Monitoring of volatile fatty acids during anaerobic digestion using a microbial electrochemical sensor

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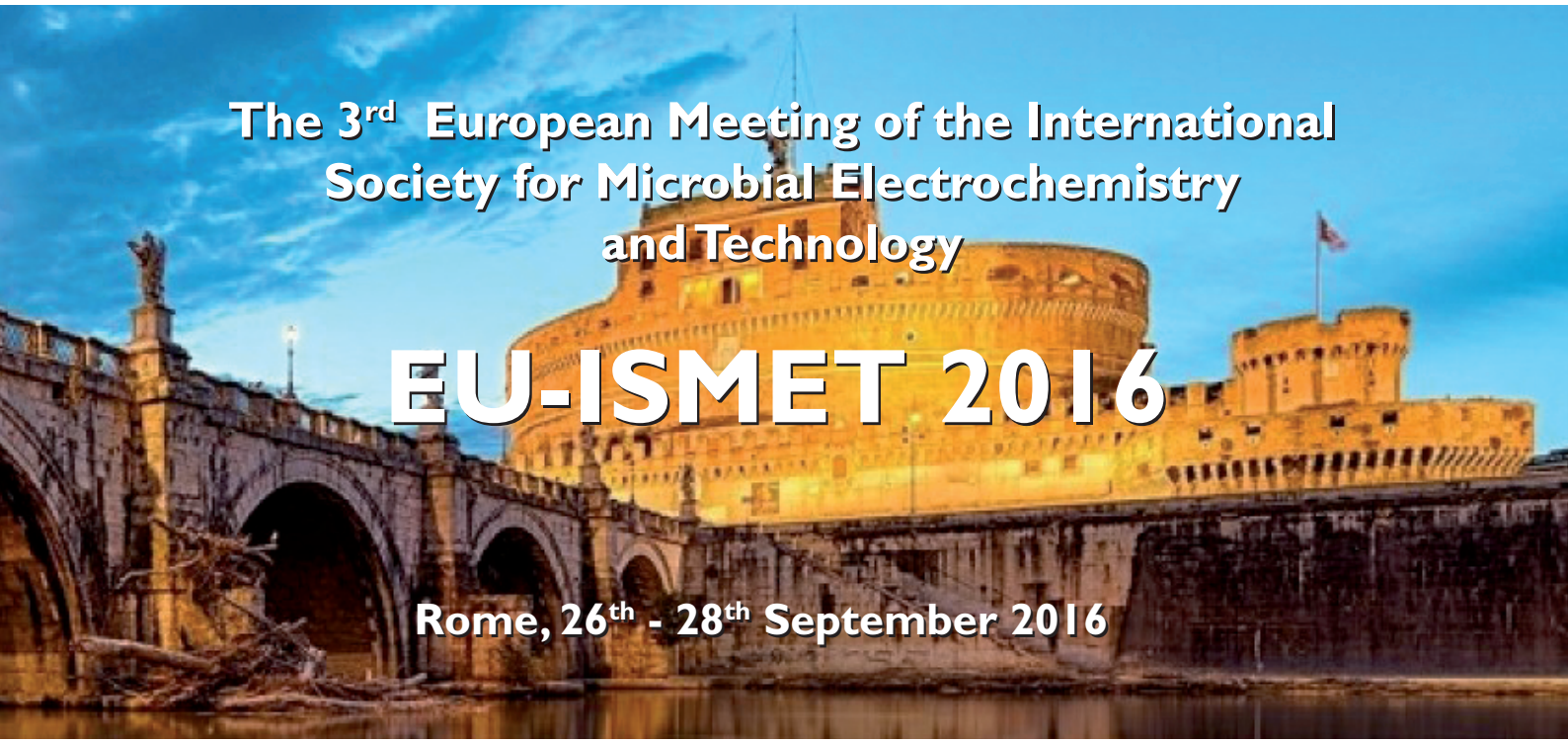

The 3rd European Meeting of the International Society for Microbial Electrochemistry and Technology

EU-ISMET 2016

Rome, 26th - 28th September 2016

Edited by

Federico Aulenta and **Mauro Majone**

Venue

Department of Chemistry (NEC)

Sapienza University of Rome

Piazzale Aldo Moro 5, 00185 Rome, Italy

Preface

The tradition of the successful European Meetings of the International Society for Microbial Electrochemistry and Technology (ISMET, www.is-met.org) continues! The 3rd EU-ISMET meeting (EU-ISMET 2016) takes place in Rome, Italy from September 26 to 28, 2016. The Water Research Institute (IRSA) of the National Research Council (CNR) and the Department of Chemistry of Sapienza University of Rome organize the meeting jointly.
EU-ISMET 2016 aims to gather scientists from multiple disciplines (from microbiology to electrochemical engineering) to exchange information, experience and achievements in the rapidly expanding field of microbial electrochemical technologies (METs), as well as to tackle the daunting scientific challenges these new and exciting technologies still pose. Along this line, besides presenting and discussing on fundamental and applied science, for the first time, the meeting hosts a special session entirely dedicated to the presentation of results of "up-scaled applications". Hopefully, the success stories herein presented and critically discussed will catalyze the interest of stakeholders and potential end-users and will contribute driving the transition of MET from the laboratories to marketable applications. We are grateful to the International Scientific Committee that has contributed in a crucial way to the definition of a high-level program. Special thanks also go the Instructors of the pre-conference workshop, Ian Head, Dino Virdis, and Deepak Pant for making it a successful event. We are also pleased to mention that, following the meeting, a selected number of articles will appear in extended form, after successful peer review, in a special issue of Fuel Cells journal (Wiley) which also generously supported an award for the best Poster presentation.

Welcome to Rome!

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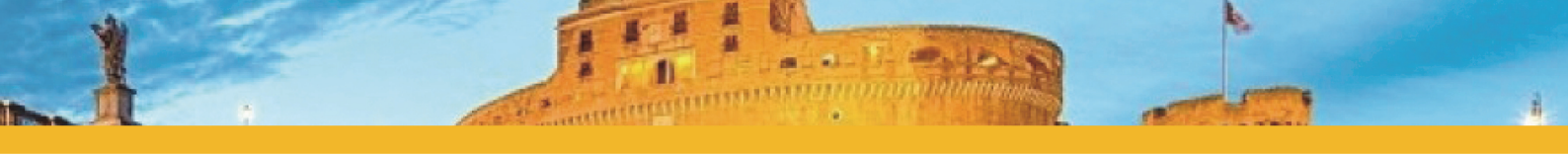

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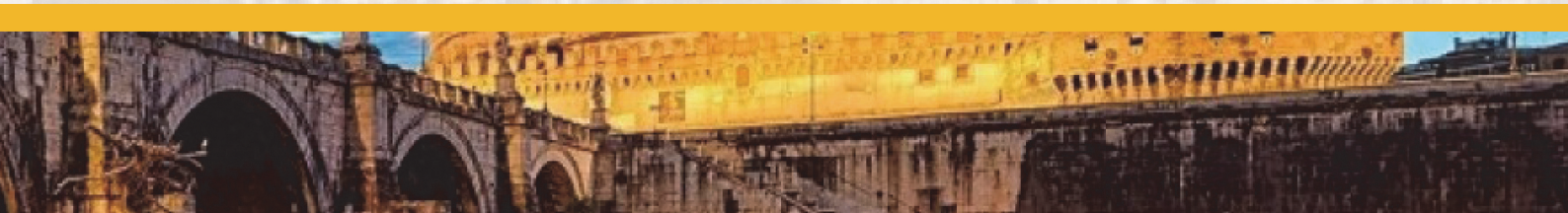

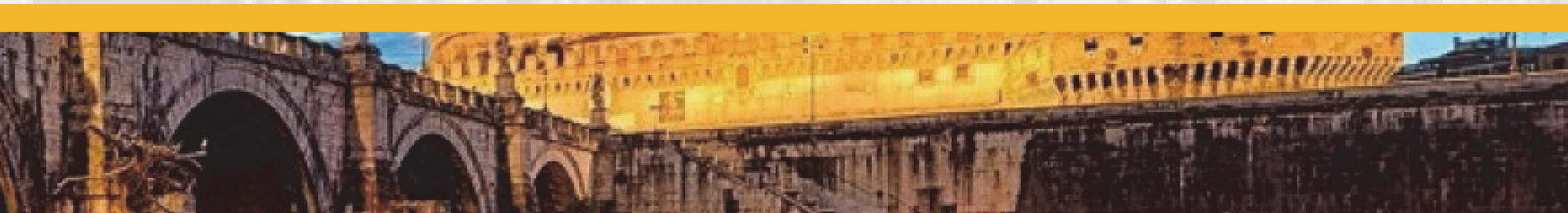

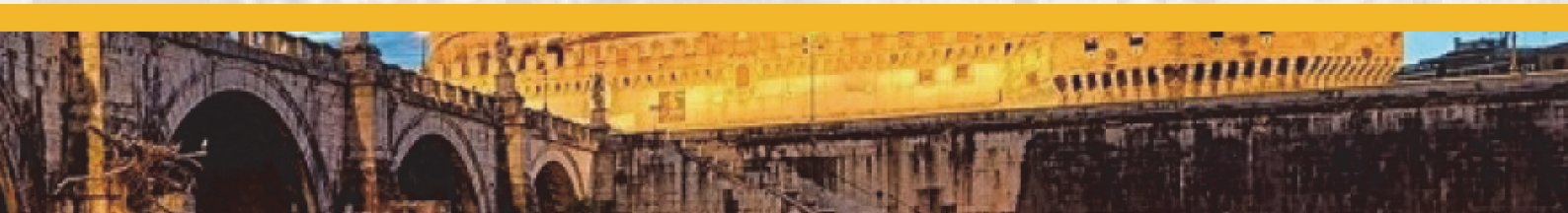

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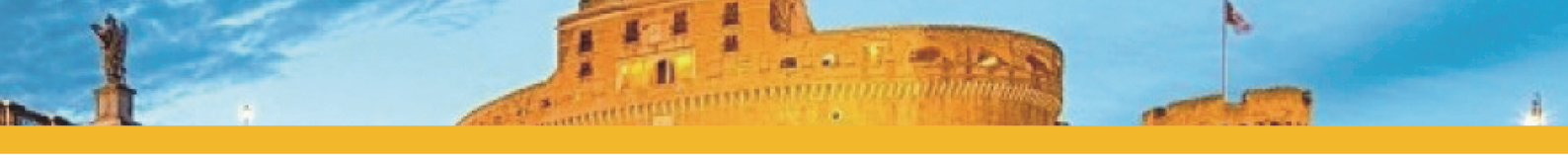

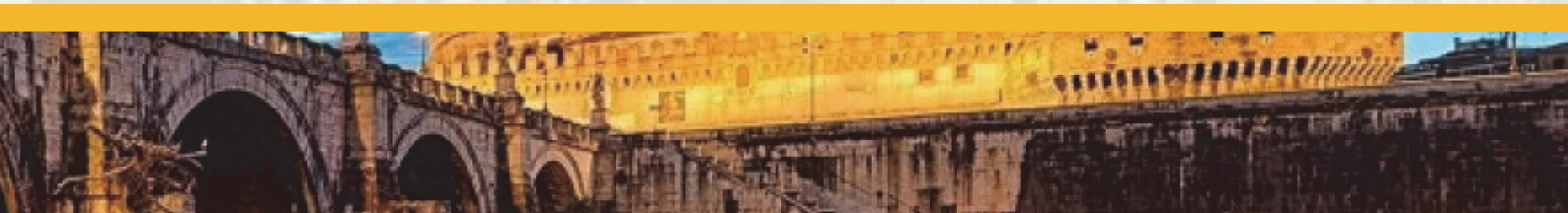

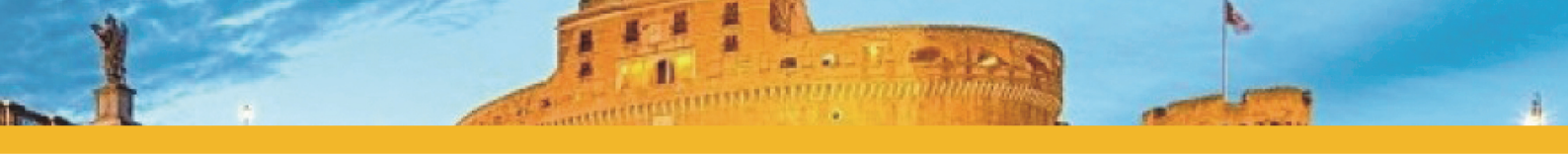

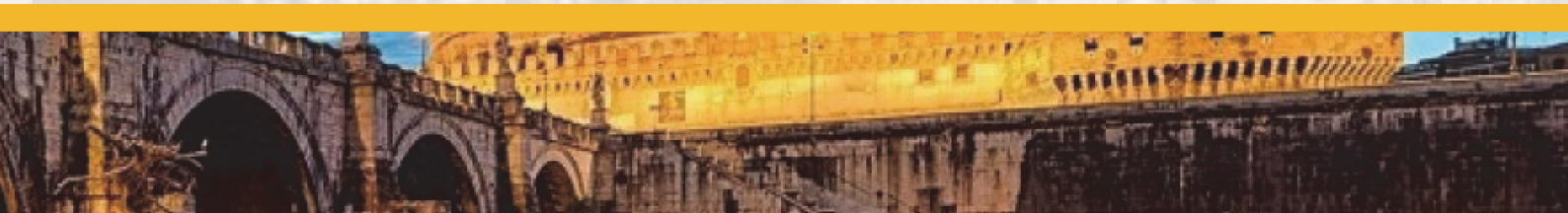

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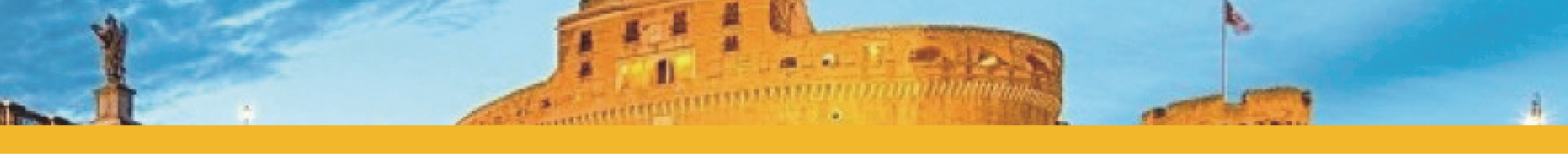
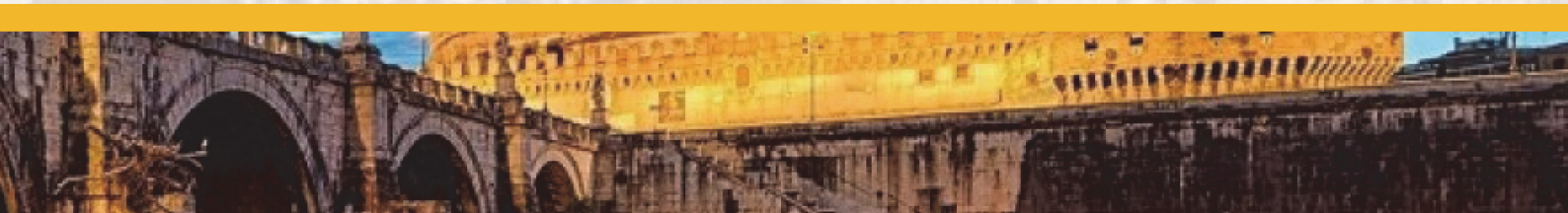
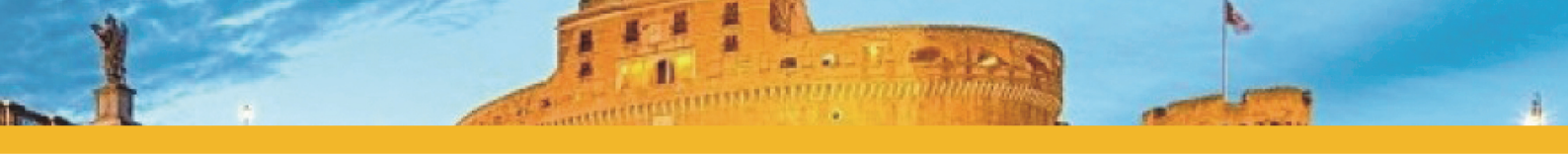
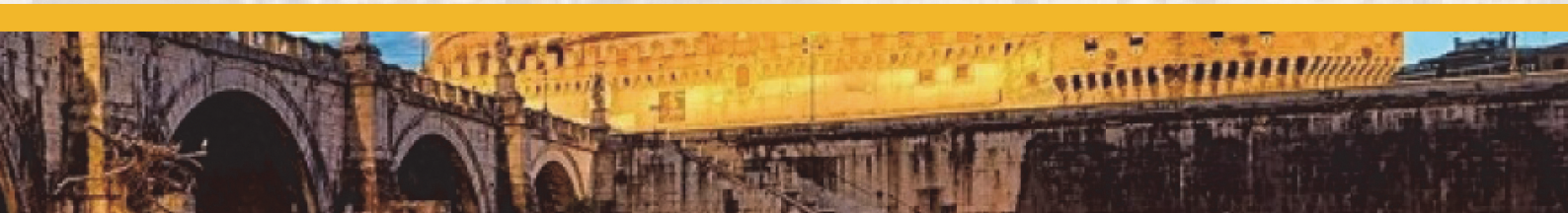
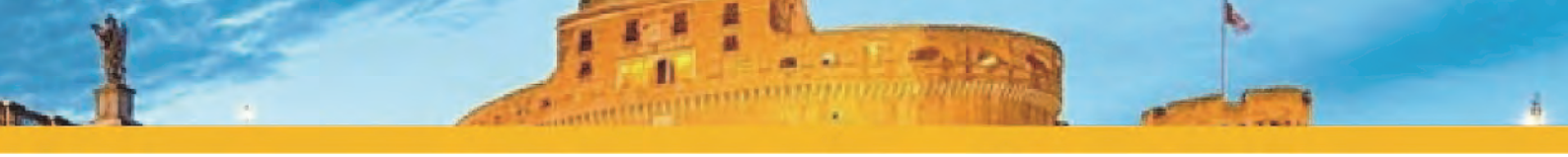


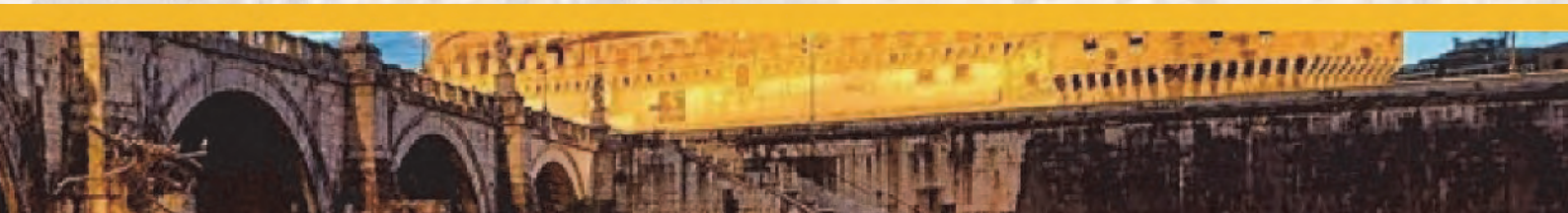








Keynote presentations



The soil biosnorkel: microbial extracellular electron transfer mechanisms of biochar

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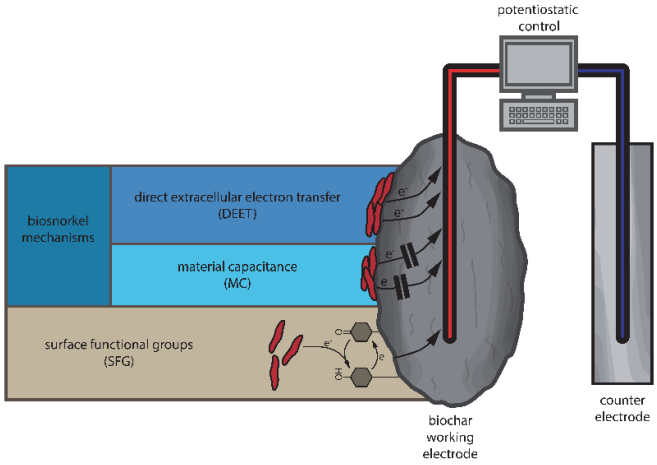

Placing graphite electrodes in wet soils had shown changes in the soil microbial ecology by reducing methanogenesis[1]. Biochar, which is carbonized wood and used as a soil enhancing material, is known to be able to transfer electrons from and to microbes extracellularly[2]. Surface functional groups with quinones have currently been identified as the likely pathway for biochar-mediated extracellular electron transfer[3]. Yet, biochar can be highly conductive, and the direct extracellular electron transfer (DEET) pathway through the carbon matrices may have been overlooked. DEET would then allow electrons to travel long distances in soils to reach different redox environments. We refer to this as the soil biosnorkel mechanism after others had published on microbial electrochemical snorkels[4]. Here, we examined the extracellular electron transfer kinetics of biochar carbon matrices by eliminating surface functional groups and by analyzing kinetic behavior to characterize DEET.

Black-walnut wood was pyrolized at different temperatures and electrochemical, bioelectrochemical, and physicochemical tests were performed. *Geobacter sulfurreducens* strain PCA was used for bioelectrochemical tests to explore the different extracellular electron transfer mechanisms. Current production confirmed that DEET processes were present. Ongoing work is focused on quantifying how active DEET, material capacitance, and surface functional groups are for overall electron transfer, and the role for biochar in natural environments and engineered conservation efforts as a soil biosnorkel.

1. Friedman, E. S.; McPhillips, L. E.; Werner, J. J.; Poole, A. C.; Ley, R.; Walter, M. T.; Angenent, L., Methane emission in a specific riparian-zone sediment decreased with bioelectrochemical manipulation and corresponded to the microbial community dynamics. *Frontiers in Microbiology* **2016,** *6*, (1523).
2. Kappler, A.; Wuestner, M. L.; Ruecker, A.; Harter, J.; Halama, M.; Behrens, S., Biochar as an electron shuttle between bacteria and Fe(III) minerals. *Environmental Science & Technology Letters* **2014,** *1*, (8), 339-344.
3. Klüpfel, L.; Keiluweit, M.; Kleber, M.; Sander, M., Redox properties of plant biomass-derived black carbon (biochar). *Environmental Science & Technology* **2014,** *48*, (10), 5601-5611.
4. Erable, B.; Etcheverry, L.; Bergel, A., From microbial fuel cell (MFC) to microbial electrochemical snorkel (mes): Maximizing chemical oxygen demand (COD) removal from wastewater. *Biofouling* **2011,** *27*, (3), 319-326.

Sporomusa ovata-driven microbial electrosynthesis: progress and perspective

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Microbial electrosynthesis (MES) is a bioenergy technology in which a microbial catalyst reduces CO_2 into organic carbon molecules with electrons from the cathode of a bioelectrochemical system. Besides being a potential measure to control greenhouse gas emissions, MES has additional attractive features. It can be used to store electricity surpluses from the power grid into the chemical bonds of valuable products such as biofuels. It can also be coupled with solar panels to become an artificial bioinorganic photosynthesis apparatus with a solar-to-chemicals conversion efficiency significantly higher than biomass-based bioproduction technologies. A promising platform for the production of multicarbon compounds from CO_2 is the gram-negative bacterium *Sporomusa ovata*, one of the most efficient acetogenic MES microbial catalysts. Still, many challenges remained before reaching a technology readiness level high enough for industrial application including the fact that: 1) *S. ovata* metabolism is not fast enough when reducing CO_2 with electrons coming from a MES cathode, 2) only a limited number of chemicals can be produced and, 3) standard electrodes are not optimal for the fast transfer of a large volume of electrons. This presentation will provide an overview of the efforts made by my group to overcome those three barriers. For instance, the recent application of a methanol-based adaptive laboratory evolution resulted in a *S. ovata* strain reducing CO_2 more efficiently into acetate by MES (Tremblay et al., 2015). Furthermore, a systematic optimization of *S. ovata* growth medium led to a more efficient MES process capable of generating ethanol beside acetate. Beyond this, it has now become possible for us to engineer *S. ovata* with a complete markerless gene editing system. On the bioelectrochemical hardware side, we developed novel graphene-based electrodes considerably more efficient for the transfer of electrons to *S. ovata* (Chen et al., 2016). In the future, combination of microbial catalysts and electrochemical reactor improvements could lead to a scalable MES system capable of producing efficiently multiple valuable chemicals.

Tremblay P-L, Höglund D, Koza A, Bonde I, Zhang T. Adaptation of the autotrophic acetogen *Sporomusa ovata* to methanol accelerates the conversion of CO_2 to organic products. Sci. Rep. 2015;5:16168.

Chen L, Tremblay P-L, Mohanty S, Xu K, Zhang T. Electrosynthesis of acetate from CO_2 by a highly structured biofilm assembled with reduced graphene oxide–tetraethylene pentamine. J. Mater. Chem. A 2016;4:8395-8401.

Microbial electrochemical technologies meet anaerobic digestion: from biogas purification and nutrient recovery to electro-fermentation

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An increasing number of studies have pointed out that Anaerobic Digestion (AD) and Microbial Electrochemical Technologies (MET) are not competing but rather complementary technologies, whose integration would offer many opportunities for improving energy and nutrients recovery from waste organic substrates under a wide range of operating conditions. As an example, integration of MET within conventional anaerobic digesters has been shown to enhance biogas output, to reduce start-up time, and to increase process resilience, although the underlying mechanisms (e.g., biomass retention at the electrodes vs. bioelectrocatalysis) are not completely elucidated[1]. Also, MET can be applied downstream of AD systems to purify and upgrade (to biomethane) the biogas as well as to simultaneously eliminate the residual organic matter contained in the digestate and to recover valuable nutrients such as ammonium[2].
Very recently, a novel concept is also being investigated which consists of employing an electrochemical approach to steer product distribution in dark fermentation, either in pure or mixed cultures. This strategy, typically referred to as "electro-fermentation", relies on the possibility to alter the intracellular redox state (e.g. $NADH/NAD^+$ balance) by using electrodes to modify the extracellular oxidation-reduction potential of the fermentation medium and/or to drive unbalanced fermentations[3]. This is particularly interesting towards a further development of two-phase AD systems, in the more general perspective of AD-based biorefineries.
In this frame, the main achievements regarding MET and AD integration will be presented and discussed based on results collected in our laboratory as well as on data reported in the literature.

1. De Vrieze J., Gildemyn S., Arends J.B.A., Vanwonterghem I., Verbeken K., Boon N., Verstraete W., Tyson G.W., Hennebel T., Rabaey K. (2014) Biomass retention on electrodes rather than electrical current enhances stability in anaerobic digestion. *Water Research*, 54: 211–221.
2. Villano M., Scardala S., Aulenta F., Majone M. (2013) Carbon and nitrogen removal and enhanced methane production in a microbial electrolysis cell. *Bioresource Technology*, 130: 366–371.
3. Moscoviz R., Toledo-Alarcón J., Trably E., Bernet N. (2016) Electro-Fermentation: How To Drive Fermentation Using Electrochemical Systems. *Trends in Biotechnology*, doi:10.1016/j.tibtech.2016.04.009.

Progress in scaling up microbial fuel cells for wastewater treatment

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Tremendous progress has been made during the last few years in the development of new architectures for air-cathode microbial fuel cells (MFCs) that use inexpensive materials, which are enabling practical applications of these systems for wastewater treatment. In this presentation, I will summarize the two main approaches for reactor architecture (tubular versus flat-modular), and the most successful materials used in these systems based on using carbon fiber brush anodes, activated carbon cathodes, and inexpensive binders. Recent work in my laboratory has focused on the design and use of flat, modular reactors for domestic wastewater treatment. We developed and tested a two liter system containing two brush anode modules (6 brushes for each module) and a single inner cathode module. Tests with domestic wastewater produced up to 400 ± 8 mW m^{-2} in fed batch tests using domestic wastewater, and *ca.* 300 mW m^{-2} under continuous flow conditions. The strength of the wastewater limited power production, as shown by an increase to 1100 ± 10 mW m^{-2} when 1 g/L of acetate was added into the wastewater. Newer results using a larger (6 liter), multi-module will also be presented with an examination of power for different numbers of cathodes connected to the anode module, and performance under different operational conditions. Application of MFCs for wastewater treatment will require downstream processes to meet discharge effluent limitations for biochemical oxygen demand. We are examining the use of anaerobic fluidized membrane bioreactors (AFMBRs) and related technologies for reducing the concentration of organic matter to levels suitable for wastewater discharge. The combination of these two technologies could enable effective and very low energy wastewater treatment at scales varying from small to large treatment plant applications.

He, W., X. Zhang, J. Liu, X. Zhu, Y. Feng, and B.E. Logan. 2016. Microbial fuel cells with an integrated spacer and separate anode and cathode modules. *Environ. Sci. Water Res. Technol*. 2:186-195.

Kim, K-Y., W. Yang, Y. Ye, N. LaBarge, and B.E. Logan. 2016. Performance of anaerobic fluidized membrane bioreactors using effluents of microbial fuel cells treating domestic wastewater. Biores. Technol. 208:58-63.

Logan, B.E., M.J. Wallack, K.-Y. Kim, W. He, Y. Feng, and P. Saikaly. 2015. Assessment of microbial fuel cell configurations and power densities. *Environ. Sci. Technol. Lett*. 2(8):206-214.

Microbial Electrochemical Technologies for energy storage

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After more than 10 years of research, Microbial Electrochemical Technologies (METs) need to prove their worth in practice. Electricity recovery from wastewater in MFCs is ready for larger-scale demonstration, and many attempts for scaling up have been done. The main challenge that still remains is to achieve sufficiently high rates and efficiencies at larger scale. In terms of efficiency, it is crucial to achieve high Coulombic efficiencies at the bio-anode. A high Coulombic efficiency can be achieved by avoiding competing processes, especially methanogenesis. To outcompete methanogens, a low substrate concentration is required, preferably still resulting in a high current. This can be achieved by using a high (specific) electrode surface area The use of fluidized activated carbon granules as a capacitive bioanode is a promising direction for scaling-up MFCs, as it combines a high specific surface are with low ohmic losses during discharge. These activated carbon granules can also be used as an additional buffer to store electricity.
In the interaction between electrochemically active microorganisms and capacitive granule, the question arises to which extent the biofilm contributes to the capacitance. We have used Fluorine-doped Tin Oxide (FTO) electrodes that have a very low capacitance, to study charge storage in the biofilm (biofilm capacitance). Two FTO electrodes were operated as bioanodes, and its performance was monitored using polarization curves and Electrochemical Impedance Spectroscopy (EIS). Analysis of EIS showed that biofilm capacitance, during biofilm growth, increased continuously . A linear relationship was found between the current during impedance spectroscopy, which is a measure for biofilm development/activity, and biofilm capacitance.
METs are not only useful for analysis of biofilm capacitance, they can also be used for storage of electricity on a longer time scale. We developed a Microbial Recheargable Battery by combining a Microbial Fuel Cell with a Microbial Electrosynthesis Cell. Duplicate runs showed stable performance over 15 days, with acetate being the main energy carrier. An energy density of around 0.1 kWh/m^3 was achieved at a full cycle energy efficiency of 30 – 40%. With this study, we show a new potential application area for METs as a future local energy storage device.

Bioremediation of contaminated water: a niche for microbial electrochemical technologies

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Water contamination with chlorinated aliphatic solvents (chlorinated methanes, ethanes and ethenes) and inorganic compounds (nitrate, sulphate, arsenic and even, uranium) is a worldwide environmental challenge. Consumption of water containing high compound levels as drinking water can cause many diseases (i.e. cancer, skin irritation, among others). This abstract contains a critical examination of the current application of microbial electrochemical technologies (METs) in the field of bioremediation of contaminated water. Data from lab-scale and pilot-plant studies will be shown to prove that in situ bioremediation is a highly promising and cost-effective technology for the sustainable remediation of contaminated sites.

