and biotechnological implications of extracellular electron transfer in lactic acid bacteria

Sara Tejedor-Sanz[1,2], Eric T. Stevens[3], Maria L. Marco[3] and Caroline M. Ajo-Franklin[1,2]

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Extracellular electron transfer (EET) has been classically associated with respiratory species. However, EET has also been discovered across many fermentative microorganisms like lactic acid bacteria (LAB)[1]. Because the bioenergetic implications are not understood, we explored the impact of EET in lactic acid fermentations employing *Lactiplantibacillus plantarum*, a LAB species found in insects, plants and mammals and essential to produce fermented foods. EET on anodes by *L. plantarum* is robust and alleviates constraints of intracellular redox balance during fermentation, generating a high NAD+/NADH ratio[2]however recent work shows some species can use mixed or alternative bioenergetic strategies. We explored the utility of a flavin-based extracellular electron transport (FLEET. This metabolic impact increases fermentation yield and flux and ATP production through substrate-level phosphorylation. This EET activity requires elements of an electron transport chain like an NADH dehydrogenase (Ndh2). Thus, EET in *L. plantarum* is a hybrid energy metabolism that blends metabolic features of fermentation and redox features of respiration. This pathway is active in physiologically niches like a food fermentation. Using *L. plantarum* as a starter culture in a kale juice electro-fermentation allows to increase the fermentation yield and acidification rate of the juice. Thus, the electroactivity of LAB also has relevant biotechnological implications as it can be used to prevent the growth of undesired microorganisms and to alter flavour and aromas in food matrices.

Keywords: lactic acid bacteria, fermentation, food, metabolism

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Bidirectional microbial biofilms with sulfur based energy storage

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Bidirectional microbial biofilms have been proposed as one the potential applications of bio-electrochemical systems (BESs), in which a single biofilm can generate power and store energy. Here, we studied underlying mechanisms involved in bidirectional biofilms developed from German coasts of North Sea and Baltic Sea through an anodic cultivation strategy, followed by a periodic potential reversal regime. Community analysis from such efficient biofilms showed the enrichment of microorganisms with different metabolic pathways such as *Shewanella* and *Desulfovibrio* species, contributing in functioning as both bioanode and biocathode. Over the long term, the bidirectional activities of the biofilms were related to microbial electrosynthesis of hydrogen, formate and acetate during the cathodic polarity and consumption of the products during the anodic polarity. However, over the short term, the bidirectional activities of the biofilms seemed to be dominated by the electrochemical conversion of sulfur formed at the electrode surfaces through oxidation of biogenic sulfide ions and reduction of sulfur to sulfide (and polysulfide). The results can open opportunities towards using a single biofilm for energy storage and release in coastal areas.

Keywords: Bidirectional microbial biofilms, coastal samples, sulfur

Design of electroactive bacteria by coupling genetic networks to redox environments

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Bacteria with the ability to transfer electrons are at the core of microbial electrochemical technologies. However, the rational design of such bacteria for the sake of controlling electroactive dynamics has yet to be explored. It has been previously suggested that a spatial redox gradient exists in anode-respiring biofilms[1]; it has also been demonstrated that it is possible for bacteria to sense their redox environment and respond with differential expression of genes[2]. We build on this interplay in order to study the fundamentals aspects of these complex dynamical system. We developed a computational model of biofilms targeted at engineering the interactions between redox gradients and discrete cells. The model allows us to explore *in silico* how redox gradients interact with cells engineered with synthetic genetic networks. In particular, we present a model of bistable gene expression coupled to local reduction potential, in which the state of this genetic 'toggle switch' can be made dependent on the cell's position in the biofilm. This shows that genetic networks can be used for engineering control strategies within bioelectrochemical systems, paving the way for new application domains.

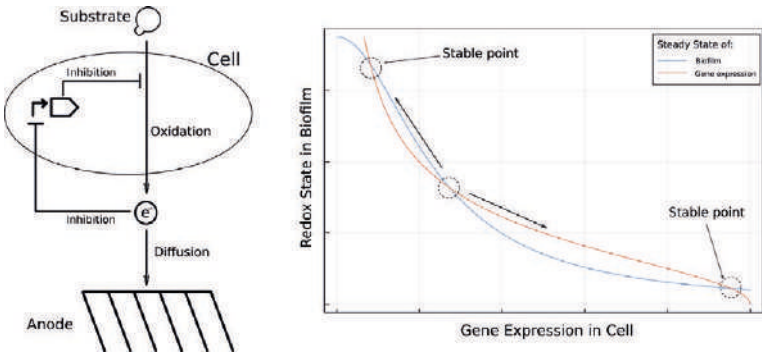

Fig. 1 On the left, a schematic of a hypothetical bistable switch coupling genetic expression and biofilm. Feedback between gene expression and biofilm reduction potential causes the bistability to emerge. On the right, fixed points of this bistable switch are plotted for a simplified model containing just a single cell. The existence and positions of the stable points in this model can then be used to guide exploration of similar behaviour in the more complex model.

Keywords: Compuational Modeling, Synthetic Biology, Biofilms, Extracellular electron transport

Funding: Comunidad de Madrid (Atracción de Talento Program, grant 2019-T1/BIO-14053), and Severo Ochoa grant SEV-2016-0672 (2017-2021)

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Haloalkaliphilic nitrate-reducing electroactive microbial biofilm enriched from the Lonar Lake sediments

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Electrotrophic microorganisms, which can take up electrons from solid-state electron donors to maintain their metabolic processes, have been barely studied from extreme environments [1, 2]. Here, we present nitrate-reducing cathodic microbial biofilm from Lonar Lake sediments, a haloalkaline environment with 9.5pH and up to 24 gNaCl/L salinity [3]. At an applied cathode potential of (*vs.* Ag/AgCl), the enriched biofilm achieved current density and nitrate reduction efficiency. It reduced nitrate *via* both partial and complete denitrification processes. The cyclic voltammetry of nitrate-reducing biofilm revealed a redox centre with formal potential of . *Pseudomonas*, *Natronococcus*, and *Pseudoalteromonas* spp. dominated the biofilm at 31.45%, 11.82%, and 9.69% relative sequence abundances, respectively. All these genera are reported for nitrate reduction [4, 5], and only *Pseudomonas* is known for electroactivity. Nitrate-reducing microorganisms produced no soluble redox mediators and did not follow hydrogen-driven nitrate reduction in bioelectrochemical reactors. These observations suggest the direct electron uptake by nitrate-reducing biofilm from cathode. The enriched microorganisms also reduced nitrate using soluble electron donors, such as acetate and citrate, found in lake sediments. This study broadens the ecological niches for electrotrophic microorganisms and suggests their potential role in N-cycling in haloalkaline environments.

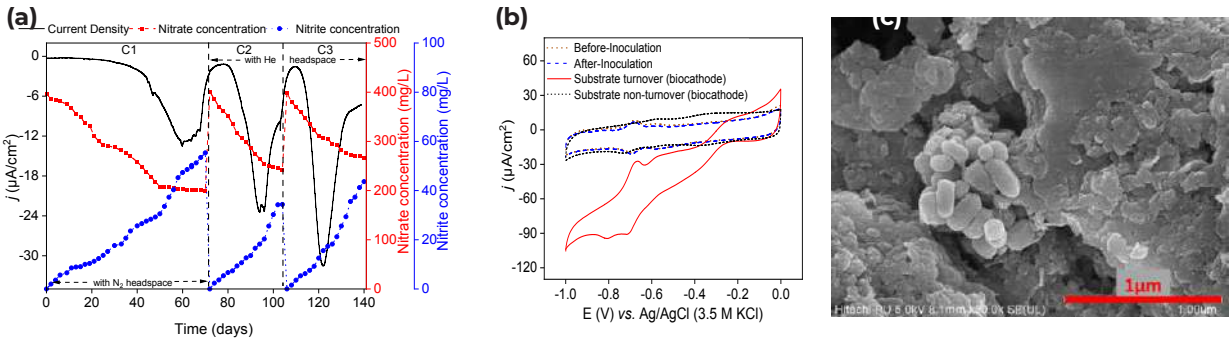

Fig. 1 (a) Chronoamperometry and nitrate/nitrite concentration profiles of the enriched cathodic biofilm. (b) Cyclic voltammograms of the abiotic cathode and biocathode recorded under different conditions. (c) Representative SEM image of the biocathode.

Keywords: Electromicrobiology, Extracellular electron transfer, electrotrophs, haloalkaline environment.

Funding: Science and Engineering Research Board, (Grant no. SRG/2019/000934), IISER Mohali and PMRF from MHRD, Government of India.

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Competition of two highly specialized and efficient acetoclastic electroactive bacteria for acetate in biofilm anode of microbial electrolysis cell

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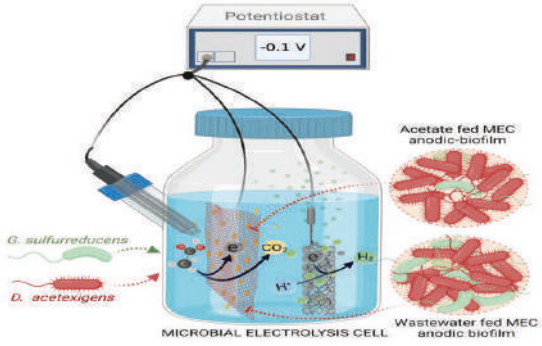
Maintaining functional stability of microbial electrolysis cell (MEC) treating wastewater depends on maintaining functional redundancy of efficient electroactive bacteria (EAB) on the anode biofilm. Here, we compare the electrochemical and kinetic properties of two efficient acetoclastic EAB, *Geobacter sulfurreducens* (GS) and *Desulfuromonas acetexigens* (DA), grown as monoculture in MECs fed with acetate. Additionally, we monitor the evolution of DA and GS in co-culture MECs fed with acetate or domestic wastewater using fluorescent in-situ hybridization. The apparent Monod kinetic parameters reveal that DA possesses higher j_{max} (10.7 ± 0.4 A/m^2) and lower $K_{S,\ app}$ (2 ± 0.15 mM) compared to GS biofilms (j_{max}: 9.6 ± 0.2 A/m^2 and $K_{S,\ app}$: 2.9 ± 0.2 mM). Further, more donor electrons are diverted to the anode for respiration in DA compared to GS. In acetate-fed co-culture MECs, DA (98% abundance) outcompete GS for anode-dependent growth. In contrast, both EAB co-exist (DA: 55 ± 2%; GS: 24 ± 1.1%) in wastewater-fed co-culture MECs despite the advantage of DA over GS based on kinetic parameters alone. The co-existence of efficient acetoclastic EAB with high current density in MECs fed with wastewater is significant in the context of functional redundancy to maintain stable performance. Our findings also provide insight to future studies on bioaugmentation of wastewater-fed MECs with efficient EAB to enhance performance.



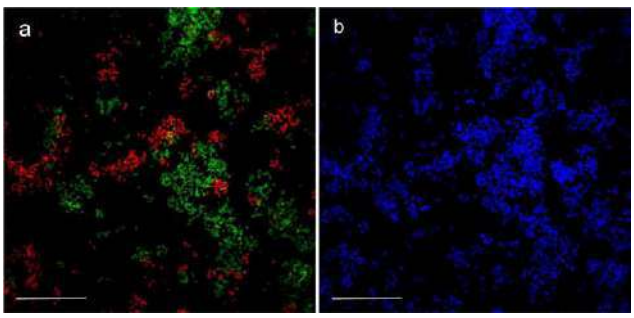


Fig. 1. Cartoon represents the highlights of the work

Fig. 2. CLSM image represent the anodic-biofilm community enriched with sewage. Red - Geobacter sulfurreducens & Green - Desulfuromonas acetexigens

Keywords: biofilm, electroactive-bacteria, acetoclastic,,*Desulfuromonas acetexigens*, *Geobacter sulfurreducens.*

Funding: Center Competitive Funding Program (FCC/1/1971-33-01)

Enhancing power generation and biocompatibility exo-electrogenic bacteria in microbial fuel cell using NiWO4/rGO as anode electro-catalyst and optimized process parameter

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Microbial fuel cells (MFCs) are bio electrochemical technologies that are able to treat wastewater and generate electrical energy simultaneously. In this study, NiWO4 impregnated on graphene oxide sheet (NWG) used as electrocatalyst for anode modification in single chambered MFC (SC-MFC). The properties of synthesized NWG nanocomposite are characterized by FTIR, XRD, EDX, TEM and SEM analysis. NWG modified anodic carbon cloth (CC) as anode is used to improve the performance of SMFC. NWG composite modified CC anode shows capacitance value of 47.27 ± 0.18 F cm^{-2} which is 295 times higher than plain carbon cloth. The maximum power density obtained by modified NWG composite CC anode was observed to be 1128 ± 42 mW m^{-2}. Further, NWG modified anodic CC based SC-MFC is used to improve the efficiency of SC-MFC by statistical optimization of operational parameters. The cumulative effect of enhancing anode electro-catalytic activity and operational parameter optimization in SC-MFC, leads to 8.5 fold higher power density (1458 mW/m^2) with respect to unmodified anode and non-optimized process parameters based SC-MFC. In addition, NWG has exposed the maximum abundance of y-proteobacteria (42.37 %), followed by NiWO4 and rGO, while CC contained the lowest amount of y-proteobacteria (11.23 %).

Keywords: Microbial fuel cell, anode electro-catalyst, statistical optimization, microbial community analysis, exo-electrogenic bacteria

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The "electro-fermentation effect" of Geobacter sulfurreducens co-cultured with Clostridium pasteurianum is not induced by electron transfer

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Interspecies electron transfer (IET) is a feature of interest in anaerobic ecosystems especially in environments where electron acceptors are lacking. Recent experiments showed that some fermentative organisms can serve as potential electron sinks in such environments. However, the exact mechanism behind this phenomenon remains unclear, especially for fermentative Gram-positive bacteria.

In a previous study[1], an interaction between the fermentative species *Clostridium pasteurianum* and the model electroactive species *Geobacter sulfurreducens* was demonstrated which resulted in a change of *C. pasteurianum* metabolic pathway on glycerol with an increase of the yield in 1,3-propanediol. The observed shift was attributed to IET, as a similar metabolic shift was observed when *C. pasteurianum* accepted electrons from a cathode. Through new co-culture setups and an RNAseq approach, we unveiled that the metabolic change in cocultures of *C. pasteurianum* and *G. sulfurreducens* is not due to an electron exchange but rather to a cobamide molecule produced by *G. sulfurreducens*, which probably interferes with the key enzyme glycerol dehydratase. Despite the lack of evidence for an electron transfer pathway, cocultures with addition of fumarate still point out to a partial IET. This work shows that *Geobacter sp.* plays a role in some fermentation setups beyond electron transfer.

Keywords: interaction, *Clostridium pasteurianum*, *Geobacter sulfurreducens*, cobamide, interspecies electron transfer

Funding: This work was funded by INRAE grant and CoNaCyT postdoctoral fellowship

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Large-scale literature meta-analysis: the impact of the bioelectrochemical process configurations on the electrochemical and wastewater treatment performances

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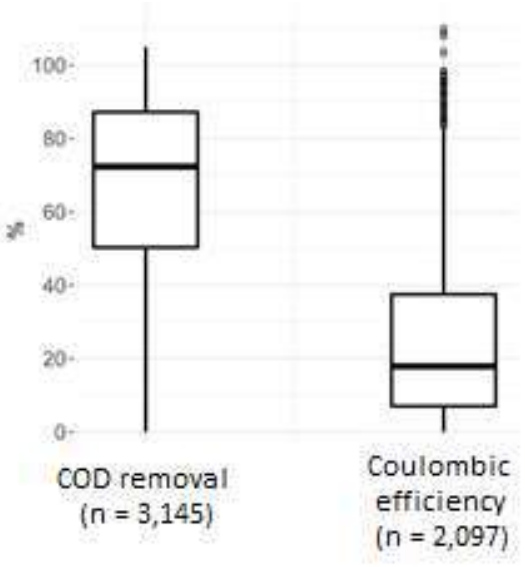
Bioelectrochemical systems (BES) represent promising technologies for wastewater treatment at low environmental impact. Since the 2000s, thousands of articles have been published on this topic, exploring a variety of process configurations, electrode materials and effluents, among others. This diversity reflects the momentum of this inter-disciplinary research topic, but also represents a major challenge for identifying the most-promising technological choices for future process scale-up.

This work presents a multivariate analysis made on 4579 observations using the database built according to the methodology developed in *De Fouchécour at al.* A focus was made on two BES performances indicators: COD removal and coulombic efficiency, describing treatment and electrochemical performances respectively.



Among all available observations, 50% reported COD removal above 70%, whereas the median coulombic efficiency was only 20%. This surprisingly low coulombic efficiency raises important questions for the bioelectrochemistry community as it reflects that many research papers report results mostly unrelated to bioelectrochemical reactions. Thus, the aim of this work is to analyse the influence of process configuration, tCOD, pH and temperature over these process performance indicators. Our results provide key technical information that will hopefully help designing more efficient BES to treat wastewater and facilitate future process scale up.

Overall distribution of COD removal and coulombic efficiency in the scientific literature. The number n represents the number of observations.

Keywords: wastewater treatment, multivariate-analysis, COD removal, coulombic efficiency

Optimization of filtering anodes for the integration of bioelectrochemical systems into anaerobic membrane bioreactors

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Previously, we demonstrated filtering microbial anodes as a promising concept for the integration of bioelectrochemical systems into anaerobic membrane bioreactors operating with brewery wastewater. In particular the so-called hybrid anode, in which polymeric membranes are combined with stainless-steel meshes, has shown a promising potential over porous stainless-steel anodes [1].

To further optimize this concept, we systematically studied a range of stainless-steel meshes with different surface area, wire diameter, and pitch with respect to their electrochemical and filtration performance. Stainless-steel meshes were first tested in non-filtering experiments using *Geobacter Sulfurreducens* in a half-cell setup with an acetate-containing medium to determine the effects of the mesh parameters on current density. Then, the selected meshes are being tested in a crossflow filtration system to observe the medium-term effects on permeate fluxes.

Ongoing experiments show that increasing mesh surface area improves current density but there appears to be saturation. On the other hand, filtration performance is minimally affected in most of the selected meshes except for the one with lowest open filtration area.

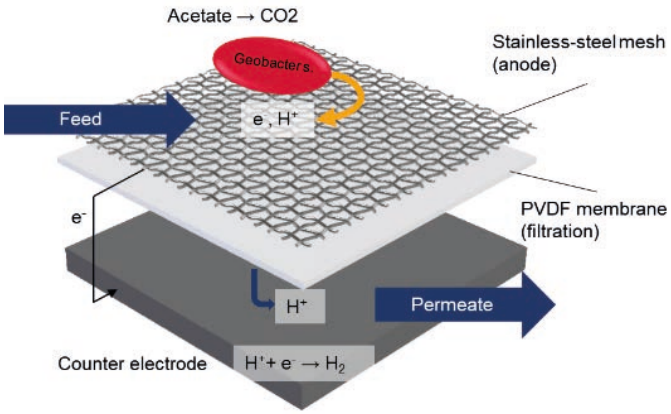

Fig. 1 Hybrid anode concept

Keywords: hybrid anode, filtering anode, anaerobic membrane bioreactor, wastewater treatment

Funding: We are grateful for the financial support from the Bundesministerium für Bildung und Forschung (BMBF) under the programs 031B0365C and 031B1053C.

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Comprehensive and long-term assessment of critical operational parameters of a pilot MEC for hydrogen production from urban wastewater

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The reported MEC current densities with urban wastewater as a feedstock are low due to its low organic matter content and conductivity. To understand the limiting factors of MEC performance at pilot scale, we present the long-term operation of 150L cassette-type pilot reactor under a wide range of different operational conditions: Voltage applied [0.8-1.1 V], residence time [1.5-5.0 d], organic loading rate [0.05-0.3 g/(L·d)] and temperature [18-30 °C], using two types of feed; raw (100 mg COD_s/L) and amended (1000 mg COD_s/L) urban wastewater (Figures 1 to 2). The reactor was able to produce up to 8.5 L H_2/d with a COD removal efficiency of 60% at optimum conditions. A comprehensive discussion of the benefits and drawbacks of all parameters will be provided in the full presentation. For instance, at different HRT_s the current density was found to be constant and only a significant reduction in COD removal efficiency could be observed. Otherwise, the COD concentration directly affected the current generation and hydrogen production. The process showed a strongly dependence on the temperature and on the applied potential, being 0.9V the optimum value.

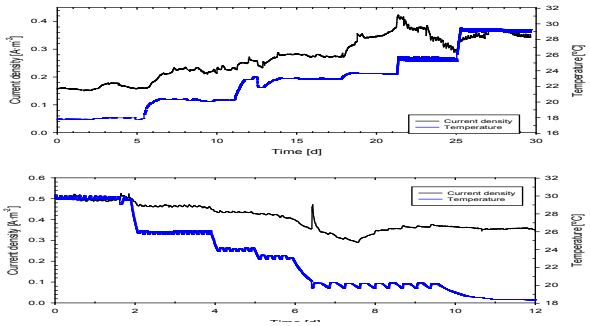

Fig. 1: Current density profile for different temperatures (raw UWW)

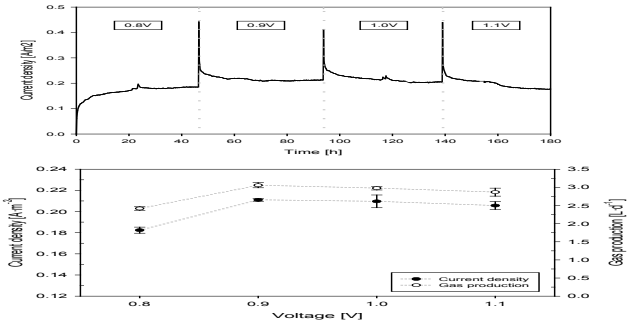

Fig. 2: Current density profile for different voltages (raw UWW)

Keywords: microbial electrolysis cell, waste valorisation, environmental engineering, water technology, bioprocess.

Funding: This work was supported by the European Life programme LIFE19 ENV/ES/000191. The authors are members of the GENOCOV research group (Grup de Recerca Consolidat de la Generalitat de Catalunya, 2017 SGR1175, www.genocov.com)

Exploring avoided environmental impacts and resource recovery from desalination brine through Microbial Desalination Cell treatment

Nastro, Rosa Anna ; Leccisi, Enrica; Toscanesi, Maria; Gengyuan, Liu; Trifuoggi, Marco; Ulgiati, Sergio

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In this work, we discuss the results of a lab-scale treatment of desalination brine by means of Microbial Desalination Cells (MDCs) operated with (MDC_3) and without (MDC_2) applied potential (fig.1). Our research took into account energy direct and indirect recovery, organic load removal from wastewater, resource recovery, and environmental impacts minimization.

The treatment in basic MDCs allowed the removal of approximately 33 g of salts (62% of the total) – including chlorides, bromides, and sulfates – from 20 mL of reject brine while the application of a 1.5V external voltage led to a decrease of 63.9% of salts content.

The MDC_2 and MDC_3 used an overall of 7.2 J and 7.9 J of energy to drive the desalination process, with a COD decrease in the fuel of respectively 77% and 7.6% in comparison to the abiotic control. We quantified the avoided life cycle human and marine eco-toxicity impacts as well as the reduction of the cumulative energy demand of recovered metals.

The main benefit in terms of avoided toxicity would arise from the mercury and copper recovery, while potential economic advantages would derive from the recovered cobalt that represents a strategic resource for many products such as battery storage systems.

Filtrating Microbial Fuel Cells for biofertiliser and energy production

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MFC processing source-separated urine can enable efficient nutrient recycling, with the additional benefit of nitrogen removal from urine. This work presents the development of urine filtration Microbial Fuel Cells, which synthesise purified catholyte in which key macronutrients such as NPK are recovered, produce energy and remove organic content. In this study, a Filtrating-MFC bioreactors using ceramic based membranes were assembled to oxidise synthetic urine generating electric current, which facilitated the movement of cationic species and water towards the cathode (Fig. 1). As a result, a purified filtrate was achieved at ~6.5 higher rate than the control, while ≥90% of organic content was removed. In addition to nitrogen, potassium and phosphorus were also recovered in the cathodic filtrate, thus demonstrating the filtration capacity of the ceramic bioreactors. This work is exploiting pH and ion splitting mechanisms that is directly related to the electric field generated by the system to include target products extraction [1] while recovering water through electroosmosis. The proposed technology allows for the additional oxidation of the organic fraction in urine on the cathode while retaining a high proportion of all other key macro nutrients for potential reuse as a fertiliser. It represents energy-positive approach towards waste valorisation.

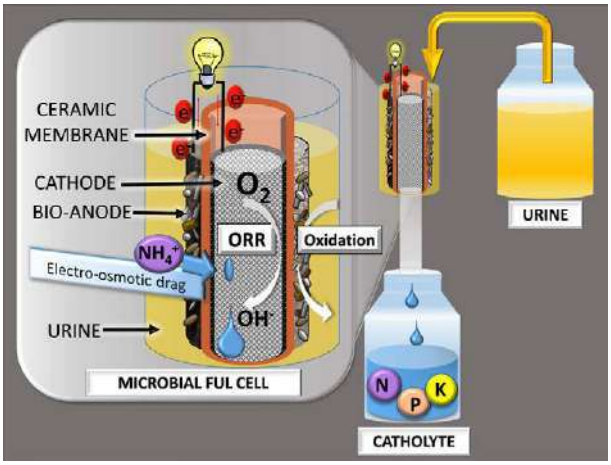

Fig. 1

Filtrating-Microbial Fuel Cell scheme with the catholyte collection system.

Keywords: microbial fuel cell, nutrient recovery, catholyte, electroosmosis, biofertiliser

Funding: Vice Chancellor's Interdisciplinary Research Fund (VC IRCF) and Bill and Melinda Gates Foundation, grant number INV006499.

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How can microbial heat help in optimising the energy conversion of Microbial Electrochemical Systems (MES)?

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Microbial Electrochemical Systems (MESs) utilise microbes as biocatalysts for transforming waste and other organics into resources, i.e., electricity, hydrogen, or chemical compounds. MESs have entered the pilot-scale realm in wastewater treatment plants [1] and off-grid locations [2]. Hence, they have attracted interest as a feasible, sustainable, and environmentally friendly technology. However, the performance of pilot-scale studies (100-1000L) is 1-3 order of magnitude lower than the lab scale (0.1-0.2L) ones. These disparities hinder the technology from full deployment and weaken industry confidence. Efforts to address this disparity and improve MES efficiencies have been heavily focused on the MES materials rather than the inherently complex biology of the system. Observing pilot-scale MES behaviour reveals that these systems are resilient in temperature fluctuations - even at 10°C - as opposed to anaerobic digestors, which also use anaerobic bacteria but struggle at temperatures below 20 °C. Hence, this suggests that the heat energy produced during bioconversion of chemical energy to electrical energy is a vital energy vector that has not been yet accounted for. Through the BIOHEAT project, we are investigating the heat energy transfers in electroactive microbial systems. Linking heat energy to growth will offer a deeper understanding of anaerobic systems' biology.

Keywords: microbial electrochemical systems, MES, microbial heat, pilot-scale, heat energy

Funding: This work is funded by EPSRC (Grant No. EP/S032517/1)

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Bacteria coated cathodes as an in-situ hydrogen evolving platform for Microbial Electrosynthesis

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Hydrogen is a key intermediate in microbial electrosynthesis as a mediator of the reduction of carbon dioxide (CO_2) into added value compounds. In the present work we aimed at studying the biological production of hydrogen in biocathodes operated at -1.0 V *vs.* Ag/AgCl, using a highly comparable technology and CO_2 as the sole carbon feedstock. Ten bacterial strains were chosen from genera *Rhodobacter*, *Rhodopseudomonas*, *Rhodocyclus*, *Desulfovibrio* and *Sporomusa*, all described as hydrogen producing candidates. Monospecific biofilms were formed on carbon cloth cathodes and hydrogen evolution was constantly monitored using a microsensor. Eight over ten bacteria strains showed electroactivity and H_2 production rates increased significantly (2 to 8-fold) compared to abiotic conditions for two of them (*Desulfovibrio paquesii* and *Desulfovibrio desulfuricans*). *D. paquesii* DSM 16681 exhibited the highest production rate (45.6 ± 18.8 $\mu M \cdot min^{-1}$) compared to abiotic conditions (5.5 ± 0.6 $\mu M \cdot min^{-1}$), although specific production rates (per unit biomass) were like those obtained for other strains. This study demonstrated that many microorganisms are suspected to participate in net hydrogen production but inherent differences among strains do occur, which are relevant for future developments of resilient biofilm coated cathodes as a stable hydrogen production platform in microbial electrosynthesis.

Keywords: electroactive bacteria, electrochemistry, electromicrobiology, hydrogen, pure cultures.

Funding: European Union's Horizon 2020 (No. 760431)

La última batalla: Interaction between Geobacter spp. dominated biofilms and anaerobic digestion effluents

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Anaerobic digestion (AD) and microbial electrochemical technologies (MET) can be combined in manifold ways, e.g., for removal of monovalent ions, effluent polishing, electrochemical biogas upgrading and sensor applications [1]. However, recent studies showed negative influences of AD effluents on the performance of pre-grown *Geobacter* spp. dominated biofilms [2]. Therefore, the questions arose, if *Geobacter* spp. dominated biofilms can be adapted to AD environments and, if the biofilm age as well as the presence or activity of methanogens affects the biofilm stability?

To answer these questions, we performed several shock and adaptation experiments using AD effluents in different concentrations (0% - 100%) and biofilms of different age. The activity of methanogens was inhibited by application of 50 mmol L^{-1} 2-BES, their presence was minimized by filtration of the AD effluent (0.2 µm pore size).

Old biofilms (pre-grown for 5 weeks) showed higher resistance and activity during shock experiments with AD effluents compared to young biofilms (3 weeks). Adaption of biofilms failed for a yet unknown reason, whereas the application of 2-BES as well as filtration of the AD effluent had positive effects on the activity and resistance of 5 weeks pre-grown biofilms [3].

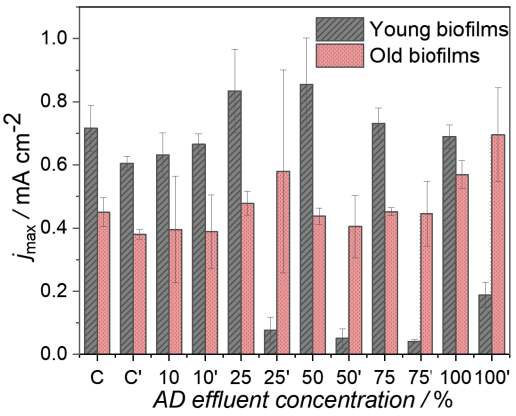

Fig.1: Maximal current density (j_{max}) during shock of young and old biofilms with increasing AD concentrations, C: control with only acetate as carbon source, ' indicates second week (second batch), n = 3, error bars indicate CI.

Keywords: bioelectrochemical systems, longevity, resistance, alternative electron acceptors

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Selection and enrichment of electroactive microbial communities for removal of pharmaceuticals in microbial electrochemical systems

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Microbial Electrochemical Systems (MESs) represent an environmentally friendly approach for wastewater treatment, where the degradation of organic matter is accompanied by simultaneous production of value-added products and/or energy. Some pharmaceuticals and other bioactive compounds are amongst the recalcitrant contaminants of highest concern, as they are continuously released into the environment and aquatic environments from discharges from pharmaceutical industries, animal farms, hospital effluents, and domestic wastewater, despite their harmful activity against living organisms. In the present study, we studied the acclimation of electroactive microbial communities to be used in the removal of three pharmaceuticals found in waters (carbamazepine, atenolol, and ketoprofen).

MESs were inoculated with anaerobic digestion sludge and subjected to receiving pharmaceuticals in the medium (500 µg/L). Maximum current densities of 215.64, 272.01 and 465.46 mA/m2 were obtained for the MESs receiving atenolol, carbamazepine and ketoprofen, respectively. Microbial community analysis revealed the abundant microbial species on anodic biofilm of MFCs.

The microbial communities enriched in MESs will be used as inocula in a system called Intimate Coupling of Photocatalysis and Biodegradation (ICPB-MES) which combines the capacity of microbial communities in MES to degrade recalcitrant compounds with another chemical approach (photocatalysis) to increase the pharmaceuticals removal efficiency.

Keywords: Microbial electrochemical systems, Recalcitrant compounds, Pharmaceuticals, Microbial communities, biodegradation

Funding: This project is an Institutional Link Project collaboration with the National Research Centre (Egypt), supported by the British Council / Newton-Mosharafa Fund (Grant 352368074).

Effect of hydraulic conditions in PFR reactors using bio-electrochemical processes to influence greenhouse gas emission

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Constructed wetlands (CWs) are an alternative to conventional wastewater treatment. However, for broader application, their performance, in particular with respect to greenhouse gas emission[1,2] (e.g. CH_4) has to be optimized. Planted fixed bed laboratory scale reactors (PFR) with electroconductive materials (biochar, graphite coke) were used for enhancement of the microbial electron acceptor availability in CWs for improved the electron flux and overall elimination performance[3]. In addition, the abundance and activity of methanogenic bacteria was analyzed.

Three PFR reactors filled with either gravel (= control), graphite coke, or biochar were operated at stable inflow conditions while varying internal recirculation flows. The electrochemical properties were measured via cyclic voltammetry (-1.0 V vs SHE, 10 mV/min). Increasing recirculation decreased the oxidation peaks in the cyclovoltammograms, indicating higher biofilm activities and relate gradients to mass transfer limitations. To assess the effect of the electroconductive materials on methane formation, the abundances of methanogenic species in water and filter-bed samples were quantified by qPCR. The relative abundance of methanogenic species declined from 81% in gravel to 24% in the biochar and 26% in the graphite coke reactor.

The results will provide information on how optimization of the hydraulic conditions superimposes the effects of electrically conductive materials.

Keywords: Bio-electrochemistry, WW treatment, greenhouse gases

Funding: ELECTRA Horizon 2020 funded by EC and NSFC (GA 826244)

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Ammonia recovery from digestates in a three-chambered bioelectrochemical system through hydrophobic membranes

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A three-chambered microbial electrolysis cell (MEC) (600 mL each compartment) was equipped with a cation exchange membrane (CEM) between the anode and the cathode compartment; and a polytetrafluorethylene (PTFE) membrane (0.45 µm pore size), between the cathode and the ammonia recovery compartment (Fig. 1). The MEC was operated in continuous mode for 115 days, feeding the anode compartment with digested pig slurry and the cathode compartment with a 0.1 g NaCl L^{-1} solution.

The MEC achieved an average ammonium removal efficiency in the anode compartment of 23%±1. The evolution of the cathode bulk solution pH (10.8±1.5) was favourable to the displacement of the ammonium-ammonia equilibrium towards the second species, and allowed a maximum flux through the hydrophobic membrane of 2.74 g N m^{-2} h^{-1}. The transferred ammonia was recovered in the third compartment by absorption in H_2SO_4 (1.8 M) and represented around 25% of the ammonia migrated from the anode to the cathode compartment.

The MEC combined with hydrophobic membranes reveals as an interesting technology for the treatment of high strength wastewater such as livestock manure. Compared to a stripping and absorption system [1], the use of hydrophobic membranes for ammonia recovery avoids electricity consumption for air pumping.

Fig. 1 Picture of the three compartment MEC. Anode, cathode and ammonia recovery chambers on the left, centre and right compartments, respectively.

Keywords: Microbial Electrolysis Cell, hydrophobic membrane, ammonia recovery, digestate.

Funding: Spanish Ministry of Economy and Competitiveness (INIA project RTA2015-00079-C02-01). CERCA Program and the Consolidated Research Group TERRA (ref. 2017 SGR 1290), both from the Generalitat de Catalunya.

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Simulation of Pilot Scale Microbial Electrochemical Technologies

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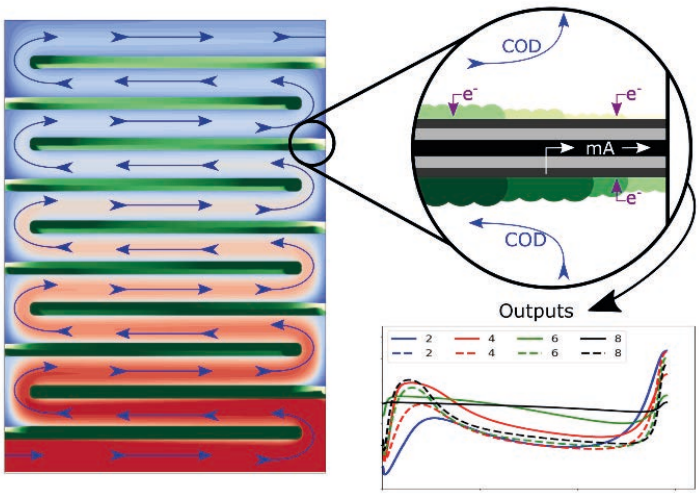
Microbial electrochemical technologies (METs) have the potential to reduce energy usage and requirements in the wastewater treatment process. However, these reactors need further refinement before implementation into the treatment process. Due to high financial cost and time requirements to develop pilot scale systems, design and optimisation process can be heavily hindered[1]. Mathematical modelling can reduce the cost and time required to design complex systems, and is being increasingly used in METs. Modelling METs for real life applications including resource recovery and wastewater treatment represents a significant challenge. To be of value, models must be complex enough to reproduce important behaviour of MET, yet simple enough to provide insight into underlying causes of this behaviour. Ideally, models must also be scalable to future industrial applications, rather than limited to describing existing laboratory experiments. We present a scalable model simulating both fluid flow and bioelectrochemical processes in MET, benchmarking against experimental pilot-scale bioreactor [2]. The model describes substrate transport through a two-dimensional fluid domain, and biofilm growth on anode surfaces. Electron transfer is achieved by an intracellular redox mediator. Finding significant spatial variations in both substrate concentration and current density. Simple changes to the reactor layout can greatly improve the overall efficiency.

Fig. 1 Graphical Summary of fluid and substrate transportation through reactor domain alongside simulation results.