Coma, M., Puig, S., Pous, N., Balaguer, M. D., and Colprim, J. (2013) Biocatalysed sulphate removal in a BES cathode. Bioresource Technology, **130**, 218–223.

Majone, M., Verdini, R., Aulenta, F., Rossetti, S., Tandoi, V., Kalogerakis, N., Agathos, S., Puig, S., Zanaroli, G., and Fava, F. (2014) In situ groundwater and sediment bioremediation: barriers and perspectives at European contaminated sites. New Biotechnology, **32**(1), 133–146.

Pous, N., Casentini, B., Rossetti, S., Fazi, S., Puig, S., and Aulenta, F. (2015) Anaerobic arsenite oxidation with an electrode serving as the sole electron acceptor : A novel approach to the bioremediation of arsenic-polluted groundwater. Journal of Hazardous Materials, **283**, 617–622.

Pous, N., Puig, S., Balaguer, M. D., and Colprim, J. (2015) Cathode potential and anode electron donor evaluation for a suitable treatment of nitrate-contaminated groundwater in bioelectrochemical systems. Chemical engineering journal, **263**, 151–159.

Williams, K. H., Bargar, J. R., Lloyd, J. R., and Lovley, D. R. (2013) Bioremediation of uranium-contaminated groundwater: A systems approach to subsurface biogeochemistry. Current Opinion in Biotechnology, **24**(3), 489–497.

Shewanella senses electrode potential for catabolic regulation

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It has been known that current generation by exoelectrogens in a bioelectrochemical system (BES) is dependent on potential of a working electrode. This phenomenon can partly be explained based on the concept of electrochemical kinetics, e.g., those expressed by the Butler-Volmer equation. In addition, it is also possible that electrode potential may affect cellular physiology and gene expression in exoelectrogens, thereby influencing current generation. Limited studies have shown that bacterial catabolic pathways are changed in association with changes in electrode potential. For instance, electrochemical characterization and gene-expression analyses have revealed that the activity of the tricarboxylic-acid cycle in *Shewanella* cells is reversibly gated by changing electrode potential (1). However, it remains to be elucidated (i) how exoelectrogens sense electrode potentials, and (ii) how they regulate catabolic pathways and energy-conservation systems depending on electrode potentials. In order to address these questions, the present study analyzed *Shewanella oneidensis* MR-1 in BESs using a combination of electrochemical, genomic and molecular approaches. We found that MR-1 senses electrode potentials and regulates its catabolic pathways using an Arc two-component system. This global regulatory system senses the redox state of membrane quinones that are electrically connected to electrodes via the extracellular electron transport pathway. The Arc system regulates the transcription of many genes (approx. 400 genes), including those coding for catabolic enzymes and energy-conservation systems (e.g., NADH/ubiquinone oxidoreductase [Nuo], pyruvate dehysrogenase, formate dehydrogenase [Fdn], and ATP synthetase), whose expression is found to be potential-dependent. Further genomic and molecular analyses suggest that formate serves as an intracellular electron mediator at low potentials, while NADH is used at high potentials. This catabolic shift facilitates electromotive force-dependent energy conservation in MR-1; namely, the NADH/Nuo-dependent electron transfer operating at high potentials is able to generate more proton-motive force than the formate/Fdn-dependent one at low potentials. We suggest that exoelectrogens are able to select appropriate catabolic pathways to efficiently conserve energy for growth by sensing electrode potentials (and external electron acceptors).

(1) Matsuda et al. (2013) Electrochemical gating of tricaboxylic acid cycle in electricity-producing bacterial cells of *Shewanella*. PLoS One 8:e72901.

Oral presentations

Recovery of copper at micromolar concentration from distillery wastewater using bioelectrochemical systems

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Bioelectrochemical systems (BESs) can be used to simultaneously remove organic contaminants and produce electricity, recover metals, or produce useful chemical products from municipal wastewater or industrial effluents. Recovery of pure metals with market value from industrial effluents gives an economic incentive for industry to adopt clean technologies which remove organic and metal pollutants as part of the circular economy. However, metal recovery using BES is hindered in real industrial waste streams by low metal ion concentration and the presence of other chemicals in the waste stream. Copper recovery from distillery spent lees is one such example, with copper deposition influenced by the concentration of copper and organics.

The feasibility of copper recovery from spent lees, a copper-containing waste stream from whisky distilling, using BES was investigated. Using BES and electrochemical half-cells with graphite plate working electrodes poised at -0.4 V vs Ag/AgCl, spent lees from different distilleries were tested for copper removal and deposition (10-42 mg). Metal deposition on the graphite electrodes was visually observed in both half cells and BES. For the best-performing spent lees, 42 mg of pure copper metal was deposited over a 7-day period, with copper removal reduced from 41±0 to 5±1 ppm in solution. A similar performance was achieved in BES with an acetate-fed anode and spent lees fed to the cathode. When the system was operated with a fixed input voltage of 0.5 V supplied from a power supply, copper metal deposition was observed. However, when the system was operated with a low external resistance, copper removal from solution was observed, but the main form of copper deposited was cuprite (Cu_2O). The difference in the form of the copper-containing deposits was attributed to differences in BES cathode potential and the presence of organics in the spent lees. In addition, the form of Cu deposited on electrodes was different when spent lees from different distilleries were used and this may reflect the presence of organic ligands in the spent lees.

Constructed Wetland-Microbial Fuel Cell enhances domestic wastewater treatment efficiency

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Horizontal subsurface flow constructed wetlands (HSSF CWs) are natural wastewater treatment systems where organic matter is oxidized mainly under anaerobic conditions. Microbial fuel cells can be implemented in HSSF CW (Corbella et al., 2014; 2015). CW-MFC can improve domestic wastewater treatment efficiency. The aim of this study was to quantify to which extent MFCs implementation in HSSF CWs can improve treatment efficiency. To this purpose, 4 lab-scale membrane-less CW-MFCs were constructed and operated for 7 batch cycles (ca. 2 months). The anode consisted of gravel wrapped in stainless steel giving an anode volume of 750 cm^3 and a projected surface of 60 cm^2. The cathode consisted of a graphite felt disc of 240 cm^2. The cathode and the anode were connected by means of stainless steel wires and the circuit was closed by implementing a 200 Ω resistance. Two of the CW-MFCs were left unconnected while the other two were left connected all along the experiment. CW-MFCs were weekly loaded with primary settled domestic wastewater collected from a municipal sewer. After each batch cycle samples were taken from the anodic area and water quality parameters such as total organic carbon (TOC), soluble organic carbon (SOC), total and soluble COD, BOD_5, ammonium, SO_4^{-2}, NO_3^--N, NO_2^--N and O-PO_4^{-3} were analysed. Results showed that connected CW-MFC (CW-MFC^+) outperformed the un-connected CW-MFC (CW-MFC^-) for almost all water quality parameters here considered. Accordingly CW-MFC^+ showed ca. 15%, 15%, 15%, 20%, 20% 25% and 35% lower effluent concentration than CW-MFC^- to the TOC, SOC, soluble COD, total COD, BOD_5, ammonium and phosphates, respectively. On the contrary, CW-MFC+ showed 25%, 20% and 10% higher effluent concentration than CW-MFC- for SO_4^{-2}, NO_3^--N and NO_2—N, respectively. However, statistical differences were only significant for total COD, ammonia and phosphates. Main conclusion of the present work is that of CW-MFCs can improve domestic wastewater treatment efficiency. However, longer study periods of pilot-scale CW-MFCs shall be further addressed to confirm the findings here reported.

Corbella C., Garfí A., Puigagut J. (2014) Vertical redox profiles in treatment wetlands as function of hydraulic regime and macrophytes presence: Surveying the optimal scenario for microbial fuel cell implementation. Science of the Total Environment, 470-471: 754-8

Corbella, C., Guivernau, M., Viñas, M., Puigagut, J. (2015) Operational, design and microbial aspects related to power production with microbial fuel cells implemented in constructed wetlands, Water Research, 84: 232-242

Two-stage bio-electrochemical process for treatment of sulfate-rich wastewaters

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In this work, a novel two-stage bio-electrochemical process was devised and tested at lab scale for the removal of sulfate, salinity and acidity from acid sulfate-rich wastewaters. The process relies on a biocathode for sulfate reduction to sulfide by sulfate-reducing bacteria (SRBs). This metabolism is known to cause microbial activity inhibition due to sulfide toxicity. This is solved by converting the produced sulfide to elemental sulfur, which also enables the effective removal of sulfate from the ongoing reduction-oxidation cycle, with no net chemical requirements. A first stage cell (bio-electrochemical) hosts the biocathode working electrode, where autotrophic sulfate reduction takes place, which may involve direct electron transfer by sulfate reducing microorganisms or indirect (H_2-mediated) sulfate reduction. In a second stage (electrochemical cell), elemental sulfur is generated by electrochemical anodic oxidation of the produced sulfide, which is subsequently recovered, while simultaneously removing sulfide toxicity on the SRBs. The process configuration is schematised in Figure 1.

Autotrophic sulfate reduction reached 29.6 ± 5.4 g SO_4^{2-}-S m^{-2} d^{-1} at -1.1 V vs SHE with a 95 ± 0.04 % current efficiency at an energy input of 6.7 kWh kg^{-1} SO_4^{2-} removed. With the incorporation of an electrochemical sulfide oxidation unit, sulfide inhibition could be avoided while simultaneously elemental sulfur is recovered.

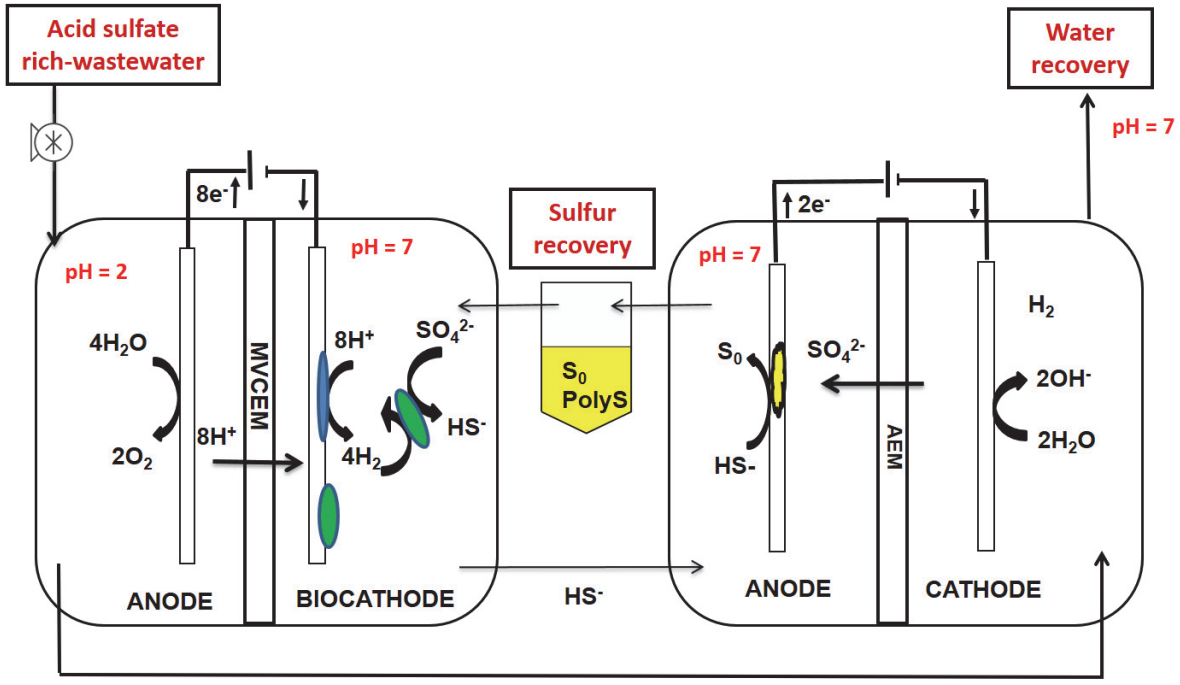

Figure 1. Proposed integrated autotrophic bio-electrochemical process for treatment of sulfate-rich wastewater.

Identifying active microbial populations in a microbial electrolysis cell coupled to a thermophilic anaerobic digester under inhibited and stable stages

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Thermophilic anaerobic digestion (AD) of pig slurry coupled to a microbial electrolysis cell (MEC) with a recirculation loop was investigated at lab-scale as a strategy to increase AD stability when submitted to organic and nitrogen overloads.

An AD reactor (4 L) operated during 118 days with an hydraulic retention time of 10 days and high organic and nitrogen loading rates (6.10±1.88 kg_{COD} m^{-3} day^{-1} and 0.35±0.04 kg_N m^{-3} day^{-1}, respectively) was connected in series with a two chambered MEC (0.5 L each compartment) for ammonia recovery, and with a recirculation loop between both reactors. The system performance was studied with the recirculation loop both connected and disconnected, in terms of AD methane production, chemical oxygen demand removal (COD) and volatile fatty acids (VFA) concentration. Furthermore, a microbial community assessment of eubacteria and archaea was performed by means of DNA and RNA-based approach (RT-qPCR and high throughput sequencing (MiSeq)), in order to determine the main active microbial populations in each phase.

Suppression of the recirculation loop resulted in the inhibition of the AD, with a reduction in the COD removal efficiency (from 40% to 22%) and in the methane production (from 0.32 to 0.03 m^3 m^{-3} d^{-1}). The restart of the recirculation increased methane production to 0.55 m^3 m^{-3} d^{-1} concomitant with a MEC maximum COD and ammonium removal efficiency of 29 and 34%, respectively. The quantitative microbial assessment of the AD biomass and the anode biofilm of the MEC showed that methanogenic population in the AD decreased by one magnitude order during its inhibition conditions, while the MEC biomass remained stable in gene copy numbers (both archaea and eubacteria). Furthermore, the most active microbial populations (RNA level sequencing) were dominated by phylotypes belonging to hydrogenotrophic methanogens as a result of the high ammonia concentrations in the AD reactor, and by *Desulfuromonadaceae* (18-19% of relative predominance at RNA level) on the anode-MEC, although not being the predominant ones according to DNA sequencing in both reactors.

As a conclusion, the recirculation loop between the AD and the MEC allowed the first one to tolerate a high organic and nitrogen loading rate that other way would result in the failure of the reactor. Besides, the MEC was able to improve the quality of the digestate during AD inhibition stages, achieving an overall AD-MEC COD removal efficiency of around 60%. Moreover, the populations of both reactors remained well differentiated in spite of the existence of the recirculation loop, increasing the biodiversity of the system and suggesting that this configuration is more robust against stress than the AD operating alone. The obtained results demonstrated that the AD microbial population was altered in response to the stress, while the biofilm of the anode-MEC stayed unaltered.

Defined co-cultures of *Pseudomonas aeruginosa* PA14 and *Enterobacter aerogenes* for enhanced current generation in bioelectrochemical systems

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Many bioelectrochemical systems (BES) employ undefined bacterial mixed cultures to recover waste energy, e.g. from wastewaters, into renewable electricity. Understanding the ecological relationships in these bacterial cultures is important to improve BES performance. Thereby, model defined co-cultures can be a valuable tool to investigate important co-culture relationships without the high complexity of undefined co-cultures. Recently, a synergistic interaction regarding current production in a defined co-culture of the phenazine redox mediator producer *Pseudomonas aeruginosa* (providing the electron shuttles) and the sugar fermenter *Enterobacter aerogenes* was observed (1). The central goal of our work is to gain a thorough understanding of the inter-microbial interactions in defined co-cultures of the key organisms *P. aeruginosa* PA14 with *E. aerogenes* for application in BES.
Therefore, we conduct potentiostatically-controlled co-culture experiments in a three electrode setup. Cultures are electrochemically and physiologically characterized including phenazine and metabolite analysis. Furthermore, species population dynamics in co-cultures of liquid samples and in biofilms were uncovered by tagging both organisms with two distinctive fluorescence proteins. To analyze the tight regulation of the diverse phenazines via quorum sensing, targeted gene deletions in the phenazine pathway of *P. aeruginosa* were accomplished.
First results show that the barely electroactive organism *E. aerogenes* exhibited strong electron transfer to the electrode when provided with synthetic phenazines. Compared to a phenazine deletion mutant of *P. aeruginosa* provided with equal amounts of phenazines, *E. aerogenes* showed a distinct stronger electrochemical response. A mixed culture comprising PA14 and *E. aerogenes* produced higher currents compared to pure cultures of both organisms. In order to stir production of the major contributing factors 2,3-butanediol and phenazines, experimental parameters as pH, inoculation time and oxygen were adapted. Especially oxygen was identified as major influential parameter, which optimization resulted in a 4-fold increase of maximum currents. By operating our BES in fed-batch mode, the collected charge was increased significantly. From the phenazine deletion mutants, we see distinct evidence for a cross-regulation between the two redundant phenazine operons, which significantly affect performance of PA14 in BES. Overall, optimization and exploration of the factors underlying the synergistic interactions provides us with information on how to tap the benefits of this ecological phenomena for BES applications.

1. Venkataraman *et al.*, (2011) Metabolite-based mutualism between *Pseudomonas aeruginosa* PA14 and *Enterobacter aerogenes* enhances current generation in bioelectrochemical systems. Energy Environ Sci, 4: 4550-4559

La tavolozza elettrica - The diversity of electroactive microorganisms

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The core of microbial electrochemical technologies (METs) is the ability of electroactive (exoelectrogenic) microorganisms to interact with electrodes *via* extracellular electron transfer (EET), allowing the wiring of electric current flow and microbial metabolism. Many microorganisms were reported for being electroactive, but besides the model organisms *Geobacter sulfurreducens* and *Shewanella oneidensis* often only sparsely characterized limiting their industrial application as well as the knowledge of the natural importance of EET based processes.
The environmental preferences of 89 electroactive species (bacteria and archaea) described in literature were combined with their physiological as well as EET characteristics. Based on 20 individual descriptors an extensive meta-analysis was performed (Koch & Harnisch).
All in all, 44 species displayed anodic, 25 cathodic (13 of them including autotrophic biomass formation) and 17 species both ways of EET with electrodes. The meta-analysis revealed correlations between some of the investigated parameters, e.g. different electron transfer characteristics (direct vs. mediated) and the preference for soluble or flexible use of soluble and solid electron acceptors as well as oxygen requirements. But no obvious linkage to any specific habitat or phylogenetic group was found.
These results strongly indicate that significantly more electroactive species exist in nature as well as in established strain collections, but current cultivation techniques certainly impede the identification of their EET capacities. The presentation will discuss microbial resource mining (of strain collections and natural habitats) based on ecological knowledge for the future developments of METs. Its enormous potential will be shown in light of specific traits required for industrial application like metabolic functions performed under defined temperature or salinity optima.

Koch, C. & Harnisch, F., Is there any ecological niche for electroactive microorganisms? (submitted)

Bioelectrochemical conversion of CO_2 to chemicals: realization and perspectives

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A recent concept of microbial electrosynthesis (MES) has evolved as the electricity-driven production of chemicals from low-value waste using microbes as catalysts. MES from carbon dioxide (CO_2) comprises bioelectrochemical reduction of CO_2 to multi-carbon organic compounds using the reducing equivalents produced at the electrically-polarized cathode. Here we are presenting an overview of our realization in the study of MES based on CO_2 reduction by mixed culture from wastewater origin, circumventing the methanogenesis. Acetate is the primary multi-carbon product of CO_2 reduction but more valuable products including ethanol, butyrate can be produced on further reduction.

Establishment of stable and robust biocathode for the bioelectrochemical CO_2 reduction at the graphite felt cathode was succeeded repeatedly under long-term operation which led to the accumulation of higher titer to extractable amount (up to 8-10 g L^{-1} of acetate). When higher titers are obtainable from the CO_2 reduction based MES, product downstream process would be feasible. But still, the rates of acetate production in MES from CO_2 using the unmodified graphite felt electrodes are insufficient for the scaling up and practical application

CO_2 reduction in MES requires continuous availability of CO_2 and sufficiently low cathode potential ($\leq$ -1 $V_{/Ag/AgCl}$) to ensure the supply of reducing equivalents/hydrogen. Importantly, the dissolution of CO_2 and mass-transfer of reducing equivalents/hydrogen are the main limitations. Improvement on the dissolution and mass transfer rate of CO_2 was explored using gas diffusion electrodes (GDEs). Gas diffusion biocathode at least doubled the CO_2 mass transfer and also able to utilize CO_2 from the diluted gas mixture (20% CO_2) to produce acetate at the rate of 240 mg L^{-1} d^{-1} when the cathode was polarized at -1.1 $V_{/Ag/AgCl}$. GDEs can enable the MES technology to be directly applicable for converting CO_2 from industrial sources.

Improvements in efficiencies associated with the current reactor system, catalyst and mass-transfer limitation are required for the full-scale application of MES. Further study and understanding on process enhancement and integration of product recovery will establish MES, an innovative sustainable technology for manufacturing renewable chemicals and biofuels and at the same time, an appropriate storage for excess electricity from renewable sources.

Anodic microbial electrosynthesis: Anaerobic production of amino acids by *Corynebacterium glutamicum*

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The important industrial bacterium, *Corynebacterium glutamicum*, has been widely used for the aerobic production of amino acids and various chemicals. However, the bacterial growth and bioproduction are highly limited by the availability of oxygen. Under anoxic conditions the cell growth is arrested and sugars are fermented to acids, mainly lactate and succinate.[1] To overcome the oxygen dependency, we introduce a soluble artificial extracellular electron acceptor, ferricyanide, to the microorganism in a bioelectrochemical system (BES) to enable anaerobic microbial growth and anaerobic biosynthesis of amino acids.

A BES allows the microorganisms to use solid-state electrodes as sources or sinks of electrons to help achieving redox balance. Applications of BES include power generation, chemical production, wastewater treatment and bioremediation.[2]

In this work, we use a BES to provide *C. glutamicum* with an extracellular electron acceptor, ferricyanide, which acts as a redox mediator and shuttles reversibly electrons between cells and anode.

An engineered strain of *C. glutamicum* gained sufficient energy for growth and production of lysine under anaerobic BES environment, which have not been observed under anaerobic conditions in absence of an extracellular electron acceptor (control). Furthermore, the glucose consumption per g cell dry weight (CDW) was 10.2 times higher, and acetate, lactate and succinate production (mmol/g CDW) was 2.6, 13.1 and 24.2 times higher, respectively, compared to the control. It appeared that providing an alternative electron sink enabled recovery of NAD^+ resulting in an increased glucose uptake and a shift in the bioproduction. Additionally, anaerobic respiration of the anode resulted likely in a proton gradient to drive ATP synthesis.[3]

We demonstrate for the first time the anaerobic growth and anaerobic production of lysine by the bacterium *C. glutamicum* using a BES and its great potential for anodic microbial electrosynthesis.

1. Okino, S.; Inui, M.; Yukawa, H., Production of organic acids by Corynebacterium glutamicum under oxygen deprivation. *Appl. Microbiol. Biotechnol.* **2005,** *68*, 475-480.
2. Lovley, D. R., Electromicrobiology. *Annu. Rev. Microbiol.* **2012,** *66*, null.
3. Kracke, F.; Vassilev, I.; Krömer, J. O., Microbial electron transport and energy conservation–the foundation for optimizing bioelectrochemical systems. *Name: Frontiers in Microbiology* **2015,** *6*, 575.

Long Term Continuous Microbial ElectroSynthesis for Production of Acetate and Secondary Alcohol from CO_2 and Current.

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Microbial ElectroSynthesis (MES) is an electricity-driven bioproduction process. This microbial electrochemical technology is an emerging approach in the context of Carbon Capture and Utilization. Several studies have shown that pure cultures and mixed microbial communities are capable for reducing CO_2 into organics with an electrode as electron donor (Patil et al., 2015). Here we present (I) an effective approach for *ex-situ* enrichment of a mixed microbial community capable of MES and (II) development of a continuous bioproduction process, producing organics from CO_2 and electrical current for over 400 days.

A strategy of quick culture transfers during the *ex-situ* enrichment phase eliminated methanogenic activity as a competing reaction (Patil et al., 2015). This enriched community was successful in producing mainly acetate from CO_2 and electrical current (5 Am^{-2} applied). Several batch cycles showed reproducible activity in terms of acetate production rates 11 ± 6.9 g $m^{-2}d^{-1}$ (n=4 reactors).

Switching reactor design to a higher electrode surface to volume ratio (32 cm^2L^{-1} *vs.* 500 cm^2L^{-1}) and operation to continuous mode, led to stable acetate production rates for over 400 days. Acetate and alcohol production (rates) could be steered based on pH and hydraulic retention time. A short residence time (~ 3d; pH 7) resulted in mainly acetate production (17.6 ± 2.3 g $m^{-2}d^{-1}$). A residence time of 5 days and a pH of 5 resulted in acetate (9.94 ± 1.7 g $m^{-2}d^{-1}$), butyrate (1.9 ± 0.6 g $m^{-2}d^{-1}$) and 2-propanol (1.16 ± 0.3 g $m^{-2}d^{-1}$) production. Interestingly, little ethanol and no butanol were detected over the course of the experiments. A decrease of the hydrogen evolution overpotential was observed due to microbial activity by means of cyclic voltammetry. The community on the electrode was dominated by *Acetobacterium* spp. (40-50% relative abundance) whereas its presence in the planktonic community decreased over time. *Acetobacterium* spp. are known homoacetogens and have been found before in cathodic biofilms (Patil et al 2015). The planktonic community became dominated by members of the Planococcaceae family (30-55% rel. abundance), the ecological function of this family in the planktonic community is not yet clear.

These results show that by electrochemically adding reducing equivalents to a biological reaction and controlling reactor operation, product spectra can be steered. We hypothesize that the selective production of 2-propanol is induced by the high partial pressure of reducing equivalents close to the electrode and the need for internal energy balancing of the microorganisms.

Further work is aimed at exploring this hypothesis and understanding the exact mechanisms of alcohol formation to further steer microbial fermentations by means of electrochemistry.

Patil SA, Arends JBA, Vanwonterghem I, van Meerbergen J, Guo K, Tyson GW, Rabaey K (2015) Selective enrichment establishes a atable performing community for microbial electrosynthesis of acetate from CO_2. Environmental Science and Technology 49:8833-8843

Electrochemical Conversion of Carbon Dioxide into Methane by using Indigenous Microorganisms in Subsurface Oil Reservoir

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As potential CO_2 geological storage site in Carbon Dioxide Capture & Storage, the use of depleted oil & gas reservoirs has been proposed. The long-term aim of this research is to establish a biotechnological system to microbiologically convert geologically stored CO_2 into methane, as energy resource. To develop a means for the conversion, we focus on technological application of bio-electrochemical reaction using microbially catalyzed electrode. On the electrode surface, methanogenic microorganisms (as biocatalysts) utilize electrical current (electrons) to reduce CO_2 ($CO_2 + 8H^+ + 8e^- \rightarrow CH_4 + 2H_2O$). Such "electro-methanogenic" system is an attractive option for energy conversion, as the bio-cathode yields methane from electrical current, which can be provided by renewable energy sources. In other words, intermittent electrical energy provided by wind turbines or/and solar cells can be stored in a stable energy form, methane.

Indigenous microorganisms originated from a formation water of a domestic depleted oil reservoir were inoculated into electrochemical-cultivation reactors. Upon application of constant voltage of 0.80 V, the reactor inoculated with indigenous microorganisms produced methane at a rate of 1103 mmol $day^{-1}m^{-2}$ (unit surface area of cathode), which was the highest electro-methanogenic production rate so far documented. Moreover, current-to-methane conversion efficiency of the reaction was as high as approx. 90-100%. Thus, we concluded that microorganisms indigenous to the subsurface reservoir are highly capable of electro-methanogenic reduction of CO_2. Electrochemical and microbial analyses suggested a reaction mechanism, in which electron-releasing bacteria mediated electron transfer from the electrode to methanogenic archaea.

Two base pairs can change it all: Comparative analysis of a spontaneous *ΔlasR* mutant of *Pseudomonas aeruginosa* for BES performance

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Besides being an opportunistic human pathogen, *Pseudomonas aeruginosa* gains biotechnological interest because of its ability to interact with the anode in a bioelectrochemical system (BES). To shuttle electrons to the anode, it employs its intrinsically produced phenazines (primarily phenazine-1-carboxylate [PCA] and its derivate pyocyanin [PYO]). In *P. aeruginosa* the production of phenazines is directly coupled to a complex multi-hierarchical quorum sensing regulatory network. This network is predominantly governed by the two transcriptional regulators LasR and RhlR.

During lab maintenance two distinct agar plate cultivation phenotypes of the electroactive strain *P. aeruginosa* PA14 were identified within our used stock culture. Electrochemical investigations of the two isolates also showed different phenotypes for BES performance. A reordered fresh PA14 culture from the German culture collection DSMZ showed the same distinct two phenotypes. After verifying the strain identity of both phenotypic populations as *P. aeruginosa* PA14, a comparative DNA resequencing of both isolates was performed. Bioinformatic analysis of the data showed a 2bp deletion within the *lasR* gene in one of the phenotypes. As shown with independent molecular alternation of the wildtype *lasR* gene, this frameshift mutation is sufficient to significantly alter the global phenotype of the strain and the electrochemical profile of PA14 utilizing different carbon sources, in our 1-chamber BES reactors.

Among other regulatory target sites, LasR is known to directly bind in front of one of the two encoded phenazine synthesis gene operons in *P. aeruginosa,* altering transcription levels of the adjacent genes. These observations give further insights for understanding the *P. aeruginosa* BES performance, which is directly coupled to the tightly regulated production of phenazines under different environmental conditions. This study shows that different phenotypes of PA14 exist or maybe easily evolve in laboratory cultivation and need to be considered independently when evaluating the strains bioelectrochemical potential.

Presence of multiple pH dependent redox peaks during anode respiration by *Thermincola ferriacetica* suggests the presence of multiple electron transport pathways

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Thermincola ferriacetica, a thermophilic, Gram-positive, anode respiring bacterium (ARB) was grown in biofilms in microbial electrochemical cells (MXCs) to investigate its external electron-transport (EET) limitations. Electrochemical studies, including cyclic voltammetry (CV), are often used to elucidate the rate limiting step of electron transport in ARB. Previously reported CV analysis of *T. ferriacetica* indicated a sigmoidal Nernst-Monod response in electrical current (j) to changes in anode potential (V). This response suggests that a single proton (H^+) coupled electron (n = 1) transport reaction is responsible for the rate-limiting step in *T. ferriacetica* metabolism. The specific protein(s) responsible for this response is thought to be a *c*-type cytochrome. However, although *T. ferriacetica* has been shown to contain 35 *c*-type cytochromes, the one(s) responsible for EET have yet to be identified. Here, we show that *T. ferriacetica*'s response under low pH growth conditions is composed of at least three separate n = 1 Nernst-Monod relationships with redox peak values at E_{p1} = -0.12 V vs SHE, E_{p2} = -0.21 V vs SHE, and E_{p3} = -0.26 V vs SHE; suggesting the presence of more than one pathway for anode respiration. We also observe a pH dependent response on the presence of E_p values, with high pH resulting in broader peaks, fewer distinguishable E_ps and an overall negative shift in E_p that is consistent with a Nernstian shift. Here, we show that *T. ferriacetica* contains more than one H+ coupled EET pathway and that EET pathways within *T. ferriacetica* are sensitive to changes in bulk pH.