Keywords: Mathematical Modelling, Computational Fluid Dynamics, Microbial Electrochemical technologies, Pilot-scale, Wastewater Treatment

Funding: EPSRC **EP/R51309X/1**

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Low energy consuming bioelectrochemical system for ammonium recovery from wastewater as liquid fertilizer

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Bioelectrochemical systems (BES) have emerged as a potential technology for nitrogen removal and recovery from wastewaters with low energy consumption. In the Run4life Project, a reactor to produce a liquid fertilizer from wastewater at low energy consumption has been developed. This consisted of a multi-chamber flat plate reactor connected to an air stripping system to recover ammonium from wastewater as ammonium nitrate in an acid trap filled with HNO_3 (0.1M). The BES system was tested under different electrochemical conditions working with synthetic wastewater. Applying an external voltage of +0.2 V and an air flow rate (for N stripping) of 150 mL·min^{-1} proved to be the best scenario for ammonium recovery, in which 15.8 ± 1.6 g N-NH_4^+·**m**$^{-2}$·**d**$^{-1}$ were removed from wastewater (corresponding to 75.1 ± 3.8% of its original content) and 9.6 ± 1.9 g N-NH_4^+·**m**$^{-2}$·**d**$^{-1}$ were recovered (45.3 ± 7.5%) at a current density of 2.5 ± 0.2 A·m^{-2}. The power consumption of the process (without considering aeration) was of 1.6 kWh per kg of removed nitrogen, which was lower than similar experiences reported in literature, making the proposed system appealing. Finally, tests with real blackwater as anolyte were performed.

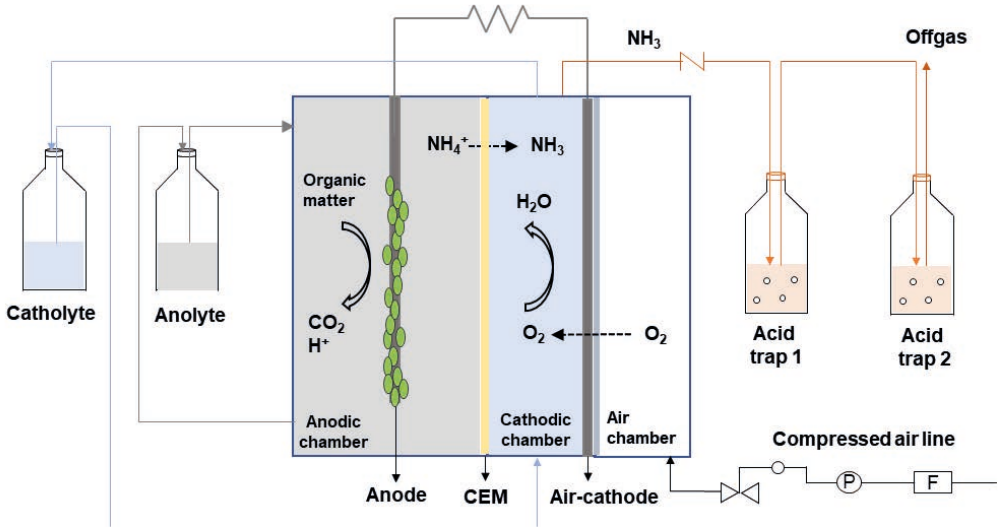

Figure 1. Experimental setup scheme used for nitrogen recovery in Run4life project.

Keywords: blackwater; electroactive bacteria; microbial electrolysis cells; nitrogen recovery.

Funding: This project has received funding from European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 730285 (Run4life).

Bioelectrochemical dechlorination of 1,2-dichloropropane by a Dehalogenimonas-enriched culture

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1,2-Dichloropropane is a contaminant which can be found in groundwater sources. In this study, we successfully degraded, for the first time, concentrations up to 1000 µM of 1,2-dichloropropane to propene using a mixed culture containing the anaerobic dehalorespiring species *Dehalogenimonas alkenigignens* [1] using a bioelectrochemical system, consisting in two independent vessels separated by a cationic exchange membrane. The 1,2-dichloropropane was reduced at the cathode of the system due to the capability of bioelectrochemical systems of providing a continuous supply of hydrogen. This results in a promising strategy in view of sustaining the long-term growth of hydrogen-dependent dehalogenating bacteria [2]. Therefore, this work studies the effect of this continuous hydrogen supply in the dechlorination rate and in *Dehalogenimonas* biomass concentration. Low cellular density is the major bottleneck when applying these systems to real environments and, thus, the achievement of high biomass concentrations would be a breakthrough for the remediation of polluted aquifers. The biomass growth was measured by qPCR and was compared to those obtained in conventional suspended cultures. A graphite brush was used in the cathode due to its high adsorption capabilities in order to maximise biomass attachment. The full presentation will also show a comprehensive study on the effect of the operational conditions (e.g. different applied cathode potentials) on the dechlorination rates obtained.

Keywords: bioelectrochemical reduction, *Dehalogenimonas*, 1,2-dichloropropane, bioremediation, dehalogenation

Funding: This work has been funded by the Spanish Ministry of Science, Innovation and Universities project CTM2017-91879-EXP.

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Electrobioremediation of Pharma pollutants is boosted in a Microbial Electrochemical Fluidized Bed Reactor

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Wastewater produced in pharmaceutical manufacturing are characterized by high concentrations of Total Organic Carbon (TOC) and the presence of pollutants that eventually will be considered as emerging contaminants (EC) once they are discharged to urban wastewater [1].

In this work, we have explored the removal of carbamazepine (CBZ), and sulfamethoxazole (SMX) from pharma wastewater in Microbial Electrochemical Fluidized Bed Reactors (ME-FBR). Different anode potentials applied to the fluid-like anode, varying from -200 mV to +600 mV (vs. Ag/AgCl), were evaluated to optimize and boost the pharma pollutant removal during the semi-continuous operation of the ME-FBR [2,3].

Results showed that adsorption phenomena or abiotic electrochemical removal were significantly lower than the electrobioremediation capacity exhibited by microorganism grown at ME-FBRs. Indeed, ME-FBR showed a remarkable capacity for removing pharma pollutants at +200 mV (vs. Ag/AgCl) anode potentials (81.45% TOC, 87.9% CBZ and 84.5% SMX removal at). It outperformed an UASB reactor, operated as control. Finally, the ecotoxicity of the treated effluent was assayed with *Vibrio fischeri*. Detoxifications of up to 70% were found at +200 and +400 mV, revealing the technological potential of ME-FBR for treating those highly polluted wastewaters.

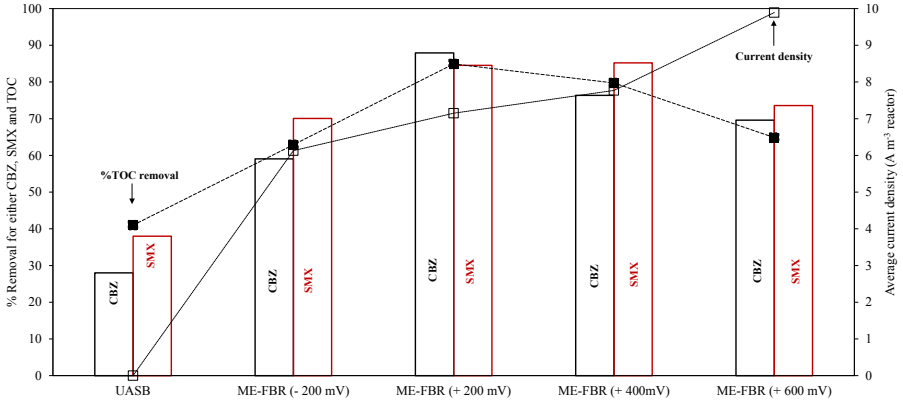

Fig.1. CBZ and SMX removal in ME-FBR at different anode potentials. Comparison with a UASB reactor

Keywords: pharma wastewater, microbial electrochemical fluidized bed reactors, carbamazepine, sulfamethoxazole, detoxification, ME-FBR

Funding: This research was supported by the Spanish Ministry of Science, Innovation and Universities, through the project MET-FLUID (RTI2018-101974-B-C21)

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Development of a four-chamber microbial desalination cell for simultaneous treatment of Cr (VI) containing wastewater and by-products recovery

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Here, we developed a novel four-chamber microbial desalination cell with different ion exchange membranes to treat Cr (VI) (2 mM) containing wastewater and by-product recovery (Fig. 1). We inoculated cathode and anode chamber with *Geobacter sulfurreducens* and the cathode was polarized at -0.8 V (vs. Ag/AgCl). Simulated wastewater containing soluble bicarbonate as carbon source and Cr (VI) (1 and 2 mM) with nutrients was added to the cathode chamber. Moreover, anode was fed with regular acetate-based substrate (2 g/l). We noted almost 100 % Cr (VI) removal in cathode in i) 24 h with a coulombic efficiency of 9.3 % when initial concentration of Cr (VI) was 1 mM; and b) 72 h with a coulombic efficiency of 13.9 %, when concentration increased to 2 mM. When acetate (2 g/l) was added to the anodic substrate, Cr (VI) reduction rate decreased significantly to 96 h (1mM) and 168 h(2mM). The pH in the acid and base recovery chambers were noted to be ≈2 and ≈12.5, respectively thus confirming a four-chamber MDC with *G. sulfurreducens* could be useful to remove high concentration of Cr (VI) from wastewater including organics removal and by-products recovery.

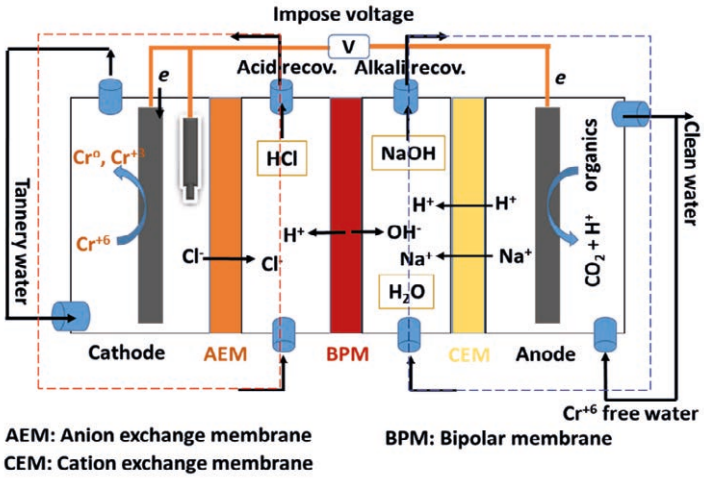

Figure1: Schematic diagram of four-chamber MES with working mechanism.

Keywords: biocathode, *Geobacter sulfurreducens,* hexavalent Chromium, microbial desalination cell.

Funding: This project has received funding from European Union´s Horizon 2020 research and innovation program under Marie Sklodowska grant agreement No. 754382

Desalination of Brackish Water Using a Microbial Desalination Cell: analysis of the electrochemical behaviour

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A microbial desalination cell (MDC) is a microbial fuel cell (MFC) integrated with an electrodialysis (ED) cell in the same device to simultaneously treat wastewater, desalinate brackish or seawater, and produce electric energy **[1]**. Most previously reported studies used oxygen reduction as the primary cathodic reaction **[2]**. In contrast, we have explored brackish water desalination (7 g L^{-1}) and energy production using a laboratory MDC system (cross-section 100 cm^2, batch mode) and ferricyanide as the catholyte. Furthermore, a rational explanation of desalination performance when using a catholyte is presented, and, additionally, the impact of producing electrical energy on desalination performance is discussed **[3]**. Interestingly, conductivity variation in the saline chamber can be used to predict electrochemical performance. In summary, this study provides the basis for the development, design, and optimisation of low-energy desalination using MDC technology.

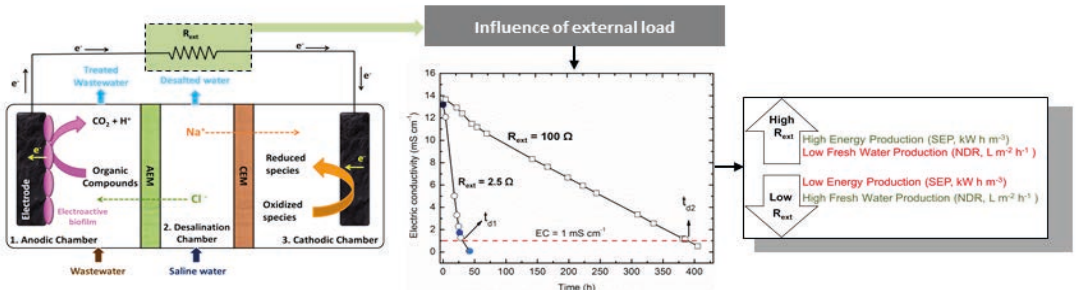

Fig. 1. Left: Scheme of an MDC. Righ: Electrical conductivity versus time for an MDC desalination cycle (7g L^{-1}/ 13 mS cm^{-1}) with 2.5 Ω (circles) and 100 Ω (squares).

Keywords: microbial desalination cell, brackish water, electrochemistry, freshwater production.

Funding: Project "MIDES – H2020" has received funding from the European Union's Horizon 2020 research and innovation programme (N° 685793). Juan M. Ortiz acknowledge the financial support of Agencia Estatal de Investigación (AEI) and Fondo Europeo de Desarrollo Regional (FEDER) (Proyecto BioDES, CTM2015-74695-JIN).

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Electrobioremediation by METland® solution differentiates among enantiomers in mixtures of pharmaceuticals and herbicides

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Removal of emerging contaminants (EC) is a growing concern of wastewater treatments. Microbial electrochemical technologies (MET) implemented into biofilters (METland® solution) are capable to treat both conventional pollutants and EC by improving the electrons flows across the microbial community [1,2]. Furthermore, chirality is an essential property of chemicals in interactions with biological systems, often overlooked in contaminants [3].

Here, two vertical upflow METland® and two conventional upflow electrode-free biofilters, were fed with different cocktails of chiral pollutants. One toxic wastewater contained six drugs of different pharmacological actions (one antidepressants, three beta-blockers, one antihypertensive and one bronchodilator) and the other contained six phenoxyacids herbicides.

Biofilters were operated at 1 day HRT for 3 months and effluents were analysed by Electrophoresis using a HP 3DCE system to calculate the enantiomeric rate of every chemical. The toxic wastewater with six chiral drugs in racemic mixture showed no differences in enantiomer fate between METland® and electrode-free control. By contrast, the wastewater with six chiral phenoxyacids herbicides showed differences in a 5-fold range for enantiomer composition after METland® treatment in comparison with electrode-free biofilter. Wastewater toxicity for microalgae aligns with this, showing no changes after METland® for drugs but a decrease for fenoxyacids.

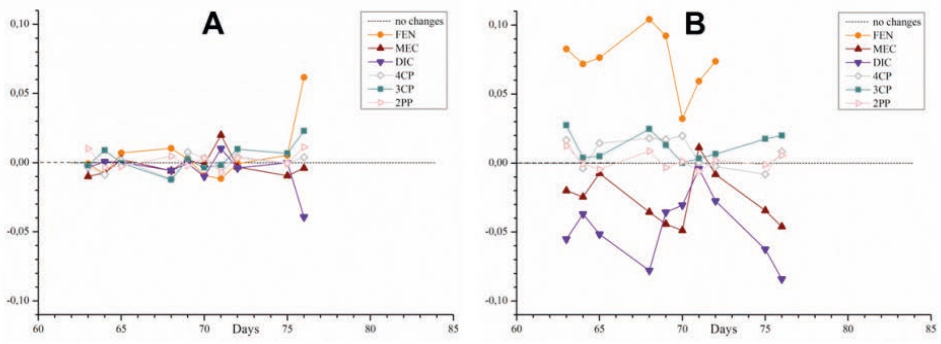

Fig. 1 Enantiomeric Fraction changes of Phenoxy acids during the experiment (A: conventional; B: METland®)

Keywords: Microbial Electrochemical Technology, Emerging Contaminants, Biofilter, Pharmaceuticals, Enantiomers

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Gira a sofre: Disentangling the sulfur cycle in one- and two-chamber bioelectrochemical systems

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Wastewaters containing high concentrations of sulfate and being usually deficient of organics are commonly produced in industrial processes and need to be treated before discharged in the environment. Cathodes of bioelectrochemical systems (BESs) have been applied for treatment of sulfate-rich wastewaters by providing reducing power. [1] However, the underlying mechanistic details of removal, for instance the electron transfer mechanism at the cathode, remained often speculative. For shedding light on this, we investigated sulfate reduction in one- and two-chamber microbial electrolysis cells (MECs). The results show that sulfate was fully reduced to sulfide that was accumulating only in two-chamber MECs. Hydrogen-driven dissimilatory sulfate reduction by microbial communities (with ongoing genetic analysis) that developed in the two-chamber system was further proven by microcosm experiments. However, no net sulfate reduction occurred in one-chamber MECs that we so far now tentatively assign to the anodic re-oxidation of sulfide to sulfate by either abiotic or biotic reactions. In principle, such an interesting sulfur cycle, while hampering sulfate removal from wastewater could be exploited to stimulate the sulfate-driven oxidation of organic contaminants in e.g., contaminated soil and groundwater, by continuously regenerating the metabolic electron acceptor.

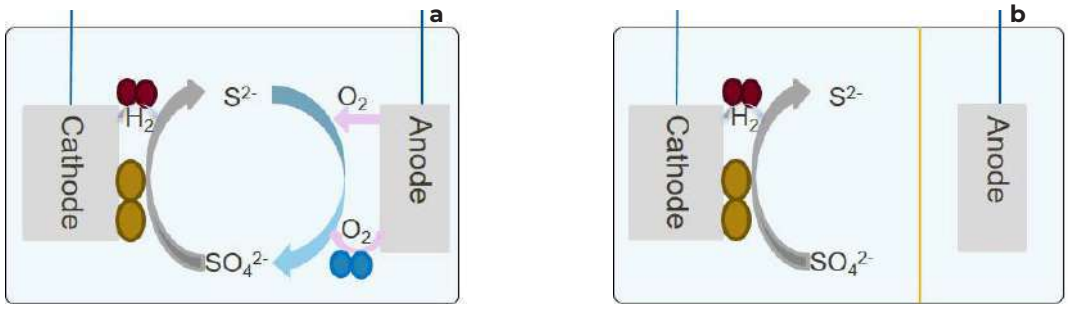

Fig. 1 Sketch of sulfate reduction in 1a in one- and 1b two-chamber MEC, with the different dots representing (so far assumed) microbial traits: brown and red dots represent sulfate reducing bacteria which are electroautotrophs and hydrogenautotrophs and the blue dots represent sulfide oxidizing bacteria.

Keywords: bioelectrochemical system, microbial electrolysis cell, sulfate reduction, sulfur cycle, performance comparison

Funding: This work was supported by the Helmholtz-Association in the frame of the Integration Platform "Tapping nature's potential for sustainable production and a healthy environment" at the UFZ. SD thanks China Scholarship Council (CSC201804910500) for 4-year granting study abroad.

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Primary Sludge-Based Blackwater Favors COD Conversion to Electrons Over Methane in Microbial Electrochemical Cells

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Microbial electrochemical cells (MEC) utilize microbial degradation of organic matter in high strength waste coupled with the metabolism of anode-respiring bacteria to produce an electrical current useful for producing electricity or other chemicals.

One application is decentralized blackwater treatment. MEC optimization relies on maximizing recovery of electrons from oxidized organics as electrical current and preventing undesirable electron diversion to alternate sinks, namely methane via methanogenic archaea. We studied conversion of primary sludge-based synthetic blackwater (PS-BW) to quantify the proportion of electrons respired to the anode versus the proportion diverted to methane. To do so, we operated five two-chamber MEC; two were fed PS-BW with high ammonia nitrogen concentrations, and two were fed PS-BW with low ammonia nitrogen concentrations, mimicking combined and source-separated blackwater, respectively.

The final MEC was an inoculum-only control. The high and low ammonia PS-BW conditions yielded 22.6% and 22.8% COD conversion to electrons, respectively, and 0.075% and 0.66% COD conversion to methane, respectively.

Simultaneously run biochemical methane potential tests demonstrated methane production was possible from the PS-BW and inoculum. Thus, treatment of PS-BW appears to favor conversion to electrons over methane. Microbial community analyses will reveal the makeup of the anode biofilm and suspension microbial communities involved.

Keywords: microbial electrochemical cells, anode-respiring bacteria, synthetic blackwater, microbial communities

Funding: NASA SC Space Grant Consortium GRA 2020-2021

Treatment and reuse of wastewater based on METland® technology

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Water scarcity is one of the main concerns for present and future generations. Indeed, the Sustainable Development Goals recognize “Clean water and sanitation” as one of the lines of action for promoting prosperity while protecting the planet. Pursuing this objective, a large-scale wastewater treatment has been developed merging the innovative METland technologies and ultrafiltration by recycled reverse osmosis membranes.

METland® is based on the integration of two concepts: Microbial Electrochemical Technologies (MET) and Constructed Wetlands (CW), systems that imitate nature to treat wastewater [1,2]. The electroactive bacteria interact with the electroconductive bed material increasing the electron availability within the system; thus, enhancing the removal of pollutants, without using external energy. Therefore, METlands are able to sustainably treat wastewater in the same place where it is produced [3], and generate reclaimed water for reuse. The full-scale treatment is divided in three phases (Fig. 1): 1) primary treatment-Imhoff tank, 2) down-flow METland® [2] and 3) ultrafiltration [4].

The performance of the system achieves an average removal of 95% for COD and 91% for TN, fulfilling the limits of discharge. To sum up, METland systems arise as a suitable and innovative alternative for treating wastewater with a possible reuse after an ultrafiltration process.

Treatment and reuse of wastewater based on METland® technology

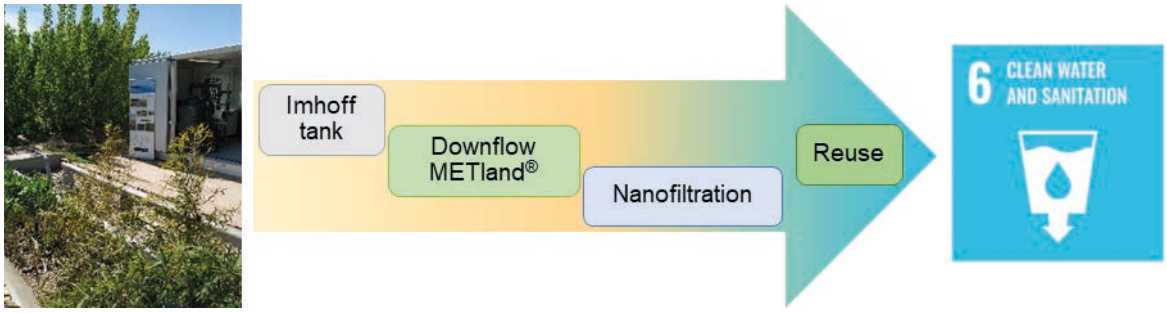

Fig. 1 Photo of the METland system in the facilities of IMDEA Water and the diagram of the wastewater treatment design.

Keywords: wastewater treatment, constructed wetland, METland, water management, reclaimed water

Funding: This investigation has received funding from the European Union's Horizon 2020 research and innovation program under the grant agreement No. 642190 (Project "iMETland"; www.imetland.eu) and No. 826244 (Project "ELECTRA"; www.electra.site). Lorena Peñacoba-Antona and Mario Jimenez-Conde were funded by the industrial PhD fellowship program from the Regional Government of Madrid, Ref. IND2017/AMB-7648 and IND2018/AMB-9197.

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Purple phototrophic bacteria in microbial electrochemical fluidized-like bed reactors for brewery wastewater treatment

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The need for sustainable reuse of resources is giving rise to a new model in wastewater treatment based on circular economy. Wastewater treatment with Purple Phototrophic Bacteria (PPB) is one of the most promising applications in the nutrient recovery framework, since they have a high protein content and synthesize added value products. Recently, it has been shown that microbial electrochemical systems are a successful platform that allows modulating the metabolism of these microorganisms [1, 2]. This work aims to apply electro-fermentation in brewery wastewater treatment using PPB. For this purpose, we studied the effect of polarization and Infrared (IR) illumination (for promoting PPB growth) in microbial electrochemical fluidized bed reactors (ME-FBR) [3].

Our results demonstrate that anodic polarization in combination with IR illumination, allows approximately a 2-fold enhancement in terms of total organic carbon removal and total nitrogen removal from brewery wastewater. The production of PPB-biomass was lower under polarization in comparison with electrode-free conditions, which suggest that anodic potential could serve to modulate biomass production. Finally, Illumina analyses showed that *Geobacter* was the electroactive genus that dominates non-illuminated ME-FBR while treating wastewater. In contrast, in illuminated ME-FBR the electroactive genus *Proteiniphilum* together *Rhodopseudomonas and Rhodobacter* outcompeted *Geobacter*.

Keywords: purple phototrophic bacteria, electro-fermentation, nutrient recovery

Funding: MET-FLUID (No. RTI2018-101974-B-C-21) from the Spanish Ministry of Science and Innovation and the industrial doctorate programme (No. IND2020/AMB-17843) from the Autonomous Community of Madrid.

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Electrobioremediation of soil polluted with the insecticide Lindane

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Lindane (C6H6Cl6, y-hexachlorocyclohexane, y-HCH) was widely used as a broad spectrum insecticide in agriculture since the 1940s. Massive amounts were produced in Sabiñanigo (Spain) between 1975 and 1992, leading to vast soil areas heavily polluted with different Lindane waste isomers including intermediates [1].

We have design and constructed a Microbial electro-remediating cells (MERCs) [2], to be implemented in outdoor mesocosm (0.5m^3) made of a real lindane polluted soil with the purpose of stimulating the electrobioremediation of the insectide. A number of electrochemical configurations were tested, together with the use of humic amendment. Our results revealed the biocathode configuration as the most efficient for removing linande (>70% in 20 weeks) and related chlorinated compounds (>90% y-HCH in 20 weeks, majority isomer). Microbial community analysis by Illumina from electrode colonizing biofilm appeared more specialized in the case of biocathode, increasing the presence of sulfate-reducing bacteria as *Desulfosporosinus*, sulfur-reducing bacteria as *Dethiobacter* and other anaerobic bacteria as *Anaerorahbdus* y *Howardella*. On the other hand, bioanode and snorkel configurations presented more biodiversity. The bioanodes show a high abundance of *Geobacter*, *Pseudomonas* and *Tolumonas* (electroactive and aromatic-degrading bacteria).

Fig. 1. Photos of A) whole systems, B) snorkel system and C) cathode system

Keywords: electrobioremediation, lindane, HCH isomers, soil remediation, real site pollution.

Funding: Andres de Deus was funded by a regional Industrial PhD fellowship from the Community of Madrid (IND2017/AMB-7703)

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Simultaneous removal of oxidable and reducible contaminants from groundwater with the "bioelectric well"

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Groundwater contamination is a critical issue that poses serious health and environmental risks. In this context, Microbial Electrochemical Technologies (METs) are being considered as an alternative to traditional groundwater remediation techniques. Indeed, they possess the advantage of avoiding addition of electron acceptors (or donors) in the aquifer to promote oxidation (or reduction) of contaminants, thus being potentially more sustainable and cost-effective [1].

Herein, a lab-scale bioelectrochemical reactor named "bioelectric well" [2–4] was continuously fed with artificial groundwater containing a mixture of oxidable (*i.e.*, toluene) and reducible (*i.e.,* chlorinated solvents) contaminants. The reactor consisted in a tubular glass cylinder where in a cylindrical graphite anode and a stainless-steel mesh cathode were concentrically placed. Anode and cathode were kept physically separated yet hydraulically connected using a polyethylene mesh. Throughout the experiment, the anode was polarized at +0.2 V *vs.* SHE.

The performances of the system were evaluated in terms of toluene and chlorinated solvents removal and coulombic efficiency, and they were compared to an open circuit control. Moreover, the microbial composition of the biofilm and of the bulk liquid was analysed by NGS methods. Our findings highlight the benefits of the bioelectrochemical system on the remediation process.

Keywords: Microbial electrochemical technologies, groundwater remediation, bioremediation, toluene, petroleum hydrocarbons

Funding: This study was supported by the European Union's Horizon 2020 project ELECTRA (www.electra.site) under grant agreement No. 826244.

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Combined Electrochemical Water Softening And Nitrate Electro Bioremediation

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Electro bioremediation of nitrate-contaminated groundwater has been successfully investigated at laboratory-scale[1solubility, and mobility. Nevertheless, the actual treatment implementation is in an ambitious transition. Groundwater hardness may generate scale deposits that irreversibly affect the treatment effectiveness. The present study aims at using electrochemical water softening implementation as a sustainable preliminary step for nitrate electro bioremediation. A single-chamber reactor was operated at a cathode potential of -1.2 V *vs.* Ag/AgCl in continuous flow-mode (HRT of 4.1h). The electrochemical reactor achieved a hardness removal efficiency of 64±4% (305±17 mg $CaCO_3$ $m^{-2}_{cathode}$ h^{-1}) over 13 days until the cathode was covered with precipitate and chemical cleaning was required. The softening life-time was increased by 48% once polarity reversal[1] strategy was applied. Softened groundwater was introduced into the nitrate electro bioremediation treatment[2], which achieved a nitrate removal effectiveness of 97±1 % (1269±30 g NO_3^- $m^{-3}_{net\ cathode\ compartment}$ d^{-1}) in continuous flow-mode (HRT_{cat} of 2.1h) under a cathode potential of -0.32 V *vs.* Ag/AgCl. The effluent reached the quality standards of drinking water in terms of concentration of nitrates and nitrite (Nitrates Directive, 91/767/EU). The estimated energy consumption of 1.4 kWh m^{-3} confirmed the competitiveness of the combined treatment, paving the ground for future scaling up the process.

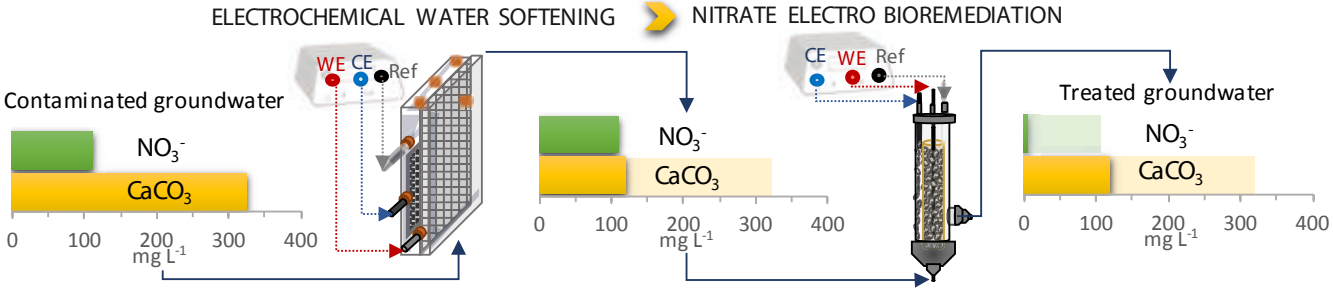

Fig. 1. Schematics of the combined treatment: electrochemical softening (left) and nitrate electro bioremediation (right). Groundwater composition (hardness and nitrate) in each step.

Keywords: Nitrate-polluted groundwater, Hardness removal, Polarity reversal, Denitrifying bioelectrochemical system.

Funding: This work was funded by European Union's Horizon 2020 project ELECTRA [No. 826244]. A.C-E. was supported by a PhD grant from the University of Girona (IF_UDG2020). S.P. is a Serra Hunter Fellow (UdG-AG-575) and acknowledges the funding from the ICREA Academia award.

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Soil Microbial Fuel Cell for Bioremediation of Recalcitrant Hydrocarbons

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Increased human, agricultural and industrial activities along with improper waste disposal leads to high levels of soil contamination and accumulation of recalcitrant contaminants. This global issue demands the use of green and sustainable technologies and soil microbial fuel cells (SMFC) can be a potential solution. In this presentation, we will give an overview of the efficacy of the SMFCs for remediation and elucidate the advantages of SMFC over natural bioremediation. We adopted minimalistic design based on low-cost carbon materials without any expensive catalyst and membrane, which makes the SMFCs suitable for upscaled in-field applications. We particularly studied the removal of hydrocarbons over time and advanced chromatographic techniques are used to analyse the fractions of total petroleum hydrocarbons (TPH) and 16 polyaromatic hydrocarbons (PAH), which are extremely hazardous to the environment and humans and listed as priority pollutants by USEPA [1]. Natural hydrocarbon remediation is time consuming and suffers from poor bioavailability. We have designed experiments to address these issues and compared the findings to understand how these modifications can facilitate the degradation process.

This study aims to elucidate the advantages of SMFCs for efficient removal of contaminants in soil with the generation of clean energy, towards a promising self-sustainable technology.

Keywords: Soil microbial fuel cells, Bioremediation, Low-cost, Hazardous, Hydrocarbons

Funding: This work is part of the project GREENER that has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 826312

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Groundwater denitrification by expanded bioelectrochemcial systems

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More than 30% of groundwater sources in the EU contain more nitrate than the drinking water standard. Bioelectrochemical system (BES) was proven to be an effective method for groundwater denitrification by autotrophs at low energy investment and without chemical addition[1,2]. However, the most commonly studied fixed-bed systems suffer from fouling issues, which prevents larger scale application. A new concept applying expanded cathode could potentially solve the problem and allow for a dramatic expansion of the working volume, where biofilmed cathodic particles moving in the catholic chamber with intermittent contact with electron donors. To investigate the impact of intermittent access of electrons on denitrification performance, two approaches were tested: 1. Periodic polarization on packed-bed biocathodes; 2. Continuous polarization on expanded-bed biocathodes. The first approach showed that the electroactive biofilm developed a higher ability for electron storage and transfer at an intermittent power supply (30s open circuit/30s polarization) at a cathodic potential of -0.4V vs. Ag/AgCl, with 86% nitrate removal at a denitrification rate of 205 g NO3--N /m3/d. However, on expanded-bed biocathodes, denitrification only occurred with H2 evolution at a cathodic potential lower than -1V vs. Ag/AgCl, with 92% nitrate removal at a denitrification rate of 155 g NO3--N /m3/d. The preliminary results from this work could provide a better understanding of the electron transfer in the bioelectrochemical denitrification process and support future reactor design.

Keywords: denitrification, BES, periodic polarization, expanded-bed biocathodes

Funding: Horizon 2020 ELECTRA Project, grant agreement No 826244.

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Electro-bioremediation of nitrate from saline groundwater and concomitant chlorine production

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Groundwater pollution and salinization have increased steadily over time [1,2], therefore a sustainable approach to the recovery of such important water resource has become essential.

A 3-compartment cell configuration was tested for the simultaneous denitrification and desalination of nitrate contaminated saline groundwater. The reactors were initially operated in potentiostatic mode to promote autotrophic denitrification at the biocathode [3], and then switched to galvanostatic mode to improve desalination in the central compartment. The average nitrate removal rate was 39±1 mgNO_3^--N $L^{-1}d^{-1}$ and no intermediates (nitrite and nitrous oxide) were observed in the effluent. Groundwater salinity was considerably reduced (average chloride removal was 63±5%). Within a circular economy approach, part of the removed chloride was recovered in the anode compartment and converted into chlorine. Production rates of 6.1 mgCl_2 $L^{-1}d^{1}$ were achieved, corresponding to a concentration of 26.8±3.4 mgCl_2 L^{-1}. The accumulated chlorine represents a value-added product, which could also be dosed for the disinfection of treated water. This proof-of-concept configuration was able to meet WHO and European legislation for drinking water in terms of nitrate and salinity, with low specific power consumptions (0.13±0.01 kWh $g^{1}NO_3^-N_{removed}$). These results pave the ground to develop a sustainable technology which tackles an urgent environmental issue.

Keywords: circular economy; denitrification; disinfectant products; saline groundwater; value-added products.

Funding: This research was funded by Fondo di Sviluppo e Coesione 2014-2020, Patto per lo sviluppo della Regione Sardegna - Area Tematica 3 - Linea d' Azione 3.1, "Interventi di sostegno alla ricerca". Project SARdNAF "Advanced Systems for the Removal of Nitrates from Groundwater", ID: RASSR53158. S.P. is a Serra Hunter Fellow (UdG-AG-575) and acknowledges the funding from the ICREA Academia award. LEQUIA has been recognized as a consolidated research group by the Catalan Government (2017-SGR-1552).

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Power Generation from Constructed Wetland Microbial Fuel Cells under Batch and Continuous Flow

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An electric current can be recovered from the metabolic activity of microorganisms through an electrochemical system in a constructed wetland microbial fuel cell (CW-MFC). Furthermore, artificial wetlands can take advantage of its own processes for simultaneously treating wastewater and producing electricity. The aim of this research was to determine whether a continuous or an intermittent flow reactor has the best performance on power generation and pollutants removal. Two experimental systems were constructed, the first one under continuous flow, and the second, in batch flow mode, and varying the external electrical resistance (5,600, 1,000, 560, and 10 Ω) over various stages (17–20 days). Results indicate that reactors operating in batch mode provided a higher organic matter removal (average 94%), while the reactor operated in continuous mode had a power output of 18.12 mW/m2, which is 1.6 times more than the power produced by the batch reactor. The highest power density occurred during the 5600 Ω of electrical resistance connection in continuous mode, indicating the external resistance influence. Therefore, operating the reactors in a continuous mode leads to stable organic matter removals and power production. Operating in batches destabilizes the electrical parameters values when the reactor is emptied and filled.

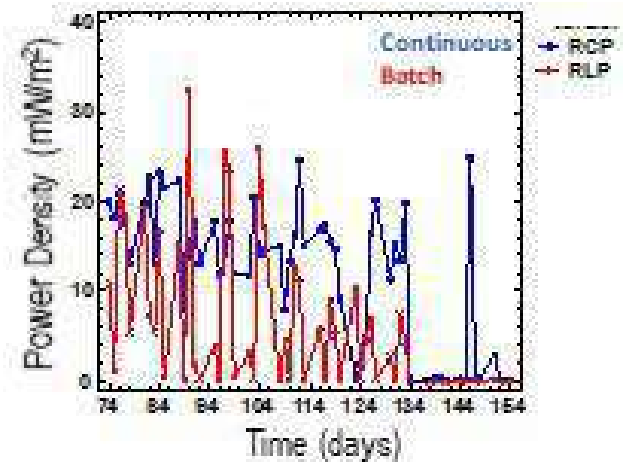

Fig. 1 Power density produced by the two experimental systems

Keywords: Constructed wetlands; Microbial fuel cells; Continuous flow, Batch mode

Funding: This study was funded by the Program to Support Research and Graduate Studies (PAIP, in Spanish) of Facultad de Química, UNAM, Code 50009067.