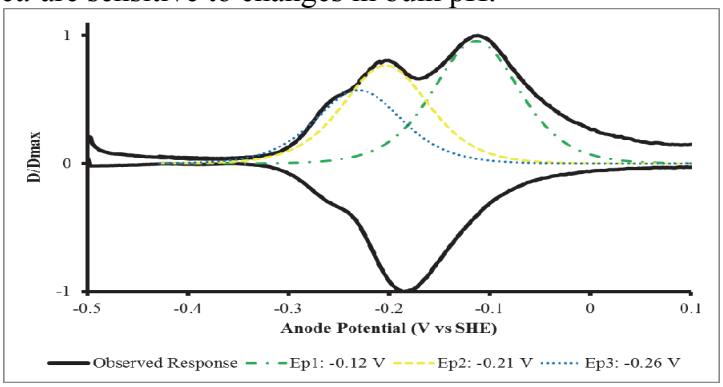

Figure 1. Observed derivative CV (1mV sec^{-1}) response (black line) of *T. ferriacetica* at pH = 5.2 shown with Nernst-Monod derivative fits for: E_{p1} = -0.12 V vs SHE (green hashed line), E_{p2} = -0.21 V vs SHE (yellow hashed line), and E_{p3} = -0.26 V vs SHE (blue dotted line).

"Dialogo sopra i due massimi sistemi del mondo" Role and mechanism of flavin-cytochrome interactions In extracellular electron transfer

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The plethora of applications of Microbial Electrochemical Technologies is underpinned by an efficient electrical contact between microorganisms and conducting electrodes in the devices. Multiheme cytochromes at the surface of electroactive microorganisms are recognized key players in this phenomenon, and their role in extracellular electron transfer mediated by small redox shuttles such as excreted flavins has been the focus of intense debate (Brutinel 2012). While on one hand binding of flavins to purified oxidised outer-membrane (OM) cytochromes was found to be weak and transient (Paquete 2014; Breuer 2015), on the other hand, electrochemical measurements of live biofilms report the presence of flavins bound to OM cytochromes (Okamoto 2013, Xu 2016). The OM decaheme cytochromes from *Shewanella* show conserved redox-active disulfide-bonds that are redox active. Interestingly, the reduction of these disulfide bridges by glutathione appears to increase the affinity of the cytochromes towards FMN (Edwards 2015), providing a way of reconciling the apparent contradiction in the literature. The evidence for the two views will be weighted in the light of novel results obtained with mutants of the OM cytochromes OmcA and MtrC from *Shewanella oneidensis* MR-1. These will shed further light on the mechanisms of cytochrome-flavin interaction and their implication in extracellular electron transfer.

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Extracellular polymeric substances play roles in extracellular electron transfer of *Shewanella oneidensis* MR-1

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It is well known that microorganism is surrounded by extracellular polymeric substances (EPS) which include polysaccharides, proteins, glycoproteins, nucleic acids, phospholipids, and humic acids. However, previous studies on microbial extracellular electron transfer (EET) are conducted on cells without extracting EPS or cells collected from log stage or early-steady stage cultures with little EPS. Therefore, microbial cells are believed in contact directly with each other or electrode. Such attempt apparently ignored the role of EPS in microbial EET, even though many components of EPS, such as DNA, humic acids and some proteins, are electrochemically active or semiconductive. Herein, we report experimental evidences of EPS role on EET for *Shewanella oneidensis* MR-1.

Atomic force microscopy clearly showed that the cell surface was cleaned and few EPS could be observed on MR-1 after the extraction (Figure 1.a and 1.b). Comparing to cells in control group, MR-1 treated at 38 °C for EPS extraction showed different electrochemical characterizations as revealed by differential pulse voltammetry (Figure 1.c). EPS extracted from MR-1 also was proved to be electrochemically active. The present study indicated that EPS play important roles in EET of MR-1.

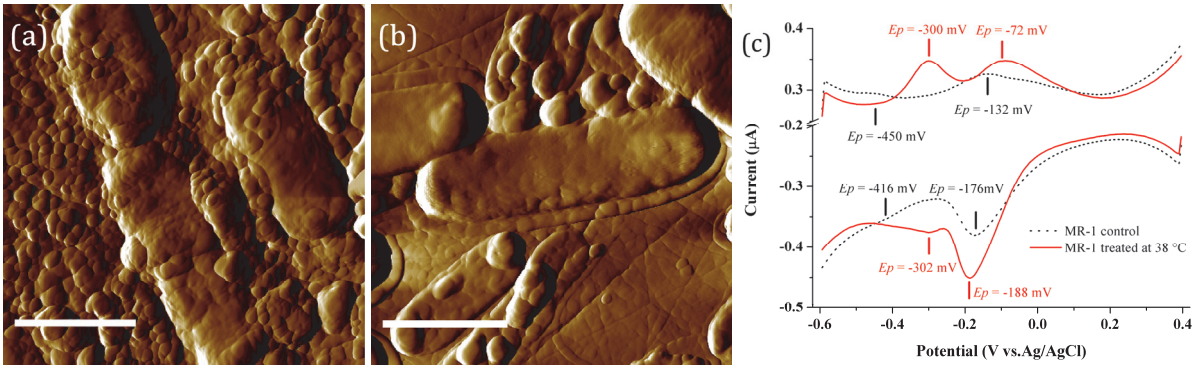

Figure 1 Atomic force microscopy shows more EPS surrounding the MR-1 cells in control groups treated 30 °C (a), comparing to those treated at 38 °C (b). Scale bar: 2 μm. Voltametric analysis of MR-1 treated at 30 °C (dotted line) and 38 °C (solid line) (c).

Electricity driven fermentations and their implications for microbial electrosynthesis

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Mixed cultures can be used to ferment carbohydrate-rich streams to alcohols and carboxylates, which can be later transformed to medium chain fatty acids (MCFA) through secondary fermentations. Microbial metabolism is sufficiently versatile to enable conversion of a wide range of alcohols as well as starting carboxylates as intermediates (Coma *et al.* 2016). Due to their toxicity, MCFA production requires both alkaline dosage and in situ extraction. When coupled to a membrane electrolysis system, the formed products can be extracted and harvested as an acid concentrate (Andersen *et al.* 2015). An advantage of such electromigration driven extraction is that it targets charged molecules, in this case carboxylates, which form the bulk of the product at typical pH values during fermentation. While this approach overcomes hydrogen and alkaline requirements, it also modifies the product outcome itself, for example thin stillage fermentation is shifted away from predominantly acetate and propionate to butyrate and caproate, and glycerol fermentations are pushed towards valerate. From this, it is clear that supply of electrons drives metabolism towards more reduced outcomes. The same goes for autotrophic metabolism during microbial electrosynthesis, where the production of alcohols is consistently found. At atmospheric pressures, homoacetogens growing with H_2 as electron donor tend not to produce ethanol, typically CO is needed to invoke this effect. This shows that the cathode can deliver stronger reducing conditions than dissolved H_2 and this results in mixtures of alcohols and carboxylates. Interestingly, this can then become the driver of a secondary series of reactions similar to the aforementioned chain elongation, leading to the formation of for example butyrate. The question remains whether butyrate can also be formed directly, as a means to limit ATP formation during high electron flux and limited growth conditions. Whichever pathway predominates, a combination of organic substrates with CO_2 and electricity may enable maximizing product benefit while limiting electron need and minimizing the formation of biomass.

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Coupling organic waste oxidation to microbial electrosynthesis for the energy-efficient production of platform chemicals: lessons from the French "BIORARE" project

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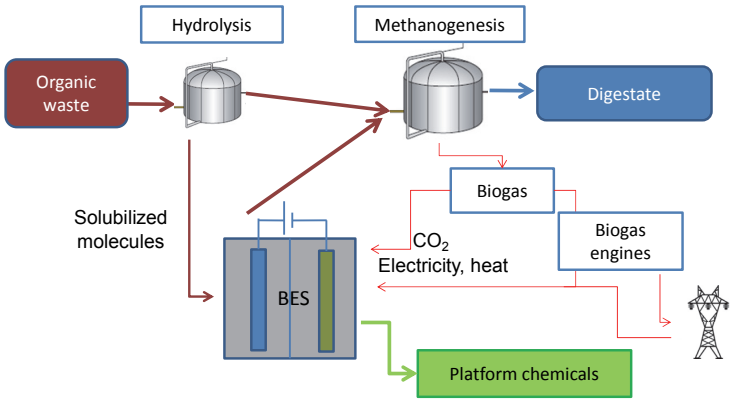

Figure 1: Mainstream scenario studied for the coupling of a BES to an existing organic waste treatment facility.

The BIORARE project started by the end of year 2011 and has gathered a consortium of 5 partners in the framework of a French "Investissement d'Avenir" program (ANR-10-BTBR-02). The objective is to evaluate how bioelectrochemical systems (BES) could be integrated into existing waste treatment facilities to promote the emergence of environmental biorefineries where oxidation of waste would be coupled to the production of platform chemicals through energy efficient CO_2 reduction. This evaluation is based not only on scientific and technological criteria (three workpackages) but also on environmental assessment of selected scenarios and first analysis of associated industrial constraints (two workpackages). The starting working hypothesis is that the BES could be coupled to a two-step anaerobic digestion process encompassing hydrolysis and methanogenesis reactors (Figure 1).

The procedures for drawing electrons out of various residual organic feedstock (sludges, biowaste) at high current densities ranging from 7 to 30 A/m^2 were established. At the cathode, the possibility of microbial electrosynthesis of multicarbon platform chemicals through CO_2 reduction by a mixed culture consortia was confirmed. Various procedures allowing the operation of BES comprising a bioanode oxidizing waste materials coupled to a cathode synthesizing platform organics were defined and patented. Stable operation of labscale BIORARE reactors were obtained during several months constituting first proofs of technological concept (TRL3). The comparison with BESs equipped with an abiotic anode, which achieved water oxidation, showed that the bioanode reduced the electric power consumption of a factor two to three. Performances of larger optimized reactors are currently being studied. Conclusions drawn from first Life Cycle Assessment performed on selected BES implementation schemes and associated industrial constraints will be discussed. Even if numerous technological, environmental and socio-economical hurdles still need to be overcome, the results of the BIORARE project also highlight the great potential of BES to become a technological cornerstone of future environmental biorefineries.

Continuous long-term bioelectrochemical chain elongation

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The ongoing transition towards a biobased economy is driven by depletion of fossil fuels, environmental pollution and energy shortages. Organic waste streams are a renewable feedstock that can replace these fossil-based fuels and chemicals. The main challenge lays in the conversion of these organic waste streams into valuable products in an economical way. Microbial electrosynthesis (MES) might prove to be a new technology for this purpose.
The objective of this study was to assess the long-term performance of continuously acetate and CO2 fed MES reactors using a mixed culture as biocatalyst. Hereby we investigated (in duplicate) the role of applied current (3.1 A/m^2 versus 9.3 A/m^2) on the performance.
n-butyrate, as the main product, was successfully and continuously produced over time. Trace amounts of propionate and n-caproate were also identified. Remarkably, no alcohols were detected during the whole course of the experiment (163 days). Figure 1 shows a selection of the data showing the volumetric production rate of a MES reactor applied with 9.2 A/m^2. CV scans indicated the process was biocatalysed. Based on the supplied electron (equivalents), identified chemicals, (un)known biochemical conversions, a theoretical framework work was developed which explained the production route to n-butyrate. Further microbial composition analysis is ongoing.
MES systems controlled with higher current, showed higher product concentration (max 590 mg/L) and 5-6 times higher volumetric production rates (540 mg/L/day) compared to the low current reactors (80 mg/L/day), at 60% and 70% coulombic efficiency, respectively. This revealed that the applied current does determine the performance of MES systems. Since not all electrons were recovered in organic products a further optimisation is possible.

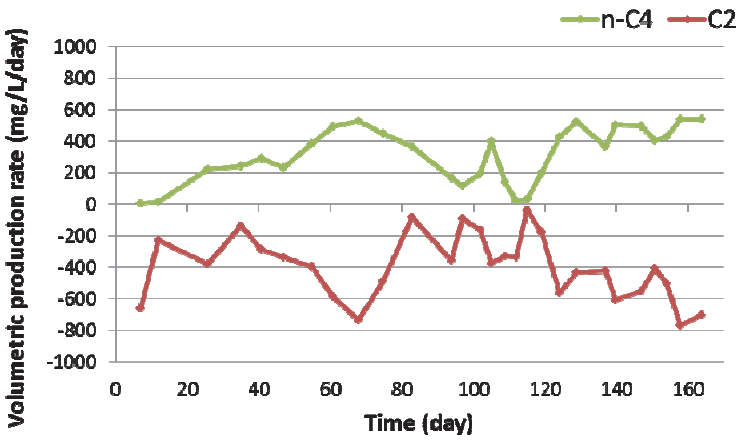

Figure 2: Volumetric production rate of MES cell applied with 9.3 A/m^2, showing n-butyrate (n-C4) production and acetate (C2) consumption during the experiment.

Microbial electrosynthesis of butyrate from carbon dioxide: selective production and extraction

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In Microbial electrosynthesis (MES), electrochemically active microorganisms use electrical current as reducing power to reduce CO_2, either by direct electron transfer or via electron mediators, such as hydrogen. To date, acetate has been the main end-product of MES, which has a low market value. Recent research in our group proved that higher value products, such as butyrate and ethanol, can be also produced by MES. However product titers were low and acetate was still the main end-product of the process (Ganigue et al., 2015). The present contribution shows our last results, which demonstrate the production of butyrate as the main end-metabolite of MES, and its selective extraction and concentration.

Experiments were conducted in a tubular bioelectrochemical system, at the cathode potential of -0.8 V vs SHE, with a total operational time of more than 90 days, divided in start-up period and 3 batch tests. Conditions were modified to build up hydrogen partial pressure and trigger the production of compounds with a higher degree of reduction. Then, butyrate was selectively extracted from the product broth through liquid membrane extraction.

In the first batch, our previous results were replicated (Ganigue et al., 2015), but with the new reactor design the concentration of products obtained increased from 55.3 to 96.3 mmol of carbon per litre (mMC) of acetate, and from 20.2 to 29.5 mMC of butyrate. In subsequent tests, operation conditions were modified, and the hydrogen partial pressure was bioelectrochemically increased, which resulted in a switch of the product spectrum. Butyrate became the predominant product of MES, with a concentration of 59.7 mMC versus 20.3 mMC of acetate at the end of the second batch. An alternative CO_2 feeding strategy was applied and 87.5 mMC of butyrate and 34.7 mMC of acetate were obtained in the third batch. Analyses of the gas phase demonstrated the crucial role of hydrogen partial pressure on the production of butyrate. Microbial analyses showed an enrichment of the biocathode bulk community, which was dominated by *Megasphaera* sp. at a relative abundance above 50%, which was putatively responsible for butyrate production. Selective extraction of butyrate from the production broth was performed with a hollow fibre membrane pre-treated with an organic phase to increase the selectivity in extraction towards butyrate. Starting from a simulated broth containing 17.9 mMC of acetate and 46.8 mMC of butyrate, a concentration solution with 15.4 mMC of acetate and 252.4 mMC of butyrate was obtained. The results open the door to MES to become a production platform of different compounds from CO_2.

Ganigue, R., Puig, S., Batlle-Vilanova, P., Balaguer, M.D., Colprim, J., 2015. Microbial electrosynthesis of butyrate from carbon dioxide. Chem. Commun. 51, 3235–3238.

Basics and principles of electro-fermentation

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Electro-fermentation system is a novel and recent bio-electrochemical approach that consists of controlling microbial fermentative metabolisms with electrodes. In this process, the polarized electrodes act like a non-soluble electron donor (cathode) or acceptor (anode) that is never the limiting reactant. This additional electron source or sink affects the redox power available for the micro-organisms during fermentations and, even with low current densities, have significant effects not only on microbial metabolism and biological regulations [1,2], but also on inter-species interactions [3] and the selection of bacterial populations [4,5] when using mixed microbial cultures.
Thus electro-fermentation systems offer a new driving tool that allows fermentation to be more specific or overpass thermodynamical limits. They provide new possibilities to better control microbial communities in mixed cultures by selecting or even adding micro-organisms capable to interact with the electrodes poised at a specific potential. In this context, electro-fermentation systems provide a well-controlled framework for the study of microbial interactions existing between electro-active and fermentative bacteria in defined co-cultures. A better understanding of these interactions would provide new insights in global metabolism related to electron transfer occurring in anaerobic mixed cultures and help to optimize future electro-fermentation systems.
In this communication, we propose to present the basics and principles of electro-fermentation systems, illustrated by an up-to-date state of the art of electro-fermentation in the literature. Our recent experimental results dealing with microbial interactions between electro-active and fermentative bacteria during glycerol electro-fermentation will also be presented to illustrate successful electro-fermentation. These results were obtained with a designed co-culture composed of *Geobacter sulfurreducens* and *Clostridium pasteurianum*. The addition of *G. sulfurreducens* during glycerol fermentation by *C. pasteurianum* showed a significant effect on the final fermentation patterns, favouring the production of 1,3-propanediol instead of other final electron acceptors. In conclusion, perspectives will be proposed in the domain of electro-fermentation and its applications.

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SmartWetland: Controlling and Optimizing the performance of a full scale Bioelectrochemical-assisted wetland

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Bioelectrochemical constructed wetland (so-called METlands) has been revealed as an excellent opportunity to implement microbial electrochemical technologies (MET) in an existent wastewater treatment technology because of the operations conditions, such as continuous flow and construction design as a single chamber, membrane-less systems. The dimensions for the lab-scale MET-Wetland were 39 cm length and 25 cm width. The full scale MET-Wetland was constructed with a length of 7 m and a width of 4 m. Lab scale MET-Wetland was explored for different flow rates and organic charges in order to get the control rules to be applied for the full scale system. Water was discontinuously pumped into the full scale METland at a total flow of 2m3/day. The full scale METland consists of a four electrode system (anode and cathode, reference electrodes placed at the anode and the cathode) controlled and monitorized by a-la-carte designed high power potentiostat. We move one step forward in the task of making METlands a really suitable technology by means of acquiring all the chemical and electrochemical information that the system can provide. We used the four electrode system to monitor on line electrochemical parameters like electrode potentials, potential difference between reference electrodes, electric current and values of redox probe. We correlate all that info with the performance and degradation capacity of the treatment based on Chemical Oxygen Demand (COD), Total Suspended Solids (TSS), pH, water temperature, Electrical Conductivity (EC), Total Nitrogen (TN), Nitrates (NO3–N), Ammonium (NH4–N). All the electrochemical parameters were remotely monitored by ICT tools.

NONIT: Scaling-up BES towards nitrate-polluted groundwater treatment application

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Nitrate (NO_3^-) presence in groundwater is a worldwide concern, particularly in waters potentially used as drinking water. Bioelectrochemical systems (BES) could become an alternative treatment by converting NO_3^- into dinitrogen gas autotrophically. Relevant knowledge has been obtained at lab-scale, but there is a risk of stagnation of the technological application if the implementation at a pilot scale is not tested. For this reason, in this work we report, for the first time, a pilot scale BES for nitrate-polluted groundwater treatment (NONIT).

A pilot plant treating 2.7$m^3 \cdot d^{-1}$ of groundwater was built. It was composed of 36 units operated in parallel with one single pump. All units were built as flow-through tubular reactors, with cathodic NO_3^- reduction (inner part of the reactor), and anodic water oxidation (outer). Cathodes were filled with granular graphite. Ti-MMO electrodes were used as anodes. Different configurations were tested: 12 units named as (A), 12 named as (B) and 12 named as (C). For each set of units, 6 reactors presented a net cathode volume (NCC) of 5.0L_{NCC} (X-10), while 6 presented 7.5L_{NCC} (X-15). The pilot plant was located in Navata (Spain), and it was fed with fresh nitrate-polluted groundwater containing 100±10 mg$NO_3^- \cdot L^{-1}$ (above drinking water standard (50mg$NO_3^- \cdot L^{-1}$)) and a conductivity of 779±64 $\mu S \cdot cm^{-1}$. The pilot plant was gradually inoculated, resulting in an operation time between 35 and 159d, depending on the unit. Each unit was fed at different flow rates of 20-400$L \cdot d^{-1}$. It implied hydraulic retention times between 0.3-6.0h. Units were operated at different cathode potentials from 0 to -400 mV vs. Ag/AgCl using potentiostats (*Bio-Logic* (France) and *nanoelectra* (Spain)). The pilot plant was inoculated with the effluent of a lab-scale 0.24L_{NCC} BES treating NO_3^- at 3760±101 g$NO_3^- \cdot m^{-3} \cdot d^{-1}$, at an HRT of 0.5h and a cathode potential of -320 mV vs. Ag/AgCl.

The highest activity was observed in reactors with 5.0L_{NCC}, being the reactors A-10 the ones presenting the highest performance with a maximum nitrate removal of 49±18% at an HRT of 4.0±0.5h (implying a nitrate removal rate around 393 g$NO_3^- \cdot m^{-3} \cdot d^{-1}$), nearly reaching the standards for drinking water. However, higher stability in terms of removal performance was observed on reactors with 7.5L_{NCC}.

In conclusion, here we present the first scale-up of a denitrifying BES for nitrate-polluted groundwater treatment at a flow rate up to 2.7$m^3 \cdot d^{-1}$. The promising results obtained, with a nearly 50% removal of influent nitrate, suggest that scale-up of denitrifying BES is feasible.

Up-scaling of Bioelectrochemical Systems for nitrogen recovery

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Conventional nitrogen removal technologies are energy intensive and represent one of the major costs in wastewater treatment plants. Besides, valuable ammonia is largely lost after sequential biological nitrification and de-nitrification in wastewater treatment plants in form of nitrogen gas. In contrast, Bioelectrochemical Systems (BESs) offer a proven, sustainable and energetically efficient recovery of ammonia from wastewaters (Arredondo et al. 2015, Kelly and He 2014). In domestic wastewater, most of the nitrogen (up to 80%) originates from urine. The high concentration of not only nitrogen, but also of other nutrients (P and K), makes urine an ideal stream to be treated for the recovery and reuse of these key nutrients.

A new treatment concept for urine has thus been developed aiming at the recovery of ammonium using a Microbial Electrolysis Cell (MEC). The principle is that biological oxidation of organic matter present in urine at a bio-anode drives the transport of ammonium towards the cathode, from where it is recovered by absorption in an acid as ammonium sulphate. Moving towards practical application, a pilot plant for the treatment of human urine has been operated for more than a year. The treatment concept includes phosphorus recovery via struvite (MAP, $MgNH_4PO_4 \cdot 6H_2O$) precipitation as a pre-treatment step, followed by ammonium recovery as ammonium sulphate via the coupling of a MEC to a TransMembraneChemiSorption (TMCS) module (Kuntke et al. 2016). Two potentially marketable fertilizers, struvite and ammonium sulphate, are the resulting products.

So far, the maximum current density attained was 2 $A \cdot m^{-2}$ at 0.5 V applied cell potential. Ammonium removal efficiencies account for 40%, while ammonium recoveries up to 80% have been achieved. Our current efforts are focused on the enhancement of the current density to optimize the removal of ammonium and therefore, its recovery.

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Kelly, P.T. and He, Z. (2014) Nutrients removal and recovery in bioelectrochemical systems: A review. Bioresource Technology 153, 351-360.

Kuntke, P., Zamora, P., Saakes, M., Buisman, C.J.N. and Hamelers, H.V.M. (2016) Gas-permeable hydrophobic tubular membranes for ammonia recovery in bio-electrochemical systems. Environmental Science: Water Research & Technology.

Start-up and successful long-term operation of a pilot-scale MEC for H_2 production from urban wastewater

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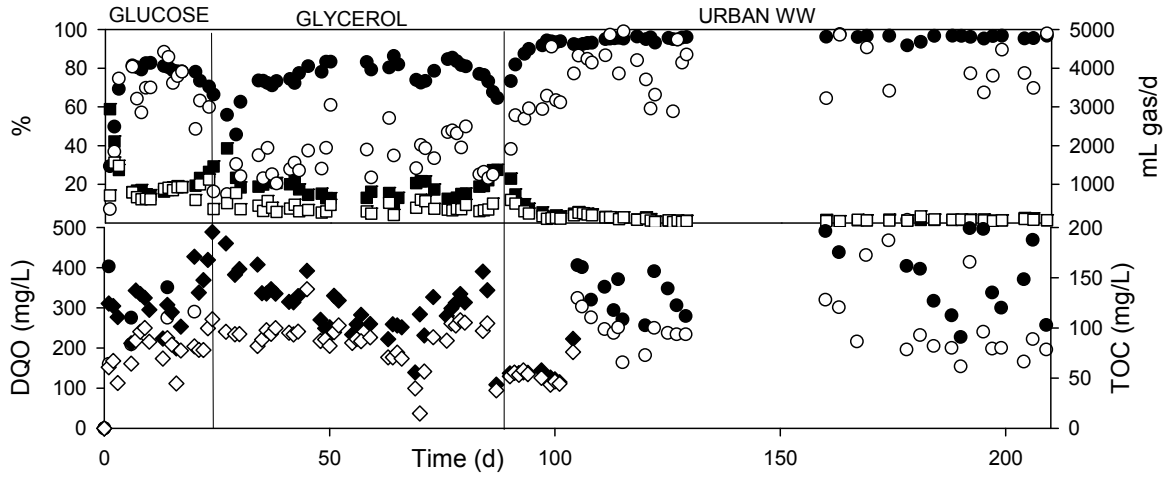
This work presents the best experimental results obtained under pilot-scale conditions (130L) with real urban wastewater and hydrogen production rates much higher than previous values reported. The key of the success of this new two-chamber configuration composed of 10 cassette type cells is the start-up/enrichment period and the optimised operation of the catholyte.

The system operation was started with glucose-based synthetic wastewater (400 mgCOD/L). The results during this period were very satisfactory (4.1 L/d H_2 production), reasonable high H_2 purity (76%) and CE (48.3%), although with low soluble COD removal. The system was then operated for two months with a dilution of crude glycerol in order to use a more complex wastewater. The H_2 production rate decreased down to 2.1 L/d with 83 % purity and 32.2% of CE. Methane was always present in the cathodic gas and electron balances showed that part of this methane was produced in the anode. Finally, after three months of synthetic operation, the plant was fed with urban wastewater (average of 450 mg COD/L) obtained from the effluent of the primary settling of a municipal WWTP (Rubí, Spain). The results obtained so far during the first 120 d of operation are exciting. Once the real wastewater was fed, a sharp decrease of methanogenesis was observed and H_2 purity increased up to more than 95%. Moreover, the amount of H_2 produced has reached values up to 5L/d (0.04 L H_2/L-reactor·d) with an average of 0.5 A of total current intensity. COD degradation was not very high leading to reasonable good CE values (up to 40%). The cathodic recovery (i.e. moles of C flowing through the circuit/ moles contained in H_2) was very high in this last period (up to 95%), which are very significant results for its posterior full-scale implementation.



Experimental profiles for the last 8 months of continuous operation of the pilot-scale MEC: UP ● (%H_2), ○ (mL H_2/d), ■ (% methane), □ (mL methane/d) and DOWN: ▼ (TOC inlet), ▽ (TOC out), ◆ (COD inlet), ◇ (COD out).

Scale up and Development of a Microbial Electrolysis Cell for Domestic Wastewater Treatment

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Much of the research to date on microbial electrolysis cells (MEC) has been carried out at a very small scale with implausible temperatures and synthetic substrates. The benefits of applying MEC technology are profound, yet the bottleneck to the technology's application lies in its scale-up. This case study details results from the third pilot scale MEC arising from this research collaboration with industry: the first of which (Heidrich *et al.,* 2013, 2014) functioned as a 'proof of concept' and the second of which failed within 6 months, providing ample opportunities for learning. In the latest pilot, a 200L microbial electrolysis cell (MEC), has been in operation for 7 months, producing 0.8 L of 95% pure H_2/day, from settled domestic wastewater at ambient winter temperatures (wastewater temperature: 5.3°C to 12.9°C). The MEC has an anodic surface area to volume ratio of 30m^2/m^3 and hydraulic retention time of 8 hours; equating to a population equivalent of 3. It removes 63.5% of the Chemical Oxygen Demand (COD) achieving the European Urban Wastewater Treatment Directive consent (of 125mg/L) 50% of the time. The technology is not yet energy neutral, with an estimated 4L of H_2/cell/day required to break even. This hurdle is surmountable and the progress to date suggests this could be achieved in the near future. This study addresses the need to recover energy from wastewater; highlights the improved performance of MEC at pilot scale; and demonstrates the technology's robustness at low temperatures, with low strength wastewater.

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Microbial Electrosynthesis of Hydrogen Peroxide in microbial reverse-electrodialysis electrolysis cell

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Microbial reverse-electrodialysis electrolysis cell (MREC) as a novel type of microbial electrochemical technologies has been proposed to produce H_2 and CH_4. In this study, we developed MREC to produce the strong oxidant H_2O_2. In the MREC, electrical potential generated by the exoelectrogens and the salinity-gradient between sea water and river water were utilized to drive the high-rate H_2O_2 production without external power supply. Operational parameters such as air flow rate, pH, cathodic potential, flow rate of high and low concentration solution were investigated. The optimal H_2O_2 production were observed at high and low concentration solution flow rate of 0.5 mL/min, air flow rate of 8-20 mL/min, cathode potential of -0.485 ± 0.025 V (vs Ag/AgCl). Under the optimal conditions, the maximum H_2O_2 yield of 778 ± 11 mg/L could be obtained. Cathode potential was found as the key factor for H_2O_2 production, which can be controlled through adjusting the air flow rate without power supply and potentiostat. This study shows for the first time high yield synthesis of H_2O_2 from oxygen reduction in a microbial electrochemical system without external power supply.

CO_2-Conversion to Methane by Microbiological Electrosynthesis

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To allow further growth of renewable energy supply the storage question needs to be solved. Chemical energy carriers will be a solution apart from local possibilities like pumped-storage hydropower plants. Microbial electrosynthesis has a great potential to directly produce easy and safe manageable energy carriers like alcohols, methane and organic acids from electricity and carbon dioxide or waste organics.
In scientific literature the topic of "microbial electrosynthesis" belongs to bio-electrochemical processes where substances are converted by the combination of electricity and microorganisms.
At the microbial electrosynthesis (MES), there is an interaction between the electrode and the microorganisms adhering thereto. For nearly one decade scientists engaged intensively with microorganisms that can transfer electrons directly to electrodes as well as taking electrons in different ways from the electrode to catalyze reduction reactions. Here we present a first set of experimental results: Mixed methanogenic cultures were isolated from digestate of a municipal sewage plant. The cultivation of mixed methanogenic cultures with H_2/CO_2 (80:20) succeeded in headspace vials at 37°C on adapted medium according to Cheng et al. The enriched methanogenic mixed cultures were tested in a two compartment cell operated in batch mode. Carbon felt was used as working electrode and a sheet of DSA as counter electrode. The catholyte solution was a medium according to Cheng et. al and a phosphate buffer (pH = 7) was used as anolyte solution. At the beginning a potential of – 800 mV (vs. Ag/AgCl) was applied to the MEC and stopped only for LSV and CV measurements. In addition experiments with a more negative potential and different operating temperatures were carried out. In sum the chamber was operated for nearly three years. The results of this long term experiment will be presented. Also a microbial characterization of the mixed culture was done three times and showed that the composition of the culture was very stable.

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Comparison of different biocathode start-up strategies and evaluation of their microbial community

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Microbial electrosynthesis (MES) is a novel technology that combines carbon capture with the production of high valuable chemicals. Carbon capture and utilization at biocathodes provides a solution to minimize CO_2 emissions meanwhile marketable chemicals are being generating from an inexpensive substrate.

The aim of this work is to develop biocathodes for MES systems capable of making use of CO_2 in order to generate valuable chemicals. The biocathodes were inoculated using two different inocula and two different strategies: inoculating the working electrode directly as a cathode or working with the electrode as a bioanode and then changing the potential to convert it into a biocathode. Hereby, the Eubacterial community composition such as the inocula in the electrodes (bioanodes and biocathodes) was investigated by means of 454-pyrosequencing.

Two chamber microbial electrolysis cells have been built for this purpose using carbon felt for both anode and cathode electrodes. The cathode was inoculated and fed with bicarbonate and nutrients, while the anode was used as pure chemical counter electrode. The applied voltage on the biocathode was -0.8V vs. Ag/AgCl, the temperature was maintained at 30ºC and the period of batch was two weeks, conditions that are within the range used by other authors (Jafary et al., 2015).

These different start-up conditions showed distinct electrical behavior. These cells were capable of achieving a production between 70-196mg/L of acetic acid from 335mg/L C-HCO_3^- in the raw feed. Regarding pyrosequencing results, microbial community enrichment were sharply dependent of the type of inoculum used. A two-fold decrease of the diversity index was observed at the cathode biofilm (1/Simpson = 12.8) compared with the initial inoculum (1/Simpson = 32.8). Besides, a clear enrichment was demonstrated onto the cathode being the dominant family *Rhodocyclaceae* (accounting up to 71.6% of the total community), which belongs to *β-proteobacteria* as it has been previously described by Jourdin et al., 2015. These results indicate that hydrogen generating bacteria are present in a substantial amount, and it is assumed that some hydrogen has been also generated although it was not quantified in this study.

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Cupriavidus necator as a production strain in microbial electrosynthesis

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The bacterial reduction of carbon dioxide and the uptake of electrons gained from electrodes can be used to form high value carbon products with bacteria (named microbial electrosynthesis, MES). In bioelectrochemical systems, where electricity preferentially generated from renewable sources enables carbon dioxide fixation, cathode-derived electrons can enter the cell by different extracellular electron transfer pathways, i.e. direct, indirect and mediated electron transfer (DET, IET, MET, respectively). *Cupriavidus necator*, formerly *Ralstonia eutropha*, is well known as production strain in biotechnological processes. The strain is also a potential candidate for MES due to its flexibility and robustness, its short doubling time up to high biomass concentrations and most importantly because of the lithoautotrophic metabolism of the "Knallgas bacterium".
The aim of this study was to investigate several topics on the applicability of *C. necator* in bioelectrochemical systems. First of all, electroautotrophic growth and isopropanol production of a genetically engineered *C. necator* strain was investigated in detail. Hydrogen can be produced electrochemically by water electrolysis, which can be used to drive *C. necator* metabolism. However, this IET is energy-intensive. In order to reduce the energy demand, alternative extracellular electron transfer mechanisms of engineered *C. necator* strains, i.e. mediated electron transfer were investigated. We could show that a strain expressing an outer membrane protein from *Pseudomonas aeruginosa* PAO1 is characterized by increased hydrophobicity, cell permeability and surface roughness. Therefore, cathode-reduced mediators could permeate the outer cell membrane more efficiently and transfer electrons to the cellular metabolism. The strain was used in a mediator containing bioelectrochemical system for further characterization of growth and product formation. Furthermore, we observed that media described for optimal growth and production do not match with electrochemical demands, e.g., type and concentration of salts. Therefore, a Design of Experiment (DoE) based media optimization was used to identify relevant factors and factor interactions regarding the target parameters: a) fast autotrophic growth and b) achievement of high biomass concentrations. The optimized medium needs to fulfill both biological demands and electrochemical suitability. On the one hand bacterial growth and isopropanol production were investigated. An improvement compared to the most common medium described in literature was achieved (Torella et al. - 2015). On the other hand suitability of the medium in bioelectrochemical systems was determined by conductivity measurement and cyclic voltammetry.

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Core microbiome of MFCs used to treat swine manure at different external resistances

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Microbial fuel cells (MFCs) are able to perform wastewater treatment with concomitant electricity production. Animal manure treatment using MFCs has been poorly explored. So far, scientific reports include the evaluation of MFCs nutrient removal capacities (Kelly and He 2014), power production (Tao et al. 2015), or microbial community characterization (Vilar-Sanz et al. 2013), which are analysed separately. Moreover, the application of an external resistance control as maximum power point tracking (MPPT) system (Premier et al. 2011), or fluid dynamic (Kim et al. 2014) are thought to heavily influence the MFC performance and the microbial community. Fundamental knowledge in these areas is required for the implementation of MFC technologies at full-scale. This study reports the bacterial community characterization of two almost identically operated MFCs fed with swine manure. A MPPT control strategy (based on dynamic resistance) was applied to one of the MFCs and compared with a control operated at fixed resistance (Ref-MFC). The microbiome was characterised by quantitative PCR and barcode-amplicon sequencing targeting bacterial 16S rRNA genes at four positions within the anodes. Variations in microbiome structure are related to the MFC fluid dynamics and the application of a MPPT system, comparing the results with Ref-MFC. Both anode chambers achieved similar organic removal rates (4.2 kgCOD $m^{-3}d^{-1}$), solids removal efficiencies (approximately 60% VSS) and gas production rates (in terms of CH_4 and CO_2). The diversity of the microbial community in the biofilm was considerably reduced and differed from the influent swine manure, indicating a selective pressure inside the MFCs. The adopted electric condition (MPPT vs fixed resistance) was more relevant than the fluid dynamics in the selection of the MFC microbiome. MPPT control positively affected bacterial abundance (a 7-fold increase compared to the Ref-MFC) and promoted the selection of putatively exoelectrogenic bacteria (*Sedimentibacter*). Exoelectrogenic phylotypes were invariably found at all sampling positions, constituting the MFC core microbiome. These differences in the microbiome were suspected to be responsible for the two-fold increase in power production and the three-fold increase in Coulombic efficiency achieved by the MPPT-MFC (5.0 W m^{-3}; 17%) compared to the Ref-MFC (2.5 W m^{-3}; 6%).

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Colonisation and development of anodic biofilms on carbon felt electrodes

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The use of emerging Microbial fuel cell (MFC) technology for combining wastewater treatment and energy harvesting has attracted enormous interests. The anode biofilm is the key to the performance of a MFC system. To understand the biofilm growth and distribution on the bioanode, a multi-electrode bioreactor was developed and applied for the study of anode biofilm development. The multi-electrode reactor allowed replication of operating conditions and avoided the need to sub-sample the same bioanode over time. The reactor was operated for 67 days at 27°C with an external resistance of 1000 Ω. Anolyte was OECD artificial wastewater which contained a mixture of complex substrates to simulate the chemical composition of real wastewater. Bioanodes were sampled in pairs over time. Anodic biofilm development on carbon felt from initial colonisation to maturity was investigated by confocal microscopy and next-generation sequencing to determine bacterial cell abundance and analyse bacteria community composition respectively. Three regions were identified in biofilms across the carbon-felt anode based on live-dead staining of cells in the biofilm: dead (red), live (green) and mixed (overlain dead and live cells). Visual examination of the images showed that in mature biofilms mixed regions are surrounded by live biomass. Such a distribution suggests growth occurs outward from the external layers of the biofilms. Images were processed to extract trends in the biovolume of dead and live cells over time and in depth profiles. Over time, the biomass distribution moved towards the periphery of the electrode suggesting that growth was limited by mass transfer. This observation suggests that electrode thickness can be decreased with no loss in performance while decreasing material costs of BESs.
Succession in the bacterial community composition was observed over time with the community composition stabilizing towards the end of the period of operation.
Biofilms developed on the lower and upper halves of carbon-felt electrode were consistently different in Community composition and bacterial cell abundance. These findings clarify some important aspects of electrigenic biofilm development and biomass distribution with time and depth and provides new insights and approaches for optimizing the geometry of three-dimensional anodes and their long-term application.

Anodic biofilm microbial communities in different microbial electrochemical cells: comparison of metagenomic analysis

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The need to better understand the composition of anodic microbial communities is of great importance in studying Microbial Electrochemical Cells (MXCs). Exoelectrogenic mechanisms in microbial species are still an open field of research, and many different microbial strains can show extracellular electron transfer capacities. Here, we compare the results of 16S metagenomics studies (454 pyrosequencing) of anodic biofilms (*Archaea* and *Bacteria*) obtained in three different set of experiments in single-chamber MXCs.

The first set studied a single-chamber MEC fed with acetate for over 4 months. Bacteria population was dominated by the genus *Geobacter* (72% of the total reads), a well-known exoelectrogen. Despite dosing high concentrations of 2-bromoethanesulfonate (up to 200 mM) to inhibit methanogens, *Archaea* population was detected and mainly belonged to *Methanobrevibacter* genus (around 98% of *Archaea* sequences).

In the second set, a microbial consortium was enriched in MXCs fed with cheese whey (CW) for producing electricity and H_2. Although *Geobacter* sp. was dominant in the community (37%), its presence was significantly lower than in the first set fed with acetate (72%). CW lactose was mainly fermented to volatile fatty acids by lactic acid bacteria as *Enterococcus* sp. (22%) and other fermentative bacteria as *Sphaerochaeta* and *Dysgonomonas* genera. Methanogenic activity was not observed in CW MXCs, without addition of specific inhibitors. Current density and H_2 productions in CW-fed MXCs were comparable to conventional acetate-fed controls.

Finally, an alkalophilic community was enriched at pH 9.3 from anaerobic sludge in the third set of experiments. *Alkalibacter* genus was predominant in MFC anodes (37%) and it was identified as a potential ARB. In MECs, *Geoalkalibacter* genus was found as main strain (43%). *Geobacter* was never detected in alkaline MXCs, while high values of *Geobacter* were present in the first set. Surprisingly, alkaline MEC gave higher H_2 production as compared to the neutral MEC built for these experiments (2.6 vs 1.2 liters of hydrogen gas per liter of reactor per day, $L_{H2} \cdot L^{-1}_{REACTOR} \cdot d^{-1}$).

Despite different experimental conditions in MXCs that enriched radically different anodic microbial communities, efficient exoelectrogenesis was observed in all trials. The results presented demonstrate that the use of different experimental conditions could be a strategy to outcompete other microorganism (i.e. methanogens in CW MXCs) that undermines MXCs performance in conventional conditions or to improve the MXCs performance (i.e. alkaline condition). Moreover, anodic biofilms with different microbial communities allow the use of real fermentable substrates or different pH conditions, which can convert MXCs in a suitable technology for direct treatment of different types of wastewaters or for the upcoming new applications, such as electro-fermentation and food-industry waste biorefineries.

Electrochemical stimulation of *thermotoga neapolitana* cultures

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Several authors demonstrated that Thermotoga neapolitana (DSM 4359, ATCC 49049), a rod-shaped, gram-negative, non-sporulating bacterium, more than the other thermotogales is able to accumulate biohydrogen and produce lactic acid from waste organic matter, in a selective environment (>80ºC). Capnophilic Lactic Fermentation (CLF) is an anaplerotic biochemical pathway, so far described only in T. neapolitana, that accomplishes chain elongation of acetate with direct reduction of CO_2, to obtain lactic acid (Pradhan et al. 2015). The whole process works as a carbon fixation mechanism but, differently from autotrophism, leads to direct elimination of fixed carbon into excreted metabolites instead of accumulating it into biomass. These characteristics make T. neapolitana an ideal candidate in recovering added-value chemicals from organic wastes (Fontana, EP14711847.5).
Here, for the first time, we present a preliminary experiment on T. neapolitana metabolism interactions with electrochemical systems. An electrochemical cell of 1 L was operated at temperature of 85°C with a culture media of T-neapolitana containing 5 g/L glucose. The media was flushed with N_2 gas to ensure anaerobic conditions and then inoculated with T. neapolitana. When the redox potential of the solution, measured with a platinum working electrode with respect Ag/AgCl electode, decreased to -650 mV , a constant polarization at -400 mV was applied to the working electrode. The counter electrode evolved H_2, saturating the solution.
Glucose fermentation was strongly inhibited under these conditions: in a 24 hours test, only 10% of glucose was oxidized to mainly acetic acid, in the molar rate of dark fermentation (glucose:acetic 1:2). Lactic acid was found in negligible concentration. Despite this, there was an apparent change in the morphology of the bacterial cells and an increase of the optical density up to OD=0.93, that suggest an increase of cell concentration. This value is typically found after efficient glycolysis (regular CLF process) (d'Ippolito et al, 2009).
These results suggest that the constant polarization was used as alternative reducing power source for cell growth. This first hint, opens future perspectives on electro-fermentation with this hyperthermophilic strain.

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MFCs biocathodes life by 3D X-ray microcomputed tomography

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The performances and full-scale application of microbial fuel cells (MFCs) are severely affected by irreversible reactions and processes on the electrodes. Membraneless Single Chamber MFCs (SCMFCs) are mainly limited by the cathodic oxygen reduction reaction (ORR), which is kinetically hindered, and further obstructed by biofilm formation and deposition phenomena on the cathode. Many dissolved salts and inorganic forms in fact tend to precipitate mainly because pH locally increase. The study of biofouling and salts-precipitation processes at cathodes is therefore crucial for SCMFCs understanding and optimization over long term operation.
In this study a number of ORR oxide-based electrocatalysts has been deposited onto the carbon-based micro-porous layer by simple adsorption. The stability of the ORR catalytic layer over biofilm growth has been investigated by X-ray microcomputed tomography (microCT).
Aiming at clarifying the involved processes, many physico-chemical and electrochemical techniques have been employed. Scanning electron microscopy (SEM) and energy-dispersive X-ray spectroscopy (EDX) were used to characterize the catalyst and fouling layer and the cathodic biofilm.
Results underlined that the biofilm growth did not affect the chemical ORR catalysis over time. Power output and cathode polarization curves, together with microCT analysis, identified a correlation between the time-dependent decrease of electrochemical performances of the MFC and the carbonate layer formed onto the cathode. Na-carbonates and Ca-carbonates were predominant on the air (outer) side and the water (inner) side, respectively.

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Bioelectrochemical enhancement of methane production in low temperature anaerobic digestion at 10°C

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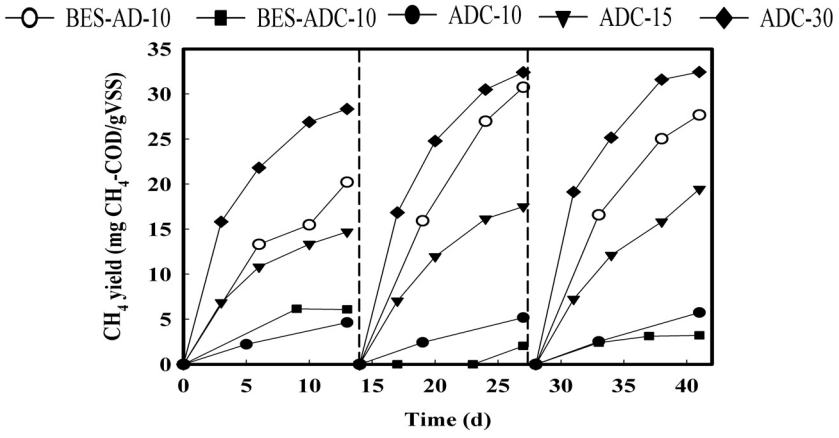
Anaerobic digestion (AD) is an attractive wastewater treatment technology that recovers energy in the form of CH_4 and has low excess sludge production and operational cast(Speece 2008). However, many wastewaters are discharged at low temperature (<20 °C) which leads to low microbial activity and consequently to low methane production rate(Zhang et al. 2013). Methane-producing bioelectrochemical systems (BESs) can produce CH_4 from CO_2 in the cathode under external electrical supply via direct (electrons and H^+) and/or indirect (H_2) pathways (Van Eerten-Jansen et al. 2012).

This study investigated whether BESs can enhance methane production from organic matter in low-temperature AD. A single chamber reactor was operated with granular activated carbon as electrodes in both anode and cathode at 10°C. Acetate was oxidized by electroactive bacteria in the anode. Our results showed that at 10°C, CH_4 yield in the BES-AD system was 5 to 6 times higher than that in the AD reactor. Methane production rate achieved in the BES-AD system at 10°C was slightly lower than the AD reactor at 30°C and much higher than the AD reactor at 10°C and 15°C, respectively. Energy input in the form of electricity in the BES-AD system was also 10 times lower than the energy for heating up the anaerobic digester from 10°C to 30 °C. To sum up, this study demonstrated that BESs has the potential to be an alternative strategy to enhance CH_4 production in low-temperature anaerobic digestion.



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Bioelectrochemical systems for BTEX removal

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BTEX compounds (Benzene, Toluene, Ethylbenzene and Xylenes) are among the main constituents of gasoline. Accidental spills can result in groundwater contamination. Since BTEX are toxic, several strategies have been developed for the remediation of contaminated sites. Both physicochemical and biological strategies can be used to remove BTEX compounds. The main issue of the biological strategies is that the electron acceptors are quickly depleted. The current strategies usually involve the stimulation of the aerobic degradation by adding oxygen to sustain the aerobic degradation. However, this approach could be expensive and technically difficult. The use of an anode as an electron acceptor in anaerobic conditions has been suggested as an alternative strategy to stimulate the hydrocarbon degradation. Although some studies showed the possibility to degrade single BTEX in bioelectrochemical systems (BES) a lack of information about the degradability of mixtures still exist.
Single chamber BES reactors (120 mL) have been set up. Pumice (2-5 mm) was used as support material in order to simulate a biobarrier for groundwater treatment. Refinery wastewater (85 mL) was used as growth medium and as microbial inoculum. Oxygen was removed by flushing with N_2. Graphite electrodes (10 cm^2, geometric area) were connected to a power supply (0.8 V, 1.0 V and 1.2 V were applied over 160 days, in duplicate). A BTEX mixture was periodically supplied as carbon source. Abiotic and open circuit controls (OCCs) were set up. Current production, SO_4^{2-} concentration and BTEX concentration were monitored over time. At the end of the experiment the microbial communities enriched in the bulk and on the electrodes surface were characterized by Illumina sequencing of the 16S rRNA gene.
Current production was associated to hydrocarbons degradation at all the potentials. The highest current peaks were observed at 0.8 V (about 200 mA m^{-2}, with a peak of 482 mA m^{-2} during the second batch cycle in one of the replicates) compared to the other potentials (about 170 mA m^{-2} and 135 mA m^{-2} at 1.0 V and 1.2 V respectively). Toluene, *m*-xylene and *p*-xylene were the most degradable compounds. The first order kinetic constants were respectively 0.4 ± 0.1 $days^{-1}$, 0.34 ± 0.09 $days^{-1}$, 0.16 ± 0.02 $days^{-1}$ at 0.8 V. In the other conditions only toluene and *m*-xylene were completely removed and the first order kinetic constants were 0.155 ± 0.004 $days^{-1}$ and 0.13 ± 0.01 $days^{-1}$ at 1.0 V, 0.20 ± 0.01 $days^{-1}$ and 0.139 ± 0.005 $days^{-1}$ at 1.2 V, 0.047 ± 0.003 $days^{-1}$ and 0.050 ± 0.005 $days^{-1}$ in the OCCs. Sulfate reduction was observed in all the conditions. The importance of the sulphur cycle was highlighted also by the microbial communities characterization. The family *Desulfobulbaceae* was the most enriched in the anodic biofilms, while the family *Desulfomicrobiaceae* dominated the cathodic communities.

Modelling multispecies glucose fed biofilm in MFC

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Bacteria ability to convert carbohydrates into electricity is the result of complex mechanisms, involving interactions between several groups of microorganisms. In a glucose-fed MFC, microbial syntrophy in a biofilm has been proposed to explicit the different metabolic steps [1] (i.e., fermentation, exoelectrogenesis, methanogenesis).
This complementarity leads to a local segregation of microbial species and metabolic groups in the biofilm, which is mainly function of substrate concentrations, specific growth kinetics, electron transfer capacity and thermodynamic equilibria.
Inspired by the first conduction-based model of electroactive biofilms [2], we proposed a two-dimensional (2D) model that represents interspecies segregation in a glucose-fed biofilm between four fractions of bacterial biomass (Xg, Xa, Xh, Xm), each one performing a specific catabolic reaction. An additional inactive fraction (Xi) represents the extracellular polymer substance (EPS) influencing the electron conductivity and diffusion mechanisms. A thermodynamic approach, based on catabolic energy (Gibbs free energy)[3], allows determination of conversion yields in anabolic reaction for each microbial type.
Local segregation of microbial groups along the biofilm length is studied by numerical simulations in different conditions, e.g., glucose concentrations, kinetic parameters and reactor length. This study provides information on the optimum organic load vs reactor geometry to run microbial fuel cell. In order to test the model hypothesis, out-situ rotating disk electrode measurements and phylogenetic studies along the biofilm length have been made.
The proposed biofilm model can be implemented in a full microbial fuel cell model and could be extended to other bioelectrochemical reactors involving syntrophic microbial communities (e.g., more complex substrates, electrosynthesis, etc.).

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In situ membrane electrolysis enables high-rate production and electrochemical pH control in microbial electrosynthesis of acetic acid from carbon dioxide

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Microbial electrosynthesis (MES) has emerged as a new bioreactor technology for the production of organics from CO_2 and renewable current. In a previous report we presented a novel reactor configuration that can uniquely couple the production and recovery of acetic acid from CO_2 through the integration of *in situ* membrane electrolysis; extraction of the produced acetate over an anion exchange membrane (AEM). This three-chambered reactor design allows the simultaneous production, extraction and concentration of the product as a single organic acid in a solid-free extraction liquid (Gildemyn et al, 2015). Although it has been shown that electricity-driven extraction of acetate over an AEM allows pure product recovery at high efficiency, the impact of the electrolytic operation on the production process still remains unknown. In this study we compared the performance of the three-compartment reactor with two conventional, two-compartment reactors that lack the intrinsic property to extract the charged products from the catholyte. Both a cation exchange membrane (CEM) and bipolar membrane (BPM) were tested as the separation barrier between the anode and the cathode in two-compartment reactors. Acetate production was cathodically driven by a mixed microbial culture at a fixed current density of 5 A m^{-2}. During a 43 days batch run in the reactor with *in situ* extraction a production rate of 13.8 g $m^{-2}_{\text{projected cathode surface}}$ d^{-1} was observed, an increase of 57% and 41% in comparison with the production rate in the reactors with CEM and BPM, respectively. This performance enhancement is obtained through the combination of a stable pH environment and a low cathodic VFA concentration, both preventing the inhibition caused by undissociated organic acids. The pH of the catholyte remained stable at 8.2 ± 0.2, in contrast to the two-compartment reactors where a pH decrease below 5.3 was observed when VFAs were produced and accumulated. Whereas a base addition up to 16 mmol $OH^-/g_{\text{acetate produced}}$ was needed to correct the pH, membrane electrolysis fully replaced chemical pH control through the *in situ* electrochemical production of hydroxide ions. This study demonstrates that in addition to pure product recovery, membrane electrolysis generates a stable pH environment in the biocathode, resulting in a zero-chemical input process at higher production rate in comparison with MES reactors without extraction.

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Some aspects of biocathode performances in membraneless microbial fuel cells fed by different organic substrates

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Lab-scale membraneless single-chamber microbial fuel cells (MFCs) equipped with open-air carbon-based cathodes were intensively investigated. A Gas Diffusion Layer, prepared from carbon powders mixed with different amount of PTFE (60, 80, 100 and 200%$_{w/w}$, with respect to carbon powder content) was applied on the air-side of the cathodes. MFCs were operated with different organic substrates (sewage wastewater from municipal treatment plants, sodium acetate, solid food wastes, highly saline synthetic wastewater, oil-field produced water) for several cycles of feeding. Most of the MFCs produced power within one week, but the average coulombic efficiency and the maximum power density considerably varied depending on substrate. Despite great differences in inoculum and composition of organic substrate, a general trend in cathode behaviour can be inferred.

All cathodes were studied by a classical electrochemical approach based on quasi-steady state cathodic polarization curves. The evaluation of the performance with respect to the oxygen reduction reaction (ORR) has been attempted on the basis of the Tafel slope, *b*. According to the Tafel law, exponential variation of the current with potential should be expected and polarization current can be related to: i) surface area effects and ii) electrocatalytic effects. In principle, Tafel slopes *b* allow to discriminate between the two factors: decrease of *b* points to electrocatalytic activation of the process. However, in bioelectrochemical systems, a complex dependence of the current on polarization potential is usually observed and hardly a linear Tafel region is unambiguously identified. In this work, a linear portion was observed in the potential range around the working potential of the cell. Tafel slopes were measured in that region. In all cases examined, in the initial stage of the MFC operation, the Tafel slope *b* was 120 mV. For cathodes with lower PTFE content (60% and 80%), after stable biofilm development, *b* decreased to near 80 mV, following the biological activity of the MFC. As the organic substrate was consumed and the MFC power output decayed, *b* turned back to 120 mV. With a new feed addition, *b* decayed again to near 80 mV. As a matter of fact, *b* varied between 120 and 80 mV depending on the content of organics, thus electrocatalysis activation and deactivation can be understood as a consequence of the biological activity in the MFC. No great differences in *b* values were detected for MFCs fed by different organic substrates, which seemed not to affect the electrocatalysis of ORR at all. On the other hand, for cathodes with high PTFE content (100% and 200%) *b* was always constant at 120 mV. So the PTFE had a detrimental effect on catalytic performances, acting as an inhibitor of ORR electrocatalysis and dramatically affecting the MFC productivity. Moreover, also the cathodes with lower PTFE content (60 and 80%) showed decreasing performances over long-time operation, because of the formation of an insoluble layer of calcium carbonate in the water side of the electrode.

Textile carbon anodes for the application of MFCs for paper mill wastewater treatment

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The development of microbial fuel cells (MFCs) as novel technology for economic wastewater treatment processes is generally known as a highly challenging task and has not yet been achieved on a commercial level for multiple reasons.
One milestone toward the successful application is the integration of a functional bioanode into the system in such a way that the electrochemical performance and wastewater cleaning capacity of the biofilm can be exploited as much as possible.
Previous studies have shown that textile carbon fiber materials exhibit a great potential for the full scale application as bioanodes in MFCs when they are custom manufactured. The Institute of Applied Microbiology is currently involved in two subsequent projects that bring together know-how and interest from research institutions, textile companies and potential customers in an application-oriented consortium. Textile carbon electrodes are developed by engineering the structure of the carbon material on fiber and woven fabric level as well as the 3D electrode configuration in the reactor. A final 20 L prototype reactor will be integrated into a local treatment plant.
The upscaling process is supported by simulation approaches in order to investigate fluid dynamics and biochemical kinetics in a model system with *Geobacter sulfurreducens* and acetate as substrate. For prototype operation, we focus on the treatment of paper mill wastewater with medium polluting load (approx. 2000 mg/l COD) as a niche application where the MFC technology has been shown to have especially promising potential[5], since no competition by established waste-to-energy technologies has to be faced. This presentation will give an overview of the project approach and a first insight into the research of specific parameters to optimize MFC functionality.

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Poly(acrylo)nitrile derived carbon based nanofiber mats as anodes in single chamber microbial fuel cells

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This work aims to optimize and enhance the performances of small size Single Chamber Microbial Fuel Cells (SCMFCs). To reach this goal, the attention has been focused on two key elements: the design of an improved fluidic system and the development of new carbon based nanofiber mats. A squared shape SCMFCs is proposed to improve the fluid dynamics, as demonstrated through the Finite Element Methods Simulation (FEM) of the working conditions of the cell. The SCMFCs is made by means of 3D printing technology, using a polymeric UV-curable material [1]. The SCMFCs are based on 3 connected compartments to form a single inner chamber: the anodic, the intermediate and the cathodic compartments. The electrodes area is 6.25 cm^2, and the inner free volume is 9 ml. To guarantee the oxygen reduction reaction the carbon electrode is modified on one side with a gas diffusion layer and with a catalytic layer on the opposite one, following the literature [2]. Simulations were run to analyze the electrolyte flowing into the reactor at different flow rates. In particular the contributes to the electrolyte flow due to pumping and to diffusion are investigated, with the aim to gain a better understanding of the mechanisms controlling nutrients and chemicals motion towards the electrodes surfaces.

Electricity generation using bacteria in MFCs, require the use of highly conductive non corrosive materials having a high specific surface area to favor biofilm growth, but with a structure sufficiently open to avoid biofouling. Carbon based nanofibers fabricated by electrospinning are among the most promising anodic materials [3,4]. In this work we evaluated the behavior of new bundled carbon nanofibers as anodes in SCMFCs, in comparison with commercial carbon felt (CF). Samples were prepared by electrospinning solutions containing 12 wt% PAN in N-N dimethylformamide (DMF). The spun nanofibers were stabilized at 280 °C in air and thermally treated at 600 °C under inert atmosphere for 1 hour to obtain the graphitic conductive carbon nanofibers, as Raman spectra confirmed. FTIR (in ATR mode) spectra demonstrate the presence of carbon-nitrogen bond on the surface. The biocompatibility of the nanofibers is confirmed by Cytotoxicity tests and by investigation of the development of a densely connected biofilm by FESEM images. The maximum value of current density obtained with nanofibers (968±12 mA/m^2) is higher than the CF (512±20 mA/m^2). The EIS characteristic confirmed also that the internal resistance is lower for carbon nanofibers than the one for CF.

This work has been performed in the framework of a NICOP project founded by the Department of the Navy, Office of Naval Research Global.

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Control of microbial fuel cell voltage using a gain scheduling control strategy

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The microbial fuel cell (MFC) research has moved on to the next level where more and more up-scaled systems have been developed by researchers in their laboratories across the world. Most up-scaled systems are modularised and so connecting MFCs electrically in series/parallel will be a natural strategy during their normal operation for harnessing the electricity. Issues such as voltage reversal and power mismatch will be unavoidable since their electrical output is highly dependent on operating and environmental conditions. Therefore, it is necessary to control these MFC voltage outputs for their improved performance and continual operation of MFCs. We have for the first time, demonstrated the control of voltage produced by MFCs, by using a simple proportional + Integral controller along with the gain scheduler. It is envisaged that the control design and the implementation will need to be simple but effective in order for the capital and operational expenses to be as low as possible. The MFC system was modelled with series of stepwise linearised models which included the system dynamics. The models were used to design the controller and the gain scheduler which was then implemented on an MFC to take care of the MFC dynamics at all operating levels. A digital potentiometer was used as an actuator and resulting voltage from the MFC was used as the controllable parameter. The results suggest that the controller was able to control the voltages from the MFC satisfactorily and reject the disturbances. Also, it was shown that the controller was transferable to similar but different power performing MFC. This study demonstrates that the control of MFC can be achieved with relatively simpler approach and could be effective when MFCs are deployed at large scale.