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Lowering energy consumption of a sequential reductive/oxidative bioelectrochemical process for CAHs removal by a galvanostatic polarization strategy

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A sequential reductive/oxidative bioelectrochemical process for chlorinated aliphatic hydrocarbons (CAHs) removal from groundwater has been developed by the combination of two membrane-less microbial electrolysis cells named reductive and oxidative reactor, respectively. In almost two years of continuous-flow operation of the sequential process, several operating conditions have been characterized including hydraulic retention time, applied potential and contaminated matrixes (mineral medium, synthetic groundwater, real groundwater). Most of the explored conditions were investigated under potentiostatic control of the cell, which in presence of side reactions like sulphate and nitrate reduction, promoted an increase of energy consumption of the process due to the side reaction contribution to the current increase. The removal of CAHs in groundwater typically requires low amounts of equivalents due to the low solubility of the CAHs, in this way, an interesting approach for energy consumption minimization is offered by the adoption of a galvanostatic approach which allows for the control of the available current in the cell. In this study, the use of a galvanostatic approach for the minimization of the energy consumption of the reductive and oxidative dechlorination has been analysed in both reductive and oxidative reactors even in presence of side reactions like sulphate and nitrate reduction.

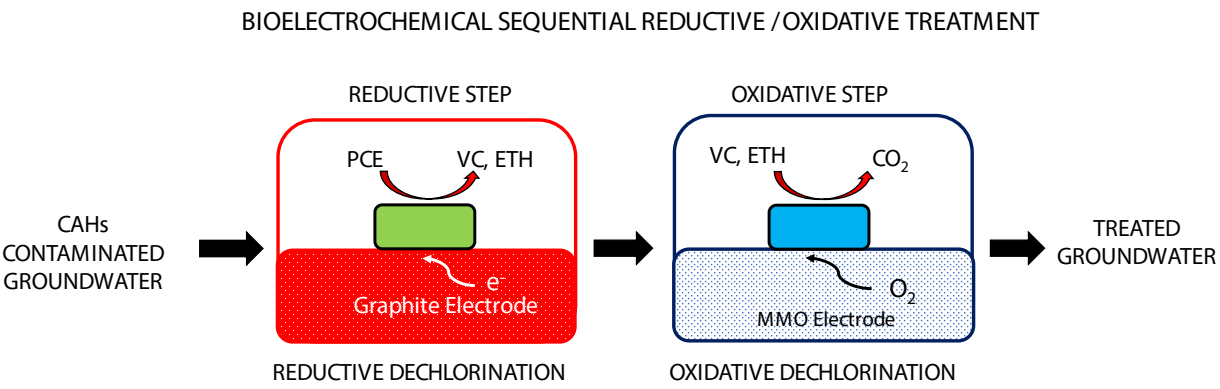

Fig.1: Schematic concept of sequential reductive/oxidative bioelectrochemical process

Keywords:, Chlorinated Aliphatic Hydrocarbons, bioremediation, galvanostatic control, potentiostatic control, reductive Dechlorination, Oxidative Dechlorination

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 826244-ELECTRA

Chlorinated aliphatic hydrocarbons (CAHs) removal from a real groundwater by a sequential Reductive/ Oxidative Bioelectrochemical process

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A reductive/oxidative bioelectrochemical process has been developed by connecting in series two membrane-less microbial electrolysis cells (MECs) equipped with an internal graphite counterelectrode[1]avoiding the use of an ion exchange membrane to separate the anodic and cathodic chamber and reducing the cost of the reactor. In the reductive reactor, a dechlorinating mixed inoculum received reducing power to perform the reductive dechlorination of perchloroethylene (PCE. The first MEC aimed at the CAHs reductive dechlorination (RD) and was constituted by a granular graphite working electrode while the second MEC, devoted to the oxidative dechlorination of low chlorinated RD by-products, utilized a MMO working electrode for the electrolytic oxygen production[2]. In the present study, the sequential reductive/oxidative bioelectrochemical process has been tested with real groundwater from a contaminated site located in northern Italy. The sequential process allowed for the complete mineralization of the CAHs contained in the real groundwater by the complete reduction of CAHs into vinyl chloride (VC) in the first MEC polarized at -450 mV vs SHE, while all the VC was successfully oxidized by the HRT increase from 0.7 to 1.7 days in the second MEC (operated at +15 mA). Biomarkers of the reductive (*Dehalococcoides mccartyi* 16S rRNA and dehalogenase genes) and oxidative (etnE, etnC genes) dechlorination in the two MECs have been monitored along with the ecotoxicity tests to assess the potential adverse effects and to provide information on the efficiency of the applied technology.

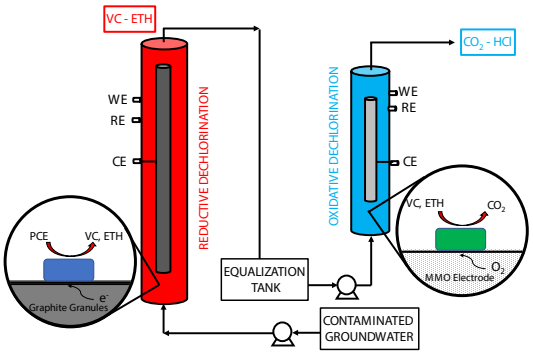

Fig.1: Schematic representation of the sequential reductive/oxidative bioelectrochemical process

Keywords: Bioremediation, Chlorinated Aliphatic Hydrocarbons, Reductive Dechlorination, Oxidative Dechlorination

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation programme under grant agreement No 826244-ELECTRA

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Promising affordable separators for microbial electrolysis cells

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Microbial electrolysis cells have emerged as a promising technology platform for wastewater treatment and the simultaneous production of hydrogen. It would be desirable to keep hydrogen produced at the cathode separated from potentially hydrogenotrophic organisms, maintaining a low ohmic potential drops across the electrolysis cell. Whereas most ion-exchange membranes can achieve these objectives, their cost is prohibitive for a scaled-up setup[1]. Thus, more affordable solutions should be analyzed to reduce the general cost and increase the applicability of this technology.

This work compares several ion-exchange membranes in terms of ohmic resistance, swelling, dimensional and mechanical stability, pH imbalance and cost. Moreover, two non-ion-exchange commercial membranes are included in the comparison set due to their low cost, potentially good transport properties and barrier effect against microorganisms. The performances of the materials are evaluated by means of abiotic experiments under realistic hydraulic operating conditions, using a semi-synthetic brewery wastewater as medium.

Results evidence the differences in the performance of the materials under the same working conditions. Cellophane and a polyethylene acid battery separator show significantly low resistances compared to state-of-the-art ion-exchange membranes (Fig. 1) which profile them as cost and possibly even performance-competitive materials.

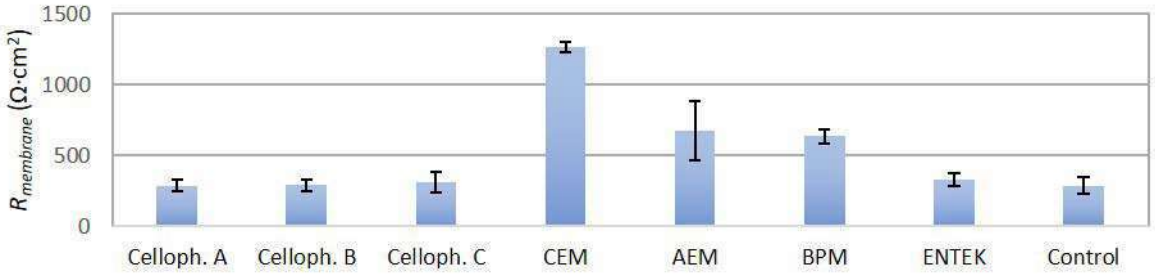

Fig. 1 Resistance of the membranes under study. Celloph. A, Celloph. B and Celloph. C represent three cellophanes provided by different suppliers and Control depicts the experiment carried out without membrane.

Keywords: MEC, ion-exchange membrane, membrane screening, low-cost separators, ohmic resistance

Funding: We are grateful for the financial support from the Bundesministerium für Bildung und Forschung (BMBF) under the program 02WER1528A.

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Modelling bioelectrochemical reactors for electrobioremediation processes development optimization

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In the frame of the GREENER Project, two mathematical models for soil and liquid BES reactors have been described. These serve as a tool to design and optimize electrobioremediation systems. On the one hand, a 3D model to describe removal of pollutants in liquid media has been built and solved. This integrates equations of charge conservation with mass transport phenomena, hydrodynamics, and kinetics of the involved processes under transient conditions, such as biofilm formation, bioelectrochemical and electrochemical reactions. Once the model was calibrated with experimental data, different scenarios were investigated, and the relevant results compared: a system with a single BES cell under fed-batch and flow conditions, and a system constituted by three BES cells hydraulically connected in-series. A model to describe tubular terracotta-based soil microbial fuel cells (SMFC) piled together for soil bioremediation was also developed. The model, calibrated with experimental data, predicts the performance of the SMFCs by combining transport phenomena in the solid, liquid and gas phase with (bio)electrochemical reactions, charge balance, evaporation and transport of water and its effect on transport of solutes. The model is also used to predict the efficacy of the piled SMFCs to biodegrade an exemplary persistent organic pesticide, such as hexachlorobenzene.

Keywords: Soil Microbial Fuel Cells, 3D model, electrobioremediation.

Funding: This work is part of the project GREENER that has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 826312

Taking the waste out of dye wastewater using microbial fuel cells

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Dye-containing wastewater (e.g. from textiles) is getting ever more challenging to treat partly due to its complexity but also due to tightening legislation regarding discharge standards. In India for example, they have a zero-water discharge policy which means the wastewater has to be treated to such a level that it can be reused. Treatment of the water for reuse is also of interest to water stressed countries. Conventional methods of treatment are however, either too costly (e.g. membrane systems) or produce sludges that create a secondary disposal problem (e.g. flocculation). I will explore the role that microbial fuel cells can play in overcoming some of the challenges of treating dye wastewater using conventional means. Case studies will include anodic vs cathodic decolourisation of dyes, integration of microbial fuel cells with activated sludge systems and scale up of reactors used.

Bio-electrochemical degradability of prospective wastewaters to determine their ammonium recovery potential

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Bio-electrochemical ammonium recovery (BEAR) can close the cycle between anthropogenic emission of reactive nitrogen and energy intensive nitrogen fixation in the Haber-Bosch process. BEAR is currently limited by the bio-electrogenic degradability of the treated wastewater. Here, we investigated the degradability of blackwater, hydrolyzed human urine, cow manure and pig manure as prospective wastewaters for BEAR in a standardized experimental design.

We found that bio-electrochemical conversion efficiencies ranged from 63% (blackwater), 42% (cow manure) and 41% (urine) to 26% (pig manure) after 5 days. These values correspond well with the relative VFA content of soluble COD for blackwater and cow manure, while additional compounds must have been converted for urine and pig manure.

The degradability of blackwater and cow manure was sufficiently high to theoretically be able to remove all TAN already after <0.5 d. The actual recovery potential (consisting of conversion efficiency and COD/TAN ratio) of pig manure was just high enough to remove all TAN. Human urine would require additional electron donor to remove all TAN in BEAR. Therefore, combining the maximum recovery potential with the relative VFA content of soluble COD can give a good estimate of the actual recovery potential of a wastewater.

	Black water	Urine	Cow manure	Pig manure
Peak current density j [A/m^2]	2.2 ± 0.6	0.6 ± 0.2	2.4 ± 0.1	1.6 ± 0.2
Conversion efficiency after peak [%]	42 ± 8	31 ± 2	36 ± 5	16 ± 2
Conversion efficiency after 5 d [%]	63 ± 4	41 ± 5	42 ± 4	23 ± 2
Acetate / COD_f [$g_{COD f,Ac}/g_{COD f}$] [%]	26	15	28	8
VFAs / COD_f [$g_{COD f,VFAs}/g_{COD f}$] [%]	63	15	40	8
BMP / BMP_{max} [%]	60 [1]	46 – 86 [2]	59 [3]	55 [4]

**Fig. 1: Summary of reproducible bio-electrochemical wastewater conversions to electricity from four replicates. BMP / BMP_{max} recalculated from maximum values from [1] de Graaff et al., 2010, [2] Barbosa et al., 2019, [3] Yao et al., 2017*and the highest methane content (56.5–75.5%*, [4] Shin et al., 2019*substantial amounts of greenhouse gases (GHG*

Keywords: bio-electrochemical systems; wastewater; ammonium recovery; recovery potential; biodegradability

Funding: Horizon 2020 research and innovation programme, Marie Skłodowska-Curie grant agreement No 665874

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Procedure for the screening of anode materials for microbial electrolysis cells operated with brewery wastewater

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The meaningful characterization of electrode materials for microbial anodes operated with complex wastewaters can be challenging[1], in particular due to changes in the medium during characterization owing to the fermentation process, change of the microbial consortium, and composition variations of the real medium used in practical applications.

Thus, a screening method is proposed where various anodes can be simultaneously compared over the time for various medium compositions. Sequential cultivation, stabilization and polarization methods are conducted to systematically compare materials over time (Fig. 1). The evaluation of the polarization curves of the anodes[2] yields insights on attainable current densities depending on the potential, limiting currents, and sensitivity towards compositional changes in the medium.

It was observed that simultaneous testing allows reliable comparison between anode materials. This strategy provides a framework to assess materials despite different degrees of fermentation of the medium and bypasses problems due to poor conductivity and buffer capacity of the medium.

First results show stainless steel wool has a competitive electrochemical performance with respect to carbon felt, providing higher current densities and limiting current densities. Furthermore, the reproducible compositional variation of the reactors in time suggest a high degree of systematization in medium-term experiments.

Fig. 1 Scheme of the developed procedure. CA = chronoamperometry, PC = polarization curve.

Keywords: Brewery wastewater, polarization curve, MEC, anode screening, anode respiring bacteria

Funding: We are grateful for the financial support from the Bundesministerium für Bildung und Forschung (BMBF) under the program 02WER1528A.

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Magnetite anodes to inhibit oxygen production and to circumvent the use of membranes in microbial electrosynthesis

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While studies on microbial electrosynthesis are mostly focused on the cathode, the anode performance is often overlooked [1]. One major product at the anode is oxygen, which is toxic to the anaerobic autotrophs that are involved in microbial electrosynthesis. Therefore, expensive membranes are used to avoid direct contact between these microbes and oxygen. Here, we tested a redox-active, mixed-valent magnetite mineral as an anodic electrode material, which acted as a pseudo-capacitor to inhibit oxygen production with the goal to circumvent membranes for microbial electrosynthesis. Abiotic experiments showed faster reaction kinetics for the oxidation of magnetite compared to the oxygen evolution. Next, we set up single-chamber bioelectrochemical systems (50-mL working volume) with magnetite-based anodes and carbon-based cathodes, and inoculated a hydrogenotrophic methanogen to produce methane at 65°C. Our results showed that the magnetite anode was able to both sustain methane production and avoid oxygen evolution to achieve an average methane production and coulombic efficiency of 21.03 $L \cdot m^{-2} \cdot d^{-1}$ and 76 %, respectively. Mineralogical analyses of the magnetite confirmed its homogenous oxidation during the passing of current. Overall, this study provided a proof-of-concept that demonstrated the suitability of magnetite-based anodes for microbial electrosynthesis, which would make this process more economically feasible.

Keywords: Microbial electrosynthesis, Magnetite anode, Oxygen inhibition, Membrane circumvention

Funding: Alexander von Humboldt Foundation by the Federal Ministry of Education and Research in Germany and the Deutsche Forschungsgemeinschaft (DFG, German Research Foundation)

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Metal-free activated biochar as an oxygen reduction reaction catalyst in single chamber microbial fuel cells

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Metal-free catalysts are promising candidates for Bio-electrochemical Systems (BESs) due to their high surface area, chemical stability, good electrical conductivity, and enhanced mass-transport capabilities. In this study, biochars derived from olive mill waste (OMW) with and without supercritical CO_2 pretreatment, and salted pistachio nut shells, were produced via pyrolysis and/or chemical and physical activation. The catalytic activity towards oxygen reduction reactions (ORR) of the biochars was investigated by cyclic and linear sweep voltammetry in neutral media. The electrochemical characterization of the samples revealed that olive mill waste biochar showed the highest catalytic activity toward ORR, in terms of reaction rate (Epr V vs. RHE= 0.537± 0.00), density of active sites, and number of electrons exchanged (ne- $E_{@}$ -0.6 V vs. RHE=3.9±0.2). These biochars were used as catalysts in air cathode microbial fuel cells. The power density obtained by MFCs equipped with an OMW cathode achieved a maximum power density of 271 ± 34 mWm^{-2} (R_{ext}=250 Ω) (Fig.1). This value was approximately 15 times higher than the power density obtained by a commercial carbon black used as control.

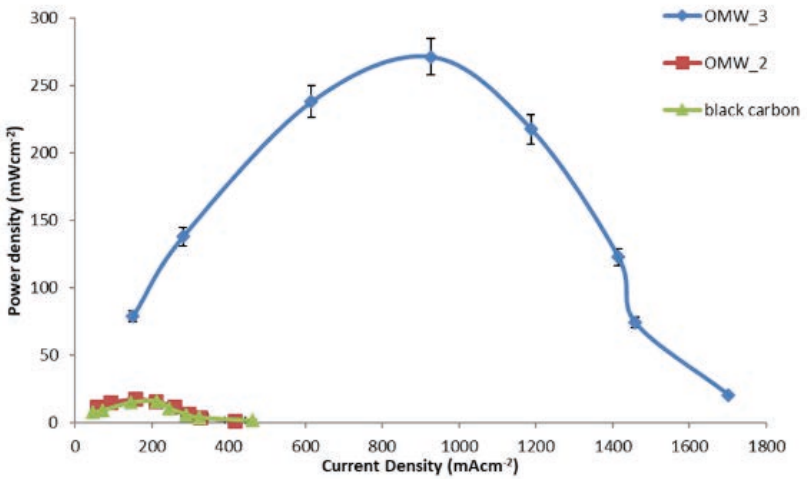

Figure 1: Comparison between power curves obtained by the different MFCs biochars reactors tested

Keywords: Olive mill waste; pistachio nutshell; biochar; activated carbon; Oxygen reduction reaction; Microbial fuel cell.

Towards improving the performance of microbial fuel cells: evaluation of inoculation strategies and anode materials

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The anode material of MFCs is crucial because its characteristics directly affect the performance of electron transfer from the bacteria to the anode. Different carbonaceous anode materials with favourable properties (carbon brush, carbon granules, thicker carbon felt (CF1), high conductivity carbon felt (CF2) and high-active-area carbon felt (CF3)) were tested in this work. Each material was fully characterized by employing different electrochemical techniques. MFC performance was studied using inoculation strategies of A) fixed 10 Ω external resistance (ER) and B) fixed anode potential (FAP) of 200 mV.

During the inoculation period, a slightly reduced start-up time and an earlier maximum current was observed for each anode using FAP. CF1 and CF2 exhibited higher maximum current with FAP. In the case of CF1, the MFC with FAP needed 33 days to get a similar current output, which was 12 days faster than the MFC operated with ER.

The full presentation will provide a comprehensive discussion on the performance and electrochemical parameters for each cell. For instance, a higher columbic efficiency was obtained when operating with FAP. Out of five anode materials, the carbon brush yielded the highest maximum current output of 7 mA with a maximum columbic efficiency of 85 %.

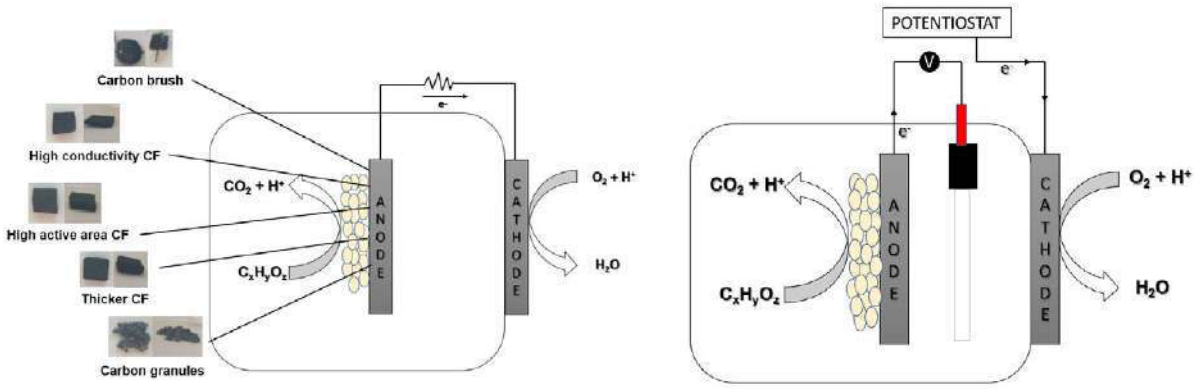

Fig. 1 Schematic diagram of MFCs with different anode materials using (A) external resistance and (B) fixed anode potential.

Keywords: Carbon-based anode materials, microbial fuel cells, fixed anode potential, anode, start-up time.

Funding: This work was supported by the Spanish Ministerio de Economía y Competitividad (CTQ2017-82404-R) with funds from the Fondo Europeo de Desarrollo Regional (FEDER). The authors are members of the GENOCOV research group (Grup de Recerca Consolidat de la Generalitat de Catalunya, 2017 SGR 1175, www.genocov.com).

Exploration of the reactivity of multiheme cytochromes and how it can be improved for (bio)electrocatalysis

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Extracellular electron transfer is a metabolism of microorganisms that can be interfaced with electrical circuits and therefore drive microbial electrochemical technologies. Multiheme cytochromes *c* are key players in this process. One of the most studied bacteria capable of extracellular electron transfer is *Shewanella oneidensis* MR-1. In this Gram-negative bacterium, electron transfer across the periplasmic space is dominated by the small tetraheme cytochrome c (STC) and flavocytochrome *c*[1]. Here, we explore the range of properties of STC that are compatible with physiological activity in *Shewanella*, and whether its functional and structural properties are optimized for electron transfer across the periplasmic space.

Our results show that a broad range of redox properties are compatible with physiological fitness as shown by the diversity of order of reduction potentials of the four hemes in STC from different organisms [2]. Our results also show that STC is not optimized for maximum rate of electron transfer. A single amino acid mutation was capable of increasing both the reduction and the oxidation rates by three fold [3].

Overall, these results provide good perspectives for manipulating multiheme cytochromes for improved performance either of their hosting organisms in microbial electrochemical technologies or as redox catalysts themselves.

Keywords: Shewanella, cytochromes, reduction potentials, redox kinetics, binding affinity

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La persistència de l'entropia: Measuring the microbial electrochemical Peltier heat of different electrode materials

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Although electroactive microorganisms (EAM) attracted considerable interests over the last two decades, their thermodynamic characterization, i.e., an analysis of the efficiency of the energy converting reactions, is still in its infancy. However, assessing the metabolic energy efficiencies and the energy fluxes during extracellular electron transfer (EET) are prerequisites for deciphering their ecological role and for estimating their biotechnological potential.

Biocalorimetry allows a thermodynamic characterizations of microbial systems by measuring its heat production. Based on a previously developed bioelectrocalorimeter[1], the heat and energy flows during cultivation of *Geobacter anodireducens* enrichment biofilms at gold, silver, and copper electrodes were analysed. By performing redox titration experiments, the respective microbial electrochemical Peltier heats (mePh) were derived. The mePh is an entropic effect at the cytochrome/solid electron acceptor interface during direct EET and represents a metabolic trade-off, as it decreases biomass yield but provides thermodynamic driving force for growth.

The experiments show that the mePh of all tested metal are substantially lower compared to graphite electrodes suggesting increased biomass yields of *Geobacter* biofilms at metal electrodes. The electrodes were analysed with scanning electron microscopy coupled with energy dispersive X-ray spectroscopy to characterize the elemental composition of electrode surfaces and to visualize the biofilms formed thereon.

Keywords: *Geobacter* biofilms, bioelectrocalorimetry, microbial electrochemical Peltier heat, metabolic energy efficiency, metal electrodes

Funding: This work was supported by the Helmholtz-Association in the frame of the Integration Platform "Tapping nature's potential for sustainable production and a healthy environment" at the UFZ.

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Role of phenazine-enzyme physiology for current generation in a bioelectrochemical system

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Phenazine natural mediators which are mainly produced by *Pseudomonas aeruginosa* are employed to shuttle electrons to the anode of a bioelectrochemical system (BES). The challenges however in exploiting the full potential of these compounds in mediated extracellular electron transfer (MET) are**:** 1) that the molecular pathways of phenazine reduction are not clarified; 2) electron mediation using phenazines is substrate dependent, and 3) only a small share of electrons is able to be mediated to the anode.

We addressed these concerns by looking out for periplasmic enzymes that reduce phenazines since we argued that phenazine reduction are favored by substrate oxidation occurring in this part of the cell as compared to the cytoplasm.

We found out that the periplasmic glucose dehydrogenase (Gcd) is able to reduce phenazines *in vitro* and that improved current was generated in the BES by engineering the over-expression of this enzyme. To confirm phenazine uptake into the cytoplasm, we developed two molecular biosensors, which can detect the presence of different phenazine variants inside the cell.

These findings therefore give a clearer picture of the molecular mechanism of MET.

Keywords: Phenazines, mediated electron transfer, bioelectrochemical system, Pseudomonas

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Microbial Biotechnology, 2021. https://doi.org/10.1111/1751-7915.13827

Degustació de pernil a diferent voltatge de l'ànode: The influence of the anode potential on single cell yield coefficients of Geobacter sulfurreducens

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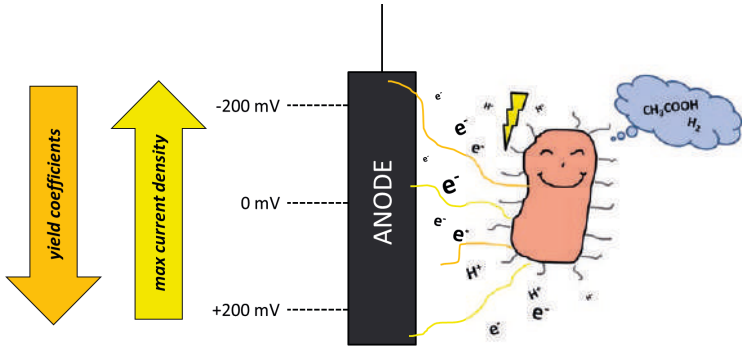
Geobacter sulfurreducens is the model organism for direct extracellular electron transfer (EET). Although several details of the molecular mechanisms of EET are deciphered [1,2], there is still a significant lack of knowledge (e.g. kinetics, yields). Here we provide information about the influence of the anode potential on coefficients at single cell level, i.e. the moles of electrons transferred to the anode, per replication for early-stage biofilm electrodes. This information is highly relevant for physiological as well as technical considerations and not known yet for *Geobacter* growing at anodes [3]. Therefore, single and double chamber BES were operated in batch mode using as anode material i) the standard graphite and ii) a microscopy slide sputter-coated with 10 nm of Chromium (Cr) and 25 nm of Gold Palladium (AuPd) enabling the analysis of the biofilms with CLSM.

We demonstrate that the anodic potential has a strong influence on the kinetics of growth as well as on the number of electrons transferred for replication, but does not influence other parameters like formal potential (E_f) of the EET and coulombic efficiencies (CE). Like in ham degustation, where the taste is used for choosing the best ham, here the anode material and potential are used.



Keywords: electroactive microorganisms, extracellular electron transfer, anode potential, yield coefficients.

Funding: This work was supported by the Helmholtz-Association in the frame of the Integration Platform "Tapping nature's potential for sustainable production and a healthy environment" at the UFZ.

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In situ and non-destructive real time microscopic investigation of multi-species electroactive biofilms on transparent microfluidic BES

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Our research interest is focused on improving the durability of multi-species microbial anodes in BES. A large number of studies have indeed documented anodic current densities that are limited or drop after a few days or tens of days of operation [1,2,3]. Identifying what occurs at the anode-biofilm interface, from the attachment of the pioneer bacteria and during the colonisation and maturation of the biofilm on the anode, might provide novel explanations for the decrease of EABs electrochemical activity. To this end, our original approach of local investigation combines microfluidic technologies, electroanalysis and real-time microscopy to focus the exploration at the micrometer scale surface of stainless steel microelectrodes. A transparent microfluidic BES was developed using ITO conductive glass and stainless steel microwire as electrodes. This design allows non-invasive and real-time investigation of local phenomena such as bacterial adhesion, cells mobility and biofilm growth by simultaneous electrode polarization, hydrodynamics and electrolyte composition (pH, substrate load) control. This approach is further complemented by more conventional destructive analyses (microbial community, cell viability, EPS composition) to link bacterial and bioelectrochemical dynamics of the biofilm. We have identified that bacterial cells require a growth state in order to express high electroactivity

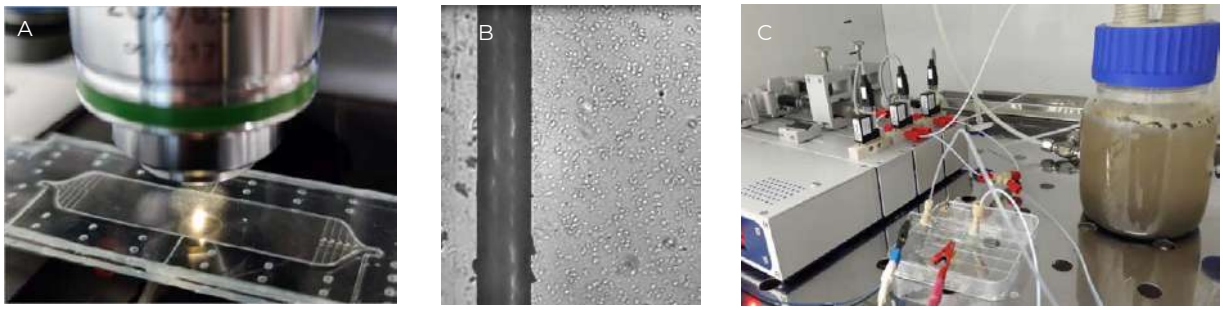

Fig. 1 (A, B) Real-time microscopic monitoring of the anode of a microBES colonized by multi-species EAB. (C) Experimental platform for the formation of multi-species EABs at the microfluidic BES.

Keywords: Electroactive biofilms, Stainless steel and platinum microelectrodes, Real-time microscopy, Microfluidic cells, Bioelectrochemical systems

Funding: Projet-ANR-18-CE05-0024

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Magnetic resonance imaging as a tool for the analysis and optimization of electroactive biofilms inside porous electrode structures

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Information about the morphology of the electroactive biofilms and the occurring transport phenomena is required to obtain a deeper understanding of limiting processes and develop improved electrode materials. Magnetic Resonance Imaging (MRI) enables the spatially resolved measurement of biofilm distribution and transport characteristics, even inside optically opaque porous electrode materials.

We present an MRI compatible reactor for the *in vivo, in situ* and *in operando* analysis of electroactive biofilms on a macro- and mesoscale (down to 50 µm). In detail we depicted electroactive model biofilms of *Shewanella oneidensis* MR-1 inside electrospun nanofiber electrodes. The structure and the transport phenomena are analyzed in terms of relative transversal relaxation times T_2 and relative apparent diffusion coefficients ADC, both normalized to their value in the bulk medium. Inside the biofilm, T_2 as well as ADC were found to be lower than in the medium.

In the future we will use MRI to quantify the biofilm density distribution, the occurring transport phenomena, and the metabolic changes of concentrations of nutrients and products. The acquired data can be used to develop accurate models of electroactive biofilms and may serve to tailor electrode materials.

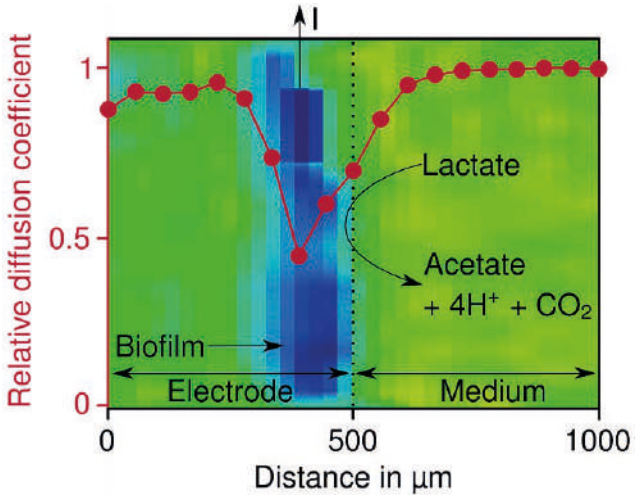

Fig. 1: Graphical abstract: Morphology of the biofilm and diffusion coefficients can be spatially obtained by MRI inside a porous electrode. The biofilm colonizes the medium facing fraction of the electrode and thus diffusion is hampered.

Keywords: Electroactive biofilm, porous electrode, magnetic resonance imaging, diffusion coefficient, biofilm structure.

Influence of different photocatalytic dopants in the antifouling performance of Nanofiltration Microbial fuel cells

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Microbial fuel cells (MFCs) have been considered as a promising candidate to tackle the contradiction between effluent treatment and energy conservation [1]. The high cost of some materials, such as membranes, has led researchers to explore alternatives. The use of ultrafiltration (UF), direct osmosis (FO) membranes has provided interesting alternatives. With pore diameters between 10 and 100 Å, NF membranes lie in a transition region between UF and FO. To our knowledge, NF membranes have never been tested in MFCs and could be considered interesting candidates [2].

Moreover, fouling of the membranes also presents a problem in all configurations. For this purpose, nature-inspired modifications using polydopamine (PDA) [3] or semiconductors such as TiO_2 and ZnO provide the membrane capabilities to oxidize organic matter that tries to adhere to the surface, which provides self-cleaning and antifouling performances.

The present work compared the benefits of bio-inspired modification of commercial membranes with PDA and TiO_2, ZnO nanoparticles, as well as the development of a ZnO photocatalyst doped with Al^{3+} and Er^{3+} to enhance visible light absorption, favoring the antifouling performance of these Nanofiltration MFC systems.

Keywords: antifouling, nanofiltration, microbial fuel cells, membranes.

Funding: (ARES) (RFCQ-CQ-SO13-186-2017)

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Development and application of conductive nanomaterials for bioelectrochemical denitrification

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Conductive nanomaterials have gained immense interest because of their distinctive properties and their potential in numerous fields. A relevant example is their potential use as electrodes in hybrid systems of biotechnology and electrochemistry, such as bioelectrochemical systems (BES). Within this work, different approaches for conductive nanostructure preparation were employed. Electrostatic spinning (Nanospider™) was used as a method for nanofiber preparation. Polypyrrole, polyaniline, Fe_3O_4 nanoparticles and aluminium were selected as conducting components for nanostructures. Nine nanomaterials were prepared and characterized by scanning electron microscopy and electrical resistance measurements. However, only three of them were able to transfer electrical current. Conductive nanomaterials were further tested as a support for electroactive denitrifying bacteria in lab-scale single cell BES reactors. The performance of the reactors with tested nanostructured electrodes was evaluated in terms of nitrate removal and bioelectrochemical activity (chronoamperometry and cyclic voltammetry), and compared to commonly used electrode materials such as carbon cloth and graphite rod. Denitrifiers were selectively enriched depending on the material used. A gradual decrease of nitrate concentration was observed when PVB nanofibers with polyaniline or polypyrrole were used as electrodes. These conductive nanomaterials have shown potential for biological removal of nitrate in low organic-content water using BES reactors.

Keywords: conductive nanomaterials, cyclic voltammetry, denitrification, microbiome selection

Funding: This work was supported by the European Union's Horizon 2020 project ELECTRA (no. 826244).

The role of bioanode-membrane interlayer in tubular Ceramic Microbial Fuel Cells (CMFCs)

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CMFCs are an interesting alternative to conventional systems, as they replace polymeric membranes with low-cost and sustainable ceramic materials. However, their development requires the optimization of some aspects such as the ohmic overpotentials associated with the membrane and the contact between components. Although, in the case of cathodes, the use of conductive carbon inks is common for improving the membrane-cathode electrical contact [1], their application in bioanodes has hardly been investigated.

In the present work, the influence of a conductive interlayer at the interface between ceramic membrane and bioanode is analyzed in tubular CMFCs. The deposition of the interlayer on the ceramic membrane was carried out by advanced ceramic processing techniques, identifying the optimal electrical and morphological conditions of the layer. Three designs of CMFCs were studied (fig. 1): carbon felt bioanode directly in contact with the ceramic membrane (MFC1); interlayer acting as anode (MFC2); bioanode and membrane connected by interlayer (MFC3, not shown). Moreover, MFC2 represents a new “one piece” configuration CMFC.

Electrochemical techniques allowed to evaluate the role of the interlayer in resistive terms. In addition, a morphological study of the different bioanodes will be carried out by SEM, paying attention to the membrane-bioanode interface and the biofilm formed.