Ultra-fast monitoring of electron transfer ability across anodic biofilm

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The mechanism of electron transfer (ET) across electroactive biofilms (EABs: *Geobacter* or anodic microbial consortia) is not fully characterized and current theories are still partially controversial. However, numerous studies have shown that the electrochemical responses of EABs are typical of a "diffusional ET" mechanism, driven by the concentration gradient of reduced redox cofactors within the biofilm[1].
As with redox polymers, which present similar ET behavior[2], the ability of ET across the EAB can be characterized by $C \times D_e^{1/2}$, where C is the concentration of redox cofactors in the biofilm ($mol \cdot cm^{-3}$) and D_e is the apparent diffusion coefficient of the electron within the biofilm ($cm^2 \cdot s^{-1}$). This product $C \times D_e^{1/2}$ has previously been determined for anodic *Geobacter* biofilms, by recording multiple cyclic voltammtries where the current peaks are proportional to $C \times D_e^{1/2}$ and increase linearly with the potential scan-rate (Randles-Ševčík relation). However, this method is time-consuming and can lack accuracy due to superimposition of the current peaks. Furthermore, it has to be performed under "non-turnover conditions" (in the absence of substrate), imply a decrease of EAB performance because of the fasting preparation step.
Here we show that the EABs response to an adequate potential step, as measured by faradaic current, follows the Cottrell equation[3], another common equation which describes semi-infinite diffusional processes in electrochemistry terms:

$$j = \frac{nFAD_e^{1/2}c}{\pi^{1/2}} t^{-1/2}$$

Via this method, similar values of $C \times D_e^{1/2}$ were obtained than with the Randles-Ševčík-based technique, but recorded only within seconds, under "turnover" or "non-turnover" conditions independently. These results further confirm that anodic EABs behave electrochemically in a similar manner as redox polymers, with a diffusional-like process for ET. In particular, this technique allows very simple and fast measurement, providing excellent opportunities for: (1) assessing the impact of numerous parameters (temperature, pH, ionic strength, bacterial strain, etc.) on the ET ability across these EABs, and (2) better understanding the overall ET from substrate(s) to the electrode surface.

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Assembly of Redox Proteins into Supramolecular Nanowires

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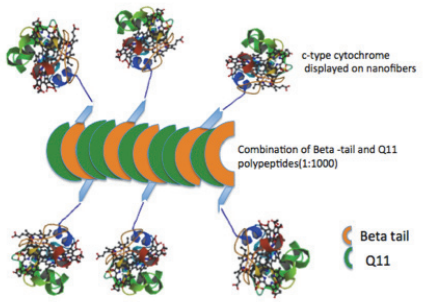

Fig: Polypeptide Nano-fiber displaying redox proteins

Many dissimilatory metal-reducing bacteria are capable of growth by transferring electrons from the cell's interior to external substrates like metal oxides. Some of these metal reducers like *Geobacter* and *Shewanell*a produce micron-scale, conductive, pilus-like appendages, that are known as bacterial nanowires and are hypothesized to play a role in this extracellular electron transfer[1]. Prior research has suggested that the conductivity along these nanowires is sufficiently high for them to serve as the conduit for electron flux from metabolism, but very little is known about the composition and mechanisms of long-distance conductivity along these structures[2]. Metal reducing organisms encode large numbers of *c*-type cytochromes and few have shown to be essential for extracellular current generation under some conditions. Thus, it has been hypothesized that c-type cytochromes may play a vital role in the conductivity of bacterial nanowires. This project seeks to construct synthetic, supra-molecular arrays of c-type cytochromes as a means to explore the electrical properties of these assemblies. As a platform to assemble a linear superstructure, we have chosen to use the β-tail/Q11 system, which has previously been used to assemble several diverse proteins into nanofibers[3]. In this approach, the "β-tail" peptide tag is expressed as a fusion to well characterized small tetraheme cytochrome (STC) from *Shewanella oeneidensis*[4]. The β-tail domain drives assembly into nanofibers in the presence of β-sheet fibrillizing glutamine rich peptides like Q11. We have successfully purified the N-terminal fusion of β-tail to STC in *E.coli* and synthesized Q11 peptide via solid phase synthesis. Pure protein and peptides samples (ratio 1:1000) were combined to assemble nanofibers. We used transmission electron microscopy to image the nanofibers and the dimensions of the nanofibers were estimated using atomic force microscopy. The conductance of the heme containing supra-molecular structure will be characterized using conductive atomic force microscopy, and the results will be compared to analogous measurements from natural bacterial nanowires. Further work will explore the impact of protein composition and orientation on electrical properties of these nanostructures. This information will not only inform models of extracellular electron transfer but may also provide a starting point for engineering synthetic biological systems for enhanced long-range electron transfer.

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Evaluation of direct interspecies electron transfer in anaerobic digestion of food waste with biochar

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Anaerobic digestion is a well-established technology for the recovery of energy from the treatment of industrial wastewater or organic wastes. Complete conversion of organic matter to CO_2 and CH_4 via anaerobic digestion requires the syntrophic cooperation between acetogenic bacteria and methanogenic Archaea. Indeed, catabolic reactions catalysed by acetogenic bacteria become energetically favourable only when produced reducing equivalents are efficiently scavenged by their syntrophic partners (i.e., the methanogenic Archaea). Typically, this interspecies electron transfer (IET) process is reported to occur via the diffusive transport of soluble electron carriers (e.g., hydrogen and/or formate) from the acetogens to the methanogens. Low concentrations of electron carriers however result in slow diffusion rates, causing IET to be often the bottleneck in the methanogenic conversion of organic substrates. Recently, direct interspecies electron transfer (DIET), in which two microbial species exchange electrons efficiently via electric currents flowing through conductive solid conduits or microbial pili, has been proposed as an alternative strategy to interspecies H_2/formate transfer, through which microbial species in a community share reducing equivalents to drive the methanogenic degradation of organic substrates.

An increasing number of studies have indicated that methanogenic conversion of organic substrates can be accelerated by the addition of electrically conductive materials, including granulated activated carbon, biochar, and naturally occurring iron minerals (hematite or magnetite), of nanometer to micrometer size. It was suggested that these electrically conductive materials promote DIET by functioning as electron conduits for direct interspecies electron transfer between syntrophic, organic matter-oxidizing bacteria and CO_2-reducing methanogens. So far, however, this interesting mechanism has been only observed in short-term batch experiments in the presence of synthetic substrates of defined composition, whereas its relevance and practical viability under conditions more closely resembling those occurring in real anaerobic digesters remains largely unknown. Here, we have investigated the impact and practical feasibility of biochar particles supplementation on the anaerobic digestion of "real" food waste. To this aim, the influence of three different biochar materials (from coppiced woodlands, from orchard and from wheat bran pellets) on the kinetics of anaerobic digestion was evaluated by conducting a series of batch tests using unacclimated methanogenic sludge as inoculum and food waste as substrate. All biochar materials enhanced the methanogenic conversion by specifically accelerating the kinetics of butyrate and propionate conversion, compared to unamended controls. In order to gain a deeper insight into the fundamental aspects of the process, an attempt was made to correlate the stimulatory effect of the used biochar materials with their chemical, physical and electrochemical properties.

La traviata energetica - The microbial electrochemical Peltier heat

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Extracellular electron transfer (EET) is the key feature of electroactive microorganisms and enables them to transfer metabolic received electrons to terminal acceptors that cannot enter the cell, e.g. electrodes, insoluble metal species and large organic molecules. Thus, EET plays an important role in nature (i.e. degradation of organic matter, biogeochemical redox cycles and interspecies electron transfer) and holds great promises for biotechnological applications including the generation of electricity, wastewater treatment and production of chemicals.
Despite the widespread distribution in nature and a plethora of possible applications the energetic evaluation of EET remained on calculated Gibbs free energies, so far. A further important state function is the enthalpy widely used for describing the energy processing of a microbial system but not applied for electroactive microorganisms, yet. We show by using an in-house developed bioelectrocalorimeter that the enthalpy balance of an electroactive biofilm performing direct EET can be assessed and subsequently the respective energy efficiency is analysed.
The results show that electroactive bacteria have to overcome an entropic barrier at the cytochrome/ electrode interface when they directly transfer electrons to an electrode. The entropy of electrons changes if they are transferred between the orbitals of a membrane-bound cytochrome and the Fermi level of an electron conductor compensated by a reversible heat flux. This heat flux is described by the microbial electrochemical Peltier effect (mePh) and depends only on the electrode material, the redox species and the electrolyte solution. This effect is long known for abiotic systems, i.e. batteries and fuel cells, and accounts for an energy loss of 20 % (Redey, 1998). In *Geobacter spec.* dominated anodes the mePh causes a heat formation of 27±6 kJ per mole of transferred electrons and therefore a loss of more than 50% of the microbial energy gain depending on the environmental conditions (Korth *et al.*, 2016). We will discuss that this energy sacrifice represents a metabolic burden for electroactive biofilms performing direct EET but only disadvantages the first layer of bacteria that are directly contacted to a solid acceptor because only at this interface changes the state (and entropy) of the electron. Overlying cell layers that exploit long-range EET mechanisms circumvent this energy investment and pass on this burden.

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Autotrophic nitrate removal from aquaculture streams in an upflow bioelectrochemical system

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Maintaining low concentrations of nitrogen compounds (either ammonium, nitrate or nitrite) in recirculating aquaculture waters is extremely important for a bigger and healthier fish production, as well as for discharge purposes. Although ammonium removal from aquaculture streams is usually done within a nitrifying step, the removal of nitrate via denitrification is still partially limited by the low availability of internal organic matter. The addition of external carbon sources can be waived by promoting purely autotrophic cathodic nitrate reduction. In this process, electrons are provided abiotically by splitting water at the counter (anode) electrode (i.e. generating oxygen) [1]. However, nitrate removal rates reported to date are still low and further improvements are necessary before this technology can be considered commercially attractive. In order to develop an easy-to-operate system which is able to achieve improved performances, a membraneless upflow cylinder reactor with total volume capacity of 1 Litre was tested with synthetic aquaculture media. The feed (5 L d^{-1}) containing 20 mg/L NO_3^- -N sequentially flowed through cathodic and then towards anodic zones (both composed of graphite granules as electrode material). The net cathodic compartment (NCC) volume was 80 mL and the cathode potential was controlled at -0.7 V vs Standard Hydrogen Electrode. The proposed technology was able to operate without added phosphate buffer, and the performance depended on buffer capacity provided by bicarbonate ions only. Furthermore, the system was able to reduce up to 0.43 kg NO_3^- -N m^{-3} NCC d^{-1} at 88% Coulombic Efficiency, which is within the highest range of removal rates observed in the literature for Bioelectrochemical systems, despite the lack of membrane. Calculated energy expenditure is 0.15 kWh m^{-3} (22 kWh kg N^{-1}), which is close to that required for nitrogen removal in activated sludge systems. Nevertheless, the system presents technical potential for simultaneous denitrification and partial re-oxygenation of recirculating aquaculture waters.

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A novel bioelectrochemical approach for chlorinated aliphatic hydrocarbons reductive and oxidative dechlorination

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Chlorinated aliphatic hydrocarbons (CAHs) are ubiquitous groundwater pollutants due to their widespread use as solvents and degreasing agents. Under anaerobic conditions, highly chlorinated CAHs, such as trichloroethene (TCE) and tetrachloroethane (TeCA), can undergo the so-called reductive dechlorination (RD), being used as final electron acceptors of the microbial respiration by naturally occurring dechlorinating microorganisms; under aerobic condition, less-chlorinated CAHs, such as cis-dichloroethene (cDCE) and vinyl chloride (VC), can be more easily removed by an oxidative microbial dechlorination (OD). The sequential stimulation of these microbial metabolisms can be a strategy for *in situ* remediation of contaminated groundwater. In recent years, our research group has deeply investigated bioelectrochemical stimulation of RD and OD, by using a flow-through sequential cathodic-anodic system (the two compartments being physically separated by a cationic membrane). Solid granular electrodes (made by graphite) have been used to create favorable redox conditions for both RD and OD (at cathode and anode compartments, respectively), while also constituting the support for dechlorinating biofilm growth as well (Aulenta et al., 2011). However, whereas RD stimulation was quite satisfactory, the following OD was poor, due to competitive O_2 scavenging by graphite bed (Lai et al., 2016). Hence, the anodic electrode and filling material have been recently replaced by mixed metal oxide electrode and silica beads, respectively, with good OD improvement (Lai et al., 2016). In this study, different cathodic potentials were investigated in order to evaluate their effect on the distribution of less-chlorinated intermediates (cDCE and VC) and the methane concentration in cathodic effluent, which in turn affect the efficiency of the following OD. It was shown that, as the applied potential becomes less negative, cDCE increased while both VC and methane decreased. Although minimising VC intermediate formation would be highly desired from an environmental point of view, the cDCE OD was quite slower than VC OD and methane resulted a key factor to support the cDCE OD, likely due to its co-metabolic nature. Hence, best performance was obtained at quite reducing cathode potential (-650 mV vs SHE). Under this configuration, 99% of influent TCE was removed (20,9±0,5 $\mu mol_{TCE} L^{-1} d^{-1}$ OLR), with no more than 4% less chlorinated products. The bioelectrochemical system is now being fed by a real groundwater from an Italian contaminated site. Besides of methane formation, main attention is given to other competitive mechanisms, such nitrate and sulphate reduction at the cathode, and related effects on OD performance at the anode.

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Towards Microbial Fuel Cell Based Volatile Fatty Acid by Applying Specific Poised Potentials

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The monitoring of anaerobic bioprocesses is complicated by the need for multiple variables to define its state, while there are few reliable online sensors. Low cost online measurement of concentration of volatile fatty acids (VFAs), key indicative variables, would be very useful to improve the operation of a number of important bioprocesses. In situ microbial fuel cell (MFC) based VFA sensors could replace the current generation of relatively complicated and expensive techniques for online VFA analysis. Cyclic voltammetry (CV) was used to determine the oxidation peak potentials for three VFA species (acetic, propionic and butyric) (Kaur et al., 2013). Subsequently, their dynamic behaviours and static sensitivities were recorded for single chambered cubic MFC (c-MFC), separately acclimated on individual VFA, while the anode was poised at a potential corresponding to the oxidation peak identified from CVs. The current responses at the fixed working electrode potentials (poised), were analysed for each VFA sensor in response to different concentration (0-250 mg/L) of the corresponding and other VFA species. When corresponding VFAs were supplied to the sensors, the range of the sensors was improved from 40 mg/L to 220 mg/L with very low or no response to other VFA species in acetate and propionate enriched MFCs; whereas the butyrate enriched MFC gave random responses. The results indicate that an increased range is achieved for the sensor by selected poised potentials. Offline testing of the samples collected from a scaled-up MFC and electro dialysis system were considered to investigate, showing that industrial application of the sensor could be plausible.

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Policyclic aromatic hydrocarbons (pahs) removal in single-chamber, air cathode microbial fuel cell: chemistry and ecotoxicology

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Polycyclic Aromatic Hydrocarbons (PAHs) in water ecosystems tend to accumulate in sediments, persisting in the environment. Nevertheless, they are present in marine and freshwaters [WHO, 1998]. Once in the aqueous compartment, PAHs are very slowly biodegradable under aerobic conditions. In this study, we investigated the influence of micro-electrogenesis on PAHs degradation and detoxification operated by a specialized microflora in water environment. A microbial pool by *Pseudomonadaceae, Bacillaceae*, *Staphylococcaceae* and *Enterobacteriaceae,* isolated in urban environment, was inoculated in a 400 ml Winogradsky saline solution containing no other carbon and energy source than naphthalene, phenantrene, pyrene, benzo(a)pyrene, in presence and absence of electrodes as described in Nastro R.A. *et al.,* 2015. MFCs performances were monitored for three months in terms of Power Density (PD), Current Density (CD) and PAHs degradation rate. The environmental toxicity of PAHs solutions in MFCs as well as in bioreactors was tested *vs Lepidium sativum, Caenorhabditis elegans* and *Daphnia magna*. Cyclic Voltammetry (CV) was used to investigate the electrons transfer at the electrodes. The results showed, for the MFCs with PAHs and microbial inoculum, a significant variability in PD and CD outputs, with highest PD of 300 $\mu W/m^3$ and 25 mA/m^3. Chemical analyses revealed, for the same MFCs, a PAHs degradation rate over 90% after one month, reaching the 98%-100% after two months. The ecotoxicity evolution as well as the presence of PAHs degradation intermediates would be discussed.

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Structural and Material Aspects of Biofilm Electrodes

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The great majority of microbial electrochemical technologies utilize electrochemically active bacterial biofilms as the electrocatalytic unit a concept that has great potential towards a technical realization. Thereby, the performance of the respective biofilm electrodes decisively depends on structural and the material properties of the underlying electrode (the *substratum*). During the recent decade, especially 3D-electrode structures have been studied intensively and new structures are proposed regularly. In this presentation, the role of microscopic and macroscopic electrode structures for the short-term and long-term performance of 3D-biofilm electrodes will be elucidated. It will be demonstrated that the relevance of the dimensions of an electrode structuring depends on dimension and the properties of the biocatalytic unit (i.e., the microbial biofilm) attached to the electrode surface.
It will further be demonstrated that, surprisingly, copper as metal that is generally considered to be antimicrobial, represents a promising electrode materials for biofilm electrodes. It allows developing scalable, high performance 3D-polymer-copper electrode structures.

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CO_2 removal in a microbial electrolysis cell: ion exchange membrane effects on transport phenomena and energy losses

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Federico Aulenta[2], Mauro Majone[1]

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The biogas from anaerobic digestion (AD) has CH_4 and CO_2 contents in the range of 50-75 % and 50-25 %, respectively. On other hand, the utilization of biogas to partially replace compressed natural gas from fossil sources (e.g. for grid injection or automotive engines), requires its upgrade into biomethane (CH_4>95%). Even if several technologies are commercially available for CO_2 removal from the biogas, they are economical feasible only for large or centralized AD plants. An innovative approach, aimed to couple the CO_2 removal to the gain of additional CH_4, can be offered by a methane producing microbial electrolysis cell (MEC).
Typically, a MEC is composed by a (bio)anode and a (bio)cathode separated by a ion exchange membrane (IEM); the IEM plays a key role by regulating the ionic transport that is needed to ensure the cell electroneutrality. Different proton (PEM) or anion (AEM) exchange membranes have a strong effect on which species (either cations or anions) are mostly involved in the electroneutrality, which in turn affects both mechanisms of CO_2 removal and related energy losses. In this frame, the characterization of transport phenomena involved in different MEC configurations (two and three chambers MEC with PEM, AEM or PEM/AEM) and their influence on CO_2 removal mechanisms and related MEC performance is presented. In particular, the presence and extent of bicarbonate transport from cathode to anode has been described by determining its diffusivity constant across different AEMs.
Moreover, the effect on the energy demand of the MEC process has been also estimated by the analysis of energy losses due to the different IEMs. Through the determination of potential drops in both biotic and abiotic tests, an integrated model has been calibrated and validated, that permits to foresee membrane energy losses through simple abiotic galvanostatic tested a function of expected MEC performances (figure 1).

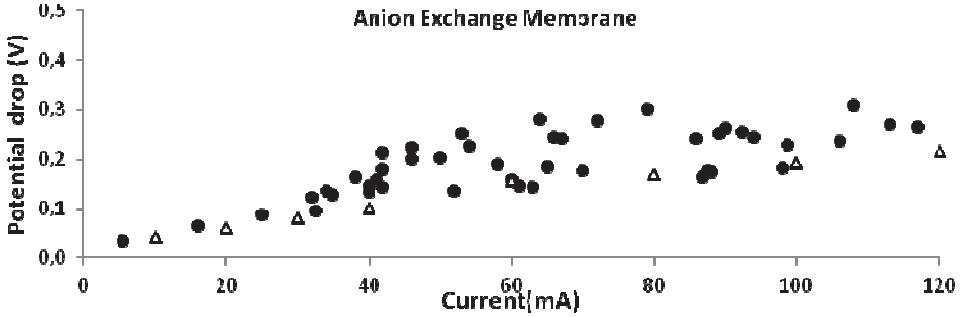

Figure 1: AEM membrane potential drop as function of current in biotic operation (black circles) and abiotic galvanostatic tests (white triangles)

Hydrophobic membranes enable transport of CO_2 and NH_3 to improve performance of Microbial Electrolysis Cells

Tom H.J.A. Sleutels[1,*], Biense J. Hoogland[1], Philipp Kuntke[1], Annemiek ter Heijne[2], Cees J.N. Buisman[1,2], Hubertus V.M. Hamelers[1]

[1] Wetsus, European Centre of Excellence for Sustainable Water Technology, Oostergoweg 9 P.O. Box 8900 CC Leeuwarden, The Netherlands. tom.sleutels@wetsus.nl
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Application of bioelectrochemical systems (BESs), for example for the production of hydrogen from organic waste material, is limited by a high internal resistance, especially when ion exchange membranes are used. This leads to a limited current density and thus to large footprint and capital costs. Ion transport between anode and cathode is one of the factors determining the internal resistance. The aim of this study was to reduce the resistance for ion transport in a Microbial Electrolysis Cell (MEC) through the ion exchange membrane by shuttling of CO2 and NH3 between anode and cathode. The transport of these chemical species was enabled through the use of a hydrophobic TransMembraneChemiSorption module (TMCS) that was placed between anolyte and catholyte circulation outside the cell (Figure 1). The driving force for transport was the pH difference between both solutions. The transport of CO2 and NH3 resulted in an increase in current density from 2.1 to 4.1 A/m2 for a cation exchange membrane (CEM) and from 2.5 to 13.0 A/m2 for an anion exchange membrane (AEM) at 1V applied voltage (Figure 2). The increase in current density was the result of a lower ion transport resistance through the membrane; this resistance was 60% lower for the CEM, as a result of NH3 recycling from cathode to anode, and 82% for the AEM, as a result of CO2 recycling from anode to cathode with TMCS, compared to experiments without TMCS.

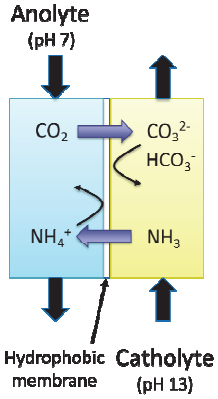

Figure 3 Working principle of the TMCS module where anolyte and catholyte of different pH are flown though the two compartments of the module separated by a hydrophobic layer. This hydrophobic layer allows for the exchange of gasses like NH3 and CO2. The driving force for this process is the pH and concentration difference of the two liquids which enables stripping of the NH3 and CO2 between anolyte and catholyte.

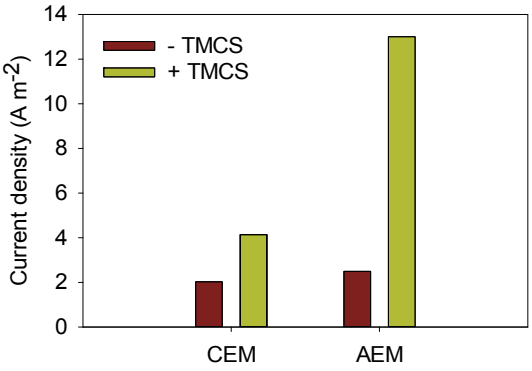

Figure 4 Produced current densities (A m^{-2}) for both AEM and CEM, with and without TMCS module. Transport of CO_2 and NH_3 via the TMCS module results in a higher current at the same applied voltage.

Mesoporous silica-based pem composites membranes for applications in single-chamber microbial fuel cells (MFC)

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Today the wastewater treatment processes are still energetically intensive, expensive and require high investments. Microbial fuel cells (MFC) are appealing bioreactors for the simultaneous electricity generation and wastewater treatment. In the particular field of the single-chamber MFC, several membranes have been tested during these last years. Among them, proton conductive membranes, which separate the anodic compartment from the cathodic one, have a high impact on power density. Nafion 117 is a typically investigated proton exchange membrane (PEM) for MFC applications, even if some questions are still open: a high cost, bacteria biofouling and oxygen crossover. Here, we describe different Nafion composite membranes ($5\%_{w/w}$) based on mesoporous silica, pristine and properly functionalized with sulfonic acid units, in order to investigate the role of the filler in the improvement of the MFC functional performances. These systems were compared with cells including pure Nafion 117 membranes. The microbial fuel cells were assembled sandwiching the membranes among a Pt-loaded GDL as cathode and analysed in municipal wastewater environment. The bioreactors were characterized in terms of electrochemical impedance spectroscopy, polarization experiments, COD determination and SEM analyses of the electrode compartments before and after the test. After 2000 hours of continuous work, the MFCs provide power densities, ranging between 96 and 282 mW/m3 depending on the composite membrane. At the same time, decreases of the MFC interfacial resistance were also observed switching from the pure Nafion membranes to the composite ones. In particular, the best results were obtained with the cell containing the Nafion composites filled with the sulfonated mesoporous silica that delivered a constant power density higher than about 300 mW/m3 . In such cases, SEM analysis showed a negligible biofouling contrary to the pure Nafion membranes, where a highly packed film made of rod-shaped microorganisms, arranged in long chains, were detected.
The same study was conducted for the main Nafion's competitor: polybenzimidazole (PBI). Due to the high mechanical and chemical stability of the PBI we obtained composites membranes with a load of inorganic filler up to $30\%_{w/w}$.
Power densities were higher and more stable than with Nafion and also biofouling resistance was quite impressing. Thanks to the cheapness of PBI and the excellent properties shown in out tests, polybenzimidazole could be an efficient alternative to Nafion or similar perflorosulfonated membranes.

Long-term stability of acidophilic tetrathionate-fed microbial fuel cell

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The process and waste waters of sulphide mineral processing facilities often contain chemical energy stored as reduced inorganic sulphur compounds (RISCs). To prevent the risk of environmental acidification due to biological oxidation of sulphur compounds, RISCs containing water streams can be treated bioelectrochemically in microbial fuel cells (MFCs)(Sulonen et al. 2015, Ni et al. 2016). Biofouling has been a significant issue in long-term operation of MFCs. Unwanted accumulation of biomass on the membrane and/or on the cathode electrode may limit the ion transfer thus increasing the internal resistance and decreasing the electrical energy yield (Miskan et al. 2016, Vogl et al. 2016). In chemolithotrophic growth, energy released in the substrate degradation is mainly consumed to synthetize cellular components, leading to lower biomass yields (McCollom & Amend 2005). Therefore, acidophilic MFCs fed with RISCs possess a lower risk for performance limiting biofouling than MFCs fed with organic compounds.

In this study, we demonstrated stable electricity production from tetrathionate ($S_4O_6^{2-}$) in highly acidic conditions with two-chamber flow-through MFC for over 2 years. The maximum current density was improved from 80 mA m^{-2} (1000 Ω) to 225 mA m^{-2} (100 Ω) by optimizing the external resistance. The average current density over the 744-day experimental period was 149 mA m^{-2}. The reaction products of tetrathionate disproportionation were sulfate and elemental sulfur. The internal resistance decreased over time and no biofouling was observed on the membrane or on the cathode electrode. The maximum current and power densities were 1,114 mA m^{-2} and 44 mW m^{-2}, respectively, in the performance analysis on day 711. This study shows that tetrathionate is a suitable substrate also for long-term bioelectricity production.

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L'odore di gas biologico - Biofilms as recognition element for vfa sensors in anaerobic digestion

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[2] Kurt-Schwabe-Institut für Mess- und Sensortechnik e.V. Meinsberg, Waldheim, Germany
[3] Technische Universität Dresden, Physical chemistry, measurement and sensor technology, Dresden, GERMANY
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Volatile fatty acids (vfa) like acetate are highly process-relevant in anaerobic digestion (AD) and are measured off-line with gas- or liquid chromatography which is time consuming and cost intensive. To establish a real time process control especially for demand driven biogas production and increased process automation we propose a microbial electrochemical sensor for on-line measurement of acetate and other vfa. The recognition element (receptor) of the sensor is based on a mixed species *Geobacter spp.* dominated anodic biofilm. The corresponding transducer consists of a carbon electrode that allows the implementation of different electrochemical measurement techniques like chronoamperometry and cyclic voltammetry.
The basic sensor parameters were characterized in three electrode arrangement using a flow cell setup. The sensor is suitable for measuring acetate within a range of 0.5 – 5 mmol L^{-1} with a measurement resolution of 0.25 – 1 mmol L^{-1} acetate depending on the provided concentration and flow rate (Kretzschmar *et al.* 2016), whereas propionate and butyrate resulted only in a low and constant signal irrespective of the applied concentration. Proof of concept experiments in stirred AD reactors (10 L) revealed a biosensor signal similar to a typical intraday vfa concentration profile. The achieved measurement range of the biosensor is so far too low for application in AD and hence has to be increased by e.g. dilution of process fluids and optimized sensor architecture. Features that promote a future application of the biosensor, e.g. the low estimated costs as well as the self-sustainability of the living recognition element, will be critically discussed.

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Towards miniature microbial fuel cells for water quality monitoring

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Thousands of different chemicals contaminate water systems. Their presence and biotoxicity must be quickly and efficiently assessed to contain the associated risks on the aquatic biota and human health.

Microbial fuel cells are devices that directly convert the chemical energy in organic matter into electricity via metabolic processes of microorganisms [1]. The current generated by an MFC directly relates to the metabolic activity of the electroactive biofilm at the anode surface [2]. Any disturbances of their metabolic pathways are translated into a change in the production of electricity.

The microbial fuel cell (MFC) technology has potential for the effective testing of water sources in real time. A single chamber (128 µL) miniature MFC biosensor for detection of the biological oxygen demand (BOD) of water systems and to detect toxicants is presented.

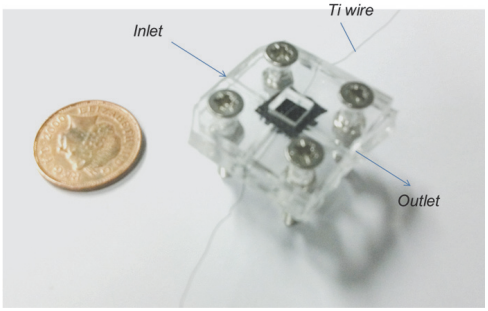

Fig. 1. Miniature single chamber MFC used in this work

The device showed a response to a change in BOD (with concentrations of acetate substrate between 10 – 600 mM) within 19 minutes. The effect of operational conditions (pH, temperature and conductivity) on current generation was shown to have a maximum sensitivity of 0.944 µA cm-2 per unit change of the operational parameter- demonstrating the importance of environmental control during sensing applications. The promise for detection of 'emerging' contaminants and toxicants is discussed.

Additionally, this work discusses avenues to increase and study the power performance of miniature MFC devices. First of all the power output of the device was enhanced by a factor of ~30 by doubling the length of the anodic chamber and doping the cathode with a sustainable biochar based catalyst. Electrochemical Impedance Spectroscopy is also discussed as a tool to study limitations of MFCs.

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Ammonium transport, removal and recovery in current-driven processes

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Complete removal and recovery of ammonium from wastewaters in (bio)electrochemical systems has proven to be a challenge. The system performance depends on several factors, such as current density, ammonium loading rate, pH in both anolyte and catholyte, and interactions between ions. The interdependence among the parameters affecting the ammonium transport is yet not well understood: insight is needed to achieve maximum ammonium recovery at minimal energy input. The aim of this study was to test the influence of applied current and ammonium loading rate to ultimately predict the behaviour of the system in terms of recovery efficiency. The system consisted of an electrochemical cell (EC) coupled to a membrane unit (Figure 1). The membrane unit contains a hydrophobic membrane through which ammonia gas (NH_3) from the catholyte can pass and be absorbed by an acid (Kuntke, 2016). It was found that one of the main factors to predict the recovery efficiency is the load ratio, which correlates the applied current to the ammonium loading rate. Furthermore, we show that coupling an EC to a hydrophobic membrane unit results in an efficient system for removing and recovering NH_3 from concentrated streams, such as urine. High fluxes (up to 433 gN m^{-2} d^{-1}) and recovery efficiencies (> 89%) were obtained. The findings can be used to optimize the operation of the system, achieving almost complete recovery at low power inputs compared to conventional nitrogen removal processes.