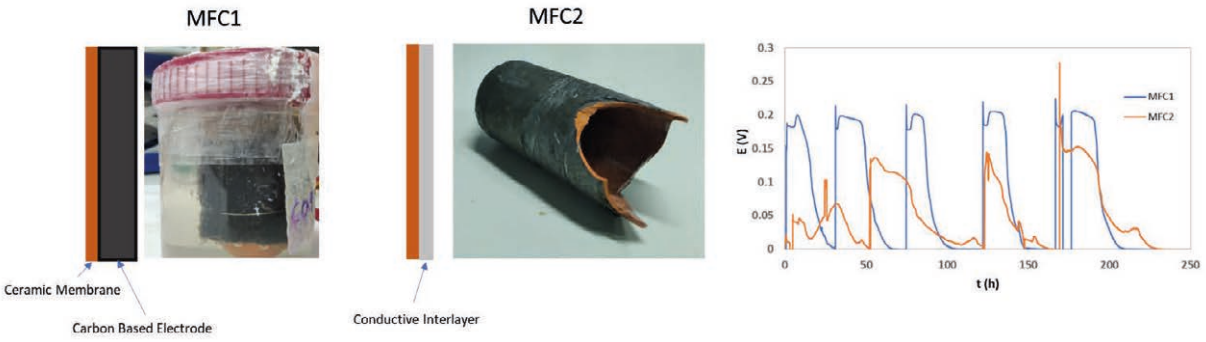

Fig. 1 Left: A) MFC 1 Design B) MFC2 Design C) Potential versus time response for MFC1 and MFC2

Keywords: Ceramic-MFC, Bioanode-Membrane Interlayer, “one piece” configuration.

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Development and upscaling of gas diffusion electrodes for wastewater treatment and electrosynthesis of chemicals

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Technologies related to gas diffusion electrodes (GDEs) offer solutions for gaseous reagents taking part in electrochemical reactions. (Bio)electrochemical processes suffer from challenges like high costs of platinized electrodes[1]; rapid catalyst degradation and low performance due to non-uniform electrode quality[2]; difficulties in upscaling. Scaling up microbial fuel cells (MFCs) requires use of large electrodes which are often difficult to fabricate without loss in quality. VITO has developed GDEs tailored for systems with aqueous electrolytes and a gas-water interface, which are characterized by controllable pore diameters in the polymer-bound active layer, mechanical robustness and low water permeability[2]. These cold-rolled (VITOCORE®) and phase-inversion based (VITO CASE®) electrodes enable reproducible quality in sizes from 10 cm^2 to 1 m^2. Large-scale VITOCORE® air cathodes were recently developed and tested in 85 L and 255 L MFCs to evaluate the impact of the cathode size on MFC performance[4,5]. For CO_2 electroreduction, GDEs based on Sn, Cu and Pd were developed and evaluated for production of formic acid and oxalic acid.

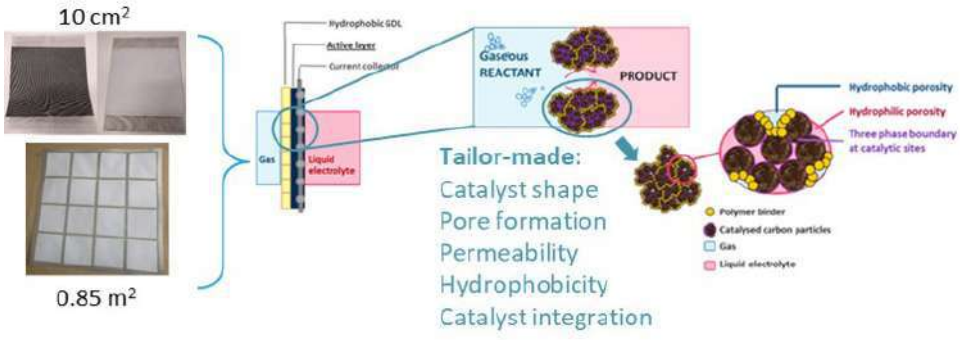

Fig. 1. VITO CORE® GDEs as air cathodes

Keywords: Gas diffusion electrodes, microbial fuel cells, microbial electrosynthesis, resource recovery, CO_2 electroreduction

Funding: E2C Project (Interreg), Bac-To-Fuel Project (Horizon 2020)

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Capacitive bioanodes in fixed and moving bed reactors

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Capacitive bioanodes can be used to improve current production in microbial electrochemical systems. Here, we studied the use of activated carbon as capacitive bioanodes in fixed and moving bed reactors. In a fixed bed reactor, most capacitive charge was recovered from the current collector closest to the membrane. Increasing bed thickness from 5 mm to 10 mm resulted in a 1.6 times higher current density per membrane area. These findings were used to improve the design of a moving bed reactor, where activated carbon granules with electro-active biofilm moved through a discharge cell and were recirculated using a gas lift. This moving bed bioanode produced a current of 43 A/m^2.

Discharge properties were further studied in abiotic experiments. Capacitive discharging was improved most by changes in potential difference between current collector and charged granules (ΔE 0.3 and 0.5 V). Discharging from both sides of the granular bed, as compared to discharging from one side, increased the transferred charge, irrespective of increasing the electrolyte conductivity. This showed that electrical resistance was more important in determining the transferred charge than ionic resistance. Analysis of the discharging process showed that discharging increased the local conductivity through the release of ions from the granules.

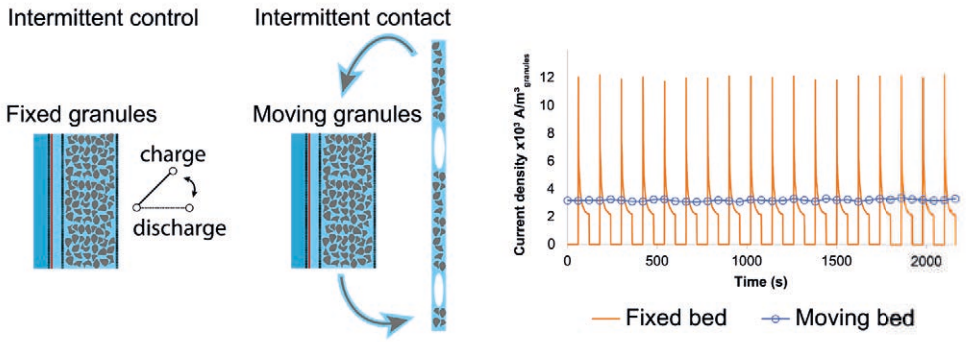

Fig. 1 The discharge behaviour of capacitive granular bioanodes was tested in fixed and moving bed reactors

Keywords: Capacitive bioanodes, moving bed, fixed bed

Graphene functionalization with metallic Pt nanoparticles: envisaging future cathodes for bioelectrochemical H_2 production

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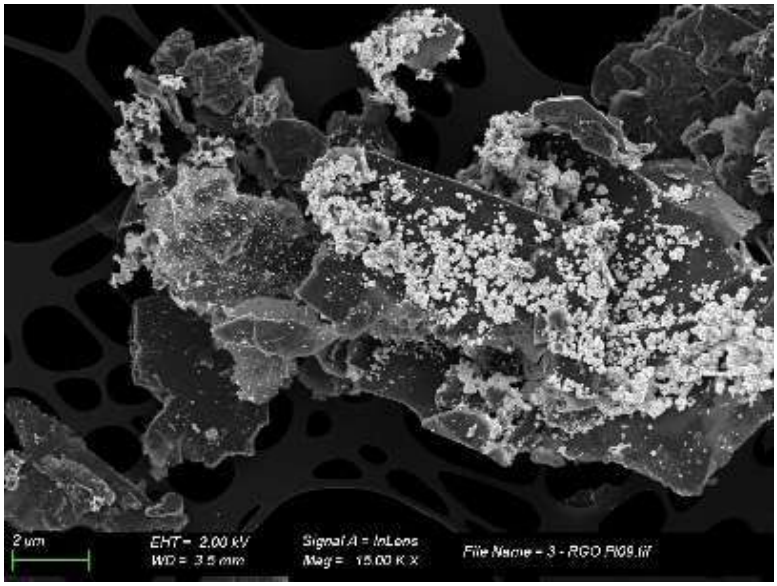
Platinum (Pt) is one of the most widely used catalysts in Microbial Electrolysis Cells (MEC) to overcome the relatively slow kinetics of hydrogen evolution. Pt is commonly applied as a commercial ink (90 % carbon/10 % Pt) [1], but this strategy is not economically feasible at large scale.

This work describes the development, application, characterisation, and optimisation of two novel inks with novel carbon materials functionalised with Pt: reduced graphene oxide (Pt@rGO) and graphitene (Pt@Graphitene). These materials showed better performance by increasing the catalytic effect over conventional inks, reaching a peak of the current density of *ca.* 0.8 A/m^2. This is almost double the average value using the same cell and commercial ink [2].

The percentage of Pt functionalization was higher than similar works: almost 20 %. The full presentation will show a comprehensive characterisation of both materials with optical (scanning electron microscopy and inductively coupled plasma mass spectrometry) and electrochemical (cyclic voltammetry and impedance) techniques.

For instance, the electroactive area was 1.14 cm^2 for Pt@rGO and 0.69 cm^2 for Pt@Graphitene and the surface area measured were 10.76 m^2/g for Pt@Graphitene and 24.40 m^2/g for Pt@rGO. These values are twice the surface area values of same materials without functionalization.

Graphene functionalization with metallic Pt nanoparticles: envisaging future cathodes for bioelectrochemical H_2 production



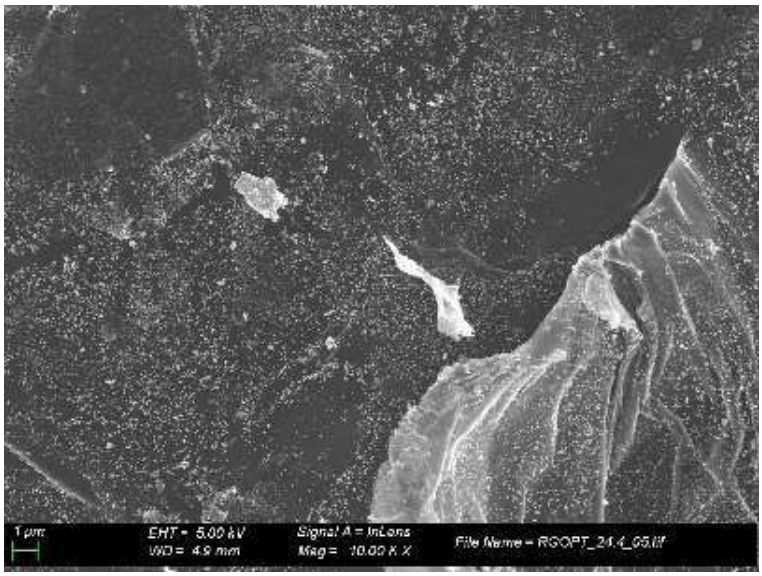


Fig. 1 SEM images of different materials. Pt@rGO (a) and Pt@ Graphitene (b).

Keywords: Platinum, Reduced graphene oxide, Graphitene, Single-chamber membrane-less microbial electrolysis cell

Funding: FI predoctoral scholarship (2018FI_B01161) from the Catalan Government (Agencia de Gestio d'Ajuts Universitaris i de Recerca). This work was supported by the Spanish Ministerio de Economía y Competitividad (CTQ2014-60495-R, CTQ2017-82404-R) with funds from the Fondo Europeo de Desarrollo Regional (FEDER). The authors are members of the GENOCOV research group (Grup de Recerca Consolidat de la Generalitat de Catalunya, 2017 SGR 1175, www.genocov.com).

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Estructures superficials com les d'Antoni Gaudi: Platinized titanium as cost-effective anode material for Kolbe electrolysis

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A circular economy is based on linking the sector of electric power production and storage with the sector of production of chemicals and fuels [1]. This link can be created by the Kolbe electrolysis allowing the electrochemical conversion of medium chain carboxylic acids (MCCA) to *n*-alkanes [2]. Pure platinum is the archetype anode material for Kolbe electrolysis. Only few other materials were studied but are not commercially available and less efficient than monolithic Pt [3–7]. On industrial scale cheaper materials being available in large quantity are required.

Hence, the suitability of five electrode materials for the Kolbe electrolysis of *n*-hexanoic acid to *n*-decane was tested. Platinized titanium stood out with a Coulombic Efficiency (*CE*) of 93.1 ± 6.7% (n=6) for *n*-hexanoic acid degradation and 48.3 ± 3.2% (n=6) for *n*-decane production which is comparable to pure platinum (89.7 ± 14.4% and 55.5 ± 3.5%; n=6). An economical evaluation showed that platinized titanium requires >36 times less capex at only <10% increased opex. Using SEM coupled with EDX structure-function-relationships could be revealed, showing that 1-3% of uncovered surface already causes a ~50% decrease in *CE*. Thus, the degree of coverage with platinum plays the most important role when using platinized anodes.

Estructures superficials com les d'Antoni Gaudi: Platinized titanium as cost-effective anode material for Kolbe electrolysis

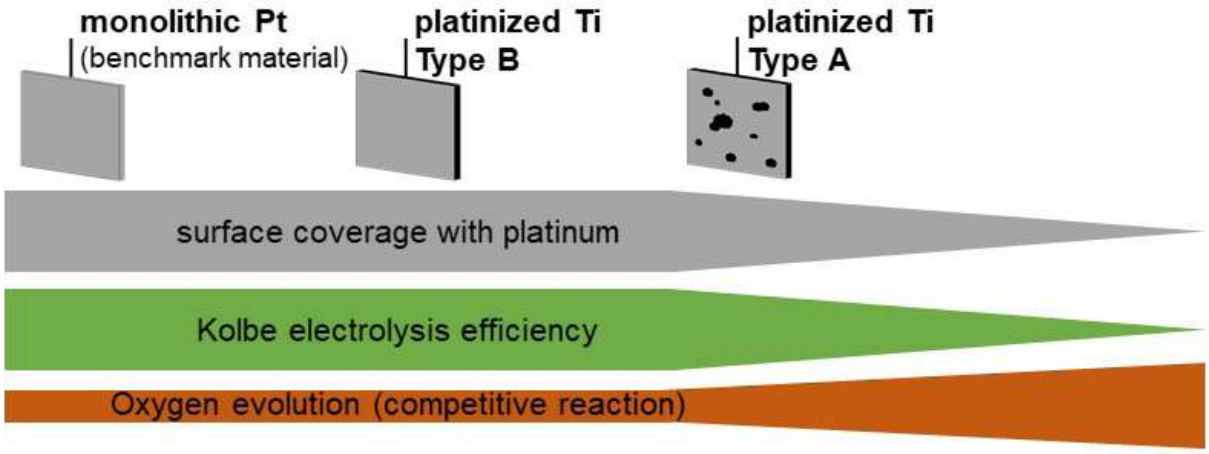

Fig. 1: Relationship between surface coverage, Kolbe electrolysis efficiency and oxygen evolution efficiency (competitive reaction) for the benchmark material monolithic platinum and two different platinized titanium materials.

Keywords: drop-in fuel, power-to-fuel, power-to-chemicals, electroorganic synthesis

Funding: This research is financed by the German Federal Ministry of Education and Research (BMBF) under the VIP+-project Molkekraft: Microbial-electrochemical exploitation of sour whey for the production of drop-in aviation fuel (Funding code: 03VP06911). This work was supported by the Helmholtz-Association in the frame of the Integration Platform "Tapping nature's potential for sustainable production and a healthy environment" at the UFZ. The authors are thankful for the use of the analytical facilities of the Centre for Chemical Microscopy (ProVIS) at UFZ Leipzig, which is supported by European Regional Development Funds (EFRE – Europe Funds Saxony) and the Helmholtz Association.

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The applicability of the Maximum Power-point of Microbial Fuel Cells: Influence of Potential Scan rate and real-time external Load

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Performance evaluation of a microbial fuel cell (MFC) is usually done with linear sweep voltammetry (LSV) [1] at a given potential scan rate (PSR) [2]. This evaluation does not often reflect the long-term performance of the MFC under real-time external loads [1]. In this study, the performance of a single-chamber MFC was evaluated with three external loads (1206, 470, and 270 Ohms) calculated from LSV maximum power point (MPP) with three PSRs (0.1, 0.5, and 1 mV/s). The estimated power from the MPP in ascending order of PSR were 61.96, 87.88, and 166.68mW/m^2 at 116.5, 229.6, and 403 mA/m^2, respectively. The average power obtained with 1206, 470, and 270 Ohms in the first two hours of operation were 73 ± 16.7, 36.3 ± 42, and 88.5 ± 120.1 mW/m^2 at current densities of 124.6 ± 14.3, 121.2 ± 73.4, and 232.6 ± 176.2 mA/m^2, respectively. The result showed that overestimation was more pronounced at higher PSRs. Although the MFC was initially underestimated at 0.1 mV/s, this PSR more accurately reflects the true and applicable estimate of the long-term performance of the MFC. These results are explicitly beneficial for electrochemical estimation of the actual performance of MFCs under real-time external loads.

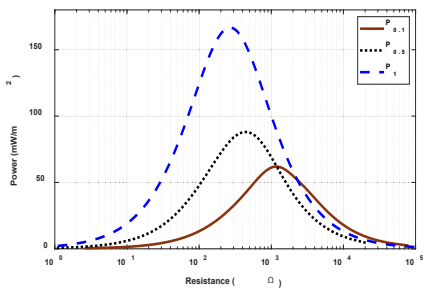

Fig. 1. Maximum power at different scan rates

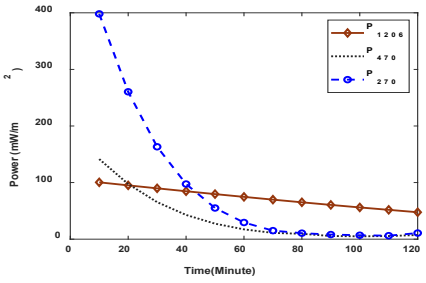

Fig. 2. Short-term performance at different external loads

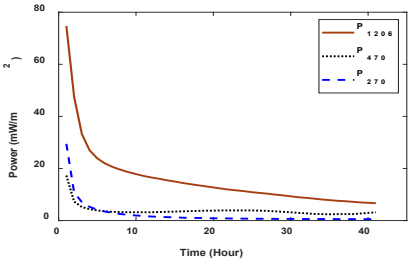

Fig. 3. long-term performance at different external loads

Keywords: maximum power, microbial fuel cell, external load, potential scan rate

Funding:Nigerian-German Postgraduate Training Programme PhD, 2018 (57401043)

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Power to Gas: newsworthy connection between electricity production trend, biochar electrodes and polarizations

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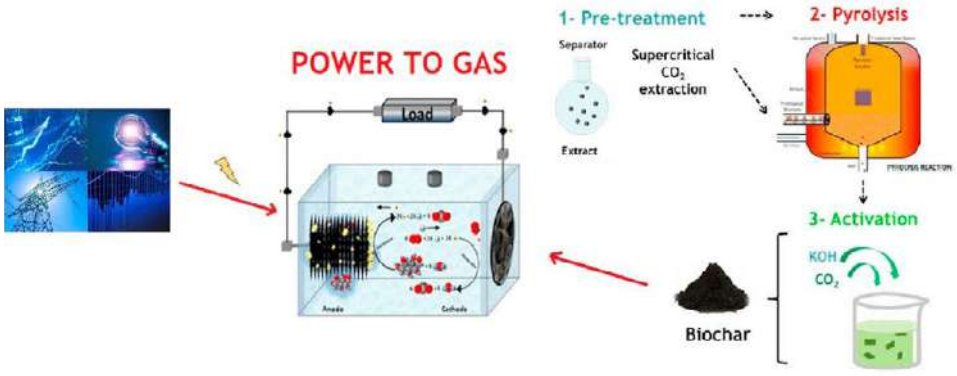
In the near future, renewable electricity produced will be considered as primary sources of energy and its cost and sustainability will soon stably meet the grid parity. Electricity storage capacity will be the real challenge, to buffer intermittent productions and consumptions (Breyer and Gerlach, 2013). Nowadays also biomasses sourced from agricultural by-products have been touted as an important feedstock for the production of high-value materials, chemicals and fuels to promote a circular economy and zero-waste production (Walmsley et al. 2019). In other hands, the hydrogen production is taking an important place into the biofuels scenario. In this study, biochars derived from olive mill waste (OMW) with and without supercritical CO_2 pre-treatment are produced via pyrolysis and/or chemical and physical activation. Different activated biochars are used to manufacture electrodes for the hydrogen production by bioelectrochemical system (BES). The electrodes produced from different biochars are characterised by a series of physical, chemical and electrochemical measurements as the BET surface area (444 - 742 $m^2 g^{-1}$). In this experiment are tested 5 different electrodes (2 different OMW activated biochar, carbon black, black pearl and stainless steel mesh) at 2 different polarizations (600-800 mV *vs* Ag/AgCl reference electrode). The study aim is the increasing of the hydrogen and methane production from organic-rich wastewater, decreasing of the energy demand to generate the cathodic reaction thanks to the biochar electrodes.



Keywords: bioelectrochemical system, power to gas, hydrogen, methane, biochar

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Copla electroquímic: How sound is Electrochemical Impedance Spectroscopy on biofilm electrodes?

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Electrochemical impedance spectroscopy (EIS) is a versatile tool to examine characteristics of electroactive biofilms that are not accessible by direct current measurements such as chronoamperometry or cyclic voltammetry [1]. Several studies show the determination of, e.g. biofilm resistance and biofilm capacitance [2-3]. However, EIS in microbial electrochemistry is sometimes applied superficially or evaluation of presented data is not comprehensive due to misinterpretation or missing data validation. This hinders a more widespread application of this powerful method, not only for determination of specific biofilm electrode parameters, but also - from a more practical perspective - as tool for in situ condition monitoring of biofilm electrodes.

With this contribution, we introduce basics of EIS on biofilm electrodes and discuss how the experimental setup, e.g. electrode material or magnetic stirrers can influence EIS data. Furthermore, we elaborate on the necessity of data validation as well as careful data interpretation using electrical equivalent circuit models. For the latter we differentiate in whitebox and greybox models that can lead to conclusive data and meaningful insights or applications of EIS on biofilm electrodes [4].

Keywords: microbial electrochemical technologies, electroactive microorganisms, exoelectrogens, alternating current.

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Electrochemical characterization of the anodic biofilm activity in a methane-producing microbial electrolysis cell

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The electro-activity of the biofilm established at the anode of a fully biological methane-producing microbial electrolysis cell (MEC) is crucial to the overall process performance [1]. Indeed, the energy required to sustain the bioelectromethanogenesis reaction at the MEC cathode is partially provided by the anodic oxidation of (waste) organic substrates [2]. Also, the understanding of the biofilm response to operational fluctuations (such as the lack of substrate availability) is of fundamental importance for the industrial deployment of this technology.

This study analysed, primarily by means of cyclic voltammetry, the time-dependent evolution of the electrogenic activity of a biofilm enriched (from an activated sludge) at the anode of a lab scale two chamber MEC (ca. 1.7 L total volume), with the cathode finalized to methane generation. Notably, after a long period of starvation (about two months), the anodic biofilm fully recovered its electrogenic activity in less than one week after the feeding solution (consisting of a synthetic mixture of glucose, peptone, and acetate) was replenished. Clearly, methane production at the cathode was strongly dependent on the electrogenic activity of the anodic biofilm. Finally, the effect of the cathode temperature on the kinetics of electrotrophic methane production is currently being assessed.

Keywords: electrogenic activity, starvation, cyclic voltammetry, bioelectromethanogenesis

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The effect of the cathode current collector on the performance of a yeast-based air cathode microbial fuel cell

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The successful deployment of microbial fuel cells depends on the development of cost-effective designs. The air cathode design is attractive since the air provides oxygen to the cathodic reaction by natural transfer mechanisms. However, the reaction kinetics is slower, limiting the cell performance [1]. The number of studies on how different current collector materials/structure can affect the oxygen transfer and the electrical conductivity is reduced [2]. Yet, current collectors are important components to fuel cells, providing electrical conductivity from the anode to the cathode, oxygen supply to the cathodic reaction and structural support to water pressure from the anode. The higher electrical conductivity of metals such as stainless steel (1.33×10^7 S/m) and titanium (1.79×10^7 S/m) should be considered over carbon [3]. Besides, metals present better mechanical strength which is an advantage in large-scale designs.

This work investigated the influence of the cathode current collector area/material on an air cathode microbial fuel cell performance inoculated with *Zygosaccharomyces bailii* and fed with a synthetic winery wastewater. Two pore sizes (3 and 6 mm) of titanium and stainless steel were tested. The best performance was achieved using a stainless steel plate with a pore size of 6 mm (41% of open ratio).

Keywords: air cathode microbial fuel cell, current collector, *Zygosaccharomyces bailii*, winery wastewater, power output.

Funding: The authors acknowledge the PhD scholarship (PD/BD/114314/2016) supported by "Fundação para a Ciência e a Tecnologia" (FCT), by "Fundos Nacionais do Ministério da Ciência, Tecnologia e Ensino Superior" (MCTES), inserted in the program "Programa Operacional Capital Humano" (POCH), cofinanced by Portugal 2020 and European Social Fund (ESF). The authors also acknowledge the national funding of FCT/MCTES (PIDDAC). POCI (FEDER) also supported this work via CEFT and LEPABE.

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Scaling Up of Electro-Stimulated Anaerobic Reactor (ELSAR™) in the industrial wastewater treatment sector

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On behalf of the EU project ULTIMATE (https://ultimatewater.eu/), Aqualia is developing the upscaling of the ELSAR™ (*Electro-Stimulated Anaerobic Reactor*) to be operated at Mahou San Miguel brewery in Lleida (Spain). The reactor designed will be able to treat up to 20 m^3/h or 2000 kg COD/d (Figure 1).

The main milestones along the ideation and development of this technology are:

The original concept of the ELSAR™ system was co-developed and patented ("Method for treating wastewater in a fluidized bed bioreactor" (Ref. EP 2927196 A1)) by Aqualia and the University of Alcalá (UAH).

Performance lab and bench tests on behalf of the LIFE Answer project (http://life-answer.eu/) showed that ELSAR™ outperforms anaerobic fluidized bed reactor (AFBR) either under standard operational conditions or under stress operational tests (COD overload, biocide dosing, long starvation periods and operation at low temperatures) [1]. Long-term operation test was carried out with a 0.5 m^3 prototype operated in real conditions.

A demo-scale 1m^3 ELSAR™ is in operation since more than a year to validate the technology in a relevant environment treating municipal wastewater in Guijuelo (Spain, Figure 1).

Fig. 1. Demo-scale plant in Guijuelo (left) and 3D model of the demonstration WWTP based on ELSAR™ (right).

Keywords: anaerobic digestion, bioelectrochemistry, hydrogen, wastewater treatment, microbial electrochemical technologies

Funding: The project leading to this application has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement No 869318.

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Competitive advantages of a circular bioeconomy based on microbial electrosynthesis and CO^2

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Since the 18th century industrial revolution, fossil fuels have been the cornerstone of society. We have however reached a critical decade where deep cuts in greenhouse gas emissions will necessitate a fundamental shift in material foundation of our society. Inspired by the natural world, a circular carbon bioeconomy holds promise for recycling carbon dioxide into chemical, agricultural and fuel products (**Figure 1**). We developed a simplified technoeconomic framework based on levelised costs of chemicals (LCCs) to critically review two approaches in this space: microbial electrosynthesis and gas fermentation (**Figure 2**) [1]. Due to fundamental limits on acetogen electron uptake rate [2], we show the only viable pathway for scale-up of microbial electrosynthesis is through decoupling surfacedependent abiotic process for electron/hydrogen delivery from volume-dependent biotic carbon fixation, which effectively is an *electrolyser-assisted gas fermentation system*. Even then, high-value, but critically also high-volume, products are needed for economic viability such as butanol and single cell proteins (SCP) for human food consumption (as opposed, for example, to low-volume caproate). In keeping with the circular carbon bioeconomy ethos, we also suggest linking waste resource recovery with gas fermentation, though new technology such as reforming of biogas, as another potentially viable scale-up strategy.

Competitive advantages of a circular bioeconomy based on microbial electrosynthesis and CO^2

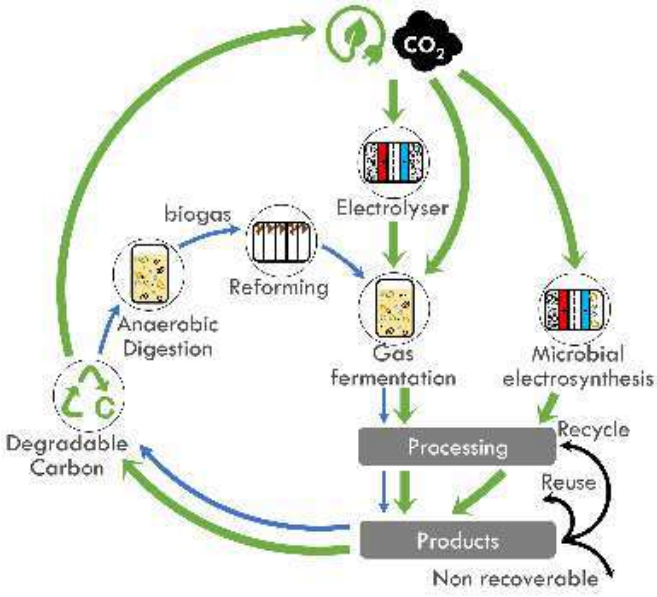

Figure 1. Circular carbon bioeconomy (green, blue), showing a closed carbon loop. There is minimal loss from the system, unlike the traditional economy.

Synthesis Technology		*1*	*2*	*3*	
Product	*Market (US$/kg)*	*LCC*			
Acetate	0.65				
Ethanol	0.48				
Caproate	3.70				
Butanol	0.92				
SCP	15.00				

Figure 2. LCC for select chemicals, colour coded according to how close the calculated value is to the market value. Production routes: **1** = microbial electrosynthesis, **2** = electrolyser-assisted gas fermentation, and **3** = biogas reforming-assisted gas fermentation. Data from [1].

Keywords: techno-economic analysis, circular economy, biogas, electron transfer

Funding: BV and ZY acknowledge the support of the Australian Research Council (ARC) for grants FL170100086 and DP160102308. EM acknowledges the support of ARC CoE in Synthetic Biology.

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Wastewater treatment with a 1000 L microbial fuel cell: a 16-month experience

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The construction of large microbial fuel cells (MFCs) and their long-term reliability are current challenges. MFCs generate power while purifying wastewater, save electricity, avoid pollutant stripping into the air and are a source of CO_2. To understand larger MFCs, a 1000-L MFC was designed. It was built from transparent polyester and electrodes were from reticulated vitreous carbon. Four power management devices were connected to an ensemble of 64 MFC units and assembled as a 12 m long MFC. Two Raspberry and a personal computer with Python programmed software automatized power management. The MFC was run for one year under maximum power point tracking (MPPT) [1]. Temperatures between 11.5 °C and 21 °C corresponded to WWTP conditions. The reactor shared electrolytes within 12 m long half-cells and 80 - 95% COD was removed generating 0.015 to 0.060 kWh/m³ with an energy efficiency of 5.8 - 12.1%. Voltage reversal were seen as potential imbalances among MFC units and all self-healing. Ammonium removal reached 48%, phosphorous was reduced to 0.59 mg/L, and micropollutants degraded by 67%. Biofilm mapping by 16S rRNA microbial community analysis indicated bi-sectorial metabolic properties. 10 Major genera were essential in the elongated scale up MFC generating electricity, reduced energy needed, and purified wastewater.

Fig. 1: 1000-L Microbial fuel cell in Wastewater Treatment Plant

Keywords: bioelectricity, scale-up, metabolism, wastewater, tracking, metagenomics

Funding: Swiss Federal Office of Energy (SI/501573-01) and “The Ark” foundation.

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Role of the C/N ratio in the feeding solution of a micro pilot microbial electrolysis cell aimed at biogas upgrading

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Microbial electrolysis cells (MECs) permit to couple the oxidation of waste organic streams (e.g., wastewater or digestate) with the reduction of carbon dioxide into products with a high market value (e.g., methane or acetic acid). MECs exploit the ability of electroactive microorganisms to use a solid electrode as final electron acceptor or donor. Here, a micro pilot tubular MEC has been set up combining the anodic oxidation of the organic matter with the bioelectromethanogenesis reaction in the cathodic chamber. Seven different synthetic feeding solutions, simulating a domestic wastewater or an acidogenic fermentate, have been used to test different C/N ratio on the performance of the MEC bioanode in the range between 25 and 0.4 (molC/molN). As a main result it was found that, under the same operating conditions (i.e. anode potential controlled at +0.2 V vs SHE and HRT of 0.5 d), a high C/N ratio (e.g., 19 mol/mol) corresponded to an average current production of 288 ± 32 mA, that decreased to 161 ± 10 mA by applying a C/N ratio as low as 0.4 mol/mol. These findings are relevant for a practical application of the technology considering the variable content of carbon and nitrogen in real feedstocks.

Keywords: Biogas, upgrading, C/N ratio, Anodic biofilm, Pilot scale

Funding:This work has been carried out with the financial support of the NoAw project. “This project has received funding from the European Union’s Horizon 2020 research and innovation programme under grant agreement No 688338”.

IoT biosensing: a microbial electrochemical sensor for monitoring water quality

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Microbial Electrochemical Technologies boosted the potential applications of biotechnology based on electroactive microorganisms. Regarding the field of microbial sensing, the use of electroactive biofilms gathers the benefits of working with whole cells (low cost) and no need of using transducer elements of the metabolic activity [1,2].

Microbial electrochemical sensors are potentially good candidates for water quality monitoring. However, there are still some challenges to face that hinder the final step into the commercialization of these devices.

Main issues are related to power consumption and the limited number of sensing analytes. Despite the possibility to be self-powered (MFC), the performance as biosensor is poorer when this configuration is chosen [3]. The reason remains in the lack of potential control.

Authors have addressed these issues designing and constructing an electrochemical biosensor so-called IoT biosensing ready for market uptake. This sensor consists of a low power potentiostat, an online data acquisition and 4G transmission module, and a three electrode bioelectrochemical system, all powered by a photovoltaic solar panel and a battery. Progress achieved during monitorization of water quality in relevant environments like real wastewater treatment plants and groundwater are shown.

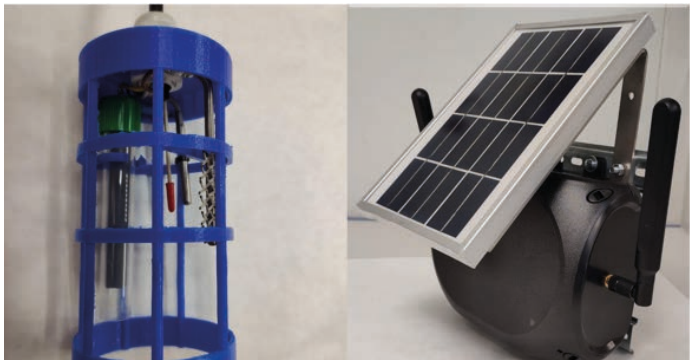

Fig. 1 A) IoT microbial electrochemical biosensor for in situ and online monitoring of water quality.

Keywords: microbial electrochemical sensor, water quality, 3D bioprinting, IoT, online monitoring

Funding: 4KET4Reuse project, Interreg Sudoe program (SOE1/P1/E0253), PRINTBIO project, H2020 ATTRACT program (N° 777222).

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Crossing the wall: extracellular electron transfer in Gram-positive bacteria

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Extracellular electron transfer (EET) is a metabolic process that enables electroactive bacteria to exchange electrons with electrodes in bioelectrochemical systems (BES). The understanding of the EET processes performed by these organisms is crucial to advance the implementation of BES, as well as advance their biotechnological application [1,2]. Gram-positive bacteria have attracted the scientific interest, given their ability to produce high levels of current in MFC, in particular at high temperatures [3,4]Thermincola ferriacetica Z-0001, was assembled at ~100× coverage from 100-bp paired-end Illumina reads. The draft genome contains 3,274 predicted genes (3,187 protein coding genes. The EET pathway of Gram-positive bacteria belonging to *Thermincola* sp. is composed by four multiheme *c*-type cytochromes: the inner-membrane decaheme cytochrome A (ImdcA); the periplasmic decaheme cytochrome A (PdcA); the cell-wall cytochrome A (CwcA) and the outer cell-wall cytochrome A (OcwA) [5,6]. The range of redox potentials in which ImdcA, PdcA and OcwA from *T. ferriacetica* are electrochemically active are adequate to extract electrons from low-potential quinone pools in the membrane and transfer them to insoluble electron acceptors, outside of the cell (Figure 1) [6,7].

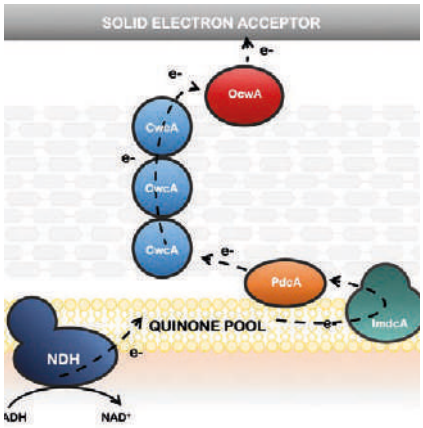

Fig. 1 Representative scheme of the EET pathway of *T. ferriacetica*

Using sequence homology modelling the 3D-architecture of CwcA is proposed, showing a novel potential pathway for electrons to be transferred across the thick peptidoglycan cell-wall. The results presented here demonstrate for the first time the EET pathway by which bacteria belonging to *Thermincola* sp. transfer electrons to extracellular electron acceptors, including electrodes in BES.

Keywords: *Thermincola*, multiheme *c*-type cytochromes, bioelectrochemical systems, electrochemical activity

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Bioelectrochemical characterization of a cytochrome belonging to the NapC/NirT family from Sideroxydans lithotrophicus ES-1

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Electroactive microorganisms have attracted significant interest for the development of bioelectrochemical systems (BES) for the sustainable production of energy and added-value products [1]. These organisms perform extracellular electron transfer which has been demonstrated to be carried out by numerous multiheme *c*-type cytochromes. One of these proteins is the membrane-bound tetraheme cytochrome *c*, CymA, a member of the NapC/NirT family of quinol dehydrogenases [2]. It has been proposed that in *Shewanella oneidensis* MR-1 CymA acts as a hub for electron transfer to multiple periplasmic partners, functioning as a quinol oxidase during Fe(III) reduction [3, 4]. Interestingly, the freshwater chemolithoautotroph *Sideroxydans lithotrophicus* ES-1 also contains a NapC/NirT family tetraheme cyctochrome containing a protein encoded by *Slit_2495* that has been proposed to function as the quinone reductase during Fe(II) oxidation based on its sequence similarity with CymA [5]. To understand the difference between these two proteins belonging to the NapC/NirT family, we heterologously expressed Slit_2495 and characterized the protein regarding its biochemical and electrochemical properties. This information is the first step in the complete elucidation of its mode of action, a knowledge that is still lacking and is crucial to use and improve this organism towards practical biotechnological applications.