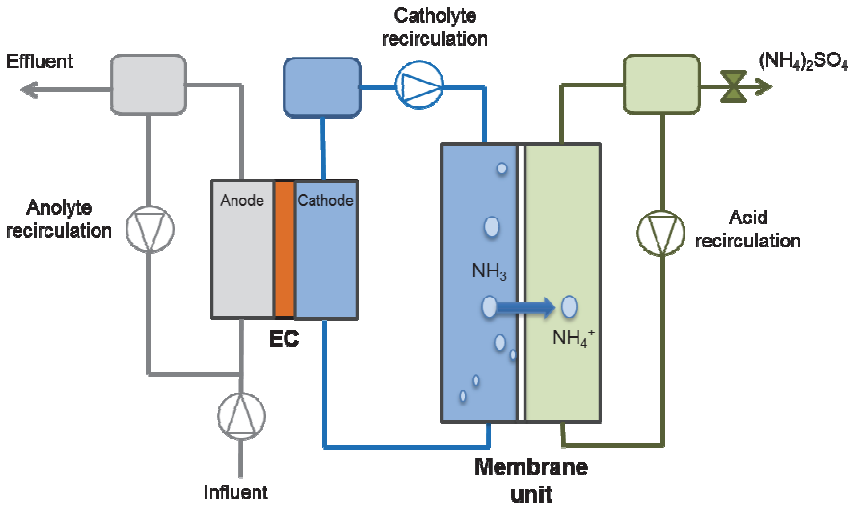

Figure 1. Schematic representation of the experimental setup. EC= Electrochemical Cell.

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Influence of cathode specific area on the performance of anaerobic electrochemical membrane bioreactor

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Recently we developed a novel anaerobic system that integrates the operating principles of a microbial electrolysis cell (MEC) with anaerobic filtration using electrically conductive, catalytic and hollow fiber membrane (HFM) cathodes to recover the energy from a low organic strength solution directly as biogas and reclaim the treated water in what is called an anaerobic electrochemical membrane bioreactor (AnEMBR) (Katuri et al., 2014). However, a major challenge of integrating MECs with membrane filtration processes is membrane fouling, which is a major drawback of all MBRs. Katuri et al. (2014) suggested that increased hydrogen production rates might reduce membrane fouling in the AnEMBR system due to the self-scouring effect of hydrogen gas bubbles on the membrane surface. Several factors including specific cathode surface area and applied voltage can affect hydrogen production rate and thus fouling propensity in the AnEMBR system. In this study we systematically evaluated the effect of specific cathode surface area (SCSA) on the performance of the AnEMBR system. Four identical AnEMBRs differing only in the SCSA (2, 4, 8 and 16 m^2/m^3) were operated in parallel using synthetic acetate solution with 400 mg/L COD. Ni-based hollow-fiber membranes were used as a cathode material for H_2 evolution reaction and for filtration of the effluent. The performance of the reactors was evaluated in terms of current density, biogas composition, volumetric gas production rate and membrane fouling. Biofilm communities on the cathode were evaluated using SEM and 16S rRNA gene sequencing. The results presented in this abstract are for the first 30 days of batch operation. Current density and biogas production increased with decreasing the SCSA of the bioreactors. Transmembrane pressure (TMP) was correlated to the SCSA with higher TMP observed at lower SCSA. Higher biofilm thickness was observed in the 2 and 4 m^2/m^3 AnEMBRs than the 8 and 16 m^2/m^3reactors. Gas production was observed after the second batch in the 16 m^2/m^3 AnEMBR, and from the third batch in the remaining AnEMBRs. Experiments are currently underway using a high-speed video camera (Phantom v2511) to calculate variations in H_2 bubble size and flow rate on the cathode surface as a function of SCSA. These experiments will provide fundamental information on the role of H_2 bubble size and flow rate in mitigating fouling.

Krishna P. Katuri, Craig MW, Rodrigo JJ, Wei Chen, Sungil Jeon, Bruce E. Logan, Zhiping Lai, Gary L. Amy, and Pascal E. Saikaly*. Environ. Sci. Technol. 2014, 48, 12833−12841

Fluidized anodes versus non-conductive fluidized beds in the treatment of a brewery wastewater

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One of the limitation of anaerobic digesters is the instability of the process due to, among other factors, the accumulation of volatile fatty acids (VFA). However, in bioelectrochemical systems this process is unlikely to occur because of the ability of electrogens to rapidly oxidize VFAs with an anode acting as an electron acceptor.
In this study, we compared an anaerobic digestion process versus another with both anaerobic digestion and electrogenic metabolism in the treatment of a brewery wastewater in a fluidized reactor configuration at continuous mode. Two identical Microbial Electrochemical Fluidized Bed Reactor (ME-FBR) were operated as a three-electrode electrochemical cell with the anode polarized to 0.2 V (vs Ag/AgCl). The anode consisted of a conductive bed made of activated carbon granules expanded by the effect of an upward flow of recirculating media. In parallel, two additional classical fluidized bed reactors (FBR) were operated with the bed made of biolite particles, a common carrier used in this kind of configuration. The treatment capacity of each system was studied and the consumption rates of volatile fatty acids in each kind of reactor was investigated in order to elucidate the metabolic limiting steps and the synergy between anaerobes and electrogens in our reactors. Interestingly, under the same scenario the ME-FBR outperformed the conventional FBR in terms of COD removal and the main differences between them were found as the organic load was enhanced. Additionally, the biofilm colonizing the bed particles and the planktonic biomass grown in the reactors were explored by scanning electron microscopy and FISH techniques. Differences in morphology, biofilm development and microbial community were found between the biomass colonizing the conductive fluidized anode and the fluidized biolite particles. Interestingly, *Geobacter* cluster was highly enriched at the inner layers of the biofilm formed on the fluidized anode.

Poster presentations

Session P1

Reductive dechlorination of 1,2-dichloroethane with an AQDS-modified electrode

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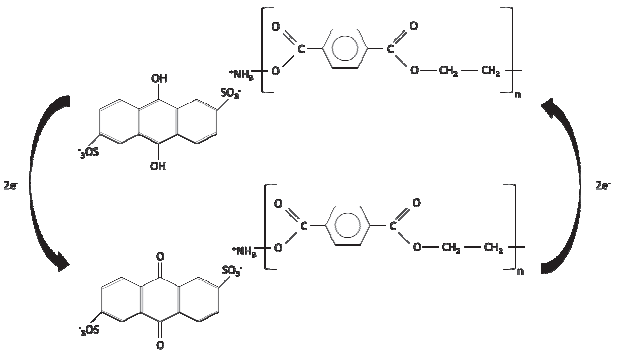
Besides many other applications, 1,2-dichloroethane (1,2-DCA) is used worldwide as a solvent and for the production of vinyl chloride. Due to improper storage, handling and disposal practices, 1,2-DCA has been detected at many locations with concentrations ranging from 0.1 to 100 mg/L. Bioremediation via anaerobic reductive dechlorination is one of the most promising approaches for the treatment of 1,2-DCA contaminated groundwater. This approach typically requires the subsurface injection of fermentable substrates that serve as electron donors to stimulate autochthonous microbial populations using 1,2-DCA as a respiratory electron acceptor. However, this approach suffers of a number of drawbacks, including the inefficient use of the added electron donors, stimulation of unwanted side reactions, and in general, poor control over reaction conditions. Recently, bioelectrochemical remediation, whereby insoluble electrodes are used as direct electron donors in the reductive dechlorination process, has been proposed as a strategy to overcome some of these limitations. The need to achieve high rates of extracellular electron transfer between the electrode and the dechlorinating microorganisms, while maintaining a highly selective process, still pose a major challenge. We have previously reported that the use of a humic-acid analogue redox mediator (anthraquinone-2,6-disulfonate (AQDS) in solution stimulates the reductive dechlorination of 1,2-DCA. In this study, we report the use of an anion exchange membrane to immobilize AQDS at the cathode. Electrochemical characterization of the bare electrode, anion exchange modified electrode and anion exchange and AQDS modified electrode was carried out in different experimental conditions. A mixed microbial culture was used in these bioelectrochemical batch experiments and characterized during the course of the experiment by fluorescent in situ hybridization (FISH) techniques targeting known dechlorinating bacteria.



Investigating the effect of inoculum and substrate type on microbial fuel cell (MFC) performance

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Nowadays, the excessive consumption of fossil fuels in human activities and consequently the excessive emission of greenhouse gasses particularly carbon dioxide (CO_2) becomes an ever increasing problem. In order to tackle this issue, bioelectrochemical system (BES) provides new insight into clean energy generation and CO_2 sequestration. Microbial fuel cell (MFC) is one type of BES able to generate electricity using bacteria as biocatalyst. Due to the importance of anode compartment for this purpose, anode chamber improvement has attracted widespread attention as its performance plays an important role in the cell performance. Therefore, 3 different sources of bacteria as bioanode (former MFC effluent, activated sludge and sediment of Tyne river in Newcastle, United Kingdom) and 3 different substrates (acetate medium, synthetic wastewater (OECD) and acetate followed by OECD) were investigated in a long-term operation.
MFC effluent with acetate substrate had the best performance in terms of voltage and current generated in the first batch cycles. However, activated sludge was able to generate more current than Tyne river sediment and more stable than MFC effluent. This fact shows that there is more variety of exoelectrogens in activated sludge and MFC effluent which accumulated in the anode chamber. Although electricity generation became stable in cells with OECD almost 24 hours after operation, the current was lower compared to cells operated by acetate which shows acetate is still the preferable substrate for bacteria and fulfils their requirement for their growth and transferring electron. Highest voltage was obtained in 7th batch cycle for cell enriched by activated sludge and fed by acetate with 225.8±21.2mV.

Monitoring of volatile fatty acids during anaerobic digestion using a microbial electrochemical sensor

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Volatile fatty acid (VFA) concentration is known as an important indicator to control and optimize anaerobic digestion (AD) process. In this study, an innovative VFA biosensor was developed based on the principle of a microbial desalination cell. The bulk substrate was dosed into the middle chamber innovatively which was separated from the anode chamber by an anion exchange membrane. The detection range can be broadened as only part of the ionized VFAs can transport through the membrane and the biofilm can be protected from inhibitors and toxicants.

The correlation between current densities and VFA concentrations was firstly evaluated with synthetic digestate. Two linear relationships were observed between current densities and VFA levels from 1 to 30 mM (0.04±0.01 to 8.50±0.32 mA/m^2, R^2=0.97) and then from 30 to 200 mM (8.50±0.32 to 10.80±1.26 mA/m^2, R^2=0.95). The detection range was much broader than that of other existing VFA biosensors. The biosensor had no response to protein and lipid which are frequently found along with VFAs in organic waste streams from AD, suggesting the selective detection of VFAs. The current displayed different responses to VFA levels when different ionic strengths and external resistances were applied, though linear relationships were always observed. Finally, the biosensor was further explored with real AD effluents and the results did not show significant differences with those measured by GC. The simple and efficient biosensor showed promising potential for online, inexpensive and reliable measurement of VFA levels during AD and other anaerobic processes. The outcomes will expand the application of bio-electrochemical system application.

Driving mixed culture fermentation with microbial electrochemical technologies

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An emerging application of the microbial electrochemical technology (MET) is "electro-fermentation", which allows using electrodes to drive the fermentation of (waste) organic substrates with both pure and mixed cultures. In principle, the presence of polarized solid-state electrodes can affect microbial metabolism through changes in the intracellular NADH/NAD^+ balance which, in turn, can influence the final spectrum of fermentation products. Here, we explored the role of a polarized electrode on the regulation of the metabolic pathways of an anaerobic sludge.
To accomplish this objective, a set of batch experiments was carried out in two-chamber cells separated by a proton exchange membrane (Nafion) with both the anode and cathode electrodes consisting of graphite rods. Glucose, acetic acid and ethanol have been used as single substrate and in mixture. All conditions have been tested either in the absence (open circuit control) or in the presence of polarization, in the latter case being the working electrode (cathode) set at -0.70 V *vs.* SHE. Highest levels of C4 compounds (i.e. butyric and isobutyric acids), with respect to open circuit experiments, were observed both with glucose/ethanol and glucose/acetate mixture. In the presence of a mixture of the three substrates, a high concentration of isobutyric acid was obtained whereas, in the open circuit tests, lactic acid was the main fermentation product detected. In addition, the effect of a redox mediator was also evaluated by adding 500μM of neutral red in the cathode chamber of the cells. Notably, when glucose was supplied as a single substrate, the presence of neutral red and electrode polarization resulted in an enhanced production of C4 acids, relative to open circuit controls. The biochemical mechanisms involved in the mixed culture electrofermentation process are being investigated in ongoing experiments aimed at identifying both the involved microbial pathways, by means of labelled substrates tests, and changes in the composition of the microbial population.

Microbial biofilm assessment of two different methanogenic biocathodes: identifying the microbial active key players

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The aim of this study was to assess the microbial population enrichment process on methanogenic biocathodes from two microbial electrolysis cells (MEC). A period time ranging from the initial inoculation to their stable operation was studied. Simultaneous total DNA and RNA extraction was implemented to identify most active microbial key players from the whole microbial community.

A pair of lab-scale two-chambered MEC (0.5 L each compartment) were operated during 95 days poising the cathode potential at -800 mV *vs* standard hydrogen electrode (SHE) to enhance the electromethanogenic process. Two different strategies were implemented for inoculum enrichment: i) direct enrichment of an anaerobic granular sludge inoculum from a full scale upflow anaerobic sludge blanket reactor (UASB) in the biocathode of the MEC (BC1); ii) external biomass enrichment of the same inoculum in a lab-scale methanol-fed UASB (BC2). The evolution of the microbial community was evaluated in terms of composition and detection of active microorganisms by the use of quantitative real-time polymerase chain reaction (qPCR) and high throughput sequencing (MiSeq) of both genomic DNA and RNA.

Results showed that current density production increase from the start-up was faster in BC2 than in BC1, and higher methane production was also achieved (maximum of 0.18 m^3 m^{-3} d^{-1}). Besides, the final eubacterial enrichment on the biocathode surface was affected by the initial inoculation strategy. Furthermore, RNA-based high throughput sequencing results revealed that the most predominant active populations differed from total DNA-based microbial community. On the contrary, the archaeal microbial community obtained on both biocathodes at the end of the assay were highly similar, dominated by present and active hydrogenotrophic methanogenic archaea such as *Methanobrevibacter* genus (*Methanobacteriaceae*), being also the most active (87 and 98% of RNA relative abundance in BC1 and BC2, respectively). Therefore, regardless of the initial composition of the inocula, the growing process on both biocathodes promoted the enrichment towards hydrogenotrophic methanogenic families, especially in the case of the inoculum of BC2, which was initially enriched in methylotrophic methanogenic archaea (*Methanomassiliicoccaceae*, 24% and *Methanosarcinaceae*, 50%, genus *Methanomethylovorans* and *Methanolobus*). Multivariate statistical analysis (correspondence analysis) performed for eubacteria and archaea community showed a clear differentiation between BC1 and BC2 initial inocula but similar final archaea enrichment was revealed in both reactors at the end of the assay.

These results corroborate that the environmental conditions in the biocathode exert a selective pressure towards specific microbial populations especially for total present and active archaea.

Microbial Rechargeable Battery

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Bioelectrochemical systems hold potential for both conversion of electricity into chemicals through microbial electrosynthesis (MES) and the provision of electrical power by oxidation of organics using microbial fuel cells (MFCs). Our study provides a proof of concept for a microbial rechargeable battery (MRB) allowing storage of electricity by combining MES and a MFC in one system. Hexacyanoferrate(II/III) was used as counter redox couple. Duplicate runs showed stable performance over 15 days, with acetate being the main energy carrier. An energy density of around 0.1 kWh/m^3 (normalized to anode electrolyte volume) was achieved at a full cycle energy efficiency of 30–40%, with a nominal power output during discharge of 190 W/m^3 (normalized to anode volume). With our study, we show a new potential application area for bioelectrochemical systems as a future local energy storage device.
Via the selection of a counter redox reaction with a sufficiently high redox potential, the relative difference between the required charging voltage and the obtained discharging voltage may be reduced, positively impacting overall energy efficiency.
Apart from the reaction at the counter electrode, the maximal achievable energy density of the MRB is directly related to the acetate concentration attained during charging as this defines the anodes' charge capacity. Acetate concentrations of 0.75 M reached previously in hydrogenotrophic reactors provide an optimistic perspective regarding further optimization of this parameter.
In conclusion, we have shown the proof of concept of a microbial rechargeable battery, using a biocathode that produces acetate from electricity and a bioanode converting acetate into electricity. Depending on the acetate concentration that can be achieved, and the counter reaction involved, the MRB could become a suitable, clean, safe, and renewable alternative to existing battery storage systems. As such, the MRB could become an inexpensive local energy storage device in the future.

This work has recently been published as:
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Optimization of anode current collector in microbial fuel cell for improved power output and bacterial development

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Microbial fuel cells (MFC) are among the most-studied bioelectrochemical systems (BES) over the last twenty years, and yet, still pose numerous difficulties when scale up is attempted at the laboratory scale. This study aimed at testing the hypothesis that external anode electrical connections play a major role in power output limitations during scale-up. At a small scale, these contacts are usually made by simple metal strip or wire connected at one point or one edge of the electrode. Scale-up strategies often do not take into account electrical contacts as possible reasons for power loss. Power losses arising due to the distance between remote anode points and single connections could appear when the anode size is increased without increasing the number of connections. We investigated the electricity production and bacterial anodic development in a 1L MFC with different electrical contact configurations on its 500 cm^2 carbon cloth anode. Ohmic resistance due to distance and electron charge transfer resistance due to potential drop distribution on the anode were thought to limit power production by shaping and restricting spatial development of microbes. Power output, electrochemical characterization by impedance spectroscopy and anodic biofilm structure through microscopy and molecular biology tools were used to examine the impact of current collector design on overall power production and microbial spatial distribution and community structure.

Electrochemical characterization of the electron-accepting and electron-donating capacity of biochar

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Recent studies have shown that addition of small amounts of biochar to anaerobic sludge is a practical and effective strategy to accelerate the methanogenic conversion of waste organic substrates. It has been suggested that the stimulatory effect may derive from the capability of electrically conductive biochar particles to function as electron conduits between acetogens and methanogens, thereby promoting a novel form of interspecies energy transfer often referred to as Direct Interspecies Electron Transfer (DIET). In essence, biochar-mediated DIET involves the conductive particles directly accepting electrons from acetogens and transferring electrons to methanogens, with the two syntrophic partners laying in close proximity one to each other, over the surface of the biochar particle. In spite of the relevant potential for application of this approach, the mechanisms through which electrons are conveyed across the biochar (e.g., metal-like conductivity or presence of redox-active groups capable to accept or donate electrons) remain poorly understood.

Here we applied a suite of chemical and electrochemical assays to characterize the electron transfer potential of biochar materials deriving from different types of biomass (wheat bran, wood, orchard pruning biomass) and produced at different temperatures (400-800 °C). More specifically, a set of electrochemical tests, based on a recently standardized protocol (Klüpfel et al., 2014), and involving the use of soluble redox mediators namely, diquat dibromide monohydrate and 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) diammonium salt (ABTS) for the targeted reduction and oxidation of quinone moietites of biochar, respectively were used to quantify the overall electron-accepting (EA) and electron-donating (ED) capacities of the selected materials. Results demonstrated that EA and ED capacity, as well as the electrical conductivity of the biochar, are highly dependent on the production material and conditions. Collectively, these findings provide new insights into biochar-based DIET processes and will possibly help devising strategies for their practical implementation.

Klüpfel L., Keiluweit M., Kleber M., Sander M. (2014) Redox properties of plant biomass-derived black carbon (biochar). Environmental Science Technology, 48: 5601-5611

Characterization of the electroactivity of phenazine-producing *Pseudomonas putida* KT2440 for bioproductions in bioelectrochemical system

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Bioelectrochemical Systems (BESs) are a promising technology that involves whole cell biocatalysts to drive oxidation and reduction (redox) reactions at solid electrodes. Recent exploration and study of natural microbial electron discharge to extracellular anodes might offer significant improvements strategies in bioelectrochemical processes for production of various useful products. However, the natural activity of biocatalysts on electrodes is limited and molecular engineering approaches are required to tailor new bioelectrochemically active production hosts. A successful initial proof-of-principle study has just been reported by Schmitz et al. (2015) proposing a new engineered strain of *Pseudomonas putida* KT2440 that is able to utilize an anode for electron discharge during oxygen limited growth via the heterologous expression of redox-mediating phenazines from *Pseudomonas aeruginosa*. In that study, phenazine-1-carboxylic acid (PCA) was found as the major phenazine synthesized to allow current production and under the most stringent oxygen levels. Biotechnologically, *P. putida* KT2440 has been already tailored to produce rhamnolipids under aerobic conditions (Wittgens et al., 2011). Yet, costly aeration and the subsequent problems with strong reactor foaming, which are technically challenging to overcome with conventional antifoam technologies, are current drawbacks. Here in this work, we are further tailoring the electroactive *P. putida* KT2440 for rhamnolipids production under oxygen-limited condition in BES. An engineered strain of *P. putida* KT2440 only expressing the genes *phzA-G* to synthesize PCA has been generated and is now investigated for biocatalytic applications. Overall, this works shows the first application of the new phenazine-producing *P. putida* KT2440 in BES.

Schmitz S., Nies S., Wierckx N., Blank L.M., Rosenbaum M.A. (2015) Engineering mediator-based electroactivity in the obligate aerobic bacterium *Pseudomonas putida* KT2440. Frontiers in Microbiology, 6 (284): 1-13.

Wittgens A., Tiso T., Arndt T. T., Wenk P., Hemmerich J., Mueller C., Wichmann R., Küpper B., Zwick M., Wilhelm S., Hausmann R., Syldatk C., Rosenau F., Blank L.M. (2011). Growth independent rhamnolipid production from glucose using the non-pathogenic *Pseudomonas putida* KT2440. Microbial Cell Factories, 10 (80): 1-17.

Enhanced biofilm growth in a novel flow field reactor for electromethanogenesis

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Methane production rates of bioelectrochemical systems are still low in comparison to other methane-producing technologies. One reason for the inefficiency of those systems is the formation of local dead zones in reactors, preventing the growth of a thick biofilm, which is necessary for high methane production rates. Therefore, the aim of this study is to further improve the concept and its key parameters like energy consumption and volumetric reactor productivity by developing new reactor designs helping to prevent the drawbacks mentioned above. In comparison to other flat-plate bioelectrochemical systems the reactor in the present case has several in- and outlets realized by a manifold, which is externally attached via capillaries to the chamber frames. This design makes flow channels unnecessary, as the capillaries allow for a better distribution of the microorganisms and the nutrients in the used medium. Moreover, without flow channels more electrode surface area is available for biofilm growth, which leads to higher production rates. Thus, the developed design is not only suitable for the electromethanogenesis process, but also for bioelectrochemical systems generating electricity or other products.
To prove the described hypotheses, the system will be *in situ* analysed electrochemically via polarization curves and cyclic voltammetry, and by measuring the gas composition. *Ex situ* examinations are focusing on the biofilm growth by using microscopic measurements (e.g. scanning electron microscopy).
In summary, the systems' outlined properties supposedly lead to denser biofilm growth in the cathode chamber compared to reactors with flow channels, as nutrition dead zones in the electrode chambers will occur to a lesser extent. With less dead zones and denser biofilm growth a high current-to-methane efficiency and especially a higher methane production rate are expected.

Enhancing electricity production from wastewater using microbial fuel cells

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Microbial fuel cells represent a promising technology for simultaneous wastewater treatment and renewable electricity production. However, the electricity recovery is still poor, typically <10% of what is theoretically possible and the extracellular electron transfer mechanisms are poorly understood.

The use of co-cultures to improve substrate (glucose) turnover rate and hence electricity recovered was investigated initially. A co-culture of *Shewanella oneidensis* and *Clostridium beijerinckii* gave a maximum power density (P_{max}) of 87 mWm^{-2} (67% COD reduction) compared to 60 mWm^{-2} for *C.beijerinckii* alone and 48 mWm^{-2} for *S.oneidensis* alone. Co-culturing *Geobacter sulphurreducens, C. beijerinckii and Saccharomyces cerevisiae* gave the highest P_{max} value of 80 mWm^{-2} (41% COD reduction) compared to other strain combinations.

Another study investigated the contribution of direct electron transfer mechanism on electricity production by physically retaining *Shewanella oneidensis* cells close to or away from the anode electrode using a dialysis membrane (as well as immobilisation of the cells in alginate). Pyruvate was used as the substrate. The outcome of this study indicated a P_{max} value of 114±6 mWm^{-2} when cells were retained close to the anode, 3.5 times more than when the cells were separated from the anode. Without the membrane P_{max} was 129±6 mWm^{-2} (57% COD reduction).

To understand the role played by c-type cytochromes MtrA, MtrB and MtrC in extracellular electron transfer in *S.oneidensis*, the genes *mtrA, mtrB*, *mtrC* and their combinations were heterologously expressed in non-electrogenic bacteria (*Escherichia coli;* glucose as substrate*)*. The *mtrCAB* transformant gave the highest P_{max} of 24 mWm^{-2} compared to 1 mWm^{-2} for the wild type although cell growth was slower.

The results demonstrate the importance of co-cultures and of the MtrCAB pathway (direct electron transfer mechanism) in improving bacterial electricity production.

Biocathodes for enhancing anaerobic growth of *corynebacterium glutamicum* with nitrate

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The industrially important *Corynebacterium glutamicum* can only incompletely reduce nitrate into nitrite which in turn accumulates and inhibits growth (Takeno et al., 2007). To find out whether cathodes could help promote anaerobic growth we supplied *C. glutamicum* with glucose, nitrate, and electrodes polarized under potentials ranging from -0.40 to -1.25 V vs. Ag/AgCl. Cell growth was inhibited and glucose consumption was limited in all cases except under the electrode potentials of -1.25 V or -1.20 V plus the redox mediator anthraquinone-2-sulfonate (AQ2S) (Figure 1). Under these conditions, nitrite reduction was more efficient and glucose consumption was up to 6 times faster. A metabolic shift was also observed, most probably due to alterations of the intracellular redox balance. Acetate (NADH producing) replaced lactate (NADH consuming) as the main metabolite and was produced at up to 11 times the yields observed in all other cases (up to 1.1 mol/mol-glucose and 5.3±0.3 g/L). Formate production (up to 3.4±0.3 g/L) was also observed under intensively reducing conditions, and we attribute this to a possible mechanism related to electrochemically triggered CO_2 bioreduction. Overall cathodes demonstrated a great potential for enhancing growth by removing inhibitory nitrite, and also for altering the metabolic end-products.

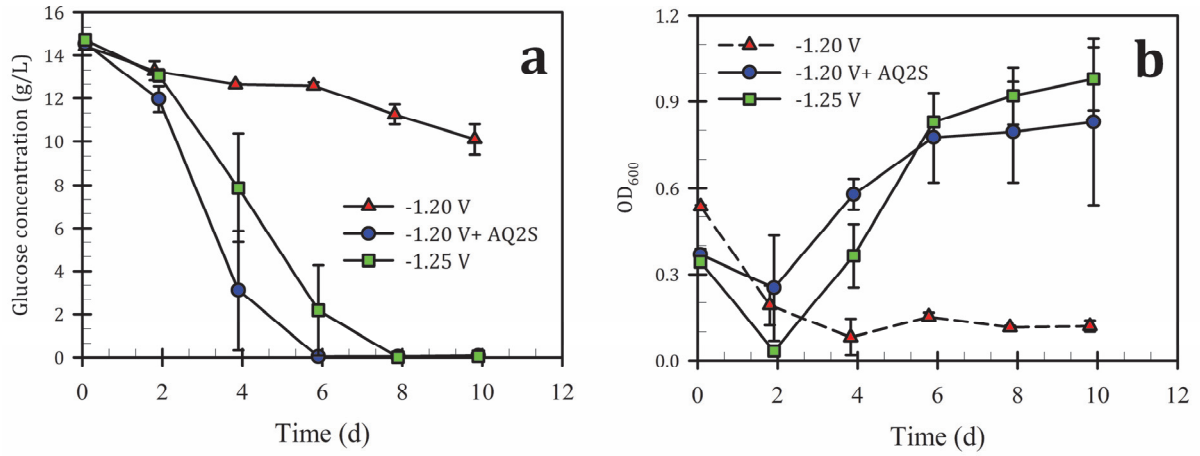

Figure 1. Glucose concentration (a) and growth (b) in bioelectrochemical cathodes inoculated with *C. glutamicum* in the presence or absence of 0.1 mM AQ2S.

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Effect of nitrogen environment on the performance of conducting polymers/CNTs nanocomposites modified anodes for microbial fuel cells (MFCs)

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The development of composite materials based on conducting polymers due to their complementary electrical, electrochemical and mechanical properties may offer improved performance in especially in the electrochemical application.
Two conducting polymers materials, polyaniline (PANI) and polypyrrole (PPY) where used to prepare the conducting polymers/CNTs nanocomposites. The nature of nitrogen environment and dominant surface nitrogen species in both nanocomposite materials was correlated with the performance of nanocomposite modified anode for MFCs. The contribution of individual nitrogen type to overall surface concentration was determined using N1s high resolution X-ray photoelectron spectroscopy (XPS). The maximum power densities were obtained for MFCs with PANI/CNT (202.26 mW/m^2) modified carbon cloth as electrode vs PPY/CNT (167.8 mW/m^2) and CNT (145.18 mW/m^2) modified carbon cloth.

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Electrochemical Study of the Extracellular Electron Transfer of *Enterococcus faecalis* to Electrodes

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There are only a few described electroactive bacteria, which are able to transfer electrons from the cell metabolism to the electrode surface directly. For most bacteria mediators are necessary.and only some of the extracellular electron transfer mechanisms have been studied in depth. Therefore more investigations and detailed studies of different strains of bacteria are needed for a better understanding and optimization of the bacterial cell-electrode interaction and electron transfer. The aim of this study was to electrochemically investigate the role of each component of the respiratory chain of the Gram-positive *Enterococcus faecalis* and accordingly find out the mechanism of the extracellular electron transfer from the cells to the electrode.
E. faecalis is a part of the natural microflora that inhabits the gastrointestinal tracts of mammals. It grows by fermentation with lactic acid as the main end product and can use more than 30 carbohydrates as substrate. This bacterium is a facultative anaerobe and aerobic respiration depends on the presence of heme, which serves as a cofactor for cytoplasmic catalase [1] and membrane bound cytochrome *bd* oxidase [2]. *E. faecalis* does not require heme to grow and lacks the genes for its synthesis but is able to take up heme or its analogues from the environment. When the cells are supplied with heme, a minimal respiratory chain is built up, including several NADH dehydrogenases, a demethylmenaquinol pool in the membrane and the heme-dependent cytochrome *bd* oxidase [3].
The wild type as well as three mutant strains of *E. faecalis* with different mutations within the electron transport chain were investigated using cyclic voltammetry and chronoamperometry under flow injection conditions and different experimental and culture conditions to identify possible ways of the cell-electrode communication.

This work was financially supported by the European Commission (project "BIOENERGY" FP7-PEOPLE-2013-ITN-607793) and the Swedish Research Council (project 2014-5908).