Keywords: *Sideroxydans lithotrophicus* ES-1, NapC/NirT protein, CymA, Slit_2495, bioelectrochemical characterization

Funding:Doctoral fellowship PD/BD/135153/2017 integrated in the PhD Programme in NMR applied to chemistry, materials and biosciences (PD/00065/2013).

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Extracellular electron transfer in a C. ljungdahlii-based platform for microbial electrosynthesis

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Microbial electrosynthesis has recently emerged as an alternative way of biologically producing organic chemicals from carbon dioxide using electricity. These processes could play a very important role in the ecological transition substituting chemical production from fossil fuels. Nevertheless, microbial electrosynthesis is currently limited by the poor control and understanding over the biological aspects of the process. In order to increase the viability and applicability of microbial electrosynthesis, a deeper knowledge about the mechanisms of electron transfer is required. In acetogens, and more specifically in *Clostridium ljungdahlii*, biofilm-dependent direct electron transfer was initially proposed[1] while the most recent studies tend to assume hydrogen-mediated electron transfer as the main electron transfer mechanism involved[2]. In our study, we aim at the clarification of the electro-physiology of *C. ljungdahlii* and its role in hydrogen evolution, using online *in situ* data collection and altering biotic factors. With a deeper understanding of biological parameters, we seek to achieve higher planktonic densities and more robust systems that decouple growth from biofilm formation. Hence, the improvement of *C. ljungdahlii* performance in microbial electrosynthesis leads to scalable and feasible electro-fermentations.

Keywords: Microbial electrosynthesis, Hydrogen evolution, *Clostridium ljungdahlii*

Funding: Deutsche Forschungsgemeinschaft

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Charge transfer parameters through biofilms of an enhanced current-producing Geobacter sulfurreducens strain reveal increased electron transfer diffusion rate

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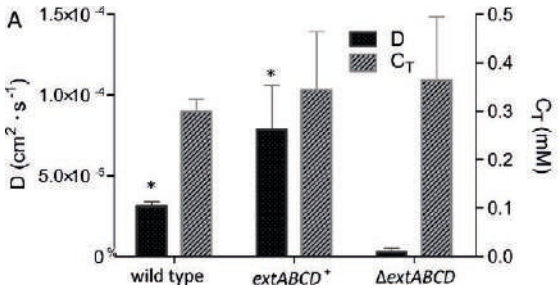
A strain of *Geobacter sulfurreducens* lacking four out of five outer membrane cytochrome complexes (strain *extABCD*$^+$) grows with a faster doubling time and produces 37% greater current density compared to wild type grown under identical conditions. Increased rates of ^{15}N incorporation and closer cell packing within the first 5 µm from the electrode in *extABCD*$^+$ biofilms compared to wild type revealed higher metabolic activity. To understand how this phenotype is sustained during extracellular electron transfer, long-distance electron transfer parameters (C_T = charge carrier concentration and D_{app} = electron diffusion rate) were measured for wild-type and *extABCD*$^+$ biofilms grown on interdigitated gold electrodes spanning 5 µm gaps. Our results reveal that *extABCD*$^+$ biofilms achieved higher current densities through an increased rate of electron transfer through *extABCD*$^+$ vs. wild-type biofilms, suggesting that denser biofilms resulting from the deletion of unnecessary multi-heme cytochromes streamlines electron transfer to electrodes. This work confirms that reducing the extracellular electron transfer pathway down to the components necessary during electron reduction is an effective strategy to construct strains producing more current per cell and, in combination with higher biofilm density and electron diffusion rates, reaching a higher final current density than wild type.



B

σ_m (S · cm^{-1})

wild type	$8.73x10^{-6} \pm 7.51x10^{-7}$
extABCD$^+$	$2.54x10^{-5} \pm 8.87x10^{-6}$
Δ*extABCD*	$9.19x10^{-7} \pm 7.86x10^{-7}$

Fig. 1 Charge transfer parameters of wild-type, *extABCD*$^+$, and Δ*extABCD* biofilms. A) Charge carrier concentration (C_T), diffusion coefficient (D), n = 3, *p = 0.0379. B) Conductivity (σ_m) of electron transport through biofilm matrix values calculated from generator-collector and square wave voltammetry measurements.

Keywords: *Geobacter sulfurreducens*, *c*-type cytochrome electron conduit

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Electron uptake in MES: How does Sporomusa ovata accept electrons at the cathode?

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Sporomusa ovata is by far the most successful microorganism in pure culture to produce acetate from carbon dioxide and current [1]. And the literature shows that improving the performance by means of choosing or manufacturing new electrode materials [2]–[4], adjusting trace elements [5], adapting the bacteria [6], [7], or varying temperature [8] is possible. But there is still the unanswered crucial question of the electron uptake mechanism: is direct electron uptake possible, or does S. ovata rely on intermediate hydrogen production on the electrode?

Analysis of the proteome (Uniprot and NCBI) of S. ovata reveals genes coding for 8 different multiheme c-type cytochromes which could contribute to a direct electron uptake pathway. But genes coding for hydrogenases and nitrogenases are also present. Therefore, an indirect pathway through the intermediate hydrogen production is also possible. Which of both pathways is used or predominant is not yet investigated.

To contribute to reveal the fundamentals of electron uptake of S. ovata, this work has thoroughly reviewed achievements of this bacterium in MES and analysed its genome. Experimental work characterizing involved protein families under different operation parameters (cathode potential and gas supply) is underway.

Keywords: Microbial electrosynthesis, Sporomusa ovata, Electron uptake mechanisms, Protein analysis

Funding: MSCA Individual Widening Fellowship (BIOCAT, Project number: 408509).

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Life cycle assessment of Microbial Electrosynthesis

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Microbial electrosynthesis (MES) offers a potentially sustainable route of producing useful platform and commodity chemicals. Here we present the environmental burdens associated with acetic acid (AA) production via MES. While downstream separation and purification remains the top environmental hotspot, membrane and reactor medium are the other significant contributors. A sensitivity analysis, assuming a more productive MES system with space time yield equal to that reported for acetogenic bacteria in a continuous gas fermenter (148 $gL^{-1}d^{-1}$) is also presented. This analysis is based on four scenarios, which involve either fed-batch or continuous operation, with electricity source as either grid power or a photovoltaic facility. Impact assessment results show that for the assumed high productivity, the environmental impact of AA production using MES could become comparable to traditional fossil-based method, but only if the energy source is completely renewable, which is a significant challenge. Among the two modes of operation, fed-batch processing leads to higher environmental impact, however, the product accumulation helps in achieving the same performance at low production rate as compared to continuous mode. A threshold production rate for a fed-batch MES reactor that could possibly lead to sustainable AA production was found to be 4100 $gm^{-2}d^{-1}$.

Keywords: microbial electrosynthesis, LCA, acetic acid, sustainability

Increasing the voltage in series-stacked bioelectrochemical systems, a road towards technology up-scaling

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Series-stacked bioelectrochemical systems (BES) are appealing in terms of energy conversion, operating at higher voltages and approaching industrial power application of BES. In case of energy-consuming BES connected in series, like microbial electrolysis cells (MEC), high-performing bioanodes can push less-performing ones in the stack outside their "working zone", resulting in unbalanced voltage drops and damage of electroactive biofilm. In this study, a passive and low-cost cell balance system (CBS) was proposed, solving this key issue for BES up-scaling.

Three double-chamber MECs were adopted and connected in series. Diodes were placed in parallel to each cell, acting as passive "bypass line" and allowing the excess current to flow outside of the individual MEC when its voltage drop exceeded the disruptive voltage of the diode. Diodes were selected for their disruptive voltage to be slightly lower than maximum voltage tolerated by individual MECs (≈1,5 V). Several tests were performed operating the stack in chronoamperometric mode (Table 1). Then, CBS was installed, and tests repeated with protected stack (Table 2).

The unprotected stack failed at a stack voltage of 4,5 V. With the protected stack, the voltage remained balanced. Developed CBS can be applied to different BES technologies, facilitating their industrial uptake.

Stack voltage	Stack current	MEC 1			MEC 2			MEC 3		
		E_{an}	E_{cat}	ΔE	E_{an}	E_{cat}	ΔE	E_{an}	E_{cat}	ΔE
3,0	3,8	-0,30	-1,40	1,10	-0,45	-1,40	0,95	-0,40	-1,40	1,00
3,5	5,4	-0,31	-1,57	1,26	-0,43	-1,51	1,08	-0,37	-1,51	1,14
4,0	6,0	-0,26	-1,75	1,49	-0,40	-1,59	1,19	-0,30	-1,59	1,29
4,5	5,9	+0,26	-1,74	2,00	-0,40	-1,59	1,19	-0,31	-1,59	1,28
3,0	4,2	-0,41	-1,40	0,99	-0,46	-1,43	0,97	-0,44	-1,44	1,00
3,5	6,0	-0,43	-1,55	1,12	-0,45	-1,58	1,13	-0,43	-1,59	1,16
4,0	7,9	-0,41	-1,68	1,27	-0,42	-1,71	1,29	-0,41	-1,72	1,31
4,5	11,1	-0,39	-1,84	1,45	-0,40	-1,87	1,47	-0,38	-1,87	1,49

Table 1 – Electrodes potentials (E_{an} and E_{cat}, in V vs Ag/AgCl), cell voltage drops (ΔE, in V) and current (mA) measured with the series-connected stack of MECs, at different values of stack voltage.

Table 2 – Electrodes potentials (E_{an} and E_{cat}, in V vs Ag/AgCl), cell voltage drops (ΔE, in V) and current (mA) measured with the series-connected stack of MECs, at different values of stack voltage, when adopting the CBS.

Keywords: cell balance system, diode, microbial electrochemical technologies, stack.

Funding: This project has received funding from European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 826312 (GREENER).

Development of Innovative Soil Microbial Fuel Cells for Energy Harvesting

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Soil microbial fuel cells (SMFCs) have great potential as an energy harvesting technology. Amongst the different types of microbial fuel cells, SMFCs are arguably the most cost attractive, as they utilise soil as the supporting matrix, the source of microorganisms, and, in membrane-less configurations, also as the separator between the anode and the cathode. Consequently, the overall design of the fuel cell is simplified and so is its operation, since no fuel continuous pumping/ replacement is required.

Nonetheless, similarly to traditional (liquid) microbial fuel cells, practical applications of SMFCs are hindered by the difficulties with power scale-up and issues with long-term stability. Finding the most practical and energy efficient strategy to operate SMFCs is crucial for successfully transitioning this technology into the market.

We focus on the design and development of low-cost SMFCs, including an extremely simple flat-plate geometry, with the anode embedded into the soil and the cathode exposed to air, as well as more complex arrangements with vertical electrodes. We also work at the development of functional electrical arrangement of multiple SMFC units to boost the power output. In particular, a net power positive energy harvesting strategy is considered for the power management system, which is based on an equivalent electrical circuit model and continuous maximum power point tracking algorithm approach, with no need for energy consuming equipment. With this approach, we show the remote powering of electronic devices, including an electrochemical reactor for water purification.

Keywords: Soil microbial fuel cells, Bioenergy, equivalent electrical circuit, maximum power point tracking

Funding: This work is part of the project GREENER that has received funding from the European Union's Horizon 2020 research and innovation programme under the grant agreement No 826312.

MIDES H2020 Project: Microbial Desalination for Low Energy Drinking Water. From lab-scale concept to Pilot Pant validation.

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The EU-H2020 MIDES project aimed to develop the World's largest demonstrator of an innovative and low-energy technology for drinking water production, using Micronbial Desalination Cells (MDC) technology as pre-treatment step for RO. The project focused on overcoming the current limitations of MDC technology such as low desalination rate, high manufacturing cost, biofouling and scaling problems on membranes, optimization of the microbial-electrochemical process, integration with RO, ceramic nano-membranes as pre-treatment, system scale up and economic feasibility of the technology. The project run from 2016 to 2020, to develop the world's largest demonstrator of the innovative MDC technology. Demonstration sites were implemented in Spain in Denia (brackish water) and in Las Palmas (sea water). This communication presents the main outcomes of the project, the process to scale-up MDC technology from lab-scale concept, and the future outlook and prospective of MDC technology **[1]**.

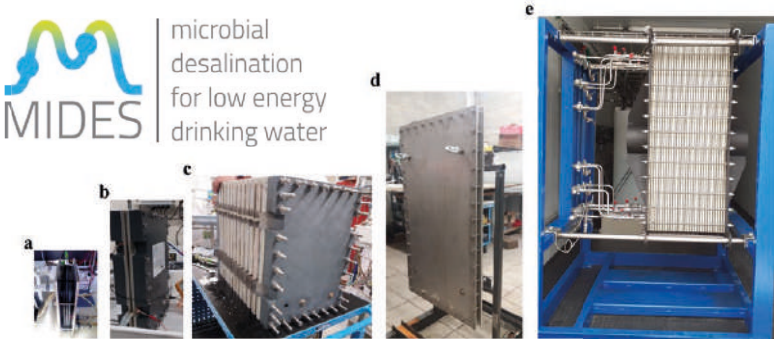

Fig. 1. Microbial desalination cells developed in the MIDES project, fabricated at the following scales: a) MDC laboratory area 100 cm²; b) pre-pilot MDC unit, area 600 cm2; c) pre-pilot MDC stack, area 600 cm² x 10; d) MDC pilot unit, area 4000 cm² and d) MDC pilot stack, area 4000 cm² x 15.

Keywords: microbial desalination cell, brackish water, electrochemistry, fresh water production.

Funding: Project "MIDES – H2020" has received funding from the European Union's Horizon 2020 research and innovation programme (N° 685793).

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Full scale operation of decentralized urban wastewater using METland® technology

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METland® represent a hybrid concept of nature-based solution by integrating MET-based concepts like CIET (conductive-mediated interspecies electron transfer) into a constructed wetland design for generating a new generation of electroconductive biofilters for treating wastewater. Different configurations and operation modes (Aguirre et la., 2020) have been tested in the last decade to eventually generate a sustainable strategy according to LCA analysis (Peñacoba et al., 2021) capable to maximize electrobioremediation rates. A number of full scale application are currently operating at different international locations. In this work we report three different succesfull cases regarding design, bioremediation performance, electrochemical performance and microbial community analysis: i) Modular METland® downflow configuration; 20-25m^3/day of sulfate-rich urban waste water from Otos municipality (Spain) were treated in 16 m^2; b) Multi stage constructed METland® downflow configuration, 100m^3/day from a Camping site (Spain) were treated in 40 m^2; iii) Constructed METland® for treating (20m^3/day) from Orby municipality (Denmark) in 80 m^2. METland® exhibited generated effluents with COD values as low as 30ppm or below 1ppm in ammonium. Electrobioremediation was correlated with electron flow measured as electric potential profile (Prado et al,. 2020). Interestingly, emergent contaminants like drugs and hormones where also efficiently removed under METland® conditions.

Fig. 1 Images of different full scale configurations for METland® technology in Spain and Denmark, and Electric Potential profile of electroconductive bed

Keywords: electrobioremediation, METland, CIET, water management, reclaimed water

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Enhancing butanol production by Clostridium beijerinckii through cathodic electro-fermentation approach

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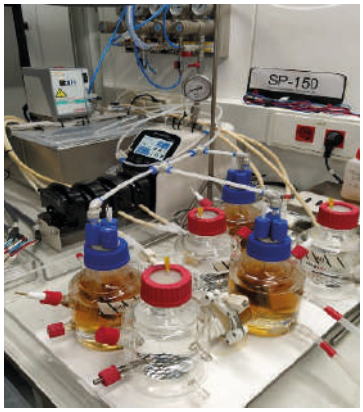
Biobutanol, produced by solventogenic bacteria like *Clostridium beijerinckii* through ABE fermentation, is a promising green fuel. Electro-fermentation (EF) can modify the oxidation-reduction potential (ORP) of the fermentation broth, manipulating the ratio of oxidized nicotinamide adenine dinucleotide to reduced nicotinamide (NAD^+/NADH), a critical intracellular factor for butanol synthesis. This study focuses on a glucose-based EF at laboratory-scale. Three replicate bioelectrochemical reactors were adopted. Chronoamperometry tests at constant cathode potential were run (E_{cat} ranging from -0.7 to -1.0 V vs Ag/AgCl). Poising E_{cat} at -0.8 V vs Ag/AgCl resulted in the optimal EF scenario, with microbial growth rate of 0.08 g/L/h (56% higher than benchmark fermentation), production of 0.13 g-butanol/L/h, yield of 0.19 g-butanol/g-glucose and selectivity of 87% (44%, 19% and 12% higher than benchmark, respectively). A titer of 5.8 g-butanol/L was reached after 46 h. At adopted E_{cat}, H_2 production was limited (HER onset at -0.7 V vs Ag/AgCl) and only 0,4% of produced solvents were attributable to electrotrophic *C. beijerinckii* metabolism. A major part of them were produced by spontaneous fermentation, with intracellular metabolism shifted towards butanol, thanks to EF approach.



Parameter	Anode	Cathode
Chamber volume	300 mL	300 mL
Electrode surface	9 cm^2	5 cm^2
Electrode material	Ir-MMO coated Ti-mesh	MnO_2 coated carbon cloth
Electrolyte	0,1 M PBS	60 g/L glucose, anaerobic cond.
Inoculum	abiotic	*C. beijerinckii*
Temperature	Room T (≈24 °C)	37 °C, constant
Separator	Cationic exchange membrane	

Keywords: bioelectrochemical systems, biofuels, butanol, cathode, fermentation

Funding: This project has received funding from the European Union's Horizon 2020 research and innovation programme under Grant Agreement no. 761042 (BIOCON-CO2).

Statistical analysis on factors affecting microbial electrosynthesis (MES)

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Carbon capture and utilization (CCU) techniques help in mitigating greenhouse gases (GHGs), mainly CO_2 and simultaneously produce multi-carbon compounds. Microbial electrosynthesis (MES) is one such technique. Advancements in MES studies can be understood by comparing their production rate. However, product titers reported in literature are not comparable directly, as they depend on many operational factors. In order to enable comparison between the different studies, a statistical analysis was performed by collecting data from studies that had results of at least two MES with only one factor varying. The MES performance (P), in terms of production rate were normalized to the maximum MES performance (P_{max}) obtained in the same study and impact of each factor was analysed individually. The analysis revealed that best performance was obtained for pure CO_2 as a carbon source as compared to bicarbonates or industrial off gas. Similarly, neutral to alkaline pH (7-10) both during inoculation and operation gave better performance. Operation of reactor in continuous mode as compared to batch mode resulted in reduced product inhibition, thereby giving a better titer. Similar statistical studies on more number of factors will help us understand MES performance better and eventually design an efficient reactor.

Keywords: Carbon capture and utilization (CCU), Microbial electrosynthesis (MES), Normalization, value added chemicals, optimization

Experimental optimization of long chain fatty acids synthesis from CO_2 using statistical design

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CO_2 emissions contributes to global warming. Biological CO_2 fixation methods using microbes as catalysts are less expensive compared to conventional carbon capture methods. Microbial electrosynthesis cell (MES) is an electrochemical technique in which chemolithoautotrophic electroactive microbes fix CO_2 to longer chain volatile fatty acids (VFA). The synthesis of VFAs from organic waste/wastewater requires optimization aiming to improve the MES performance for industrial feasibility. This study was conducted in serum bottles to understand individual and interdependence of factors impacting VFA synthesis. The optimized parameter values will be adopted for further MES studies. Central composite design (CCD) and response surface methodology (RSM) were used to optimize total pressure, pH and ethanol concentration for VFA synthesis from synthetic wastewater in a fed batch reactor process. Enrichment of cultures was done using CO_2:H_2 gas (20:80, v/v). The optimum condition obtained from RSM was 1.11 atm pressure, pH of 7.149 and ethanol concentration 4639 mg/L. The rates of total VFA production obtained from the experimental study was 38.12 mg L^{-1} d^{-1} under this optimized condition, which agreed with the predicted value of 35 mg L^{-1} d^{-1}.

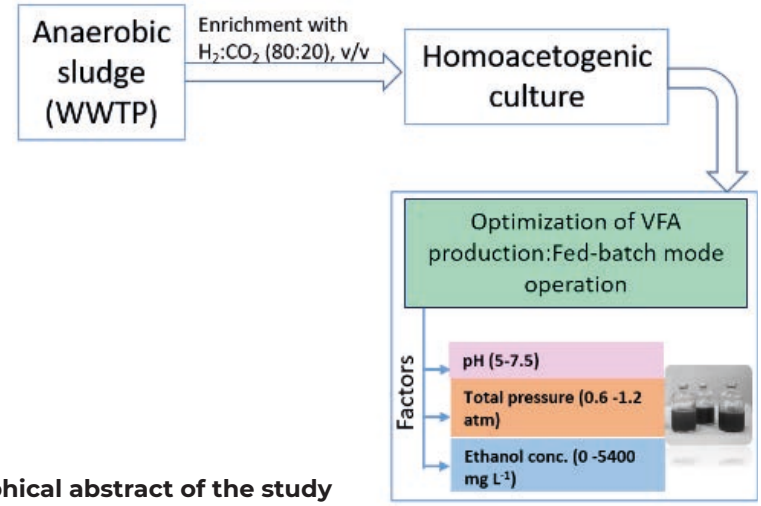

Fig. 1 Graphical abstract of the study

Keywords: Total pressure, Ethanol concentration, Response surface methodology, Optimization, Long chain fatty acids

Funding: Science and Engineering Research Borad (SERB) (SRG/2019/000757 Dated 10.12.2019)

Voleu crear models per fer una drecera? Development of a model for microbial electrosynthesis with planktonic cells and mediator based electron transfer

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Despite many functional systems, reactors for microbial electrochemical technologies are mostly made by individual laboratories for their respective processes. They are hardly scalable and optimised, thus there is a lack of comparability among processes and scales [1–4]. Reactor infrastructure and aligned reactor and process models are required, that jointly enable rapid process development across scales, especially for microbial electrosynthesis (MES). As an initial step, an exemplary MES process model, the 0D model presented in this contribution, describes the synthesis of chiral alcohols from ketones using planktonic cells and mediator based electron transfer. It is implemented in COMSOL Multiphysics and tested against different experimental setups and scales. Model input parameters were gathered from previous publications [5] or optimised via partial-least-square-analysis. For validation, different input parameter combinations were modelled and compared to experimental parameter values [6]. The process model properly predicts the experimental output for different input parameter combinations on the 250 mL H-cell and for a 1 L batch and fed-batch process using CSTR-based electrobioreactors [7]. Thus, a model generated from one dataset on small scale (H-cell) can successfully predict output parameters of up-scaled (CSTR) setups, qualifying it as a tool for further work in analysis and engineering of similar MES processes.

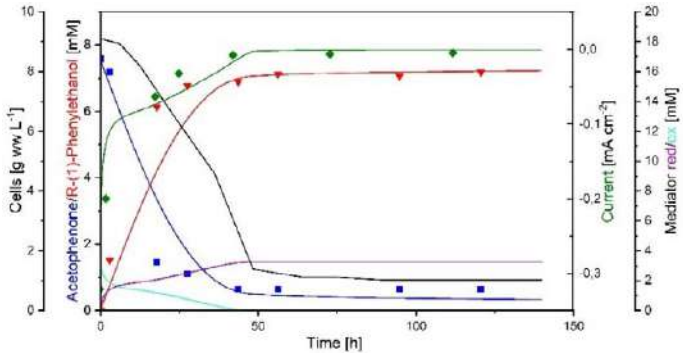

Fig. 1: Modelling of the batch 250 mL H-cell experiment published in Mayr et al. [6]. Concentration of Acetophenone (blue) and R-phenylethanol (red), reduced methyl viologene (MV, pink), oxidised MV (turquoise) in mM, current in mA (green) and cell concentration (black) in g L-1 over time. Curves represent the modelling results, symbols represent the experimental results; Initial Acetophenone concentration 7.58 mM; 8.97 g L-1 E. coli JG622_LbADH; 3.26 mM MV; TEA buffer (pH 7.5); potential: -503 mV vs SHE; electrode surface area: 39.27 cm2. Experimental values are averaged and calculated from three independent biological replicates shown in Mayr et al. [6].

Keywords: Scaling, electrobioreactors, modelling, electrobiosynthesis, Power-to-X

Funding: This work was supported by the Helmholtz-Association in the frame of the Integration Platform "Tapping nature's potential for sustainable production and a healthy environment" at the UFZ.

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Insights in the electro-fermentation process with ^{13}C-labelled experiments supported by NMR spectroscopy analysis

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The electro-fermentation (EF) process is an interesting and promising approach to control the spectrum of products deriving from microbial anaerobic fermentations, in terms of both composition and concentration. In EF a polarized electrode, functioning either as an anode [1] or a cathode [2], is used to modify the intracellular redox balance so as to redirect the carbon flux towards the desired end-products. To date, however, it is still not clear whether an electron transfer between microorganisms and the electrode is involved or if the process is simply triggered by a change in the oxidation-reduction potential of the reaction medium.

Here, in order to gain a deeper understanding on the effect of the polarized electrode on the microbial metabolism, EF experiments have been performed by supplying ^{13}C-labelled glucose, along with ethanol and acetate, to anaerobic mixed cultures inoculated at the cathode chamber of H-type reactors with the electrode potential controlled at -0.70 V (*vs.* SHE). The fermentation pattern has been tracked over time with the nuclear magnetic resonance (NMR) spectroscopy, that is the elective technique for ^{13}C-labelled analysis. Parallel control tests (under identical conditions but in the absence of electrode polarization) have been carried out and compared to EF experiments.

Keywords: electro-fermentation, mixed microbial cultures, redox balance, nuclear magnetic resonance (NMR)

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Power-to-algae: carbon dioxide to bio-oil in a BES-supported microalgae biorefinery

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Microbial electrosynthesis (MES) for bioelectro carbon dioxide (CO_2) recycling is an interesting and sustainable opportunity to exploit spent off gases from industrial facilities as a feedstock to be converted into valuable energy sources. In this proof-of-concept study, a bioelectrochemical system (BES) coupled with a heterotrophic microalgae reactor was used to convert acetate synthetized from CO_2 into bio-oil. Two BESs were operated in batch mode at an applied cathodic potential of -0.8 V vs SHE (standard hydrogen electrode) for 95 days. The systems reached a concentration of up to 13 g L^{-1} of acetate (at a maximum production rate of 21.2 g m^{-2} d^{-1}). In a second stage, the effluents from the biocathodes were transferred to microalgae reactors containing *Auxenochlorella protothecoides* to assess oil production. Bio-oil content obtained was up to 25% w/w (dry weight), enough to encourage further exploration of microalgae-to-oil recovery in the future. The overall balance resulted in 0.207 kg bio-oil per kg acetate produced, corresponding to 0.03 kg bio-oil per kgCO_2 captured, that can be further processed to produce an EU biodiesel requirement compatible bio-oil. Altogether, this research provides new alternatives to improve overall MES economic balance through the direct integration with microalgae biorefinery.

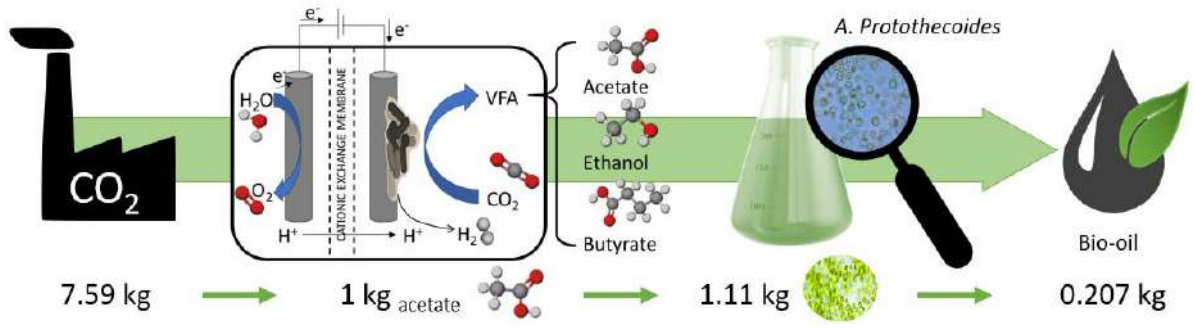

Fig. 1 – Schematic of the combined BES-microalgae biorefinery process.

Keywords: Microbial electrosynthesis, Biodiesel, *Auxenochlorella protothecoides*, Acetate production, Biocathode.

Funding: This research was funded by the Spanish Ministry of Science through the grant RTI2018-098360-B-I00 and the Agency for Business Competitiveness of the Government of Catalonia (ACCIÓ) through the DigesTake Project (COMRDI16-1-0061). S.P. is a Serra Húnter Fellow (UdG-AG- 575) and acknowledges the funding from the ICREA Acadèmia award.

Characterization of fluidized vs. fixed granular activated carbon beds as cathodes for microbial electrosynthesis of carboxylates from CO^2

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Microbial electrosynthesis (MES) is a promising green biotechnology, which can use renewable electrical energy to drive the microbial conversion of the greenhouse gas carbon dioxide (CO_2) into value-added multi-carbon products [1]. To achieve high production rates in MES it is crucial to consider how the microorganisms can be sufficiently supplied with nutrients, CO_2 and reducing power. Therefore, the cathode material and its configuration play a key role in the optimization of MES systems.

Granular activated carbon (GAC) is an attractive cathode material due to its high biocompatibility, conductivity and high specific surface area [2]. In addition, the fluidization of the GAC bed may improve mixing and mass transfer and result in the enhanced conversion of CO_2 into multi-carbon products.

Therefore, the authors compared fluidized with fixed GAC bed cathodes for MES of acetate from CO_2 and observed clear differences in acetate production rates and biofilm formation between the different GAC bed configurations.

One of the challenges MES research needs to face is the efficient scale-up of the technology. In this regard, fluidized bed cathodes can be a promising possibility to bring MES one step closer to real-world applications.

Keywords: Fluidized bed reactor, microbial electrosynthesis, granular activated carbon bed electrodes, cathodic biofilms, mass transfer.

Funding: This research was supported by the Academy of Finland (grants no. 316657 and 319910).

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Insights into electro-fermentation of caproate from CO_2 and ethanol

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Although the thermodynamics of bioelectrochemical systems is fundamentally deterministic, the end-products spectrum can be unpredictable when dealing with ever-changing circumstances. Two methacrylate bioreactors were set-up, inoculated with an open culture and fed with CO_2 and ethanol for simultaneous microbial electrosynthesis and chain elongation to valuable compounds[1]and the resulting legislative pressure is pushing the chemical and energy industries away from fossil fuels. Microbial electrosynthesis (MES. pH was adjusted to 7 and acetate to ethanol molar ratio was set at 1:1. In the first batch, mainly acetate was produced until enough butyrate was accumulated to trigger caproate formation (Fig.1). In the second batch, samples were taken at irregular time intervals to decipher the different chain elongation phases. This could have modified the thermodynamics towards caproate, but also hinted the fluctuation of organics from C1 to C8 depending on the time elapsed between sampling and feeding points. Maximum caproate production rate, titter and selectivity were 14,3 $g \cdot m^{-2} \cdot d^{-1}$, 4,8 $g \cdot L^{-1}$ and 36%, respectively. In the third batch, when samples were taken regularly again, only acetate production was observed. Nutrient depletion was avoided by adding a concentrated mineral solution and the maximum undissociated caproic acid content (4mM) was considered non inhibitory[2]. Thereby, this study provides insight into key operational parameters influencing the bioreactor's dynamics that may exhibit intermediates formation of more reduced compounds.

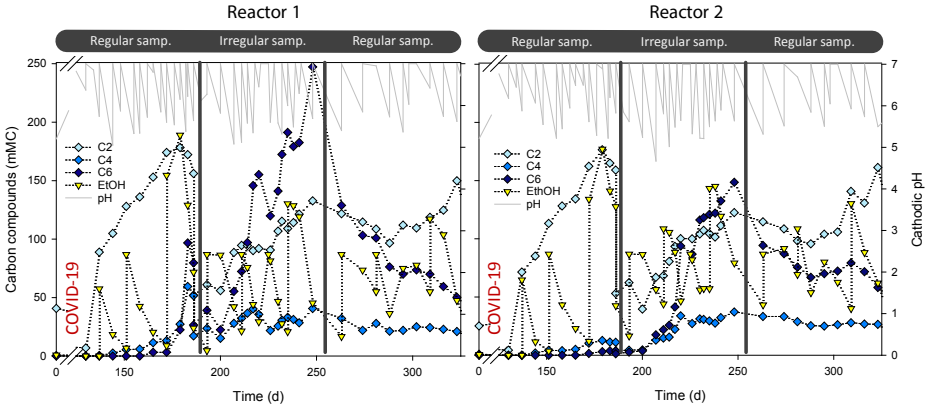

Figure 1. Organic compounds evolution and pH over time of two bioelectrochemial reactors.

Keywords: Bioelectrocatalysis, carboxylate platform, chain elongation, CO_2 valorisation, open culture.

Funding: Catalan Government (2018 FI-B 00347) in the European FSE program (CCI 2014ES05SFOP007)

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Theory of transport and recovery in microbial electrosynthesis of acetate from CO^2

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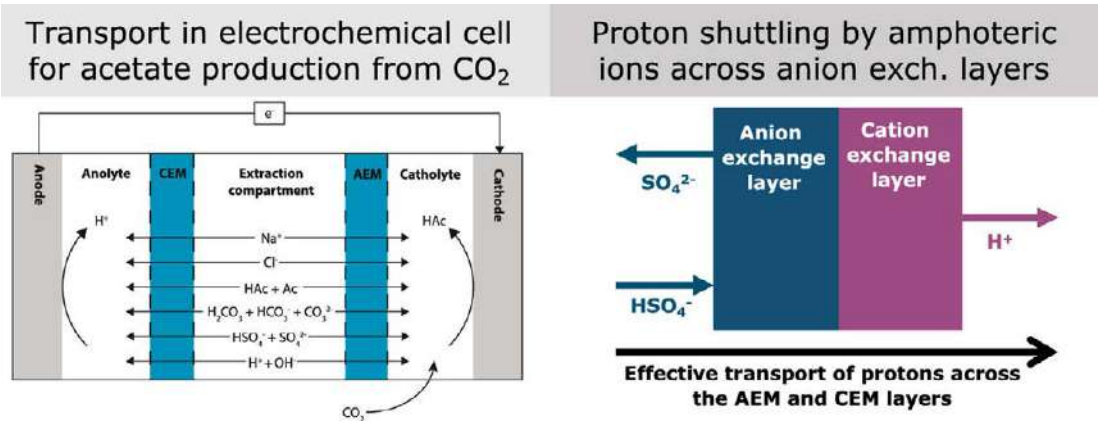
Microbial electrosynthesis (MES) provides a route for the conversion of CO_2 into acetate and other organics. The conversion of CO_2 takes place at the biocathode. Since both charged and uncharged species participate in the reaction, understanding the transport of these species through the ion exchange membrane, is of key importance. We develop a theory for ion transport and conversion, which includes ion transport, acid-base reactions, as well as electrochemical reactions at the electrodes. We first analyze a cell configuration including three compartments, in which the acetate recovery compartment in the middle is separated from the outer compartments by one cation exchange membrane and one anion exchange membrane, and we compare with experimental data from literature. Analysis of ion transport across the membranes revealed that acetic acid/acetate and carbonic acid/bicarbonate species were used as proton shuttles between the catholyte and the recovery compartment. We also analyzed a system including a bipolar membrane (BPM), and showed that a commonly made assumption that in BPMs the charge is carried by protons and hydroxyl ions, produced inside the BPM is not generally correct. In our calculation charge is mainly carried by bisulphate and sulphate ions in the anion exchange layer of the BPM.



Keywords: bio-electrochemical systems, multi-component mass transport, amphoteric ions, ion exchange membranes, bipolar membranes.

Screening for hyperthermophilic electroautotrophs for the microbial electrosynthesis of organic compounds

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Microbial electrosynthesis is a promising novel technology to sustainably produce organic compounds: an electrotrophic biofilm grows on a cathode, using electricity as energy source and fixing CO_2 into metabolites. The technology is so far limited by the low value of the end products. While most studies focus on the molecular engineering of model mesophilic bacteria, we aimed in this study to identify new electrotrophs from extreme environments, harboring atypical metabolisms potentially leading to new pathway for electrosynthesis. In this study, we screened 12 hyperthermophilic pure cultures and different communities from hydrothermal vents to evaluate their potential as novel electrosynthesis biocatalysts.

The experiments were performed in H-cells maintained at 75°C, with the cathode polarized at -600mV vs. SHE, in presence of different electron acceptors (NO_3, SO_4, S_2O_3, FeOH, O_2 or CO_2), depending on their metabolisms. After few days of culture, the production of organic acids was analyzed by HPLC and compared to the consumption of electron acceptors and electrical current. Depending on the biocatalyst, the main products were formic, acetic, propionic, succinic, isobutyric, butyric and phenylacetic acids and glycerol from 0.16 to 1.59 $mg \cdot day^{-1} cm^{-3}$. The biofilms obtained at the end of each experiment were characterized by Microscopy, qPCR, and Metabarcoding.

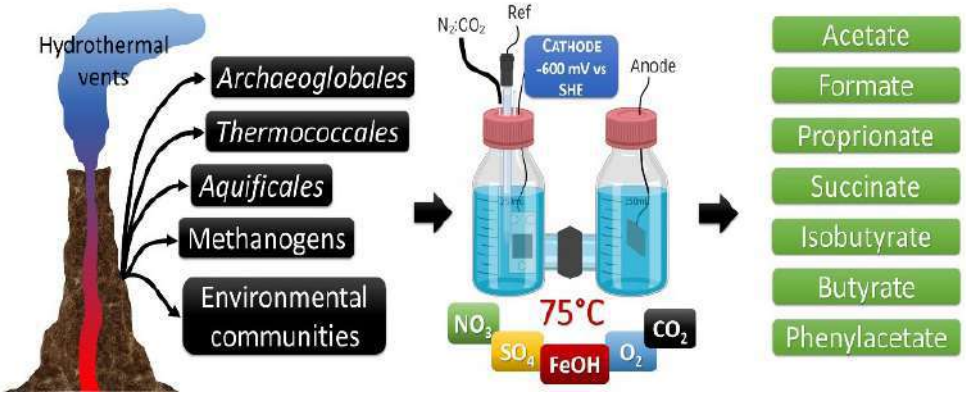

Fig. 1: Schematic of our approach to screen for hyperthermophilic electroautotrophs producing valuable compounds.

Keywords: electrosynthesis, hyperthermophily, electroautotrophs, hydrothermal vents

Biophotovoltaics for hydrogen production using cyanobacteria

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Hydrogen plays a critical role in decarbonizing our industry. Biophotovoltaics (BPV) provides a unique carbon-sink solution for bio-hydrogen production in terms of resource and energy efficiency. Water is split by oxygenic *cyanobacteria* using light in anode chamber and the released electrons are transferred to the cathode to reduce protons into hydrogen. CO_2 is the sole carbon source for biomass maintenance. Despite the distinguished features, BPV is still poorly understood, and the typical diverse operating parameters and the miniaturized scale of reported BPV systems hinder systemic understanding and rational optimization.

Here we present the first technical-scale BPV system with optimized and defined parameters[1]. Long-term reproducible light-dependent current output of *Synechocystis sp* PCC6803 (hereafter *Synechocystis*) was demonstrated by systematically examining potential interfering factors with biotic and abiotic controls. While using ferricyanide as the redox mediator in the system, a specific current density of 52 μA/nmol$_{Chla}$/m^2 was achieved, the highest value reported so far for similar conditions. To further assess the theoretical capacity and potential constraints, a genome-scale model of *Synechocystis* was established[2]. Impacts of physical and physiological parameters on the BPV performance were simulated, including overall production capacity, extracellular electron transfer pathways, seasonal light source and biomass turnover rate, etc.

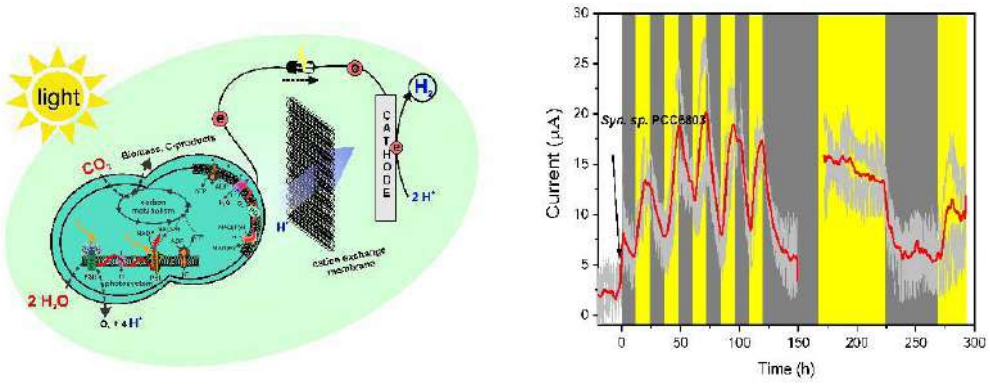

Fig. 1. The Biophotovoltaics concept for hydrogen production from water driven by sunlight (left) and representative results (right).

Keywords: biophotovoltaics, cyanobacteria, genome scale model, systems design, H_2 production

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Electrifying biotrickling filters for the treatment of aquaponics wastewater

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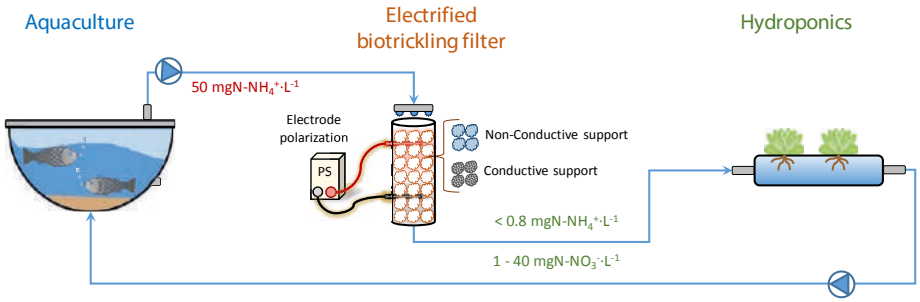
The combination of aquaculture (fish farming) and hydroponics (soilless plant cultivation) is a promising method for providing food while reducing the environmental impacts of the individual peers (i.e. water consumption, nitrogen loads, land use). However, ammonium-rich aquaculture effluents cannot be directly used for hydroponics, which requires almost ammonium-free water but with a certain amount of nitrate as nutrient (1 - 34 mgN·L^{-1}). Thus, for aquaponics a system providing full nitrification and a controlled denitrification is required. For this reason, this work explored the potential of biotrickling filters (i.e. common aquaculture depuration technology) to be electrified for improving nitrification/denitrification rates and provide a tuneable denitrification by means of Microbial Electrochemical Technologies (MET).

Four different easy-to-assemble and easy-to-use designs were tested[1]. The highest ammonium and nitrate removal rates (94 gN·m^{-3}·d^{-1} and 43 gN·m^{-3}·d^{-1}, respectively) were reached by combining an aerobic non-conductive zone with an electrified anoxic zone (Fig.1). The effluent criteria for an aquaponics application was reached at low energy cost (8.3 × 10^{-2} kWh·gN^{-1}) without neither external aeration nor addition of chemicals. Electrified biotrickling filters have demonstrated to be promising sustainable alternative for aquaponics and potentially for the treatment for organic carbon-deficient ammonium-contaminated waters.

Fig. 1 Introduction of an electrified biotrickling filter in an aquaponics loop



Keywords: Circular bioeconomy, Bioelectrochemical nitrogen removal, Biocathode, Blue economy, Power-to-food.

Funding: This research was carried out in the Project "Wireless Aquaponic Farming in Remote Areas: A smart adaptive socio-economic solution" (WAFRA) funded within the 7th Framework Program (ERANETMED) by the Spanish Ministry of Science (PCI2018-092946) and the German Federal Ministry of Education and Research (BMBF) (01DH18003). S.P. is a Serra Hunter Fellow (UdG-AG-575) and acknowledges the funding from the ICREA Academia award.

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Metabolic Pathway Involved in CO_2 Fixation under Photo-Bioelectrochemical conditions by a Purple Phototrophic Bacteria Biofilm

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Bioelectrochemical systems are a promising technology to treat wastewater and fix CO_2 via electrosynthesis [1]. We demonstrated in previous work the capacity of a mixed biofilm of purple phototrophic bacteria (PPB) to degrade organic matter and fix CO_2 under electrochemical conditions [2]. However, the specific metabolic pathway involved in this process is still not known. In this work, we explore it through a comprehensive analysis by metaproteomics and MiSeq 16S-rRNA gen sequencing analysis of different samples of a cathodic mixed biofilm grown under irradiation and different voltages (0, -0.2, -0.4, and -0.8V vs. Ag/AgCl) and dark conditions at non-voltage.

Preliminary results show an inverse correlation between chemical oxygen demand and CO_2 consumption rates (Fig 1a), suggesting that the negative polarization enhances the use of CO_2 for the PPB growing instead of organic carbon. The results from MiSeq analysis (Fig 1b) show that the polarization of the cathode strongly enhances the growth of PPB. It also suggests a synergic relationship between different genus of PPB and genus *Wolinella*, which may be related to direct interspecies electron transfer. The metaproteomics analysis will explain whether the electron target reaction responsible for the CO_2 fixation process is anaplerotic or belongs to the Calvin cycle.

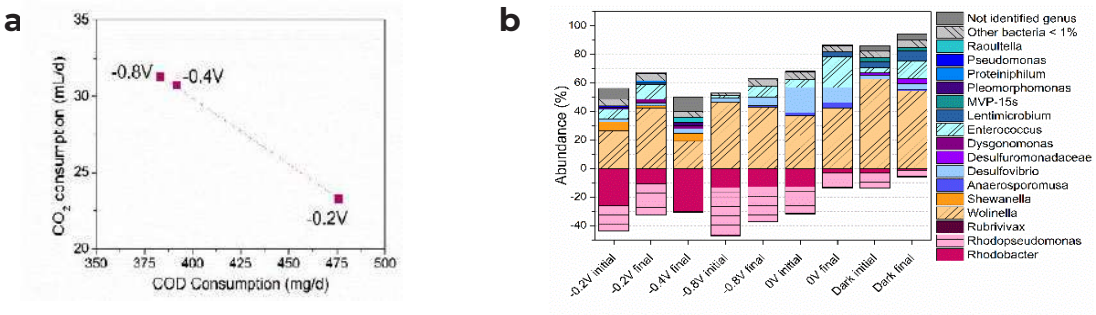

Fig. 1 (a) Correlation between CO2 and chemical oxygen demand (COD) consumption rates and (b) percentage of different genera that form part of the biofilm from BES.

Keywords: Bioelectrochemical, purple phototrophic bacteria, carbon dioxide

Funding: FOTOBIOELECTRO (CTM2017-91186-EXP), REMTAVARES-CM (S2018/EMT-4341), BIOTRES-CM (S2018/EMT-4344) and R&D Young Researchers Fund 2019 (SUN-BIOELECTROMAT) projects, and Ramon y Cajal grant. The bioprofiling platform used for the proteomic analysis was supported by the European Regional Development Fund and the Walloon Region, Belgium. B. Leroy work was supported by the FNRS under the CDR project "Redox homeostasis in purple bacteria"

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Hyperthermophilic hydrogen production by iron reducing Archaea in microbial electrolysis cells (MECs)

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Utilization of (hyper)thermophilic microorganisms was suggested to improve reaction rates and insoluble pollutant degradations and minimize risk of contamination in bioelectrochemical systems. Only a small group of hyperthermophilic microorganisms were identified with the ability to produce current in such systems and here we present a new culture that fits to this description. This iron reducing archaeal culture originally isolated from a hydrothermal structure, produced 1.53 A/m^2 ± 0.24 current density in MECs operated at 80 °C. Hydrogen production rate was found as 0.50 ± 0.09 mL H_2 / mL active volume in the 5 mL Mini-MEC reactors fed with 10mM acetate. Resulting current density is 2.5 – 3 times higher than the previously reported electro-active behaviour of closely related archaeal species of *F. placidus* and *G. ahangari* with the exact reactor configuration. There was a visible biofilm formation on the anode and 6 cycles of biohydrogen generation coupled with current generation was measured. After 6th cycle biofilm started to peel from the electrode surface and both hydrogen and current generations were decreased. Cyclic voltammetry (CV) analysis produced a sigmoidal catalytic wave with a mid-point potential of−0.35 V (vs. Ag/AgCl) and Scanning Electron Microscopy showed thick biofilm with multiple layers of cells.

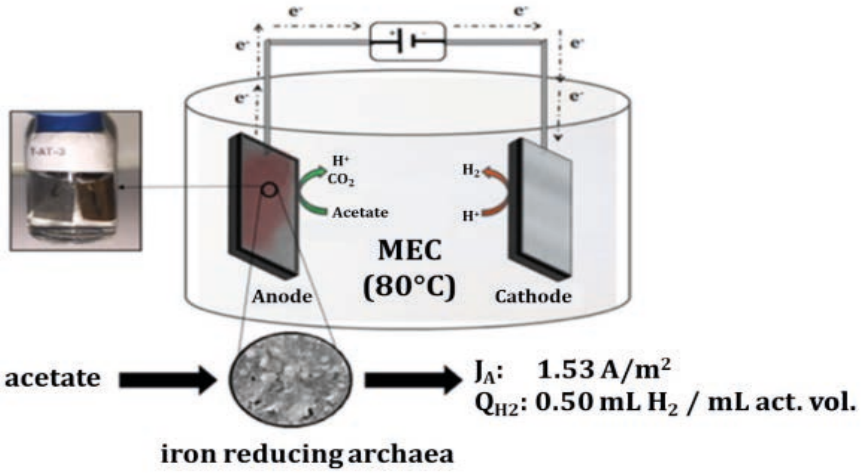

Fig. 1 Iron reducing archaeal culture in microbial electrolysis cell (MEC) operated at 80 °C

Keywords: iron reducing archaea, hyperthermophilic, microbial electrolysis cells, biohydrogen

This study was funded by The Scientific and Technological Research Council of Turkey (TUBITAK) grant no 119C011.

Alliance of microbial electrochemical technologies and fermentation for the conversion of carbon dioxide into elongated chemical building blocks

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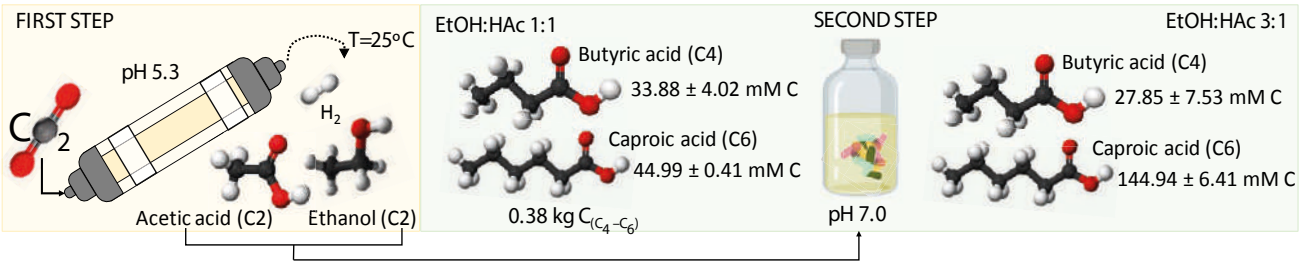
This work presents the combination of microbial electrochemical technologies and fermentation in a two-step process for the electro-bioconversion of carbon dioxide (CO_2) into elongated organic compounds. The electro bio-reduction of CO_2 was coupled to an elongation fermentative step. Key parameters considered in the first step were pH, hydrogen and CO_2 partial pressures [1,2]. Concerning the second step, selected parameters were pH, ethanol to acetate ratio, and hydrogen availability. The first step reached an ethanol to acetic acid (EtOH:HAc) ratio above 1-to-1 when fed with CO_2, under pH around 5.3 in an H_2-mediated microbial electrosynthesis. Formed products allowed the follow-up chain elongation processes. The fermentation step reached up to C_6 compounds at pH 7.0, when fed with EtOH:HAc 3:1 or 1:1, CO_2 and H_2. Outcomes demonstrated that pH was a crucial factor throughout the process. Elongation conversion efficiencies were above 80 % in all studied conditions. The overall process carbon conversion efficiency, when fed with EtOH:HAc 1:1, was 38 % being the limiting step of the bio-electro CO_2 transformation. These results pave the ground to selective production of reduced commodity chemicals from CO_2 and electricity.

Fig. 1 Schematic representation of the proposed platform.



Keywords: Chain elongation; CO_2 valorisation; electrochemistry; gas fermentation; solventogenesis

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3D bioprinted MES biofilms enhancing the acetate production rate of Sporomusa ovata

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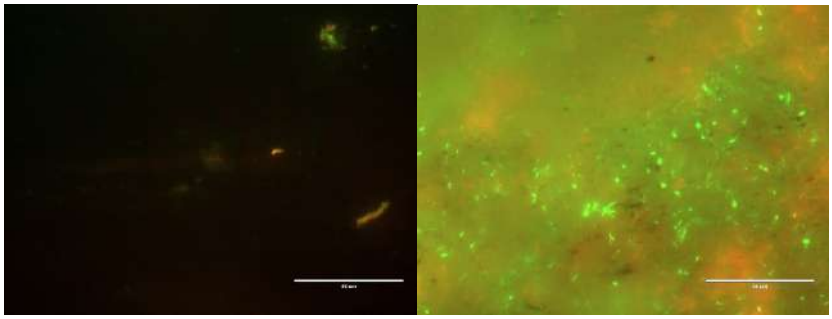
Microbial Electrosynthesis (MES) is an emerging and promising technology for carbon capture and utilization. Achieving highly efficient electron transfer from the cathode to microbes is, however, one of the main challenges hindering the development of MES reactors. It is, therefore, essential to improve biofilm growth for direct electron uptake-based bioconversions [1,2]although a techno-economic-driven roadmap towards validation and large-scale demonstration of the technology is lacking. In this work, two main integrated systems were modelled, centered on (1. Here we show the use of 3D bio-printing for MES systems, creating a "synthetic biofilm", containing *Sporomusa ovata*. The synthetic biofilm greatly improves the acetate production rate while drastically decreasing start-up time. By bioprinting on a carbon cloth electrode, we obtained an average acetate productivity of 3156 ±338mMday^{-1}m^{-2} (5.12 ±0.55 mMday^{-1}) when poised at -0.8V vs Ag/AgCl. This is an order of magnitude higher than typical *S. ovata* production rates. Furthermore, a high production rate was achieved almost directly after the start-up (±40 hours) without requiring media exchanges. Cyclic voltammetry data showed similar current densities, much higher potentials than in the control reactors, (achieving similar currents at approx. 300mV higher than the controls). Improved growth of *S. ovata* cells at the cathode surface and throughout the gel was shown using SEM imaging and fluorescent microscopy, supporting an increased electron transfer efficiency and potentially improving the bio-catalyzation of H_2.



Bioprinted Control

Fig. 1 Acetate production and "Live/Dead" fluorescent microscopy images of MES reactors using 3D bio-printed "synthetic biofilm" cathodes compared to MES cells using suspended cell inoculums as a control

Keywords: Bioprint; biofilm; *S. ovata*; microbial electrosynthesis; bioelectrochemical

Funding: Swedish Research Council (VR), (No. 2018-03875)

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Bioinorganic electrosynthesis of single-cell protein from CO_2 and green electricity

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Climate change and food shortage due to the growing world population are two of the biggest challenges for the transition to a low-carbon economy in the coming decades. In this study, we propose a promising two-stage bioinorganic electrosynthesis process that can first convert CO_2 and renewable electricity into methane and then synthesize single-cell protein, as an alternative solution to address these challenges. With an external voltage of 3.5 V and a CO_2 inflow rate of 50 mL·d^{-1}, it was possible to produce methanotrophic biomass of 118.7 ± 9.2 mg·L^{-1} with an amino acids mass-content of 54.6 ± 8.3%, resulting in nitrogen assimilation and CO_2 conversion efficiency of 91.0 ± 1.3% and 71.0%. The applied voltages, CO_2 inflow rates, and O_2 supply were found to affect the process significantly. The outcomes will lead to sustainable protein production, CO_2 mitigation, and valorization of renewable electricity, which are perfectly in line with the United Nations Sustainable Development Goals (2, 6, 7, 8, 9, 13, 14, 15).

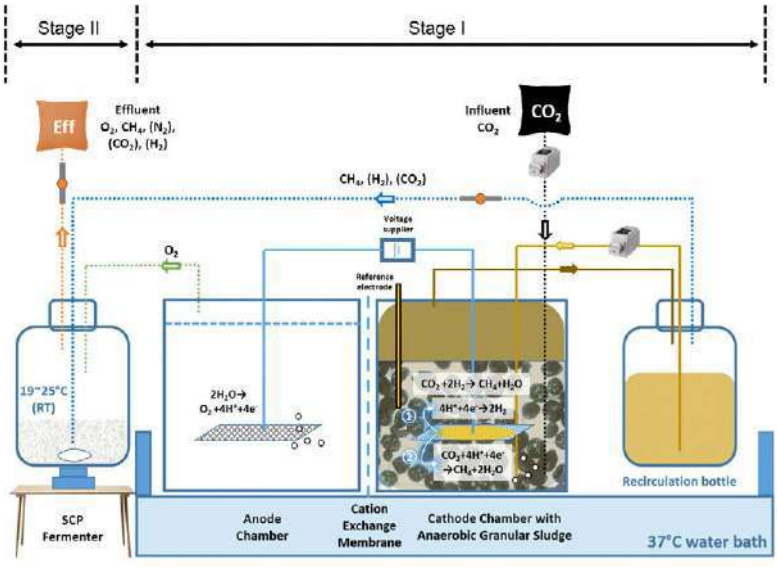

Fig. 1 Schematic illustration of the proposed BIES for SCP production

Keywords: Microbial electrosynthesis, Single-cell protein, Carbon capture and utilization, Hydrogenotrophic methanogens, Aerobic methane oxidation

Funding: the Novo Nordisk Foundation (NNF16OC0021568, Denma
the Carlsberg Foundation Distinguished Fellowships (CF18-0084, Denmark)
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the Otto Mønsted Fond (Denmark)

In-situ production of microbial protein with reclaimed ammonium in a microbial electrochemical recovery conversion cell

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Microbial protein (MP) is regarded as a promising alternative to plant/animal based protein to feed the world because of its low environmental impact. In-situ and cost-effectively recovery of nitrogen from wastewater is one of the key challenges to economy of MP production. We therefore proposed a two-chamber microbial electrochemical recovery conversion cell to meet the challenge. A representative MP producer, *Cupriavidus necator*, could fix carbon dioxide as protein-rich biomass in the cathode chamber with ammonium recovered from wastewater in the anode chamber through diffusion and electroosmosis and in-situ generated hydrogen and oxygen through water electrolysis. MP was successfully obtained with an external power supply of 3 ~ 5 volt and the MP yield was 0.81 g/L in 36 h at 5V in batch mode. *C. necator* could grow in cathode even when the anode chamber was filled with ammonium solution in a broad range (0.05 ~ 8 g N/L). Highest MP yield 0.9 g/L was achieved in 36 h at 2 g N/L. The system was also proved had the ability to produce MP with reclaimed ammonium from real wastewater streams (e.g., influent from municipal wastewater and digestate) and the protein yielded had a high quality on amino acid profile.

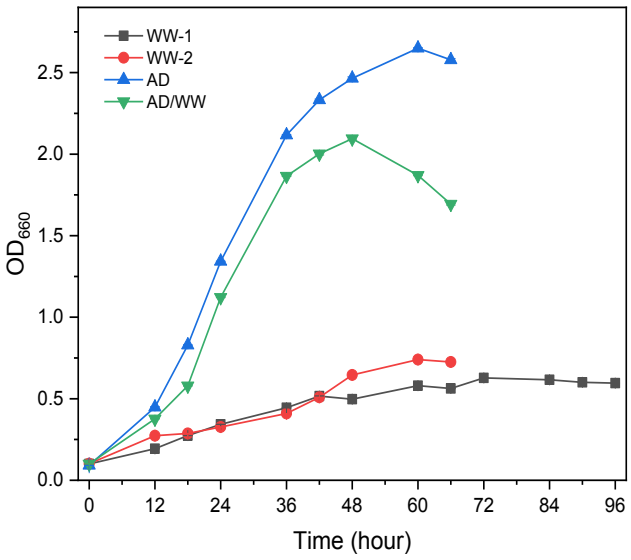

Fig. 1 *C. necator* was cultivated in the microbial electrochemical recovery conversion cell with different wastewater streams, WW, influent from a municipal wastewater, AD, digestate from an anaerobic digester, AD/WW, a mixture of the former two wastewater streams.

Keywords: single cell protein, hydrogen-oxidizing bacterium, ammonium recovery, microbial electrochemical system

Funding:This work was supported by the Novo Nordisk Foundation (NNF16OC0021568, Denmark) and the Carlsberg Foundation Distinguished Fellowships (CF18-0084, Denmark). The authors would like to acknowledge the financial support from the China Scholarship Council (China),

Towards bioelectrochemical degradation of hydrophobic wastes coupled with synthesis of biosurfactants

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In recent decades, bioelectrochemical systems (BES) have undergone significant improvements and numerous exciting applications have emerged. This technology has great potential to contribute in solving multiple global issues such as environmental contamination. There are, however, some limitations in the efficiency of treating hydrophobic wastes such as petroleum contaminants or waste cooking oil.

In our research, we aimed to couple the bioelectrochemical degradation of these substrates with *in-situ* production of biosurfactants in microbial fuel cells (MFCs) and potentiostatic – controlled BESs. Several approaches have been taken for the enrichment of microbial consortia capable of bioelectrochemical synthesis of surfactants. The microbial communities were derived from environments with various contact with hydrocarbons such as contaminated soil, municipal environments, river sediments, activated sludge, mud volcanoes, and glacier environments. Each of the communities revealed unique electrochemical properties and tolerance to petroleum hydrocarbons. The amount of biosurfactants detected in the process varied and their highest activity was reported when using waste cooking oil and external load used as a selective factor in contrary to various poised potentials, where biodegradation was accompanied with negligible changes in surface tension. The established electrofermentation led to the synthesis of biosurfactants and increased bioavailability of hydrophobic substrates for their further biodegradation.

Keywords: MFC, BES, waste, petroleum, oil, biosurfactants

Funding: This work was supported by the Polish National Agency for Academic Exchange – Polish Returns grant (PPN/PPO/2018/1/00038) and National Science Centre (Poland) OPUS grant (2019/33/B/NZ9/02774).

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Biogas upgradation through CO_2 conversion into acetic acid via microbial electrosynthesis (MES)

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Biogas is considered one of the major biofuel sources to address the issues associated with fossil-fuels through sustainable approaches. Due to the presence of CO_2 in biogas, its calorific value decreases considerably. Various biogas upgradation technologies are under intense investigation to increase the methane content to desired level []. In this study, we demonstrate methane content enhancement in biogas through CO_2 conversion into acetic acid *via* MES process. In the MES reactors inoculated with *Acetobacterium* dominated mixed culture [2], biogas was fed continuously at different flow-rates (0.5,0.3,0.2ml/min). At a fixed cathodic potential of -1 V (*vs.* Ag/AgCl), a considerable increase in methane content along with acetic acid production was achieved in all cases. The highest flow-rate (0.5ml/min) was found to be least effective both for methane content increase (from 61±3% to 86±2%) and acetic acid titer(2.0±0.25g/L). In comparison, the lowest flow-rate of 0.2ml/min was the most effective for the intended process (methane upgradation from 62±7% to 93±3% and acetic acid titer(3.9±0.6 g/L). The bioproduction and biogas upgradation occurred at an E_{cell} of 3.3±0.35V. The demonstrated approach offers the advantage of obtaining two products; one in the bulk phase and the other in the off-gas from the biogas feed.

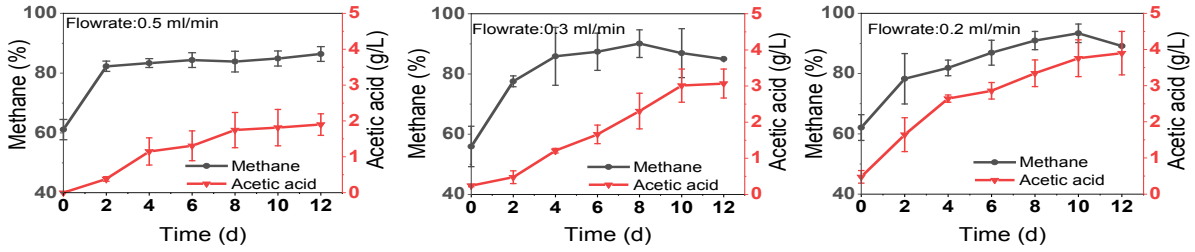

Fig.1 Methane and acetic acid concentration profiles at different flow rates of biogas.

Keywords: Biogas upgradation, Microbial electrosynthesis, *Acetobacterium*

Funding: Department of Biotechnology (DBT), Government of India (Project No. BT/PR28914/BCE/8/1441/2018)

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CO_2 conversion by combining a Cu electrocatalyst and microorganisms

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Electrocatalytic conversion of CO_2 can utilize renewable electricity to valorise this waste greenhouse gas. Both abiotic (e.g. metal) catalysts and microorganisms (MOs) can catalyse this reaction. Metals achieve high reaction rates, but often deactivate fast and produce short carbon chains. In microbial electrosynthesis, MOs have a lag phase, but are self-replicating, can operate for long periods and produce longer carbon chains. Based on their advantages and disadvantages, metals and MOs could complement each other if combined. Metals can produce intermediates at high rates, which can be further elongated by MOs. On the other hand, combination can result in biofouling, catalyst deactivation due to microbial growth nutrients, and/or metal toxicity to MOs.

Here, we present the first reports on combining a copper (Cu) electrocatalyst and MOs for CO_2 conversion. Microbial growth medium not only allowed, but in fact enhanced the catalytic activity of Cu for formate production, compared to buffer (Fig.1a).[1] MOs did not hinder the catalytic activity of Cu, despite biofilm growth on the electrode (Fig.1b).[2] MOs, evidently not toxified by Cu, were able to consume CO_2 and formate, producing acetate as final product (Fig.1c).[2] Despite its antimicrobial properties, Cu is a promising catalyst to combine with MOs.

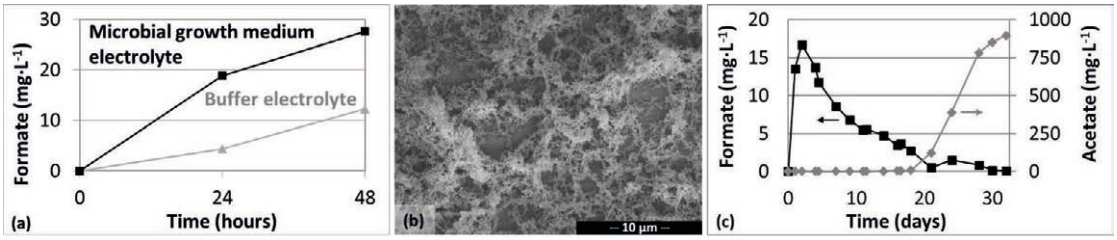

Fig. 1: (a) Electrocatalytic formate production over time with Cu electrodes in microbial growth medium (black line) and buffer (grey line) electrolytes, in the absence of MOs. (b) Biofilm growth on Cu electrode at the end of microbial electrosynthesis experiments. (c) Formate production by Cu electrode (black line, left axis) and simultaneous consumption by MOs, accompanied by acetate production by MOs (grey line, right axis).

Keywords: microbial electrosynthesis, copper electrocatalyst, CO_2 electroreduction, catalytic cooperation

Funding: Wageningen Institute for Environment and Climate Research; Graduate School of Food Technology, Agrobiotechnology, Nutrition and Health Sciences

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Biocathode and photoanode development for solar energy-driven microbial electrosynthesis

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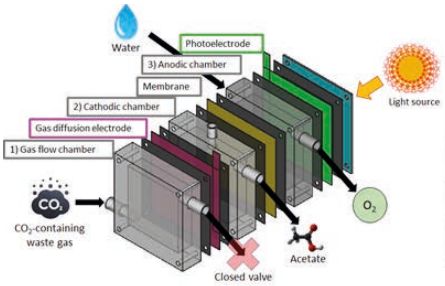
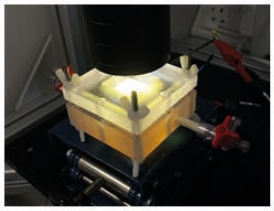
Microbial electrosynthesis (MES) is a promising technology for CO_2 recycling into valuable products such as volatile fatty acids and alcohols [1]. However, the high capital (particularly the anode material) and operation costs (mainly electric energy consumption) are hindering its adoption in industry [2]. This study focuses on the development of a MES cell equipped with a low-cost photoanode that exploits visible light to decrease the electric power input required to drive bio-cathodic CO_2 reduction. A three-chamber hybrid cell prototype has been designed and 3-D printed for laboratory-scale experiments, where the bio-cathode and photo-anode parts are currently being optimized separately. The highest acetate production rate (53 g/m²/d, 0.85 g/L/d) was achieved in the cell equipped with a gas diffusion biocathode operated in continuous mode (100% CO_2, 2 mL/min) at room temperature under galvanostatic mode (1 mA/cm²). This resulted in an average specific production of 38.7 $kg_{acetate}/MWh_{el}$, highlighting the need of reducing the electric power input to make the process sustainable. Photoanodes were fabricated by deposition of a thin layer (60 µm) of $BiVO_4$-based photocatalyst on a fluorine tin oxide transparent electrode, achieving the highest stable photocurrent of 0.15 mA/cm² when exposed to a simulated AM1.5G solar spectrum at 1 V bias.





Current density (mA/cm²)	Acetate production rate (g/m²/d)	CE (%)	CO_2 removal (%)	Specific production (kg/MWh)
0.25	5.4	31.9	17.0	31.0
1.00	38.0	56.4	24.0	38.7

Fig. 1. From left to right: schematic overview of the hybrid cell; picture of the BiVO4-based photocatalyst under testing; table summarizing average production rate, coulombic efficiency, CO_2 removal efficiency and specific production obtained at an applied current of 0.25 and 1.00 mA/cm².

Keywords: Bioelectrochemical system, CCU, Microbial electrosynthesis, OER, Photocatalysis

Funding: Science Foundation Ireland (SFI) Pathfinder Award on "Hybrid Bio-Solar Reactors for wastewater treatment and CO_2 recycling" (nr. 19/FIP/ZE/7572PF).

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A general model for biofilm-driven microbial electrosynthesis of carboxylates from CO_2

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Up to date, computational modelling of microbial electrosynthesis (MES) has been underexplored, but is necessary to achieve breakthrough understanding of the process-limiting steps. We developed a general framework for modelling microbial kinetics in a MES reactor. A thermodynamic approach is used to link microbial metabolism to the electrochemical reduction of an intracellular mediator, allowing to predict cellular growth and current consumption. The model accounts for CO_2 reduction to acetate, and further elongation to n-butyrate and n-caproate. Simulation results were compared with experimental data obtained from different sources and proved the model is able to successfully describe microbial kinetics (growth, chain elongation, product inhibition) and reactor performance (current density, product titre). The capacity of the model to simulate different system configurations is also shown. Model results suggest CO_2 dissolved concentration might be limiting existing MES systems, and highlight the importance of the delivery method utilized to supply it. Simulation results also indicate that for biofilm-driven reactors, continuous mode significantly enhances microbial growth and might allow denser biofilms to be formed and higher current densities to be achieved.

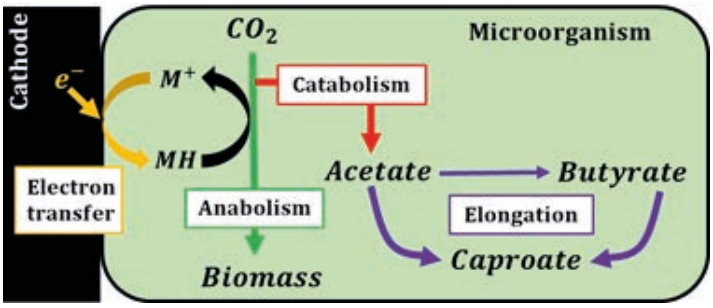

Fig. 1 Schematic representation of the modelled reactions within the microbial cell.

Keywords: Microbial electrosynthesis, Microbial kinetics, Mathematical model, CO_2 reduction, Chain elongation.

Funding: This activity is co-financed by Shell and a PPP-allowance from Top Consortia for Knowledge and Innovation (TKI's) of the Dutch Ministry of Economic Affairs and Climate in the context of the TU Delft e-Refinery Institute.

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The impact of cathode acclimation methods on electrotrophic biofilm formation and performance of electromethanogenic cells

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In this work, we investigated the impact of changing substrate on the formation of biofilm in electromethanogenic cells. The objective was to compare the performance of two different acclimation substrates, namely, acetate and cattle manure in single-chamber MECs for methane production. The experiments consist of three groups; in the first group, cattle manure was used both as inoculum and carbon source; in the two other groups, seed from anaerobic digester was used as inoculum, and cattle manure and acetate with different organic loads were used as carbon sources. A voltage (0.7 V) was applied to all the reactors cyclic voltammetry was used to test activity. The maximum current density for reactors acclimated with acetate and then supplemented with cattle manure reached to 0.19 mA/cm^{2,} with 102 ml$_{CH4}$/L, 53% coulombic efficiency (CE) and 38% COD removal. In the reactors, fed only with manure, the current density reached to 0.16 mA/cm^2 with 108 ml$_{CH4}$/L, 51% CE and 32% COD removal. Hence, we conclude (i) it is possible to start-up an MEC using cattle manure as the only inoculum and carbon source and (ii) change in the type of substrate in the acclimation period has no effect on the reactor performance.

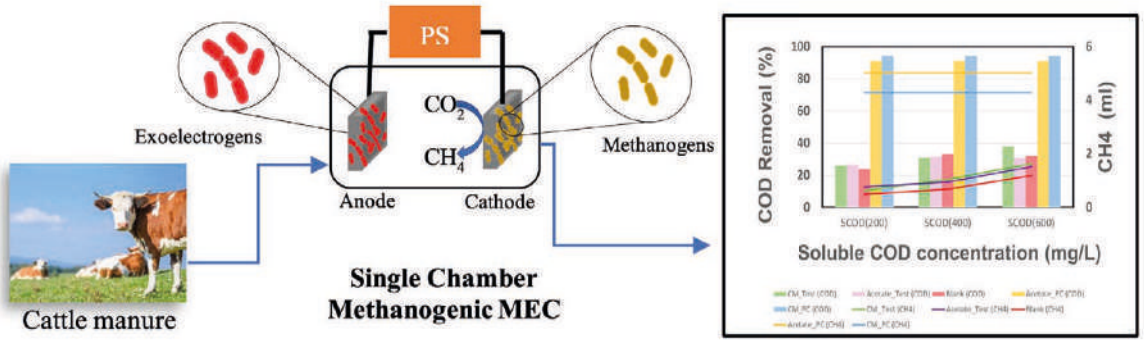

Fig. 1 Schematic representation of a microbial electrolysis cell (MEC) for methane production from cattle manure

Keywords: acclimation, methane, electromethanogenesis, cattle manure, microbial electrolysis cell

Funding: This study was funded by the Office of Sponsored Research at METU grant number 10776.

Strategies for increasing production of bioproducts from CO_2 by Microbial electrosynthesis (MES)

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In this study, we investigated different strategies to increase acetate and longer chain bio-products from MES. At -1.0 V Ag/AgCl applied potential [1], and dissolved CO_2 as carbon source through.
1) addition of external electron donors in additional to cathode [2]

2) application of continuous operational mode [3];

3) modification of cathode with PANI polymer [4].