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Monitoring microbial communities dynamic during start-up of microbial fuel cell by high-throughput screening techniques

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The dynamics and changes of the bacterial community in the anode biofilm and planktonic broth of an acetate fed batch single chamber air cathode MFC, has been studied during initial stages of the biological process (from 0 to 16 days) by using two screening techniques: flow-cytometry and Illumina sequencing. Flow-cytometry has been rarely used to monitor the evolution of microbial communities in MFC (Koch et al., 2013; Koch et al., 2014) but it represents a useful investigation tool to comprehend the on-time microbiota structure change. In addition, high-resolution sequencing methods can increase the knowledge about the composition and the role of the complex microbial communities involved in the evolution of MFC voltage (numbers and quantity; Song et al., 2015). In this study, the microbial population dynamics and the evolution of an electroactive biofilm over the anode (and planktonic phase) was investigated by flow cytometry quantifying the viable population and the level of cells membrane polarization. The microbial population dynamic was monitored until the voltage obtained was stable for four voltage cycles (0.5V). Flow cytometry analysis evidenced a dynamic change in microbial complexity of both planktonic and anode communities, that decreased during process. Considering the planktonic communities, seven populations were detected, basing on cell size and DNA content, just few hours after the activation of the MFC, and only one main population after 70 h, i.e. when the voltage reached its maximum at 0.4 V. Similarly, basing on cell membrane polarization, the three main populations detected at the beginning of the process, then decreased to one when voltage reached its maximum. From 70 h to 16 day of MFC activity, microbial communities changed from one to a maximum of three, such as revealed by DNA content and from one to two based on the cell membrane polarization [revealed by $DiOC_6(3)$ probe]. The *16S rDNA* gene profiling confirmed the shift in microbial communities, being *Acinetobacter* (39.34 %) *Azospirillum* (27.66 %), *Arcobacter* (4.17%) *Comamonas* (2.62%) as the most abundant genera at the beginning of the MFC activation. Contrarily after 70h the main genera detected were *Azospirillum* (46.42%), *Acinetobacter* (34.66 %), *Enterococcus* (2.32 %) and *Dysgonomonas* (2.14 %) together with the well-known exoelectrogen *Geobacter* (0.6%). The overall data obtained clearly indicated that a multi-parameter flow cytometry is a useful tool able to monitor "online" the changes in microbial communities during the evolution of MFC voltage. Here we showed also that the monitoring of planktonic population, instead of the less accessible anode biofilm, was in good agreement with the MFC voltage evolution.

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Direct external electron transfer from a cathodic surface to a non-hydrogenotrophic methanogen

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Electromethanogenesis is a process in which reducing equivalents in the form of an electrical current are supplied to a methanogenic culture to enhance methane production from carbon dioxide (CO_2). Electromethanogenesis could be applied to upgrade biogas to natural gas using renewable electricity. However, the underlying molecular mechanism of electromethanogenesis remains elusive, as successful attempts have been mostly made with mixed cultures and with hydrogenotrophic methanogens. In this study, we demonstrate the production of methane (CH_4) by a non-hydrogenotrophic methanogen, *Methanosarcina horonobensis* incubated for several months with an electrode poised at -0.6 V (vs. Ag/AgCl, 3.4M KCl) as sole electron donor, which indicates direct electron transfer to this organism. To examine if H_2 is electrochemically generated at -0.6 V (vs. Ag/AgCl) in the cathode chamber, we are now monitoring H_2 evolution with a newly developed H_2 sensor. Spent cell-free media controls are also tested in order to rule out enzymatic H_2 or formate generation at the cathode. At the end of the incubation we are using cyclic voltammetry to find out if the electroactive properties are associated with the cathodic biofilm or with cells in suspension. At last, the transcriptome of *M. horonobensis* grown on cathodes will be compared with that of cells grown on acetate, to learn if certain surface proteins are specifically expressed in cells grown on the cathode. This is the first study showing direct electron transfer from a poised electrode to a non-hydrogenotrophic methanogen.

Fe-decorated Grafene Oxide Nanoplatforms as Oxygen Reducing Electrocatalysts in Microbial Fuel Cells

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The Microbial fuel cell (MFC) technology is most promising for sustainable waste management and energy supply from bio-convertible substrates. As a commonly used catalyst for oxygen reduction reaction (ORR) in MFCs, platinum is not appropriate for commercial development due to its high cost and fouling effect. Therefore, developing effective and low-cost Pt-free catalysts for ORR has aroused extensive research interest. Iron phthalocyanine (FePc) is particularly well suited to meet MFC requirements, due to its low cost, availability and good catalytic activity toward ORR. On the other hand, to mitigate aggregation phenomena and poor conductivity, the immobilization of FePc on carbon nanostructures is recommended [1].
Aim of this study is to develop electrocatalysts based on iron phthalocyanine (FePc) supported on electrochemically exfoliated Graphene Oxide (GO) derivatives. This was carried out by chemical, physical, and electrochemical entrapment strategies, respectively. In particular, electropolymerization offers several advantages, as *i)* no time consuming fabrication of the engineered graphene composites; *ii)* no loss of materials because the entire product is "*ready to use*" in a single step, avoiding additional post-synthesis manipulation, and *iii)* cost minimization thanks to the mass production of graphene obtained by electrochemical exfoliation of the graphite anode [2]. The synthesised nanocomposites are characterized under a morphological/topographic and structural point of view, by using High Resolution Transmission Electron Microscopy, Fourier Transform Infrared Spectroscopy, and Raman Spectroscopy. The electrochemical characterization was carried out by Cyclic Voltammetry and Rotating Disk experiments in neutral pH electrolyte solution to evaluate catalytic activity towards ORR of the prepared materials. The main important results show high polydispersion of FePc on GO nanoplatform (avoiding aggregation), enhanced electron transfer in ORR, and long term stability. This can be ascribed to the high electrical conductivity of graphene as well as high surface nominal area. In addition, the graphene 2D-structure enhances the π-stacking interactions and alignment between the planar aromatic structure of the macrocycle and the graphene surfaces. The FePc-based nanocomposite materials can be applied for the assembly of ORR in single chamber air-catodes MFCs configuration, where the power and voltage parameters are quantitatively detected.

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A tailor made process for the treatment of municipal waste water using a bioelectrochemical system

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The implementation of bioelectrochemical systems (BES) in wastewater treatment processes is a promising approach to further increase their sustainability. So far the removal of carbon and nitrogen compounds from wastewater is mainly realized by energy intensive activated sludge processes. In contrast BES based processes allow to directly convert the chemical energy captured in the wastewater into electrical energy. Although this technology offers a tremendous potential it did not reach broad scale applications so far. This is most probably due to constructional challenges but also to treatment site specific features like the composition of the wastewater or the present microbiome that influence the BES-performance.
Within this context, this study focuses on a tailor made bioelectrochemical process for a municipal wastewater treatment plant. Here a BES was optimized by adapting it to different site-specific characteristics. Initially three central aspects were investigated: First the effluent composition of the preliminary clarification was analyzed. The second central aspect was the isolation of adapted, potentially exoelectrogenic microorganisms from the primary clarification. Finally, different bacterial strains could be isolated and identified as promising candidates for anode interaction. The third aspect was the development of a robust flow BES which was capable to overcome the prominent problems of wastewater associated systems like membrane fouling, for example.
The next step was the combination of these three aspects in order to test the BES function under laboratory conditions: Based on the previously analyzed composition an artificial wastewater was created to feed the system. After inoculation using the isolated strains the BES generated an average current density of 55 mA/m^2 under controlled anode potential conditions and 40 % of the TOC were removed. Interestingly the average current density of the BES could be tripled (at comparable TOC removal rates) under field study conditions at the pilot plant, using real waste water as feed. Further experiments revealed that a stepwise decrease of the anode potential decreased the current production and reduced TOC removal. These results indicate an anode dependent oxidation of organic compounds under controlled electrochemical conditions. Furthermore the described approach shows that using a well-adapted bacterial community has a positive influence on the system's performance. In the next period of the project the full-cell performance of the system and its long-term stability will be investigated.

Electrocatalytic oxygen reduction by porphyrin and corrole iron-complexes in microbial fuel cells

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Microbial Fuel Cells (MFCs) are being explored as a technology for energy recovery and wastewater treatment, based on electricity generation from wastewater organics using exoelectrogenic bacteria. One of the major barriers to the widespread diffusion of this technology is the use of costly platinum as catalyst for oxygen reduction reaction (ORR). Although platinum is a highly effective ORR catalyst, its activity is decreased by a variety of poisoning substances present in wastewater [1]. In the last decade, the development of non precious ions, coordinated to tetrapyrrolic macrocycles and supported on nanostructured carbonaceous materials, has opened a window on platinum replacement [2]. Anyway, the exact nature of active sites for ORR in non-platinum-group metal catalysts has generally not been clearly understood and further investigation is needed to clarify on that.

In this work, we synthesized, and supported on carbon nanotubes (CNTs) by physical adsorption, two different tetrapyrrolic macrocycles: tetraphenylporphyrin iron(III) chloride (FeTPP) and triphenylcorrole iron(III) chloride (FeTPC), since these complexes are among the most active tetrapyrrole catalysts [3]. To facilitate the catalyst dispersion and obtain effective ORR active sites, the supports were modified by a two-steps treatment with nitric acid and ammonia gas. This treatment aimed to introduce oxygen and nitrogen functionalities on the carbon surface, while maintaining an extensively developed porous structure. Porphyrin and Corrole iron-complexes were also covalently bound to CNTs support to explore the effect of catalyst/support interaction on the materials properties.

Cyclic voltammetry and rotating disk voltammetry experiments were used to test the electrocatalytic activity of the prepared materials. The catalytic activity of the two composites has also been tested at different degrees of pyrolysis.

The performance of Air-cathode MFC assembled with CNTs/FeTPP and CNTs/FeTPC as cathodes was evaluated by acquiring polarization and power density curves, as well as voltage generation cycles as a function of time.

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Potentiostatic enrichment of electrochemically active bacteria from polluted sediments and soils

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The majority of studies on electrochemically active mixed microbial consortia investigate domestic wastewater[2,3]. While this is relevant for industrial applications such as microbial fuel cells (MFCs), it is likely that only a small fraction of electrochemically active microorganisms is found in domestic wastewater. Because of the abundance of microbial consortia in soils and sediments, combined with higher insoluble metal and mineral concentrations which can be used as external electron acceptors, soils and sediments are likely to host a much larger diversity of electrochemically active bacteria . There is also potential to discover novel microorganisms capable of Extracellular Electron Transfer (EET) mechanisms with relevant application to biogeochemistry, biosensors and bioremediation[1]. In this study, we will investigate the potentiostatic enrichment and preliminary characterization of electrochemically active microorganisms from contaminated soils and sediments.

Soils and sediments from heavy metal and PAH polluted and pristine sites will be used as inoculum for enrichment of electrochemically active bacteria, enriched by growth on carbon felt working electrodes poised at 0.2 V vs Ag/AgCl in 120 mL stirred anaerobic reactors at 30°C. Carbon felt was selected because of its large accessible surface area for biofilm formation. The microbial composition of the sediment inoculum and the enriched biofilm will be determined by metagenomic analysis. The biofilm's electrochemical properties will be measured by voltamperometric methods and electrochemical impedance spectroscopy.

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Obvious and hidden limiting steps in microbial fuel cells

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The power produced by microbial fuel cells (MFC) remains modest because of several bottlenecks, some of which are well-known, others not so obvious. The purpose of this presentation is to point out some limiting steps and to show how engineering can help to overcome them.
It is known that oxygen reduction on the cathode is slow at the neutral pH required by microbial anodes. A simple way to implement a given bioanode optimally is to increase the surface area of the cathode. An original MFC device was designed with a central bioanode surrounded by 1 to 4 air-cathodes. This configuration increased the power production from 2.2 W/m^2 (referred to the bioanode area) with one air-cathode to 4 W/m^2 with four air-cathodes. A numerical model was developed, based on the secondary distribution of the electrostatic potential inside the cell. It was validated on the experimental data and then used to predict the highest performance that could be reached with a given bioanode included in an optimal MFC architecture.
Another MFC prototype was designed that allowed the air-cathode to be removed in case of biofouling without draining the electrolyte or affecting the bioanode. The air-cathode could thus be replaced by a new one in the course of the MFC operation. Such experiments showed the great impact of initial biofouling of the air-cathode. During the MFC starting phase, fast biofouling of the air-cathode was masked by improvement of the bioanode kinetics. As a result, the power produced by the MFC was stable. In contrast, replacing the air-cathode by a new one after a few days of operation immediately increased the power by a factor of 2.8. It can be concluded that biofouling of the air-cathode during the MFC starting phase occurs more commonly than suspected, which may have resulted in MFC performance being considerably underestimated so far.
Biofouling of the cathode surface can be mitigated in a two-compartment MFC equipped with a cation-exchange membrane. Such a membrane prevents the transfer of acetate to the cathode compartment and thus hinders microbial growth on the cathode surface. Ion transport in MFC electrolyte was analysed by using the concept of transport numbers. This analysis evidenced that protons did not contribute to the carrying of electricity through the electrolyte. Consequently, a proton exchange membrane, as still often used, constitutes a considerable hindrance. In order to avoid the transfer of acetate to the cathode, a cationic membrane may be justified but the theory shows that it is very detrimental to the pH balance between the two compartments. It is consequently suggested to turn towards the promising concept of a “microbial separator”, which could decrease the acetate flux to the cathode without being detrimental to pH balance.

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High-rate microbial electrosynthesis of acetate from carbon dioxide using an enriched mixed culture: role of sulphate

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This work shows the results of two parent MES cells aiming at producing acetate from CO_2 and electricity using a mixed anaerobic culture. The cell configuration and the pH control in the cathode allowed for a very high acetate production when compared to similar reported works. The cathodic potential of the cathode was varied during the operation and the results seemed to indicate that most of the acetate production was using hydrogen as intermediary.

Two parent MES cells with an abiotic anode were inoculated with a mixed anaerobic consortium in the cathode . The cathode was poised at -1V and the system was operated under batch mode. Every week, the cathodic medium was fully replaced to disfavour the planktonic activity and CO_2 was sparged in the solution. The pH of the solution was controlled at pH=6 and 2-bromoethanesulfonate (BES) was dosed to avoid methane build-up. Under these conditions, the cells gave reasonably very good results (2.3 mM/d, Figure 1A) when compared to similar works in the literature. However, after some cycles of operation, a surprising acetate decrease was observed at the end of experiments (Figure 1B). This acetate depletion was gradually increased through the cycles. Acetoclastic methanogenesis was discarded due to the BES presence and was not measured in the bag containing the gases collected. Moreover, an experiment with no power addition and a high initial acetate concentration (Figure 1C) showed that acetate degradation was not linked to any reductive process involving electricity to reduce acetate to other organic compounds. Then, acetate degradation should be related to some electron acceptor present in the medium. However, no oxygen leakages were detected and no nitrite/nitrate was added. We unexpectedly observed sulphate presence in our systems despite it was not added in the initial medium (Figure 1D) and this caused acetate depletion. We detected in several cycles sulphate build-up followed by sulphate degradation. We observed that sulphate appearance was due to BES degradation in our medium. Hence, we removed BES from our system and operated both cells for months with very successful long-term results. Acetate production rate increased up to 3.1 and 4.1 mM/d in each cell without methane build-up.

These results are very high when compared to similar results in the literature and could represent a promising breakthrough in the field. The microbial composition of the cell was analysed through Illumina sequencing giving a high proportion of *Acetobacterium spp.* (up to 65%) in

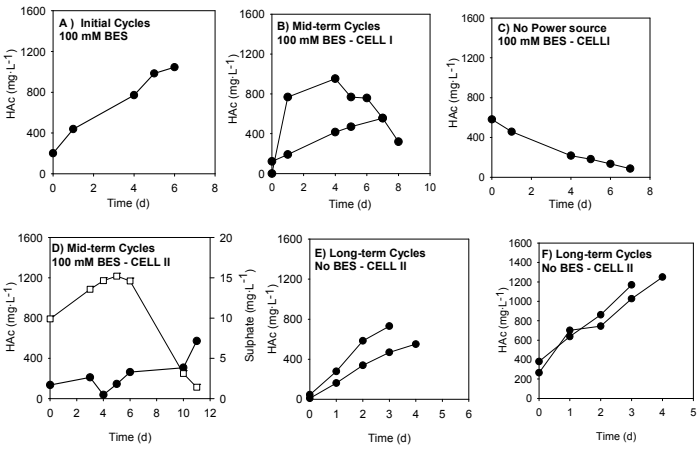

Figure 1. Examples of cycles from each of the experimental periods (● acetate, □ sulphate)

Alternate switching between MFC and MEC for H_2O_2 synthesis and residual removal in Bioelectro-Fenton system

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Sustainable H_2O_2 supply and elimination of residual H_2O_2 are two key challenges to the Fenton processes treating recalcitrant contaminants. In this study, an innovative Bioelectro-Fenton system capable of alternate switching between microbial electrolysis cell (MEC) and microbial fuel cell (MFC) mode of operation was developed to meet the challenges. In the MEC mode, H_2O_2 was electrochemically produced which reacts with Fenton's reagent (Fe II) to form hydroxyradical. The residual H_2O_2 (unused H_2O_2) is removed as electron acceptor by switching the system to MFC mode. Complete decolorization and mineralization of 50 mg L^{-1} methylene blue (MB) was achieved in the MEC mode with apparent first order rate constants of 0.43 and 0.22 h^{-1}, respectively. After switching to the MFC mode, residual H_2O_2 of 180 mg L^{-1} was removed at a removal rate of 4.61 mg L^{-1} h^{-1} while generating a maximum current density of 0.49 A m^{-2}. The MB degradation and residual H_2O_2 removal were affected by external resistance, cathode pH and initial MB concentration. Furthermore, the system performance was enhanced under stack operation. This study provides a proof-in-concept new system for efficient and cost-effective H_2O_2 control and recalcitrant pollutants removal.

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Integration of electrodes into a conventional bioreactor for microbial electrosynthesis

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Green and sustainable processes are a key factor for the upcoming novel energy strategy. Energy derived from renewable energy sources such as solar power plants and wind power plants needs to be stored. Microbial Electrosynthesis (MES) enables the storage of electric energy by converting it into chemical energy while fixing carbon dioxide (Nevin et al. – 2010). Electroactive organisms are able to grow on electrodes as a biofilm utilizing electrons to convert carbon dioxide into valuable products (e.g. acetate, alcohols). Besides, also unbalanced fermentations are a very interesting field allowing the production of compounds from unusual substrates using an electrode to balance the redox equivalents in the cell (Flynn et al. – 2010).
In contrast to Microbial fuel cells (MFCs), where no autoclavable reactors and controls are needed, because usually sludge from waste water is used as a mixed culture, for MES autoclavable reactor systems with control units (e.g. pH, temperature) are needed (Krieg et al. – 2014). Here we represent an electrode integration strategy into a standard bioreactor. The system was evaluated using a *Shewanella oneidensis* based testing system showing current production rates depending linearly on the anodic surface area. The system was enhanced by a separated counter electrode chamber to conduct separated electrochemical measurements. Computational fluid dynamics showed that mixing was not affected negatively by the developed assembly. Furthermore, a cell voltage analysis of the system showed that the medium (electrolyte) was responsible for the highest electrochemical losses.
The assembly allows a flexible adjustment of electrode surfaces hosting plate electrodes of almost any kind. It is easy to apply to any bioreactor and is ready to conduct electro-biotechnological conversions.

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Electroactive biodiversity from deep hydrothermal vents in bioelectrochemical systems

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Microorganisms play a key role in biogeochemical cycles and, consequently, in the functioning of ecosystems. Microorganisms preferentially grow in biofilms composed of diverse microbial species, forming communities with strong inter-species interactions (mutualisms) and a complex trophic chain. Recently, a new type of mutualism called "electronic" mutualism was discovered. Electro-active microorganisms that have the ability to transfer electrons to and from inorganic and insoluble compounds insure this mutualism. This electro-active feature has been studied through microbial bioelectrochemical systems, where microorganisms adhere to the surface of electrodes and form ElectroActive Biofilms (EABs). Currently, there is very little data on the electroactive extremophilic biodiversity.

Thus, to assess the importance of electroactive extremophilic biodiversity, we aimed to study the electroactive biodiversity present in deep hydrothermal vents located in ultramafic contexts with more than 90% of minerals rich in iron and magnesium. Samples of these hydrothermal vents were used as inoculum in anoxic basic media at 72°C in a BioElectrochemical System (BES) with a polarized anode as final electron acceptor. At the same time, flask enrichments were made on acetate, glucose, or yeast extract with iron oxide as an insoluble final electron acceptor. The results in electrochemistry (current density over time) were obtained using a potentiostat. The biodiversity in the BES (EAB and free in the media), and in the flasks were carried out by the MiSeq method.

During these experiments, the current obtained in the BES confirmed the presence of electroactive hyperthermophilic microorganisms from hydrothermal vents, which had never been shown before. The microorganisms involved in these extracellular electronic transfers have been identified predominantly as *Archaea* and are mainly found in the EAB covering the electrode (*Thermococcales*, *Archaeoglobales*). Conversely, the planktonic cells of the BES consist essentially of microorganisms in the domain of *Bacteria*. Similarly, the enrichment in flasks with an insoluble electron acceptor (iron oxide) led essentially to the selection of bacteria from the phylum *Firmicutes* (*Clostridiales* and *Bacilli*). The main hypothesis that emerges is that the polarized electrode allows the development of a specific biodiversity essentially composed of *Archaea*. To support this premise, specific experiences of re-enrichment in a BES of flasks enrichments, showed that we were actually able to re-enrich *Archaea* on polarized electrodes by promoting the development of genus *Geoglobus*, *Ferroglobus* and *Thermococcus*.

To conclude, we can say that culture in polarized BES at 72°C allows the enrichment of electroactive microorganisms (hyper)thermophilic from deep hydrothermal vent to obtain a larger global biodiversity than in our growing conditions in flasks. The results will certainly give a new dimension to microbial ecology based on the study of biodiversity built around obligatory "electronic" mutualism. Finally, these findings should lead over time to innovative applications on energy and CO_2 capture.

The effect of storing inoculum on the start-up time and the electricity production of the microbial fuel cell

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Microbial fuel cells (MFCs) require sometimes a long start-up time. Especially, if the inoculum is not taken from an MFC operated under similar conditions, the lag time before power density reaches a relatively stable level, can be 20-40 days. In our preliminary experiments with an up-flow MFC, electricity production started after operating the reactor for 40 days at 37 °C with xylose as substrate and anaerobic sludge from municipal wastewater treatment plant as inoculum. Also old MFC anolytes stored at -20 °C for 1-2 years were tested as inoculum, but only very low power densities (less than 10% of the power density obtained in successfully enriched MFCs) were obtained. As it is not always possible to start MFCs with fresh inoculum from similar operating conditions the aim of this study was to assess the effect of storing anolyte solutions of working MFCs for different time periods.

Electricity generation was studied in 3-chamber MFCs (one anode chamber between two cathode chambers) equipped with two carbon brush anode electrodes and two carbon cloth cathode electrodes. Aerated buffer solution (pH 7) was used as catholyte, and the cathode chambers were separated from anolyte (500 mL) with Pt:Ni (1:1) coated CMI7000 membranes. Pt:Ni coating was in close contact to both membrane and cathode electrode. MFCs were fed with 1.0 g/L xylose in buffered solution with feeding intervals of 7-8 days. The reactors were heated by circulating the anolyte via bottles in 37 °C water bath. A mixture of effluent and anolyte from an operating up-flow MFC was used as inoculum in duplicate MFCs without any storing and after storing it at -20 °C for 1 and 6 months.

The lag time for reaching a cell voltage of 100 mV (0.2 W/m^3) was 2 days for the MFCs with fresh inoculum, and 6-9 days after storing the inoculum for one month. After storing the inoculum for 6 months, the cell voltage remained below 30 mV (0.02 W/m^3) during the whole experiment (28 days). No significant differences in stable power density values were measured between the MFCs with fresh inoculum and the inoculum stored for 1 month (0.5-0.6 W/m^3 and 0.4-0.65 W/m^3 respectively). Linear sweep voltammetry results (measured after the last feeding) enforced the findings, by showing significantly lower current densities for the MFCs inoculated after storing the inoculum for 6 months compared to the other MFCs. VFA concentration measurements revealed that acetate and butyrate accumulated in anolytes of all the MFCs, especially when electricity production was low. These results suggest that long-term storing of the inoculum at -20 °C reduced the activity of exoelectrogenic microorganisms, but had less significant effects on activity of the bacteria fermenting xylose to VFAs.

Investigations of the molecular mechanism behind 2,3-butanediol interspecies communication and synergistic electroactivity in *Pseudomonas aeruginosa* co-cultures

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Microbial communities evolve into consortia characterised by intricate interactions and synergisms. Such consortia have also been shown to occur in undefined bioelectrochemical systems (BES) cultures where the synergistic interactions confer or lead to increased electro-activity. The composition of such communities has been investigated and phenazines that enhance mediated electron transfer have been reported to play a major role in conferring electro-activity. *P. aeruginosa*, one of the dominant members of these communities produces increased amounts of the phenazine redox mediators in a synergistic interaction involving the transfer of fermentation products, and more recently, 2,3-butanediol (2,3-BD) has been reported as one of products that enhance the production of not only phenazines but also many of the other *P. aeruginosa* virulence factors. However, the mechanisms by which 2,3-BD influences the phenazine redox mediator production are yet to be fully understood. Gaining a deeper understanding of these mechanisms will provide a basis of defining co-cultures that effectively utilise this natural phenomenon for many applications besides current production. We therefore endeavour to understand the mechanisms behind the influence exerted by 2,3-BD and consequently increased phenazine production. Since the production of phenazine is one of the events controlled by the extensive quorum sensing (QS) and virulence generation circuit, we explore the overall production of virulence factors in order to understand this interspecies communication. Using mutants of specific pathways for 2,3-BD synthesis, utilization, and signalling and subsequent determination of the levels of virulence generation, we seek to provide insight into the role of 2,3-BD signalling or metabolism in the enhancement of phenazine production and virulence of *P. aeruginosa*. Our findings will be applicable in optimisation of BES ecological conditions, especially to foster synergistic interactions towards increased current production. Since these interactions influence the overall virulence factor generation in *P. aeruginosa*, understanding the mechanisms of interactions will not only be fundamental in improving performance of microbial fuel cells, but also help understand the physiology of *P. aeruginosa* in other ecological niches.

Bacterial competition for the colonization of anodic surface in microbial fuel cells

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Microbial fuel cells (MFCs) have been recognized as a potentially sustainable biotechnology that enables the direct conversion of organic matter from wastewater into electricity using bacterial biofilms as biocatalysts. Currently, one of critic step in MFC efficiency is the initial colonization of the anode which affects the final stabilized electricity production. During this initial colonization, electroactive bacteria (EAB) compete with the other members of the bacterial community to adhere on the anode. Many studies show the impact of hydrophobic/hydrophilic surface characteristics on bacterial adhesion on non-conductive surfaces, however the impact of electrical parameters such as the anodic potential - which is a critic parameter in MFCs- on bacterial adhesion is poorly understood. One of particularities of EABs, such as *Geobacter* or *Shewanella,* is that they have a more electronegative surface than most bacteria. The anodic potential might play a role in colonization of the anodic surface by modifying electrostatic forces between the bacteria and the anode. In order to study the bacterial adhesion under different anodic potentials, a MFC with a stress shear chamber configuration was designed. The shear force on the anodic surface is controlled by the intensity of the flow. The adhesion of bacteria on a graphite anode was monitored at three different shear stresses (1mPa, 10mPa and 50mPa) and two anodic potentials (-200mV and +200mV vs Ag/AgCl). The colonization of the anode by bacteria from activated sludge was evaluated as a function of the time by fluorescence microscopy. For each time point, DNA from anodic surface was extracted to determine the dynamic nature of the bacterial community structure. This analysis was compared with the MFC electricity production in order to understand anodic-bacterial interaction and electron transfer. These results will help us understand the effect of the anodic potential on bacterial colonization in order to improve MFC performance.

Microbial Electroreduction of Biomass Intermediates to Tailor-Made Fuels

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Our work on microbial electroreduction is part of the German cluster of excellence "Tailor-Made Fuels from Biomass" at RWTH Aachen University. The overall goal of this interdisciplinary collaboration is the analysis of novel and the optimization of established methods to produce tailor-made fuels from biomass. The usage of whole cell microbial biocatalysts with solid state electron donors/ acceptors seems to be a very promising strategy to combine certain production steps for target biofuels to avoid energy intensive precursor purification and the use of non-renewable H_2 as reductant. Our main goals are to understand microbial electrophysiological processes, to design and to manipulate the important transformation pathways, and to utilize them for new bioelectrochemical productions. Thereby, itaconic and levulinic acid are being used as model target compounds to be reduced into fuel precursors in bioelectrochemical systems.

Since only a few microorganisms with electroreduction abilities have been identified, the screening and characterization of new cathodic biocatalysts plays an important role in the development of the field and we recently presented new candidates.

In another part of this project, we want to enhance the electroreduction activity of the well characterized cathodic biocatalyst *Clostridium ljungdahlii* by controlled evolution. Therefore we constructed a mutator plasmid, which gives us the possibility to perform a rapid whole cell evolution. This is a powerful tool to enhance the growth and product formation under reactor conditions and to adaption to important process parameters. With this approach, we were able generate a new variant of *C. ljungdahlii*, which readily growth at pH 3.5 – the target pH for itaconic acid conversion.

The third part of this project deals with the genetic engineering of *C. ljungdahlii* to equip this biocatalyst with reduction pathways for itaconic acid transformation to fuel components. In a first step, we introduced genes for the activation of itaconic acid with Coenzyme A. At the moment several options for the further reduction of itaconic acid are under investigation. One very promising way is the reduction with reductases that are ferredoxin-independent or ferredoxin-dependent.

Combined, these parts should generate an efficient biocatalyst that uses electrons from the cathode to produce new biofuels from itaconic acid.

Organic Dye Degradation from textile industry waste in Microbial Fuel Cell

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Microbial Fuel cell (MFC) has the ability to generate power from bioremediation of organic wastes from wide range of wastewater, including wastewater from animal sources, domestic and food processing but potentially also from organic waste from textile industries. In this study MFC is employed to degrade the toxic azo dye: Reactive Orange 5 (RO5). RO5 is selected on the base of its chemical nature and molecular structure, i.e. azo bond para position to sulphonated electron withdrawing group, which helps the withdraw of electrons from dye compound during electrochemical oxidation-reduction reactions. RO5 cannot be degraded aerobically directly due to complex aromatic structure. Current study was undertaken using sequential anaerobic-aerobic treatment. Eventually metabolites formed by anaerobic treatment replace organic substrate required for aerobic growth and mineral efficiency is enhanced. The goal is to degrade azo dye bonds anaerobically in the microbial fuel cell to form lower molecular weight aromatic amines that can be further degraded by aerobically degradation. For the study, a flow cell has been developed using passive air cathodes based on activated carbon-PVDF porous electrodes[1] and anodes of electrospun carbon fiber felt from lignin source[2] or cellulosic composite carbon anode. The biofilm at the anode is composed of a geobacter culture. The electrochemical performance and degradation efficiency is measured using electrochemical techniques and UV-vis. The novel cell-design has potential to be scaled-up for industrial use because of the low cost materials.

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Elucidating the extracellular electron transfer mechanisms of gram positive bacteria

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Extracellular electron transfer (EET) has been gaining increasing interest in the development of bioelectrochemical technologies (BETs), either for energy production in the case of microbial fuel cells (MFC) or for the synthesis of added value compounds in microbial electrosynthesis. Most of the studies in these systems have been concentrated on devices operating at 20-30ºC and the corresponding mesophilic organisms. However MFCs operated at elevated temperature hold much promise due to potentially higher rates of metabolic activity, the easier maintenance of anaerobic conditions and the elimination of pathogenic organisms [1,2]. *Thermincola potens* JR and *T. ferriacetica* are both electrogenic thermophilic Gram-positive bacteria capable of generating current from acetate on anodes of a MFC operating at 65ºC [1,2]. Interestingly, like the well-studied Gram-negative mesophilic bacteria, these organisms contains in their genome several genes that code for multiheme *c*-type cytochromes [3,4], which were proposed to be involved in EET. In order to understand the mechanism by which Gram-positive bacteria transfer electrons across the thick cell wall made of peptidoglycan and are capable of performing EET, it is necessary to characterize these multiheme cytochromes. Toward this aim, we developed a strategy to over-express heterologously multiheme cytochromes from Gram-positive bacteria [5], allowing their structural and functional characterization. This knowledge is crucial not only to understand how Gram-positive bacteria perform EET, but also to guide the rational improvement of BETs, in particular MFCs for energy harvesting and wastewater treatment.