The effect of hydraulic retention time (HRTs) was investigated with 3 and 7 days (Fig 1). For HRT of 3 days, pH was 5.2 and acetate was the dominant product with the highest production rate of 651.8 ± 214.2 ppm day^{-1}, while for HRT of 7 days, pH was lower at 4.5, lower but stable acetate production rate 280 ppm day^{-1} was obtained, and more diverse longer chain, C4-C6 products were observed. With additional formate and ethanol to the MES reactors, although acetate was still main product, more diverse and longer chain bio-products were achieved. Further enhancement by using PANI modified biocathode. Reactors were operated during 60 days and PANI-GDEs led to an acetate concentration of 6.85 ± 0.9 g L^{-1} and 0.20 ± 0.05 g L^{-1} butyrate. It is possible to control MES process and products by applying different parameters.

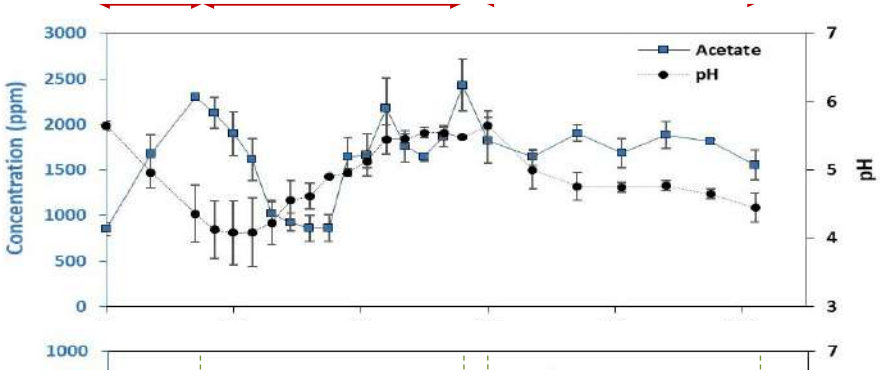

Fig. 1 Acetate production at HRT 3 days and 7 days

Keywords: CO2 conversion, MES, HRT, additional electron donner, GDE, polymer modification

Funding: **EPSRC** EP/N009746/1**,** EP/R021503/1 **and EP/V011863/1**, NERC MeteoRR (NE/L014246/1) and NBIC 002POC19034

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Bioelectrosynthesis of organic acids from CO_2 using fluidized bed electrodes in a 3 phase reactor.

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Microbial electrochemical technologies (MET) are becoming more interesting to design and provide solutions to the society pollution issues. One of them is the release of greenhouses gases, as CO_2, in industrial processes. In this study, we investigate a new microbial electrochemical design for the process of Microbial Electrosynthesis (MES). In this context, the CO_2 is microbially reduced using electrons released in the fluid-like electrode of a Microbial Electrochemical Fluidized Bed Reactor (ME-FBR). ME-FBR was operated as a 3 phase reactor (solid-liquid-gas) where working electrode (biocathode) was fluidized by gas, and polarized at different potentials (-0.6, -0.8 and -1 V vs Ag/AgCl). Microorganisms were enriched from real wastewater under anaerobic conditions in presence of 2-bromoetanosulfonic acid (BES) to avoid methanogens growth. Acetate, and other volatile fatty acids, such as formate and propionate, were detected when the reactor was operated. The electrons needed in the reduction of CO_2, were provided by hydrogen produced either electrochemically as well as mediated by microorganisms using the fluid-like biocathode.

Keywords: microbial electrosynthesis, fluidized bed, biocathode, bioelectrochemistry, CO_2 fixation.

Funding: MET-FLUID project (RTI2018-101974-B-C21)

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Posters

5th EU-ISMET ONLINE

5th European Meeting of the International Society for Microbial Electrochemistry and Technology

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Large-scale meta-analysis methodology for facilitating the scale-up of bioelectrochemical systems for wastewater treatment

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Bioelectrochemical systems (BESs) hold great promises for energy-efficient wastewater treatment and they have undergone a quick development since 2003. Notably, over the past few years, the number of BES scale-up reports has increased. To help steering technology development, there is now a clear need for a more thorough joint assessment of the great variety of configurations and operational conditions investigated so far. To that extent, dealing with the inhomogeneity in the way to report results, despite some valuable proposals [1,2], remains a major challenge.

In order to tackle these challenges, a meta-analysis was conducted based on the systematic literature review methodology [3]. This poster details the successive steps of the study: scoping, selection of which data to collect, articles search and selection, database constitution and cleaning, and quantitative analysis. This allowed the exhaustive and reproducible selection of 1,074 articles from which data was extracted. Collected data included both explanatory variables (eg. effluent characteristics, anodic configuration, inoculum ...) and performance indicators (eg. COD removal and coulombic efficiency).

This poster is a methodological complement to the work of Larzillière et al. that will discuss the main conclusions of the meta-analysis.

Keywords: methodology, wastewater treatment efficiency, process scale-up, systematic review

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Dual-cathode configuration and headspace gas recirculation enhanced microbial electrosynthesis from CO_2 using Sporomusa ovata

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High-rate production of acetate and/or other value-added products from the bacterial reduction of CO_2 in microbial electrosynthesis (MES) can be achieved with high reducing power where H_2 appears as a key electron mediator. Due to the low solubility of H_2, the availability of H_2 remains limited to bacteria. H_2 evolution using metal cathode can enhance the availability of H_2 to support high-rate microbial reduction of CO_2. In this study, we investigated the performance of *Sporomusa ovata* for CO_2 reduction when graphite rod and titanium mesh were used combinedly in dual-cathode configuration to promote hydrogen production. At the same time, the headspace gas was recirculated to increase the H_2 availability to the bacteria. High-rate CO_2 reduction was observed at -0.9 V versus Ag/AgCl with double cathode configuration and gas recirculation as compared to the performances of individual cathodes in MES. High titer of acetate (up to ~11 g/L) with a maximum rate of 0.68 g/L/d at -0.9 V vs Ag/AgCl cathode potential was observed, which is higher than the production rate (0.3 g/L/d) obtained under H_2:CO_2:N_2 (60:20:20) gas fermentation with *S. ovata*. High H_2 availability supported the high-rate acetate production from CO_2 with diminished electricity input.

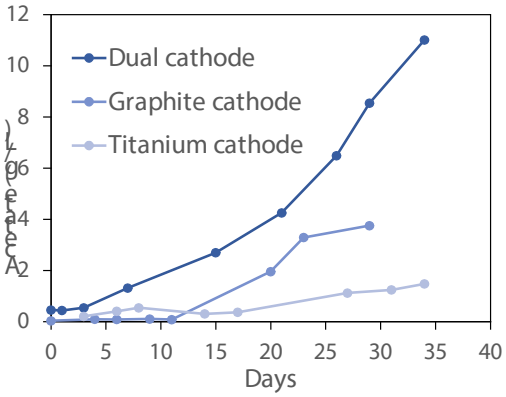

Fig. 1 Graphite rod and Titanium mesh as dual cathode configuration (left). Acetate production in dual cathode and single cathode MESs (right)

Keywords: Microbial Electrosynthesis, CO_2 reduction, Dual-cathode, Hydrogen evolution, gas-liquid mass transfer, gas recirculation

Funding: Swedish Research Council (BEAM no. 2018-03875)

To the extreme: the effect of temperature on psychrotolerant *Shewanella loihica* and its electrical output in a microbial fuel cell

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Temperature is a determining factor in the performance of bioelectrochemical systems[1]. Microbial fuel cells (MFCs), for instance, convert chemical energy into an electrical current with bacteria called exoelectrogens. As most bacteria are mesophilic, more extreme operating temperatures often lead to lower power outputs. Nevertheless, such harsh temperatures can have additional value such as an improved bacterial metabolism and increased conductivity at higher operating temperature[2], or higher coulombic efficiency at lower operating temperature[3]. To broaden the working temperature interval of MFCs, extremophilic exoelectrogens can be used. These bacteria can withstand more extreme environments such as cold (psychrophiles) or hot (thermophiles) environments. Here, the effect of temperature on growth and output of psychrotolerant species *Shewanella loihica* was investigated. *S. loihica* was cultured at 5°C, 15°C and 25°C and growth curves were established with OD_{600} measurements; of each growth phase the morphology was investigated with atomic force microscopy (AFM). MFCs with *S. loihica* operated and monitored for 30 days at identical temperatures. The temperature effect was investigated through morphology of planktonic and biofilm cells (AFM), biofilm formation (confocal laser scanning microscopy), mediator quantity (fluorescence spectroscopy) and electrical output (polarisation/power curve). These results aided in uncovering *S. loihica*'s potential to broaden the MFC temperature range.

Keywords: S. loihica, psychrotolerant exoelectrogens, microbial fuel cell, temperature dependence

Funding: BOF Doctoral Fund (Hasselt University)

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Design, process control, and online measurement for a microbial fuel cell system targeting upscaling in wastewater treatment

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The power density of a microbial fuel cell (MFC) is influenced by its system configuration and the process conditions contributing directly to the efficiency of electroactive biofilms [1]. Commercialization of MFCs for wastewater treatment requires larger scale MFCs to handle greater wastewater flows [2].

The tubular 2-liter single-chamber MFC, adapted for brewery wastewater treatment, was designed considering scalability, process control and instrumentation. The scale-up potential and design limits were calculated taking into account key factors (e.g. electrode packing density) and the clogging tendency of carbon fiber brushes when used as anode electrodes and in combination with VITO CORE® cathodes. The parallel use of the automated MFC system provided the opportunity to compare batch and continuous flow under the same process conditions. Online sensors (mass flow, pH, conductivity) enabled analysis of the wastewater during long-term MFC operation on a laboratory scale, allowing conclusions to be drawn for the necessary wastewater management for the use of MFCs in breweries.

Keywords: microbial fuel cell design, process control, brewery wastewater, scale-up potential

Funding: German Federal Ministry for Economic Affairs and Energy (grant number: IGF 20789 N/1)

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Anaerobic cultivation of genetically engineered Pseudomonas putida in a bioelectrochemical system

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In order to establish bio-based processes, the optimization of material- and energy-efficiency is one of the key performance indicators. Therefore, a focus is the establishment of anaerobic processes. These processes could offer many advantages over aerobic processes, such as bypassing oxygen limitations, higher carbon yields and less foaming [1].

Here, a *Pseudomonas putida* F1 strain is used for anaerobic cultivation in a bioelectrochemical system (BES). The anode respiration via a mediator was already described [2]. Here we investigated the heterologous expression of electron transfer proteins from *Shewanella oneidensis* in *P. putida* to intensify the productivity of 2-ketogluconate (2-kga). For the expression of CymA and MtrA from *S. oneidensis*, two vectors were synthesized and transformed into *P. putida*. CymA could be identified via expression analysis and numerous other heme-containing proteins were detected in wildtype and genetically modified organism (GMO). In the BES, no benefit was found for the GMO in terms of the productivity of 2-kga. It is shown, however, that the wildtype can carry out an “overflow” metabolism in the BES, which delivers an increased current and is possibly limited by the available amount of oxidized mediator. The fermentation supernatants also showed the accumulation of acetoin as a novel byproduct.

Keywords: Anoxic metabolism, redox mediators, mediated extracellular electron transfer, Pseudomonas putida F1
Funding: Federal Ministry of Education and Research – BMBF (031B0523)

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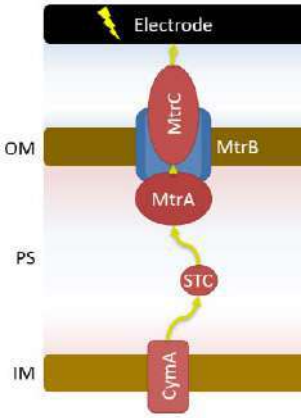

Fig. 1 Electron transfer proteins from *S. oneidensis*.

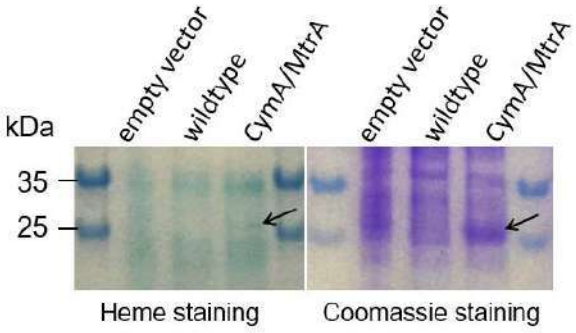

Fig. 2 Expression of CymA and other heme-containing proteins.

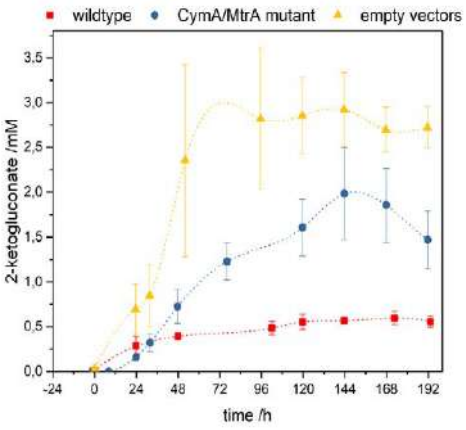

Fig. 3 Productivity of 2-ketogluconate with CymA/MtrA mutant, empty vector control and wildtype.

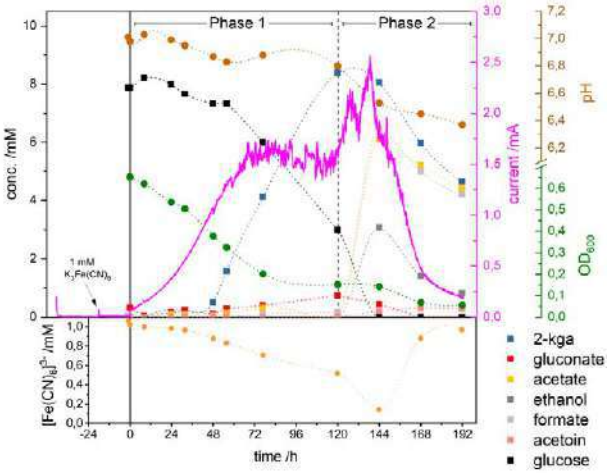

Fig. 4 Metabolic shift in BES cultivation together with depletion of oxidised mediator.

Utilization of phenazines (pyocyanin and phenazine-1-carboxylic acid) by Enterobacter aerogenes

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Aerobic respiration is considered the most energy efficient form of metabolism. However, oxygen is toxic to several industrially relevant biochemical products, e.g. redox enzymes, and therefore, cannot be utilized for their production (1). Inside an anaerobic bioelectrochemical system (BES), *Enterobacter aerogenes* (a facultative aerobe) is capable of utilizing an anode as terminal electron acceptor by using phenazine redox mediators; electrochemically active, secondary metabolites produced by *Pseudomonas aeruginosa* (2, 3). By doing so, it induces a partial shift in its metabolism from fermentation to energetically beneficial, electrode-based respiration resulting in higher biomass yield (3).

In this study, the process of electro-respiration in *E. aerogenes* as an alternate approach to aerobic respiration was investigated in more detail. Multiple concentrations of phenazines (pyocyanin/PYO and phenazine-1-carboxylic acid/PCA) in BESs were tested under anoxic conditions.

An increase in the biomass production was observed with increasing concentration of phenazines. However, the expected simultaneous decrease in fermentation by-products was not seen. PCA was found to be energetically less beneficial than PYO due to its more negative electrochemical potential.

Keywords: Bioelectrochemical system, Phenazines, Electro-respiration

Funding: This research is funded by the European Research Council (ERC) consolidator grant #864669.

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Pintant biofilms com Dalí: Light sheet microscopy reveals growth of electroactive biofilms in 4D

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Light sheet fluorescence microscopy (LSFM) enables 4-dimensional imaging of eukaryotic cells and tissues [1,2]. This imaging technology allows large specimen to be investigated in vivo under physiological conditions in a non-destructive way. We show that the advantages of LSFM are also highly valuable for prokaryotic analyses by studying label-free natural microbial biofilm development on the example of an anaerobic electroactive biofilm [3]. Therefore a bioelectrochemical system was implemented in the LSFM. The biofilm was grown on a graphite electrode and the current production as a measure of activity was monitored over time. This is the first time that by label-free and non-destructive imaging the growth could be linked to the production of current in vivo by monitoring an anaerobically grown electroactive bacterial biofilm with high spatial and temporal resolution. After exponential growth a homogeneous biofilm (thickness of 9 µm) was formed. This was followed by a stratification of the biofilm including formation of 3D structures (see Figure 1). Light reflection was sufficient to visualize the biofilm structure and development over time. The morphology was confirmed using fluorescent staining and thus LSFM can paint biofilms as (sur)realistic as Dalí.

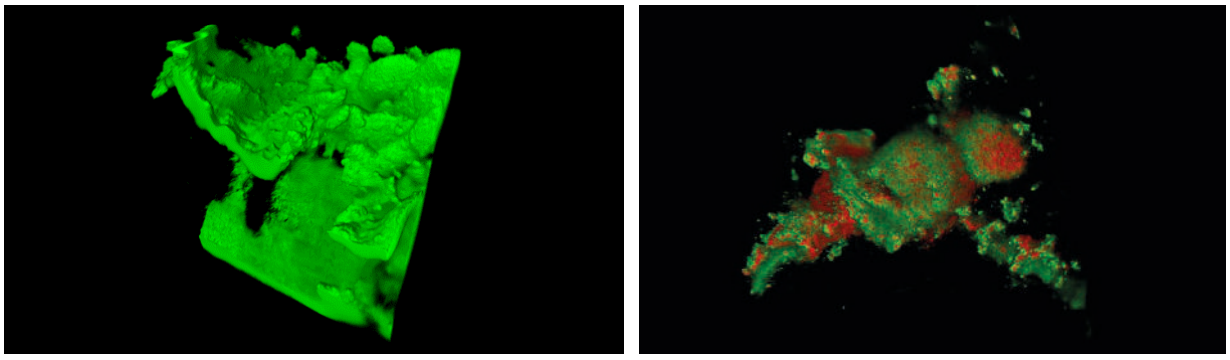

Fig. 1: Painting biofilms as Dalí: A) Light reflection (excitation with 405 nm) is sufficient to visualize the 3-dimensional biofilm surface structure. B) LIVE/DEAD nucleic acid fluorescent staining confirms the organization of the individual cells and the three-dimensional organization of the biofilm.

Keywords:
microscopy, biofilms, electrophysiology

Funding: This work was supported by the Helmholtz-Association in the frame of the Integration Platform "Tapping nature's potential for sustainable production and a healthy environment" at the UFZ.

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Reversing the Castells principles:

The activity stratification in electrified biotrickling filters

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Recently, electrified biotrickling filters were introduced as sustainable microbial electrochemical technology (MET) for treating nitrogen-contaminated waters while neither requiring addition of chemicals nor external aeration[1]. The simultaneous degradation of ammonium and nitrate to dinitrogen was achieved by establishing a redox potential stratification in the filter constituted of glass beads (non-polarized aerobic zone) and graphite granules as conductive filling material (polarized anoxic oxidative/reductive zones). It was also demonstrated that this MET can be applied in aquaponics as it provides an appropriate effluent quality.

For revealing the stratification of activity within electrified biotrickling filters, the electrified biotrickling filters were modified by tailor-made sampling systems allowing a minimal-invasive sampling of graphite from the filling. Subsequently, the granules were electrochemically analyzed with the recently developed e-Clamp[2] for providing thermodynamic information about the redox centers responsible for the extracellular electron transfer and participating in the bioelectrochemical degradation of ammonium and nitrate. Thereby, several redox signals in the range of −500 to +100 mV (vs. Ag/AgCl sat. KCl) were observed that were distinctively different than previous reported redox signals of similar processes[3,4]the elucidation of the underlying extracellular electron transfer (EET. A genetic analysis (T-RFLP and metagenomics) revealed a microbial stratification being putatively linked to the stratification of activity within the individual zones.

Keywords: bioelectrochemical nitrogen removal, cyclic voltammetry, redox potential stratification, circular bioeconomy, biocathode

Funding: This research was carried out in the project “Wireless Aquaponic Farming in Remote Areas: A smart adaptive socio-economic solution” (WAFRA) funded within the 7th Framework Program (ERANETMED).

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Bioelectrochemical Methanation of CO_2 from Untreated Steel Mill Gas

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Steel production is one of the most energy-intensive processes in which 7% of global CO_2 is emitted. Therefore, the project LOCON (low energy CO_2 conversion and utilization at the example of steel industry) focuses on the biological methanation of CO_2 from untreated steel mill gases by usage of a bioelectrochemical system (BES) and geomethanation. In a BES, at least one electrode is covered by an electroactive biofilm, which catalyses oxidation or reduction processes. In LOCON we want to take a closer look on a BES with a bioanode and a biocathode. At the bioanode microbial oxidation of organics (e.g., wastewater) occurs, whereby electrons are produced which are supplied to a CO_2 reducing biocathode (Fig. 1). To test the biological methanation of CO_2 from untreated steel mill gas, a lab-scale BES was set up. During long-term operation different process parameters like chemical oxygen demand removal, current generation and CH_4 production were monitored. In the first stage the biocathode was flushed with pure CO_2. After optimization of process parameters and successful methanation, the CO_2 conversion of untreated steel mill gas was tested, and the biofilm community composition was analysed.

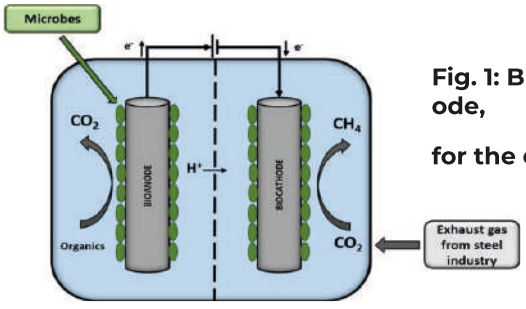

Fig. 1: BES, consisting of a bioanode and a biocathode,

for the conversion of CO_2 rich exhaust gas to CH_4

Keywords: bioelectrochemical system, bioanode, biocathode, CH_4 production, CO_2 conversion

Funding: This project has received funding from the Austrian Research Promotion Agency (FFG).

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Revealing the potential exoelectrogenic activity of bacteria mediated by their phenazine production

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The synthesis of redox-active molecules acting as extracellular electron shuttles, such as phenazines, has been predicted based on the presence of genes involved in their biosynthesis (*phzA-G*)[1] or detection (*soxR*)[2]. Phenazine synthesis has been confirmed in a limited number of genera and only few species are employed in bioelectrochemical systems (BES), mainly pseudomonads, native phenazine producers[3,4]. Here, the potential exoelectrogenic activity mediated by phenazines and their performance within BES was evaluated in diverse species under varying conditions such as medium, oxygen availability, addition of synthetic phenazines, etc. This is the first report indicating that species of genera *Pseudomonas*, *Streptomyces*, *Nocardiopsis*, *Brevibacterium*, and *Burkholderia* are electroactive bacteria employing phenazines. Electron discharge to the anode and therefore, the produced electric current correlated with phenazine synthesis, phenazine-1-carboxylic acid being the dominant redox mediator in BES reaching concentrations of 76 mg/L and anodic current of 14 µA/cm^2. Phenazine-1-carboxamide and pyocyanine were produced in lower concentrations but also contributed to current generation. Phenazine addition to the BES decreased the biomass production and increased current in selected cases. Elucidating the phenazine usage by bacteria will improve biotechnological applications of oxygen limited BES with single or mixed cultures, allowing for the discovery and synthesis of new bioproducts.

Keywords: exoelectrogenic bacteria, phenazine, bioelectrochemical system, electric current.

Funding: This research is funded by the European Research Council (ERC) consolidator grant # 864669.

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Investigation of electrosynthetic acetate production by the novel cathodically active bacterium Desulfosporosinus orientis

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During recent years, Microbial Electrosynthesis directed attention towards the exploitation of CO_2-rich waste gas streams for electrosynthesis of value-added products. We investigated the acetate bioelectrosynthesis of the cathodically active, sulfate reducing bacterium *Desulfosporosinus orientis*. Acetogenesis was observed during electroautotrophic growth, previously [1]. However, since sulfate respiration is energetically more favorable [2], CO_2 fixation was low. Therefore, Adaptive Laboratory Evolution towards reduced sulfate utilization (25 % and 50 % of the optimal concentration) was performed. New strains were analyzed during autotrophic growth in serum bottles and in bioelectrochemical H-type reactors. Additionally, acetate bioelectrosynthesis was confirmed through stable isotope labeling experiments. The highest electrosynthetic acetate titer was 1.91 mM (50 % sulfate-strain). The 25 % sulfate-strain performed less well in terms of titers, but had higher yields of acetate per biomass. These results showed that enhanced acetate production by restricting sulfate respiration in *D. orientis*, come at the expense of reduced biomass formation. During autotrophic growth in serum bottles, enhanced acetogenic titers and formation of a new by-product, butyric acid, were observed [3]. This highlights that the potential of the strain was not fully tapped in the electrochemical reactors, yet and deeper exploration of electroautotrophic CO_2 fixation harbors further expandable possibilities.

Keywords: Microbial Electrosynthesis, Acetogenesis, Adaptive Laboratory Evolution keyword

Funding: ERC Consolidator Grant 2019, Project: e-MICROBe

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Real-time monitoring and selective detection of micropollutants using a single chamber microbial fuel cell-based biosensor

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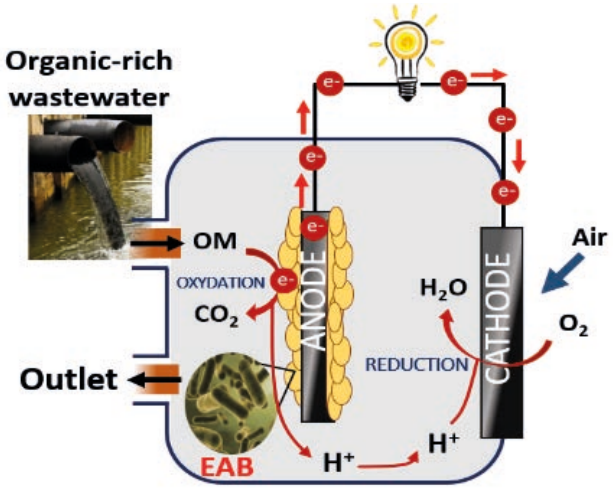
On-line monitoring of water quality plays a central role in providing safe drinking water and in the operation of wastewater treatment plants**.** Biosensors based on microbial fuel cell (MFC) platform have recently been received increasing attention [1]. MFCs are bioelectrochemical devices that utilize microorganisms as catalysts to convert chemical energy to electrical energy [2]. Biofilms composed of electrochemically active bacteria (EAB), oxidize organic matter in the anodic compartment to produce protons and electrons. The resulting electrons are captured at the anode and transferred to the cathode through an external circuit producing electricity. MFC-based biosensors rely on a change in the output current for any disturbances on the metabolism of the EAB's activity. They offer the advantages of being self-powered and directly generate electrical signals without any need for a separated transducer. However, it is challenging to use MFC biosensors for toxicity monitoring when a high specificity is required. In this study, a new configuration of single-chamber cube biosensors based on MFC was investigated to further improve the MFC sensor's specificity. A differential analysis approach (DAA) based on the difference in responses of MFC biosensors based on micropollutant-adapted and nonadapted biofilms was investigated to detect specifically the target analyte.



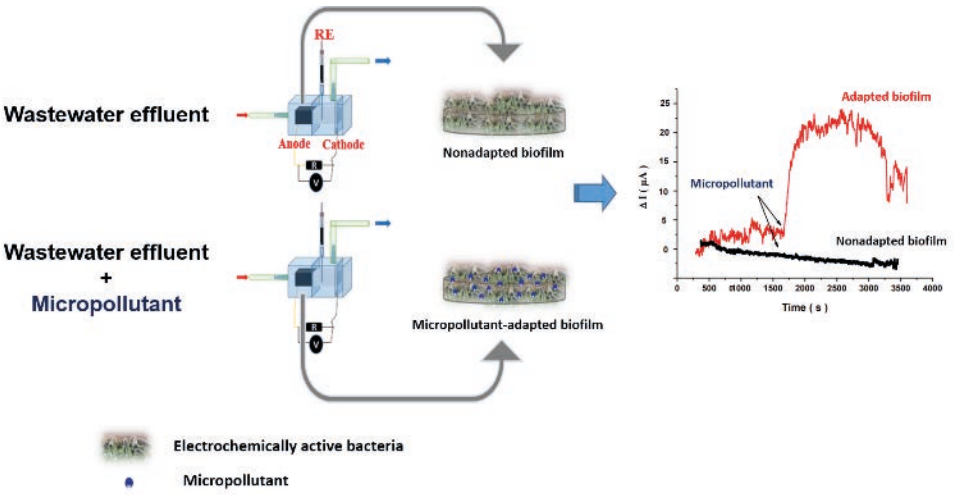


Fig. 1.
A) Schematic of a MFC-based biosensors,
B) illustration of micropollutant-adapted and nonadapted biofilm and change in the output current

Keywords: Adapted-biofilms, biosensor, microbial fuel cell, micropollutant, real-time detection, differential analysis approach

Funding: The authors greatly acknowledge the PHC Maghreb 2019 project **N°41382WC** for its support to do this work.

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Sustainable Remediation of Crude Oil Contamination by utilizing a Bio-Electrochemical System (BES)

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Sustainable remediation has become a fundamental approach in the development of remediation technologies. Bio-Electrochemical Systems (BES) are an innovative method which is considered as a prime candidate for the bioremediation of contaminated soil that are also good candidates as sustainable remediation technologies. Crude oil contaminated land is a challenge for BES due the toxicity and recalcitrant characteristics of crude oil. Parameters such as BES configuration and electrode materials must be considered for BES platform to be adopted as a sustainable remediation approach. The effect of ionic strength applied to a BES to test resolution of BES electrical output and effect of changes in operating procedure (concentration, temperature). The electrochemical difference between anode and cathode produces an electrical signal that is a linear relationship with concentration. This work considers a BES chamber that focuses on the degradation of Kuwaiti soil contaminated with crude oil. Initial data indicates that there is a slow change in the voltage and power output suggesting it might take longer time for the biodegradation and enrichment of the electrogenic microbial communities. The controlled lab-scale BES have been identified to assist civil engineers for designing and scaling up BESs for the enhancement of semi-passive medium term remediation systems.

Fig. 1 (BES configuration)

Keywords: Bio-electrochemical system (BES), Crude oil contaminated soil , Sustainable remediation, Microbial ecology, Biodegradation.

Funding: Queen's University of Belfast

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RedStat: Leveraging open-source projects for laboratory infrastructure

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Laboratory infrastructure often require computers for interfacing and managing data. Moreover, it is often required to have several machines working synchronously. It is possible to use proprietary platforms to sync infrastructure which might involve limitations on the machines utilized or sync data afterwards which can be error prone.

We propose a different scenario by using a local area network and low-cost computers. Due to the memory limitation challenges of using low-cost computer, we present a flexible and easy-to-deploy wi-fi server-client platform. It allows using low-cost Raspberry Pi computers to interface hardware in the laboratory and use up-to-date software tools such as time-series databases and online data visualization tools. This facilitates long-term monitoring and data management. RedStat consists of an installation script that helps configuring the LAN hostname and installs the open software tools required to interface and manage the data monitoring. Node-red acts as the orchestrator of the software integration and RedStat supplies three different example programs to interface 3 open hardware projects: the microcontroller Arduino, the hardware incubator “OpenTCC”[1]heating MET using water jacket recirculation can raise safety issues, whereas heating coils may affect the results of electrochemical analyses.

The proposed open-source temperature-control chamber (OpenTCC and the open-source potentiostat “dStat”[2]\” giving limited information about their circuitry and behaviour which can make development of new measurement techniques and integration with other instruments challenging. Recently, a number of lab-built potentiostats have emerged with various design goals including low manufacturing cost and field-portability, but notably lacking is an accessible potentiostat designed for general lab use, focusing on measurement quality combined with ease of use and versatility. To fill this gap, we introduce DStat (http://microfluidics.utoronto.ca/dstat.

RedStat provides an infrastructure to develop similar programs interacting different type of serial communication devices.

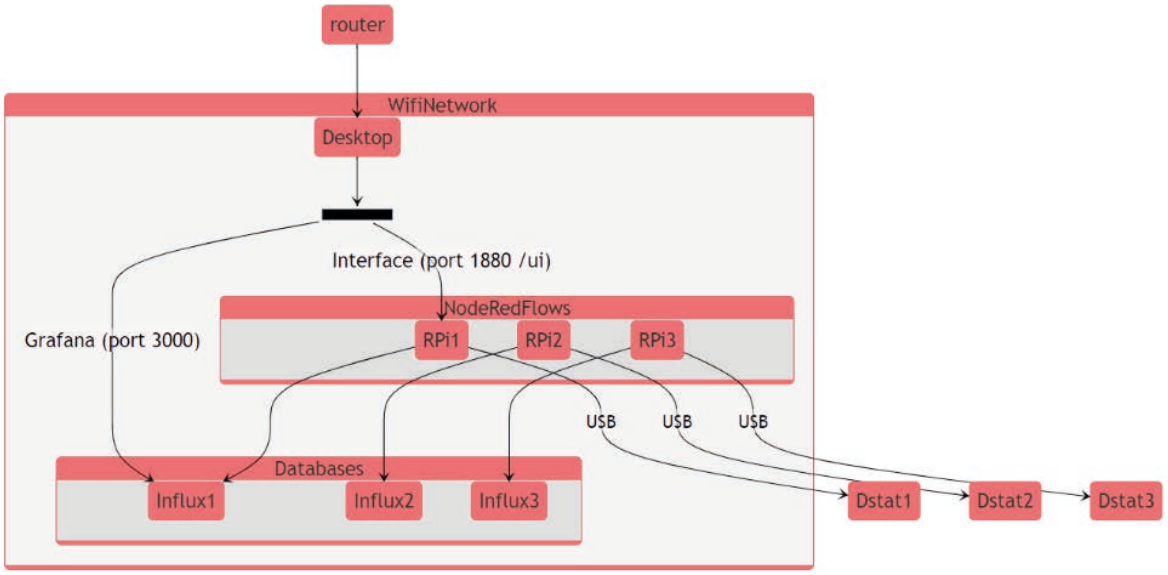

Fig. 1 Schematic of the RedStat platform showing the wi-fi LAN with several Raspberry Pis, the time-series database InfluxDB and the serial communication devices dStat.

Keywords: Raspberry Pi, time-series, databases, potentiostat, wi-fi.

Funding: Science Foundation Ireland (SFI) Professorship Programme *Innovative Energy Technologies for Biofuels, Bioenergy and a Sustainable Irish Bioeconomy* (IETSBIO3; grant number 15/RP/2763)

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CO_2 conversion to formate – insight in electrolyte conditions

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The sustainable production of value-added chemicals remains one of the major goals of the fossil resources based chemical industry. Reduced C1-compounds, such as CO and formate are promising molecules towards a chemical industry decoupled from fossil feedstock. Moreover, the highly selective electrochemical CO_2 reduction using electricity from renewables creates synergies between energy and chemical production sectors. CO and formate are versatile precursors to more complex carbon compounds. Compared to CO, formate provides several advantages for carbon and energy storage, such as high stability, low safety requirements, good storage characteristics as salt, and its application as a sole substrate for the synthesis of bio-based products. For instance, methanogens can produce methane and high-value products such as isoprenoids, coenzymes, amino acids, and carboxylic acids from CO_2. However, especially when thermophilic methanogens are desirable to use, a preceding CO_2 conversion to formate could circumvent low-solubility issues of H_2 and CO_2 in high-temperature aqueous solutions. Here, we present insights into the electrolyte composition in order to realize drop-in reaction systems. Formate was produced in different electrolytes and a methanogen medium, which provide physiological conditions (salinity and pH) with Faradaic efficiencies as high as 60 %.

Keywords: CO_2 conversion, formate, bioelectrochemistry, methanogens

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The role of the quinol dehydrogenase ImcH in Geobacter sulfurreducens energy metabolism

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Electroactive bacteria are strong candidates for new biotechnological applications, namely for energy production as bioelectricity in microbial fuel cell devices. The development of these devices is still a work in progress, with their practical implementation limited by low power densities or low production rates. To optimize the performance of the electroactive bacteria used in such devices, we must understand and improve their metabolism, specifically the mechanisms used for energy production.

Geobacter sulfurreducens is a model electroactive bacterium frequently used in electrochemical devices, due to the highly conductive biofilms it generates. It is capable of extracellular electron transport of electrons from carbon metabolism to the cell exterior, towards insoluble minerals or electrodes. Two proposed quinone-interacting membrane cytochromes, ImcH and CbcL, were proven to be essential for this extracellular electron transfer to high and low redox potential electron acceptors, respectively [1]. the CbcL-dependent pathway operates at or below redox potentials of -0.10 V vs the standard hydrogen electrode, whereas the ImcH-dependent pathway operates only above this value. Here, we provide evidence that G. sulfurreducens also requires different electron transfer proteins for reduction of a wide range of Fe(IIIImcH has an N-terminal membrane domain with three transmembrane helixes and a C-terminal domain with seven *c*-type hemes.

In this work, we cloned, overexpressed and purified *G. sulfurreducens* ImcH. The recombinant protein was characterized using biochemical, spectroscopic and electrochemical techniques. Electron transfer experiments with ImcH and quinones are ongoing to understand its role in energy conservation and extracellular electron transfer.

Keywords: Anaerobic Respiration, Energy Metabolism, Quinol dehydrogenase,

Funding: Fundação para a Ciência e Tecnologia (Portugal) through grant PTDC/BIA-BQM/29118/2017 (to AGD and IACP) and R&D unit MOSTMICRO-ITQB (UIDB/04612/2020 and UIDP/04612/2020). European Union's Horizon 2020 research and innovation program (Grant agreement no. 810856).

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Voltammetric analysis of the extracellular electron transfer process: Diagnostic criteria for understanding and quantification.

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A key factor in bioelectrochemical systems is the extracellular electron transfer (EET). This process occurs when electrogenic microorganisms (EM) receive or deliver electrons to conductive electrodes, according to their metabolic cycle. Currently, it is recognized that EM can use two pathways for EET: 1) direct extracellular electron transfer (EETD); 2) indirect extracellular electron transfer (EETI) [1]. In recent years, important advances have been made in understanding EET at the biological level. However, models are still needed to explain EET at the electrochemical level [2]. The development of equations through finite element analysis to simulate the electrochemical behaviour of EET in cyclic voltammetry is a strategy that allows us to understand biological processes at the electrochemical level (Fig. 1). This allows the quantification of important kinetic parameters such as the electron transfer rate, relationship between substrate consumption and microbial electrocatalysis, cell growth effects and others [3]. Therefore, the present work uses differential equations to simulate the EET in cyclic voltammetry, which allows proposing strategic experimental arrangements for the understanding and quantification of the EET.

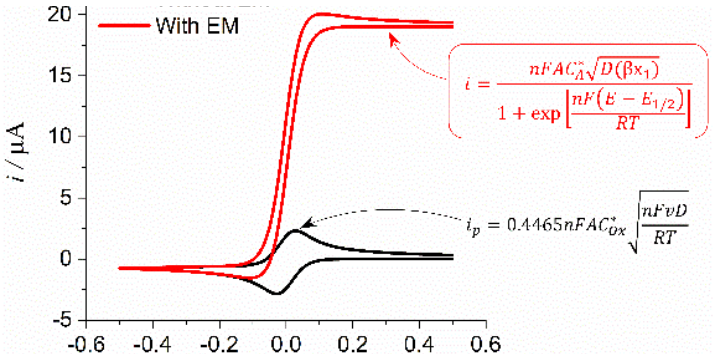

Fig. 1 Voltammetric profiles of a redox mediator and equations, in the presence and absence of EM [4].

Keywords: Voltammetric analysis, Electrochemical simulation, Extracellular electron transfer.

Funding: CONACYT-SENER-Sustentabilidad Energética (Project 246052) and CONACYT-SEP-Ciencia Básica (Project 258159).

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Effect of applied voltage on co-digestion of cattle manure and wastewater biosolids in Anaerobic Digestion-Microbial Electrolysis Cell (AD-MEC) systems

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The application of integrated anaerobic digestion MEC (AD-MEC) system was studied for co-digestion of cattle manure (CM) and wastewater biosolids (WBS) composed of primary sludge and waste activated sludge. In the experimental design, three different voltages (0.3, 0.7 and 0.9 V) and different substrate mixing ratios were used in single chamber AD-MEC systems for enhanced methane production. The reactors consisted of conventional AD, open circuit, and applied potential reactors with different CM to WBS mixing ratios of 100:0, 30:70, 70:30 and 0:100. Reactors were operated in duplicate under mesophilic conditions (35 °C) with no mixing, and current production was monitored continuously throughout the study. Results showed that, there was no significant impact of applied voltage on methane production in the WBS reactors, yet in CM reactors applied voltage showed a significant enhancement on methane production. Methane yield of CM reactors at an applied voltage of 0.9V was 684.19 mL CH_4/g $VS_{removed}$ which is about 1.85 times higher than conventional AD reactors (370.28 mL CH_4/g $VS_{removed}$) and 1.37 times higher than OC reactors (500.98 mL CH_4/g $VS_{removed}$). Integrated AD-MEC system fed with CM presents a significant opportunity to enhance the effectiveness of conventional AD systems with a relatively simple modification.

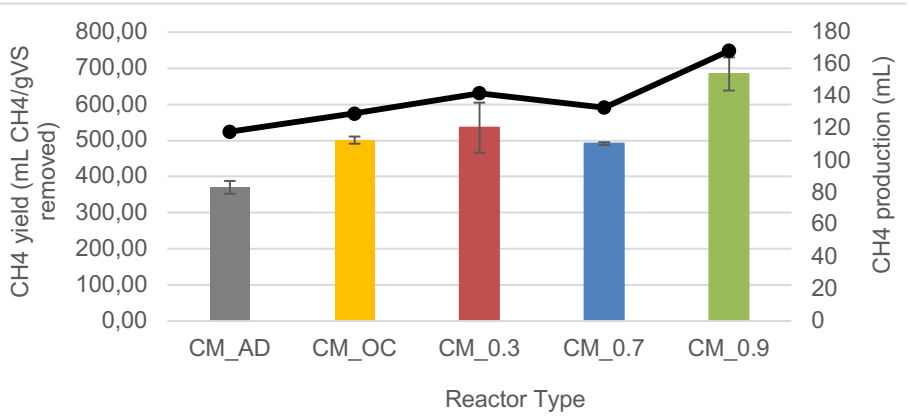

Fig. 1 Methane yield graph for cattle manure reactors (bar chart shows yield, line shows production)

Keywords: co-digestion, methane, cattle manure, microbial electrolysis cell, AD-MEC system

Funding: This study was funded by The Scientific and Technological Research Council of Turkey (TUBITAK) (No: 217M854)

Converting the offgas of hydrotermal carbonization into methane by means of electromethanogenesis

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Hydrothermal carbonization (HTC) is a sustainable and cost-effective solution for waste biomass management. The gaseous phase resulting from HTC of organic waste is mainly composed of CO_2 (between 85 and 95 vol.%) and traces of other gases such as CO, CH_4, N_2 or H_2. This gas is usually seen as a waste stream and released into the atmosphere. Valorising this CO2-rich waste stream through electromethanogenesis would avoid GHG emissions while adding extra value to the overall bioprocess. In the present work, HTC offgas effectively substituted CO_2 gas in the feeding of mixed-cultures biocathodes poised at -1V vs. Ag/AgCl. HTC offgas exhibited a certain negative effect on the electrochemical part of the process, decreasing current density and maximum methane yield by 44.2% and 17.5% respectively. Removing the applied electrical potential diminished methane yield by 91.1%, revealing the electrical dependence of the process.

Keywords: Bioelectrochemical systems, Hydrothermal carbonization, Electromethanogenesis, Biocathode

Co-metabolism of benzene and glucose in microbial fuel cells by synthetic communities

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Soil and groundwater environments are often exposed to contamination by benzene, which is toxic and carcinogenic. Microbial Fuel Cell (MFC) technology can be employed in biodegradation of this pollutant, generating electricity as a by-product[1].

The aim of this study was to investigate the optimal composition of microbial consortia made up of pure bacterial cultures isolated from environments contaminated with petroleum products. Biodegradation of benzene and glucose was investigated in single-chamber MFCs. Five different, both gram negative and positive bacteria were used: *Pseudomonas aeruginosa*, *Streptococcus pyogenes*, *Shewanella putrefaciens*, *Pseudomonas spp.* and unidentified strain *LSK*. Inoculum was prepared in MSM medium with 0,07% benzene and 0,1% glucose concentration.

The growth dynamics of microorganisms was observed by real-time voltage monitoring and linear sweep voltammetry measurements of individual MFC electrodes using Ag/AgCl as the reference electrode. The highest power density was observed in MFCs dominated with*: Pseudomonas aeruginosa* 5,383 W/m^3, while the lowest value was observed in MFC dominated with *LSK* strain: 0,6909 W/m^3. The NMR analysis of the anolyte samples indicated, that both of the substrates – glucose and benzene were utilised by microorganisms. Nevertheless, analysis of metabolic profiles of each community revealed predominance of fermentation products, which indicated strong presence of non-electrogenic pathways.

Keywords: benzene-degrading microorganisms, MFC, co-metabolism, pure culture

Funding: This work was supported by the Polish National Agency for Academic Exchange – Polish Returns grant (PPN/PPO/2018/1/00038) and National Science Centre (Poland) OPUS grant (2019/33/B/NZ9/02774)

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Synthesis of biosurfactants from waste vegetable oil in air-cathode microbial fuel cell

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Microbial fuel cells (MFC) are devices that use microorganisms as biocatalysts for the oxidation of organic matter to generate electricity and/or synthesise valuable compounds. One of the possible bioconversion products are biosurfactants that are of great interest due to their wide range of applications and unique properties. In this study, the potential of biosurfactants and electricity production from waste vegetable oil was assessed in single-chamber air-cathode microbial fuel cells. The MFC was investigated using surface tension measurements, real time temporal analysis, and linear and cyclic voltammetry measurements. The electroactive biofilm capable of conducting the process of biosynthesis once it reached stable current. The biosynthesis process has been noticeable after 5 weeks of feeding MFC's with waste cooking oil. In the anolyte, a decrease in surface tension to 47.9 mN m^{-1} was reached, while at the same time, a maximum power density of 4.83 W m^{-3} was generated. In this study, we showed that simultaneous production of biosurfactants and electricity through electofermentation process can be achieved by using waste vegetable oils in single-chamber system.

Keywords: Microbial fuel cell, waste vegetable oil, biosurfactants

Funding: This work was supported by the Polish National Agency for Academic Exchange – Polish Returns grant (PPN/PPO/2018/1/00038) and National Science Centre (Poland) OPUS grant (2019/33/B/NZ9/02774)

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On the use of MEC technology to treat exhausted vine stock wastewater

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In this work, we try to explore the ability of a single-chamber microbial electrolysis cell (MEC) to produce methane (CH_4) from an exhausted vine stock wastewater. Carbon felt electrodes were used as bioanodes and stainless steel as cathode (working volume of 0.5 L, see Fig. 1). The cell worked in a two-electrode configuration at an applied potential of 1 V with a feeding ratio of 30/70 (30% substrate to 70% synthetic medium). In addition, an identical MEC operated in open circuit was used as a control. Although carbon removal was similar in the two cells (in the range between 70 – 76%), biogas recovery was three times higher in the connected cell (17.1 L vs 6.0 L) along 10 cycles of operation (7 days per cycle), achieving CH_4 concentrations of up to 79%. In addition, the electrical capacitance of the connected anode doubled that of the non-connected cell (as measured using electrochemical impedance spectroscopy), containing 31% more Bacteria and 77% more Archaea. In summary, the results here reported ratify the advantages of using MEC for the treatment of organic liquid wastes.

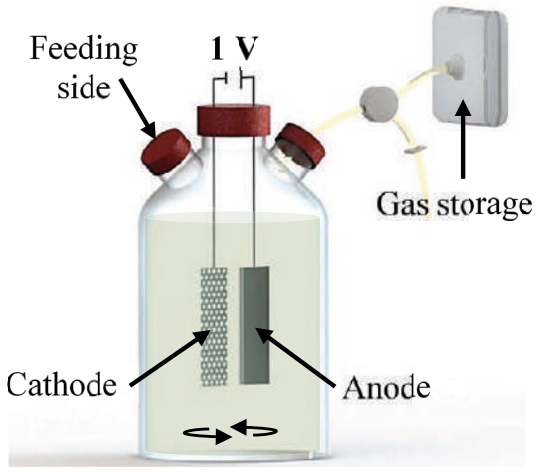

Fig. 1 Microbial electrolysis cell (MEC) configuration.

Keywords: Biogas recovery, methane, microbial electrolysis cell.

Funding: This research was possible thanks to the financial support by FEDER and Interreg V-A España-Portugal (POCTEP) 2014-2020. Project: Desarrollo de una estrategia transfronteriza para la valorización ecosostenible de biomasas residuales del sector vinícola y vitivinícola en Biorrefinerias integrales para la producción de Biocombustibles y Bioproductos. (No.0688_BIOVINO_6_E).

Prospecting for electrochemically-active hydrocarbon-degrading microorganisms for use in bioelectrochemical remediation of petroleum hydrocarbons.

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Petroleum hydrocarbons pollution is commonplace in the environment owing to accidental spillages, leakages and indiscriminate disposal. Globally, it is estimated that between 1.7-8.8 million tonnes of oil is released into the aquatic environment annually; and from 1970-2018, 5.8 million tonnes of oil were spilled as a result of tanker incidences. Remediating these spills is a great priority due to their negative impacts on the environment e.g. irreversible habitat loss and threat to the survival of living organisms and public health e.g. genotoxic, mutagenic and/or carcinogenic effects.

Work is currently underway to enrich and select electrochemically active hydrocarbon degraders for use in bioelectrochemical remediation of target petroleum hydrocarbons – benzene and phenantherene. Inocula from contaminated matrices (soil, sediment and groundwater) was taken from the Niger Delta region, Nigeria, which was highlighted by a 2011 UNEP report as being highly contaminated with petroleum hydrocarbons. Selected isolates will be identified and inoculated into microbial fuel cell as pure cultures and as microcosms to determine their hydrocarbon removal efficiencies and rates.

Bioelectrochemical remediation has promise in speeding up the degradation process while reducing the amount of energy and chemicals used both of which are current impediments to conventional bioremediation processes applied to petroleum hydrocarbons.

Keywords: Bioelectrochemical, Remediation, Environment, Petroleum, Pollution.

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Potentiostatic enrichment of electroactive biofilm communities derived from soil contaminated with petroleum products

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The technology of bioelectrical systems is gaining increasing attention for its sustainability. These systems are capable of generating electricity, but also of treating various types of wastes. In principle, soluble wastes are readily accessible for microbial degradation, while insoluble, hydrophobic wastes reinforce particular MFC design and operation.

Our aim was to enrich electroactive biofilm from soil derived from petroleum-contaminated environment, capable of degradation of such compounds. The enrichment was done by using chronoamperometry, to accelerate the growth of biofilm at the anode. Customised BES design was built using a polypropylene chamber and acrylic lid supplied with sampling and reference electrode ports.

During the experimental period, the following anodic potentials were kept constant -0.3; 0,0 and 0,3 V (vs Ag/AgCl). After reaching stable, reproducible current, the cyclic voltammetry and linear sweep voltammetry were used to characterize electroactive biofilm. The observed maturing phase of biofilm growth lasted 8 weeks. The highest current was observed for the potential of -0.3 V, and reached approximately 100 µA.. Two main oxidizing peaks were also detected during CV studies, their height was 27.1 µA (-0.230 V) and 158.6 µA (0.389 V). Thus, we have selected the most beneficial growth conditions for microorganisms degrading petroleum products.

Keywords: bioelectrochemical system, chronoamperometry, petroleum products

Funding: This work was supported by the Polish National Agency for Academic Exchange – Polish Returns grant (PPN/PPO/2018/1/00038) and National Science Centre (Poland) OPUS grant (2019/33/B/NZ9/02774).

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Unveiling the role of H_2 in the cathodic electron uptake by acetogenic bacteria

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Some acetogenic bacteria have the capacity to use cathodes as electron donor for the reduction of CO_2 into acetate or other organic compounds. This capacity can be applied for innovative bioelectrochemical technologies, such as microbial electrosynthesis (MES). However, the rates of MES remain too low, while the mechanisms used by acetogens to take up cathodic electrons are still unclear.

We investigate the role of H_2 as an intermediate in the cathodic electron uptake by acetogens. We hypothesize that the capacity of acetogens to take up cathodic electrons depends on their H_2 consumption characteristics, including their H_2 threshold (1). We experimentally determined the H_2 threshold, i.e. the H_2 partial pressure at which acetogenesis halts, of several acetogenic strains. We found the highest H_2 thresholds for acetogenic *Clostridia*, while *Acetobacterium* spp. had lower H_2 threshold. The lowest H_2 thresholds were found for *Sporomusa* spp. These H_2 thresholds inversely correlate with cathodic electron uptake rates described in literature: highest MES rates for *Sporomusa* spp. for which we found the lowest H_2 threshold. We will further investigate if and how the H_2 partial pressure maintained by the acetogens at the cathode surface affects the cathodic H_2 evolution rate.

Keywords: acetogens, H_2 thresholds, extracellular electron transfer mechanisms, cathodic H_2 evolution , microbial electrosynthesis(Up to 5 keywords, Font: Arial, 10.5 pt.)

Funding: Starting Grant of the Aarhus University Research Fund (AUFF)

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Stimulating Biofilm Formation of Sporomusa ovata for Improved Attachment on Cathode in Microbial Electrosynthesis

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In microbial electrosynthesis, acetogenic bacteria reduce CO_2 to organic compounds using a cathode as electron donor. Microbial electrosynthesis rates remain low and need to be improved. We hypothesize that improved biofilm formation and higher cell numbers on the cathode can increase microbial electrosynthesis rates.

We performed adaptive evolution with a selection pressure for biofilm formation on *Sporomusa ovata*, an acetogen well known for its microbial electrosynthesis activity. In a long-term serial transfer experiment, *S. ovata* was grown in a $H_2/CO_2/N_2$ headspace on plastic carriers incompletely covered by liquid medium. Activity was followed by measuring the decrease in headspace pressure (indicating consumption of H_2 and CO_2). At regular time intervals, one or two carriers were transferred to bottles with fresh carriers and fresh medium. By transferring only *S. ovata* cells sticking to the transferred carriers, a selection pressure for biofilm formation was created.

After eight serial transfers, this resulted in an adapted population of *S. ovata,* which has a significant higher biofilm formation than the initial strain, based on crystal violet assays in well plates under heterotrophic conditions. In addition, we will present results of microbial electrosynthesis activity by the adapted *S. ovata* and its biofilm formation on the cathode.

Keywords: Microbial Electrosynthesis, Biofilm, Adaption, Acetogens

Funding: Novo Nordisk Fonden Biotechnology-based Synthesis & Production Research. Project Grant 'Let them stick'.

*Random genetic mutagenesis in **Geobacter Sulfurreducens** induces high cytochrome activity to tolerate with hexavalent chromium*

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Geobacter Sulfurreducens can reduce Cr (VI) by c-type cytochrome (*c-Cysts*). However, high concentration of Cr (VI) imparts lethal effect on *G. Sulfurreducens,* which moderates their cell metabolism. Thus, we improved Cr (VI) tolerance of wild type *G. Sulfurreducens* by chemically induced random mutagenesis. Selected *G. Sulfurreducens* mutants grew in medium containing 2-times higher concentration of Cr (VI) (0.2 mM) as compared to wild type. Mutated cells harvested at late exponential growth phase (0.7 x 10^7 CFU/ml) removed 90 % Cr (VI) from medium containing 0.5 mM Cr (VI) in 2.5 h with cell normalized rate of 1.3 x 10^{-9} mg/CFU.h, which was higher than the wild type (69 %, and 1.0 x 10^{-9} mg/CFU.h). We also analysed total Cr distribution in the system and noticed that a significant portion of total Cr was assimilated in the biomass as Cr (III), thus confirming biologically assisted Cr (VI) reduction. Both type of bacterial genes showed typical UV-VIS spectra with six coordinated low-spin hemes, however, the reduced *c-Cysts* absorption band was large in mutants. Cyclic voltammetry analysis showed large redox current response for mutants as compared to wild type, confirming high electron flux in mutants, which enhanced the bioreduction of Cr (VI).

Keywords: Bioreduction, *Geobacter sulfurreducens,* hexavalent Chromium, Mutagenesis

Funding: This project has received funding from European Union´s Horizon 2020 research and innovation program under Marie Sklodowska grant agreement No. 754382

Electrobioremediation of aromatic hydrocarbons using downflow METland® technology

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The versatility of the electromicrobiology makes it suitable to face the most challenging environmental problems through electrobioremediation actions (Wang et al., 2020). In this context, METland® technology constitute a sustainable solution where concepts from Microbial electrochemical Technologies (MET) are integrated into constructed wetlands (Peñacoba et al., 2021) by replacing inert gravel with electroconductive granular material.

In this study, a heterogeneous mixture of hydrocarbons was treated in a lab-scale METLAND® operated downflow with a hydraulic loading rate of 265 mm d^{-1}. In this configuration oxygen was passively supplied with no energy cost and aerobic metabolism occurred in combination with anaerobic electroactive bacteria (Aguirre et al. 2020). The removal capacity of the treatment was determined by measuring COD, TOC and benzene and toluene as specific hydrocarbons (12ppm in inlet wastewater). Our results showed an effluent free of benzene & toluene after the treatment. The microbial community analysis (Illumina) of electrode-associated biofilm revealed the presence of halophiles like *Halomonas*, *Oceanicaulis (consistent with the saline nature of our wastewater); and Alishewanella*, *Pseudomonas*, *Parvibaculum, Azoarcus and Thauera* (known to degrade hydrocarbons or outcompete in hydrocarbon polluted sediments).

Keywords: Wastewater treatment, Hydrocarbons, Electroactive biofilters, Microbial electrochemical technologies

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SMFC as a tool for the removal of hydrocarbons and metals in the marine environment: a concise research update

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Marine pollution is becoming more and more serious, especially in coastal areas. Because of the sequestration and consequent accumulation of pollutants in sediments (mainly organic compounds and heavy metals), marine environment restoration cannot exempt from effective remediation of sediments themselves. It has been well proven that, after entering into the seawater, these pollutants are biotransformed into their metabolites, which may be more toxic than their parent molecules. Based on their bioavailability and toxic nature, these compounds may accumulate into the living cells of marine organisms. Pollutants bioaccumulation and biomagnification along the marine food chain lead to seafood contamination and human health hazards. Nowadays, different technologies are available for sediment remediation, such as physicochemical, biological, and bio-electrochemical processes. This paper gives an overview of the most recent techniques for marine sediments remediation while presenting Sediment based Microbial Fuel Cells (SMFCs). We discuss the issues, the progress, and future perspectives of SMFCs application to the removal of hydrocarbons and metals in the marine environment with concurrent energy production. We give an insight into the possible mechanisms leading to sediments remediation, SMFCs energy balance, and future exploitation.

Bacteria Recognition-Extraction from Wastewater (BREW) for next generation microbial electrochemical technologies

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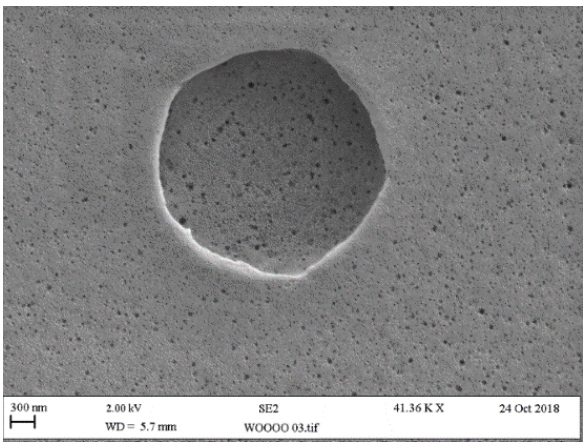
Performance of microbial fuel cells (MFCs) strongly depends on the anode, particularly on the attachment of (electro)active bacteria to this electrode. To target high costs and limited bacterial adhesion associated with carbon electrodes, we will use a technique referred to as molecular imprinting, which creates artificial cavities complementary to the template molecule [1]. The binding sites generated can be engineered to selectively extract the bacteria of interest (in this case *Geobacter* [2]) from wastewater, expecting improved colonisation will enhance the MFC's performance. This is complicated as a balance needs to be struck between polymers that can grab the bacteria while retaining the bacteria's ability to proliferate.

Bacterial imprinting is a template polymerisation technique; polymerisation occurs in the presence of bacteria [1]. Following extraction, binding sites remain on the surface that are complementary to the size, shape and chemical functionality of the target bacteria. It is possible to discriminate between different strains of bacteria due to the differences of antigens on the surface (such as proteins or sugar groups) that can interact with the binding sites in different ways [3]. An example of a bacterial binding site is depicted below for *S. aureus* and *E. coli*, indicating the binding site has the same shape and dimension as the original target.



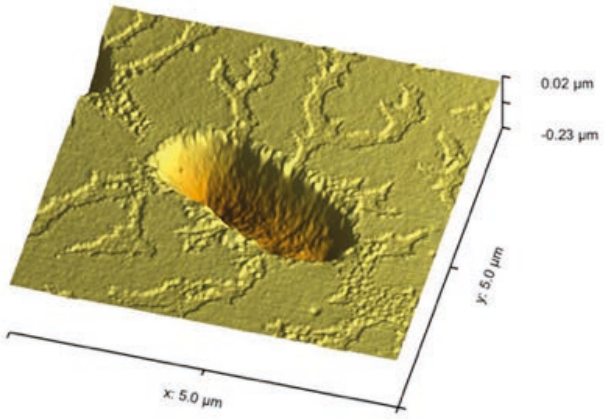


Fig. 1. (A) A typical binding site for *S. aureus* (visualised by scanning electron microscopy). (B) Profile analysis of a cavity complementary to a single *E. coli* bacterium, made on glass (visualised by 3D atomic force microscopy) [4].

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Sense Fuet no són tapes: Bioelectrosynthesis scale-up and optimization - a fed-batch story

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Microbial electrosynthesis (MES) is a dynamic and exciting research area originating from the combination of microbiology and electrochemistry. To bring MES to industrial application, reactor infrastructure is needed that meets the requirements of both, biological and electrochemical engineering. Our upgrade kit for turning commercial bioreactors, based on batch stirred tank reactors (BSTR) into electrobioreactors, has been previously benchmarked [1] and was applied to a potential upcoming Industrial process based on another industry work horse, *E. coli*, as a microbial bioelectrosynthesis chassis for the production of chiral building blocks for pharmaceutical industry, that was also recently demonstrated in small scale [2]. By optimizing the process conduction from batch to fed-batch processing in the 1L bioelectroreactors, we have increased the titer of (*R*)-1-phenylethanol produced from acetophenone to 12.8±2.0 mM, with a demonstrated Coulombic efficiency of near to 100%, and further improvements are still possible!

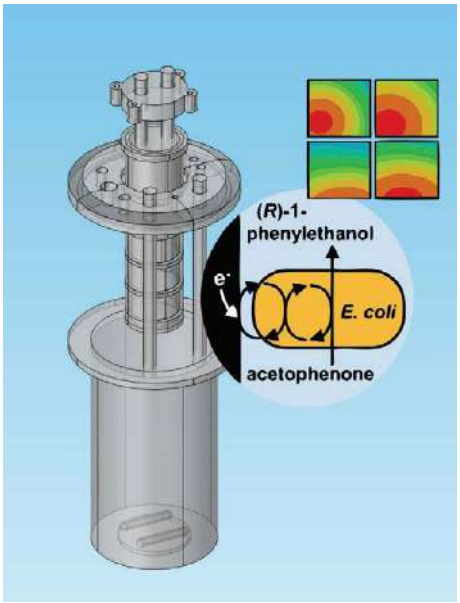

Fig. 1: Schematic depiction of the process/system.

Keywords: Bioelectrosynthesis, Electrobioreactor, Design of Experiments, Fed-Batch.

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A Modelling Analysis of pH Change in Microbial Electrosynthesis (MES) Catholytes

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Microbial electrosynthesis (MES) is an emerging technology for converting CO_2 to industrially useful organics by microbial catalysis. The study's main aim was to create a model of pH change in the MES catholyte, whose methodology can be used as a basis for the development of models with increasing accuracy for the prediction of experimental results.

The model achieves its aim through a reliable method for calculating pH based on relevant acid-base species concentration. A dynamic method using kinetic rate law, alongside reaction equilibrium, and stoichiometry is considered. Microbial processes are treated as a black box. It was found that all ions participating in acid-base reactions must be well considered to predict pH change in MES. Fluxes through membranes, particularly the flux of acetic acid, should be included for a more accurate fit with experimental results. Then, kinetic rate constants should be chosen to fit with experimental results through a rigorous trial and error process.

Sensitivity analysis on the impact of coulombic efficiency and hydraulic retention time (HRT) on pH found that a continuous system may be advantageous over batch for maintaining optimum pH. Model results found significantly lesser fluctuation in pH over 7 days using a continuous system, even at HRT of over 10 days for modelled parameters.

These results imply that the use of continuous operation over batch could allow for control and optimisation of organic production rates. The findings show that further investigation of pH change in MES through modelling would allow cost-effective and time-efficient targeting of MES experimentation, accelerating technology readiness levels.

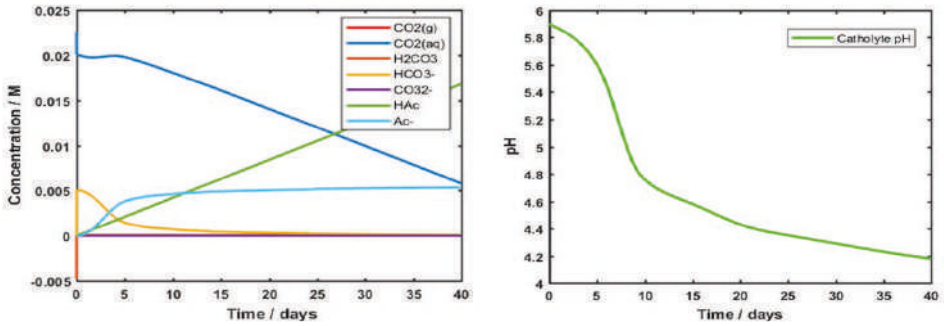

Figure 1 Model output plots for th with a current density at 0.5 x10^{-3} A cm^{-2}. A Coulombic efficiency of 0.1 over a period of 40 days was applied A) Plot of concentration of key species concentration (M) against time B) Plot of pH against time.

Key words: MES, acid-base reactions, pH change, Hydraulic retention time

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Electrochemical behaviour of carbon nanofiber/PDMS composite used as a corrosion –resistant coating for copper in microbial fuel cells

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Surface modifications of electrode materials are essential for improving the performance of microbial bio-electrochemical systems [1]. The use of copper (Cu) to functionalize electrodes as anodes in microbial fuel cells (MFCs) is a new process to improve their interfacial electron transfer, biocompatibility, and corrosion resistance. Cu anodes are prepared by surface modification with a composite made of polydimethylsiloxane (PDMS) doped with commercially carbon nanofiber (CNF). In our study, four-Cu anodes with CNF-PDMS with different thicknesses have been tested in MFCs. Three MFCs are equipped with modified Cu by CNF-PDMS with a thickness of 250 µm, 500 µm, and 1000 µm, respectively, and one MFC uses Cu anodes unmodified. The electrical characterization demonstrates that the maximum power density generated by MFCs with Cu / CNF-PDMS anodes is more stable. The Cu /CNF-PDMS electrode with a thickness of 500 µm shows the best performance. The cyclic voltammetry measurement indicates that the electrochemical activity of the modified anode significantly increases after ten days and that the electron transfer is facilitated by CNF-PDMS modification. These results prove that CNF-PDMS modification with an optimal CNF-PDMS thickness is a donating approach for improving the properties of anodic materials for applications in MFCs.

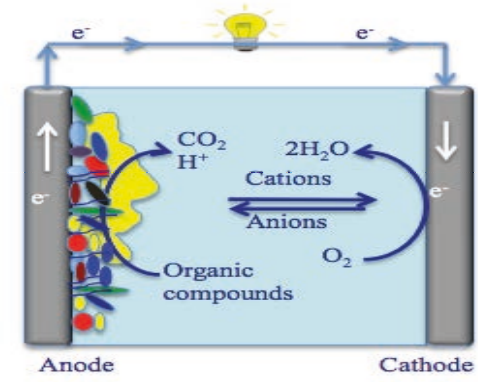

Fig. 1. ***Microbial fuel cells principle***

Keywords: microbial fuel cell (MFC),CNF ,PDMS, anode functionalization, corrosion resistance ,biofilm

Funding: The "Hubert Curien Program" through the PHC MAGHREB Project number 19MAG23/41382WC financially supported this work.

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Whole bacterial cell surface modified screen printed nitrate biosensor based on direct electron transfer of *Bacillus* sp. electropolymerized within polyaniline films

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Screen printed electrode (SPE) sensing is low cost, precise, rapid, sensitive and provide easy in situ quantification (1). Electropolymerization of whole bacterial cells (WBC) on SPE offers a constitutive expression system where the target analyte is sensed and the signal is converted to a measurable output (2). In the present work, *Bacillus* sp. possessing a periplasmic nitrate reductase was used as a recognition element to develop a nitrate biosensor. The bacteria was embedded within polyaniline electroconductive matrix via electropolymerization on miniaturized carbon screen printed electrodes (SPE) at 100 mV/sec and scan rate from −0.35 V to+1.7 V. Characterization was performed through different transduction modes (cyclic voltammetry, chroamperometric of cathodic current, and impedometric spectroscopy). Optimal bacterial density was OD_{600} 1.3. The developed sensor showed sensitivity over different working pHs (5-8) with wide dynamic linear detection range 0.5-50 ppm NO_3-N. It showed acceptable reproducibility and multiple usage up to 4 times after storage at 4°C over a duration of 2 months. Low cost and simple preparation allows the biosensor to be mass produced as a disposable point of care device (POCD) for water analysis. *Bacillus* sp. cells immobilized polyaniline is a good candidate for the improvement of amperometric biosensors for quantification of nitrate in aqueous solutions.

Keywords: Screen Printed Electrode, *Bacillus* sp., Nitrate, Whole Bacterial Cell Biosensor, Polyaniline

Funding: This work is part of ERANETMED3 project funded by Science Technology and Development Fund # 31321

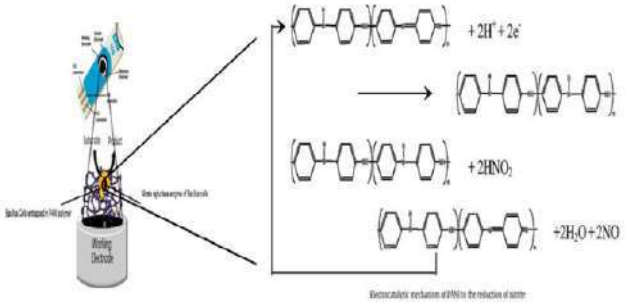

Fig. 1 Biomimetic electrochemical sensor based on Bacillus doped in PANI on the surface of SPE for nitrate detection

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Microbial Electrochemical Snorkel (MES) for Nitrate Reduction

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MES is a short-circuited Microbial Fuel Cell which operates at the maximum current for effective water treatment. The electrons released in the oxidation of organic matter by bioanodic side of MES are transported by solid electrode to the biocathodic side, where they are used to reduce electron acceptor (Fig. 1). Several applications were studied, such as enhancing wastewater treatment [1].

Here, we built a MES consisting of stainless steel electrodes placed in sediment and in wastewater. Keeping the proper ratio between anodic and cathodic parts allows to reach the specific potential [2] which for nitrate-reducing MES should be adequately low (in literature between -100 and -300 mV vs SHE) [3]. Our MES had the potential always in this negative range, with no external potential applied. The reactor was open to air, so oxygen - competing electron acceptor - was present. The addition of nitrate (C_0 = 33.8 mg N-NO3-/l) caused increase of current from -0.5 mA to -1.5 mA which decreased when nitrate was consumed, therefore it was corresponding to nitrate reduction. The nitrate-reduction peak was observed on the CV, beginning at +0.25 V vs SHE (Fig. 2). The nitrate reduction rate was increased in the presence of MES.

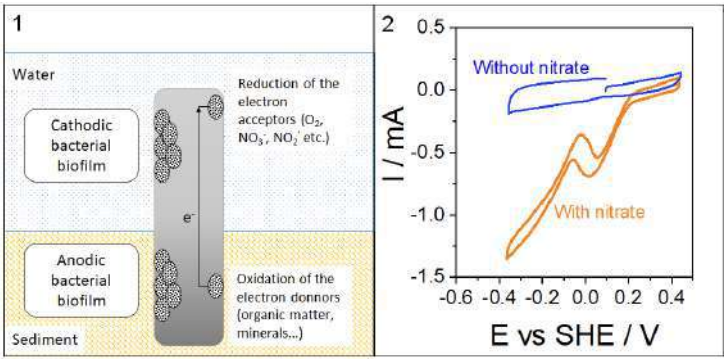

Fig. 1 The scheme of a MES.

Fig. 2 The cyclic voltammetry of a biocathode without (blue) and with (orange) nitrate.

Keywords: Microbial Electrochemical Snorkel, Microbial Fuel Cells, Biocathode, Denitrification

Funding: Financial support from The French National Research Agency (ANR-17-CE04-0004) is gratefully acknowledged.

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Comparison of different air cathode designs

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Air cathode is basic part of MFC or MFC/biosensor devices. If air cathode is rate limiting step in MFC, bioanode cannot breathe properly. It can lead to decrease metabolic activity and low COD, VFA removal rate. In case of MFC type biosensor weak air cathode will decrease detection limit of sensor.

We compared two types of air cathodes: 1st design developed in our lab for wastewater treatment using hydrogel cation exchange membrane (CEM) on ceramic support [1] separating bioanode from catalyst **Fig 1a**. 2nd air cathode with diffusion layer design, made by Toyo Ink, where catalyst is in direct contact with bioanode part of reactor **Fig 1b.** The comparison was made by measuring linear sweep voltammetry.

Both designs have advantages and disadvantages. In 1st case catalyst is protected by membrane from anolyte solution which can lead to longer half-life of catalyst (no biofouling) and air cathode is mechanically strong, which is allowing to use it for scale up projects. But proton flux is limited by membrane properties. In 2nd case catalyst layer is in direct contact with anolyte and oxygen flux is limited only by oxygen diffusion layer, but this air cathode is difficult to use in scaleup projects.

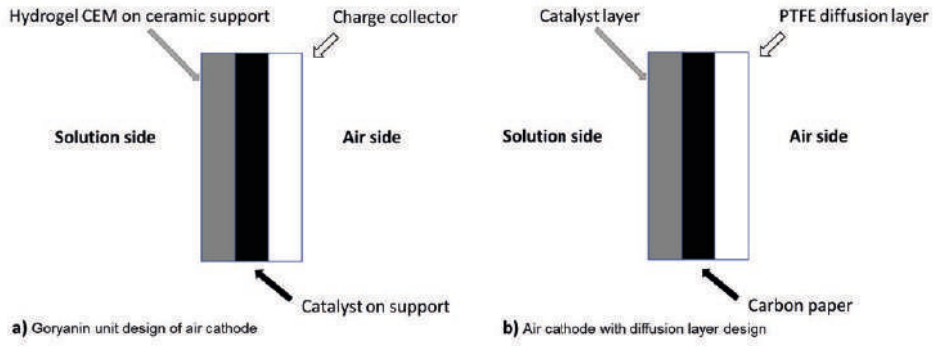

Fig. 1

Keywords: Air cathode, MFC, Scale up

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