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Electrobioleaching applied to a pegmatite lithium ore

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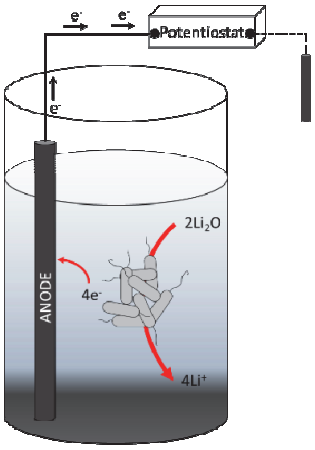
The recovery of lithium from lepidolite (1.1% Li_2O) by electrobioleaching is investigated. The present study arose based on the experience gained by research in bioleaching of a lithium ore from the central-north part of Portugal. Electrochemical bioleaching or electrobioleaching is an advanced hydrometallurgical process that has been tested with *acidithiobacillus* bacteria in the treatment of high grade complex sulphide ores, namely copper and zinc. Lithium is the lightest metal with a wide range of applications (industrial, technological and energy). Its high economic importance due to increase demand and small global abundance, has boosted the need for technical and environmentally alternatives to the conventional hydrometallurgical processes. Bioleaching has been proven to be a potential alternative method to common hydrometallurgical processes. However, it presents the disadvantage of very slow kinetics that are prohibitive for the requirements of industrial scale. The results obtained with native heterotrophic microorganisms in the lepidolite bioleaching tests are encouraging when compared to the results recently published by Marcinčáková et al. (2015). However, because we are committed to increase recovery and reduce the process time for competitive and manageable levels required at industrial scale, we intend to accelerate the kinetics of lithium electrobioleaching using a bioelectrochemical system. To this aim, batch experiments were carried out to evaluate the feasibility of using insoluble electrodes to stimulate mediated microbial lithium catalysis.



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Improving the performance of microbial fuel cells using laccase-based biocathodes

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Microbial fuel cells (MFCs) hold great promise for the simultaneous treatment of wastewater and electricity production. However, their performance is currently hampered by high cathodic potential losses and cost. Laccases are multi-copper oxidoreductase enzymes that could be used as cathode catalysts in MFCs to improve the cathode performance and reduce the cost of catalyst used.

In the present study laccase from *Trametes versicolor* was immobilised on electropolymerised polyaniline (PANI) via cross-linking in the absence of redox mediators. A comparison of performance of an MFC utilising laccase in the freely suspended state with the enzyme immobilised was studied. Characterisation of the laccase/PANI/carbon biocathode was carried out using the ABTS assay (enzyme activity), FTIR analysis (molecular analysis) and SEM (morphology). Electrochemical measurements were used to determine MFC performance.

FTIR spectra indicated clear cross-links between laccase and PANI, characterised by the disappearance of peak at 1042 nm and 1167 nm due to the COOH functional group of glutaraldehyde cross-linking with laccase. The activity of laccase on the surface of the electrode was 25mU of enzyme per cm^2. The SEM image showed that PANI formed a uniform coating over the carbon microfibers with no change in morphology on laccase cross-linking. Laccase/PANI/C biocathode showed higher stability and activity, producing a power density of 34 mW/m^2 compared to 6.5 mW/m^2 for the freely suspended enzyme. Laccase activity measurements indicated that for the freely suspended enzyme, the enzyme remained active for 2 days after which there was constant deactivation. The immobilised enzyme biocathode was stable for a period of 5 days after which it started to decrease. The results suggest that immobilisation of laccase by cross-linking with PANI is a useful way of improving the performance of cathodes in microbial fuel cells.

Microbial electrosynthesis of acetate with reduced graphene oxide–tetraethylene pentamine-modified cathode and a novel *S. ovata* adapted to reduce CO_2 faster.

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Microbes can reduce CO_2 into multicarbon chemicals with electrons acquired from the cathode of a bioelectrochemical reactor via a process termed microbial electrosynthesis (MES). Two fundamental challenges to achieve highly-productive MES are to increase electrons transfer rates from the cathode to microbes and develop microbial catalysts reducing CO_2 into multicarbon compounds more efficiently. In this project, carbon cloth cathodes modified with reduced graphene oxide functionalized with tetraethylene pentamine (rGO-TEPA) were readily self-assembled in the cathodic chamber of a MES reactor. Electroactive biofilms with unique spatial arrangement were subsequently formed between *Sporomusa ovata* cells and rGO-TEPA nanoparticles resulting in more performant MES process. The acetate production rate from CO_2 was increased 3.6 fold with the formation of dense biofilms when wild type *S. ovata* was combined with rGO-TEPA. When a recently-developed strain of *S. ovata* adapted to reduce CO_2 faster (Tremblay et al., 2015) was used as the microbial catalyst instead of the wild type, acetate production rate was even higher with an augmentation of 11.8 fold. Furthermore, a highly-structured biofilm including multiple bioinorganic spherical structures, probably consisting in networks of rGO-TEPA and bacterial cells was formed. The three dimensional biofilms observed in this study enabled highly-effective electric interaction between an upgraded *S. ovata* strain and the cathode, demonstrating that the development of dense cathode biofilm and better microbial catalyst can be combined to improve MES productivity.

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Profumo di microbi- What is the essence of microbial electroactivity?

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Microorganisms performing extracellular electron transfer (EET), so called „electroactives" are the fundament of primary microbial electrochemical technologies (MET). However, only a few microorganisms, like *Geobacter* or *Shewanella,* are studied in detail, e.g. for their electron transfer mechanisms. Many more species are only globally assigned to be electroactive, but mechanistic knowledge is generally missing. In this respect, the question arises: What is the essence of microbial electroactivity?
Therefrom specific questions arise, for instance: Which kind of interactions between microbial cells and electrodes have to be considered? How relevant are these interactions for the microbial metabolism? Starting from these questions we will illustrate that a concerted action by researchers in the field is necessary to define standards or at least a common sense of microbial "electroactivity" for addressing fundamental questions and fostering the development of MET.

Or in other words: *The scent of electroactive microbes needs to be distilled*.

La scuderia bioelettrochimica - Bioelectroreactors as platform for engineering METs

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Microbial electrosynthesis is the recent offspring of microbial electrochemical technologies (METs) (Schröder *et al.* 2015). The MET technology platform allows the bidirectional conversion of electric energy into chemical energy stored in biomass as well as potential platform and fine chemicals with its reactors being usually termed bioelectrochemical systems (BES).
The reactor systems used for microbial electrosynthesis, i.e. BES-based bio-production, so far reported in literature (Harnisch *et al.* 2015) are relatively small in scale and highly diverse in on their architecture and modes of operation. The often diverging requirements of the electrochemical and the biological processes and the interdisciplinarity of the field make the engineering of these BES a special challenge. This has led to multiple, differently optimized approaches of reactor vessels, designs and operating conditions making standardization and normalization or even a systematic engineering almost impossible. This lack of standardization, scalability hence knowledge driven engineering is the driving force for this work. The different prototypes of our BES up-grade set (Harnisch *et al.* 2015) will be shown, and we will go through the design process and evolution of our prototypes for integration with existent bioreactor/ fermentor systems. These systems allow interfacing into the existing infrastructure of conventional bioreactors for running bioelectrosynthesis, with the added electrochemical control of the introduced electrodes. Our results growing pure cultures of *Shewanella oneidensis sp.* (Rosa *et al.* 2016) as well as other model organisms clearly show that these systems can be used to control, monitor and scale up microbial bioelectrochemical processes.

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Le nozze elettrochimico di Figaro – Interfacing microbial waste degradation with electroorganic synthesis

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Natural resources are becoming scarce and hence, there is an increasing demand for bio-based liquid fuels and chemicals harvested by sustainable resource management strategies. Among others, microbiome-based fermentation technologies to upgrade low grade biomass to value added products are a promising platform technology. Short- and mid-chain volatile fatty acids (VFAs) that can be produced by microbiome-based anaerobic digestion of waste were rediscovered as valuable platform chemicals. One particular process of interest is the mid-chain VFA production (chain length of C_4 to C_6) based on ethanol (C_2) and acetate (C_2). Similar to biotechnology, electroorganic synthesis distinguishes themselves by mild operational conditions (e.g. pressure, temperature) and by replacing hazardous reactants by electrons from conventional petrol-based technologies. Mid-chain VFAs from the fermentation broth can be further upgraded by applying electrochemistry, i.e. Kolbe-and non-Kolbe reaction, to yield easily storable and energy-dense gaseous and liquid hydrocarbons and esters. Therefore, these two technologies are merged in microbial electrorefineries, a promising conversion and storage concept that can be used to buffer the highly fluctuating demand of electric energy.

In this study, enriched reactor microbiomes were applied in lab scale bioreactors (2.2 L working volume) for the conversion of acetate (C_2) and ethanol (C_2) into a mixture of VFAs. Within only 3.5 days 370 mM ethanol and 175 mM acetate were converted into 121 ± 3 mM butyrate (C_4) and 118 ± 2 mM caproate (C_6). A higher product concentration especially of caproate was hindered due to end-product inhibition by the protonated form of caproate. In order to avoid the inhibition of microbial activity, an effective *in-situ* removal of carboxylic acids from the fermentation broth *via* reactive extraction is envisaged.

Within this study we show the feasibility and evaluate the production of carboxylic acids during fermentation, the extraction efficiency, and the performance of the electroorganic synthesis of alkanes and esters to engineer a bioelectrorefinery cascade. The stable and fast production of butyrate and caproate by the used reactor microbiome combined with the fast electrochemical upgrading of VFAs thereby shows the high potential to become an efficient storage concept for electrical energy in liquid fuel.

Effect of the applied voltage and the initial concentration on the mec performances for h_2 production

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The biohydrogen yield can be increased when dark fermentation is coupled to microbial electrolysis cells (MEC). In this work we focused on the effect of variation of applied voltage and initial concentration on the performance of a single-chamber MEC. The substrate was a mixture of volatile fatty acids simulating a dark fermentation effluent (30% acetate, 13% propionate and 57% butyrate respect to COD). The anode was made of graphite felt whereas the cathode was made of nickel foam. Anode was colonized with anaerobic sludge. MEC were operated at 30 °C and pH 7 with a hydraulic retention time of 48 h. The experiment was conducted varying the applied voltage (E_{ap}) (3 cycles per condition) following the next order: 0.5, 0.2, 0.5, 0.7 and 0.3 V, and maintaining the initial COD concentration constant at 2g/L. Another set of experiments were carried out varying the initial concentration at 0.6, 1.0, 2.0, 1.0 and 0.6 mg-COD/L, maintain the E_{ap} constant at 0.5 V. The performance of the MEC was evaluated (potentiostat/galvanostat VSP, BioLogic Sci Inst). High hydrogen production rates (up to 1.51 m^3H_2/m^3-d) were observed. The H_2 purity was 84%.No effect on the MEC performances was observed when the E_{ap} was varied (Fig. 1) up to 0.5 V. At 0.7 the performance decreased. This was attributed to a cathode contamination by the formation of contaminant compounds as nickel hydrides (Jeremiasse et al. 2010). It was observed that the initial substrate had no significant effect of the MEC performances, except for the last conditions (Fig. 2). As in the previous set of experiments it seems that the major effect is the operating time. For both cases, irrespectively of the initial condition the MEC performances decreased after 32 days. These low performances are result to the consumption of acetate and H_2-produced by methanogenic microorganisms and is compounded by the oxidation of hydrogen at electrons in the anode leading to increased coulombic efficiencies (C_E) over 100 % (Lee et al., 2009). All experiments presented a C_E higher than 100% (151-541 %).

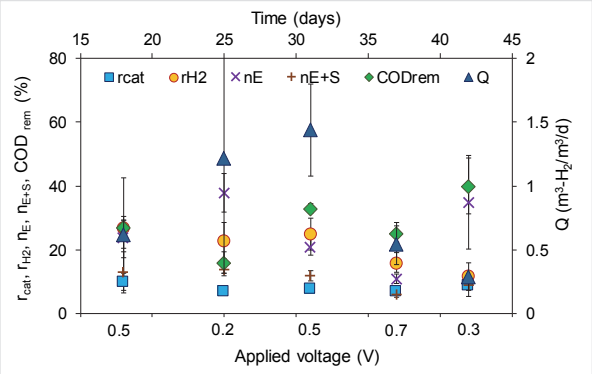

Fig 1. Effect of E_{ap} variation

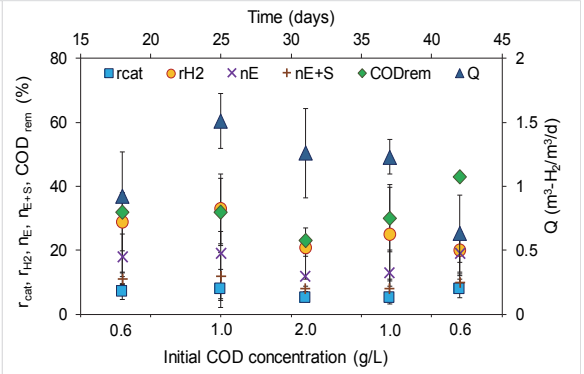

Fig 2. Effect of initial concentration variation

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Low-cost and efficient mfc materials for bioelectricity production from waste materials

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For microbial fuel cells (MFCs) to be commercially viable for large scale bioelectricity production from waste streams, low-cost and efficient electrode and separator materials are required. These materials must be compatible with the microorganisms used and with the waste streams treated as well as easy to scale up for large scale systems. The electrode materials should also have high electrical conductivity, high surface-to-volume ratio and good corrosion resistance. The separators should allow efficient proton transfer but prevent oxygen diffusion from cathode to anode.

Low-cost and efficient MFC materials for bioelectricity production from waste materials (Bio-e-MAT) is a collaborative project between Tampere University of Technology (Finland), Yildiz Technical University (Turkey), CSIR-Indian Institute of Chemical Technology (India) and Indian Institute of Technology Delhi (India) funded under the INDIGO funding programme. The specific aims of the project include: 1) developing low-cost and efficient electrode and membrane materials for electricity production in MFCs, 2) developing a novel MFC design, 3) testing the biocompatibility of the electrode materials with exoelectrogenic cultures, and 4) optimizing electricity production from different waste materials and studying the suitability of the new MFC materials with different waste streams. The project is expected to result in MFC technology that is more feasible for industrial applications and that will improve environmental protection through enhanced treatment of waste materials and by decreasing fossil fuel based energy production.

Recycling of food waste by inoculation of vermicomposted organic matter into mfc (microbial fuel cell) energy harvester

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Food waste is an emerging source of environmental pollution, however, there is no definite solution to treat it other than to process into animal feed. Vermicomposting is a good alternative to flushing into sewage or making animal feed since the earthworms in the vermicomposting facility can digest food waste into organic compost which is greatly beneficial to plant cultivation as well as they reduce the volume of waste.
There is plenty of organic matter in the vermicompost, which means that it could possibly be utilized to production of electric energy if it is feed into the Microbial Fuel Cell (MFC) [1]. Moreover, among many microbiologic species living in the compost, it is highly likely that there live the same geobacter species which are employed in the MFC to produce electricity, because they are usually seen in the soils and many organic wastes as well.
In this study, we proposed a novel energy harvesting from food waste by inoculation of vermicomposted organic matter into MFC in order to produce electricity while decomposing organic waste by catalytic activity of geobacter species which already live in the compost. We designed and implemented a prototype of vermicomposting MFC energy harvester. and measured its output voltage for 4 weeks. The voltage-time profile and current-voltage (CV) characteristic showed a maximum open-circuit voltage of 0.75 volts and maximum power of 0.41mW as shown in Figure 1. The power scale of MFC is suitable to be used in the practical applications like wireless sensor systems.
Further study will focus on its applications which are energy self-sustainable so that it may encourage more installation and utilization of the proposed food waste energy harvester. The electric energy produced by MFC will supply power for the embedded sensor module, which realize energy self-sustainable device. The proposed system is expected that it can contribute to the popularization of a sustainable recycling of food waste.

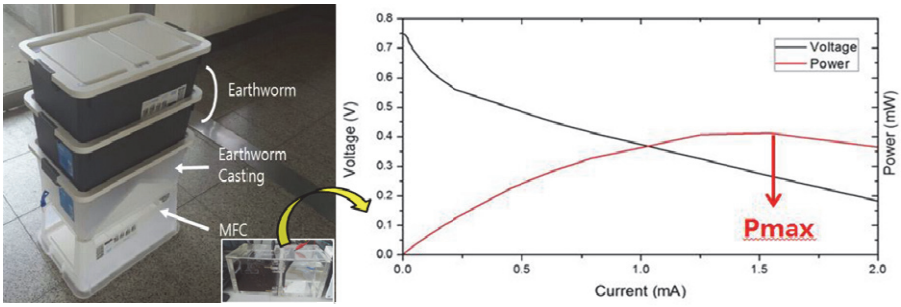

Figure 5. Vermicompost MFC system and its output characteristic

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Benthic microbial fuel cell powering an autonomous sensor node

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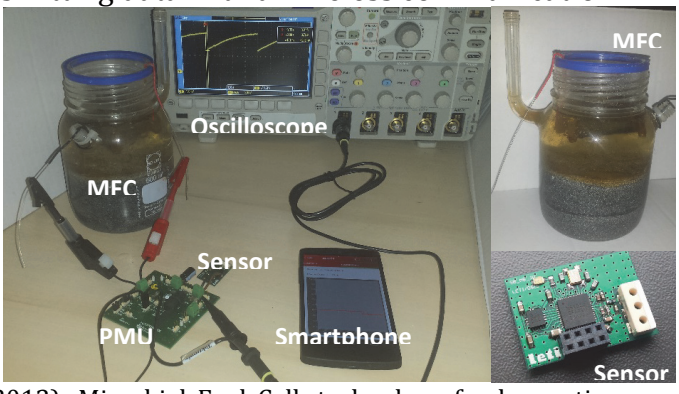
Harvesting energy in the surrounding environment is an advantageous alternative to conventional batteries for powering autonomous remote sensors. In aquatic environment where solar, thermal and vibration sources are inadequate, Microbial Fuel Cell is a promising energy harvester to durably power sensors [1, 2]. The MFC is a relatively mature concept but the generated power is not directly usable to power continuously low power sensor node. Different approaches are used to overcome this problem. MFCs can be stacked in series and parallel in order to have higher voltage and power. Another possibility consists in using just one MFC and boosting the voltage with electric converters. Some researchers propose dedicated circuits to efficiently harvest the MFC energy [3] but these circuits are not commercially available for a company which would like to massively deploy these harvesters.

The aim of this paper is to demonstrate the efficient association of a centimeter-scale, low-cost and close-to-real-conditions benthic MFC, with a commercially-available circuit designed for other scavenging sources, in order to power a sensor node measuring and wirelessly transmitting useful data in seafloor environment. Our goal is also to give the first steps of methodology to configure the interface circuit to autonomously extract the maximum power from the MFC.

We will explain the fabrication of our low-cost MFC and present its electrical characteristics. In steady-state, the maximum power produced by the MFC was 101µW under 320mV. We have characterized the commercial off-the-shelf electric converter used as interface circuit and show how to adapt its operation to extract dynamically the maximum power of a MFC. The interface circuit, configured to extract the maximum available energy, provides 47µW at 3V in steady-state, which allows to power a wide range of environmental sensors. We finally used the association of the MFC with the interface circuit to power autonomously a sensor node, consuming 100µJ every 4s for intermittently transmitting data with a wireless communication.



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CO_2-Conversion to butanol by Microbial Electrosynthesis

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The project CO2TRANSFER aims the synthesis of alcohol like butanol by using CO_2 and electrons in a microbial electrolysis cell (MEC). This new approach addresses two problems: (I) Up to now it is not possible to effectively store electricity on a large scale. (II) The emission of carbon dioxide contributes to the greenhouse effect. The new technology offers a possibility of storing electricity from renewable energies like wind, water and solar energy in an environmentally friendly way. For the reduction of CO_2 to butanol 24 electrons are needed. Acetone-butanol-ethanol fermentative microorganisms are saccharolytic clostridia normally grown on glucose or complex carbohydrates. In this project the metabolic pattern of these microorganisms will be observed when they are provided with CO_2 and electrons. Beside pure also mixed cultures will be studied. Microorganisms will be tested for their ability to grow on electrode materials and take up electrons directly from the cathode. If the direct approach turns out not being possible, it will be tried to produce butanol via an intermediate using a co-culture. *Sporomusa ovata* produced acetate via direct electron transfer, which was proved by Nevin et al, 2010. In a second step acetate will be the source for the production of alcohol by microorganisms. All experiments are performed in two compartment cells (2 x 250 mL) with anode and cathode chamber separated by a Nafion membrane, allowing proton transport. A carbon felt (2.5 x 9 x 0.6 cm), with a titanium wire as electrical contact, serves as working electrode and an Ag/AgCl in 3M KCl electrode is applied as reference electrode.

Nevin P.K., Woodard T.L., Franks A.E., Summer Z.M., Lovely D.R., 2010. Microbial electrosynthesis: feeding microbes electricity to convert carbon dioxide and water to multicarbon extracellular organic compounds. mBio 1(2):e00103-10. doi:10.1128/mBio.00103-10.

Electrochemical analysis of electron transfer mechanisms in mixed-community microbial biofilms from freshwater sediment

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Electrochemical impedance spectroscopy (EIS), which imposes only very small potential perturbations to the system under study, represents a powerful non-intrusive method for the testing and the diagnosis of bioelectrochemical systems (BES)[1].
The majority of EIS studies on BES have been done in whole cell configuration and in open circuit condition, rarely giving useful information about electroactive microbial biofilms[2]. Interestingly EIS can have a tremendous potential for direct investigation of bioanodes performing measurements in 3-electrodes set-up and closed circuit conditions.
The aim of this study is to investigate the electron transfer mechanisms that occur in mixed-community biofilms through EIS analysis.
In this work an environmental sample from freshwater sediment (Valle d'Aosta, Italy) was used as inoculum to obtain mixed community electroactive biofilms. In particular, two different chemical enrichment methods (Fe(III)-Citrate medium and general anaerobic medium) were employed to enrich and select two different microbial consortia, which were inoculated in BES.
Polarization Tests, Electrochemical Impedance Spectroscopy and Cyclic Voltammetry characterizations were carried out in order to study the different processes occurring inside the anodic compartment of BES, and their dependence on the diverse microbial communities. In particular bioanode EIS measurements were performed in 3-electrode configuration under different working conditions, with various external load ranging from 8.2 to 0.1 kΩ. Moreover impedance spectra were recorded also in non-turnover and abiotic conditions, to give a better interpretation of the obtained EIS experimental data.

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Electrochemical analysis of permselective properties of anodic biofilms in microbial fuel cells

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Microbial fuel cells (MFCs) employ electrochemically active bacteria to capture the chemical energy contained in organic substrates and convert it to electrical energy. Bacteria adhere on the MFC electrodes and form biofilms, allowing diffusion of substrates and products, which facilitates extracellular electron transfer (EET). In this biofilm structure, bacteria are enclosed in a self-developed polymeric matrix of a primarily polysaccharide material that can hinder the diffusion of species. It has reported that the biofilm produces more current when the biofilm thickness is not too thick or too thin. If the biofilm is too thick, it acts as insulation and reduces both electron transfer and organic substrate diffusion. On the other hand, if the biofilm is too thin, it has too few bacteria to extract the electrons from the organic substrate.
The main objective of this work was to study the influence of biofilm permselectivity on the electrical production of MFCs. The permselective properties of different structures of biofilms formed on anodic electrodes were studied by means of electrochemical voltammetric and impedance techniques. The biofilm permeability to molecular species was evaluated using a negatively-charged redox species $(Fe(CN)_6)^{4-}$, a positively-charged redox probe $(Ru(NH_3)_6)^{3+}$and neutral redox probes (ferrocenemethanol, dopamine). The model used in this study was based on membrane permeation assuming the biofilm as a viscous, concentrated polyelectrolyte solution bonded to the electrode in which electroactive species penetrate and diffuse. The value of the effective diffusion coefficient of the different probes within the biofilm was determined. The biofilm permselectivity was studied according to the physical structure of the biofilm (thickness, density, bacterial distribution…) and its microbial community structure. We also investigated the influence of the biofilm permselectivity on the electricity production of MFCs that were operated under similar conditions.

Challenges in upscaling microbial electrochemical reactors for copper recovery from acidic leachates

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Microbial electrochemical reactors have been examined for energy-efficient recovery of metals from wastewater (e.g. Ter Heijne et al. 2010). Metal recovery from acidic leachates of municipal solid waste incineration fly ash and contaminated soil is particularly interesting because of the high metal content and the urgent societal need to manage these materials. In previous studies, we have demonstrated that ml-scale systems with over-dimensioned anodes can be used (Modin et al., 2012; Fedje et al., 2015). In this study, we investigated a continuous litre-scale reactor with scalable design for Cu recovery from highly acidic leachate. The anode was fed with municipal wastewater amended with $NaHCO_3^-$ and $NaCH_3COO$, and the cathode was fed with a synthetic leachate containing NaCl, HCl, and $CuSO_4$. An anion exchange membrane (AEM) was applied as separator between the reactor chambers. In a separate test, we examined diffusion of Cl^- through the AEM in an abiotic reactor.
The most challenging aspect in the operation of the reactor was to control the pH in the cathode and anode chambers. The cathode should have a low pH, which favors reduction to metallic Cu and prevents precipitation of e.g. $Cu(OH)_2$. The anode needs to be operated near neutral pH to facilitate efficient bioelectrochemical activity. The diffusion test showed that Cl^- accompanied by H^+ rapidly diffused through the AEM and caused acidification of the anode compartment. This restricted the use of a very acidic catholyte as cathode feed. Initials attempts feed small volumes of 1M HCl resulted in a rapid drop in anolyte pH and a sharp decrease of bioelectrochemical activity (Fig. 1). When the reactor was operated with less concentrated acid in the catholyte, the catholyte pH rose to >5 leading to blue $Cu(OH)_2$ precipitates in the cathode chamber. Thus, careful control of catholyte pH is important.

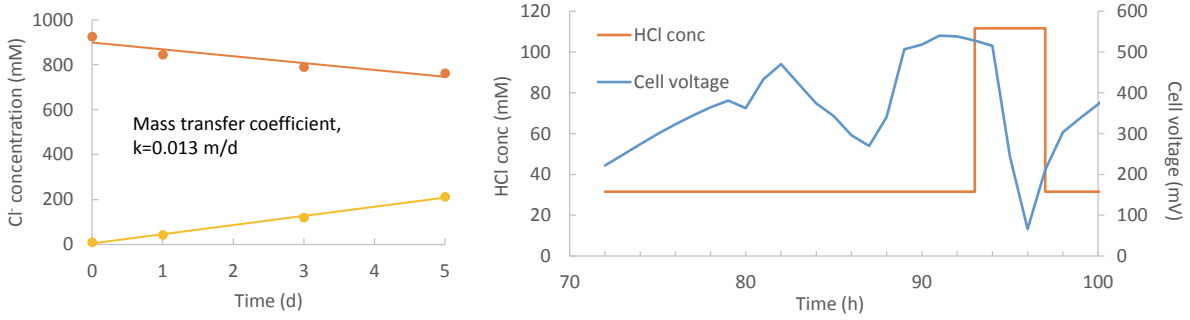

Fig. 1. (Left) Diffusion of Cl^- through the AEM. (Right) HCl concentration in catholyte and cell voltage over a 100 Ω resistor.

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Ter Heijne, A., Liu, F., van der Weijden, R., Weijma, J., Buisman, C.J.N., Hamelers, H.V.M. (2010) Copper recovery combined with electricity production in a microbial fuel cell. Environmental Science & Technology, 44: 4376-4381

Preconditioning electroactive biofilm communities to improve treatment and energetic performance of microbial electrochemical technologies

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This study mainly focuses on two aims: Firstly, on approaches for sophisticated biofilm conditioning procedures in a complex artificial wastewater to achieve preconditioning effects concerning real wastewater applications; secondly, the effects of the conditioning procedure with regard to energetic aspects of microbial electrochemical technologies.

The preconditioning of electrochemically active microorganisms leads to enhanced and sustained electrocatalytic biofilm performance [1]. This study addresses the transfer of this principle towards real wastewater applications. For biofilm growth during MEC mode operation, the potential of the anode does not necessarily have to be positive (vs. SHE) [2, 3]. While for acetate as the sole substrate (which is mainly used in common synthetic media) the anode potential does not have a distinct effect on the microbial community composition [3] but has been shown to effect the electrocatalytic performance; its differing influence on biofilms grown in a more complex media is examined. This is then related to improving energetic efficiency of microbial electrochemical technologies with regard to specific cathodic operating conditions.

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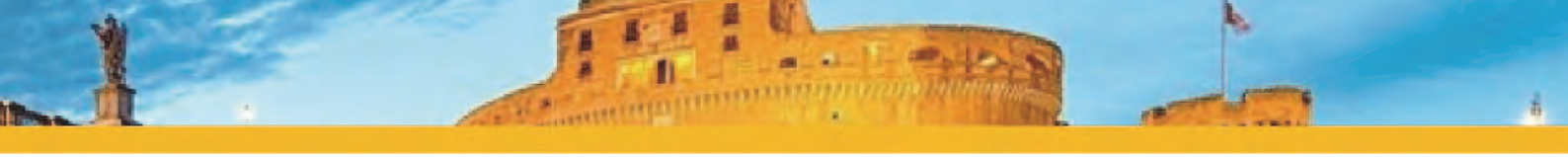

Poster presentations

Session P2

Light dependent exoelectrogenic activity of *Synechocystis* sp. PCC 6803 and production of sustainable electricity in a bio-photovoltaic system

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Biophotovoltaics (BPV) technology is a microbial electrochemical technology that harvests solar energy through biological activity of photosynthetic organisms. It is a rapidly evolving field and holds great promises for a self-sustainable, clean as well as green technology allowing chemicals and electricity production without the need of organic compounds. The exoelectrogenic activity of photosynthetic microorganism *Synechocystis* sp. PCC 6803 was explored using a BPV platform. Light dependent exoelectrogenic activity was quantified and compared to dark conditions. Difference between light and dark condition was clearly elucidated with an absolute current output of 10 µA during illumination. Current densities of above 100 mA m^{-2} (anode geometrical surface area) were achieved on a small scale (25 mL). In the presence of the site specific photo-inhibitor, DCMU (3-(3, 4-dichlorophenyl)-1, 1-dimethylurea), which blocks binding of Plastoquinone (PQ) to photosystem II preventing reduction of PQ, the output current generated by the system was significantly inhibited and thus underlined the water photolysis reaction as the main source of electrons during illumination. These findings can set a basis for further exploring photosynthetic extracellular electron transport mechanisms. Subsequently, scale-up of a BPV reactor module was designed and continuous electricity production was realized over a period of more than 25 days. Current densities as high as 40 mA m^{-2} were achieved in an open-single chamber BPV whereas a current density of only 7 mA m^{-2} could be obtained when the system was completely closed. The improvement in the current output might have resulted from the facilitated CO_2 exchange with the atmosphere. This biological approach with much smaller environmental footprint holds a great potential to be a competitive technology in future. However, significant improvements are possible to enhance the overall energy conversion efficiency and current density of BPV systems if the technology is to be of interest outside of the laboratory.

Study of the capacitance of granular activated carbon and graphite for their application in bio-anodes

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Microbial Fuel Cells (MFCs) are devices that can produce current from the bacterial oxidation of organic matter in wastewater. The configuration of classical MFCs encounters many limitations and only allows direct production of electricity (no storage). One possibility to increase performance of MFCs is to integrate capacitors inside the cell; in fact, it was proven that capacitive MFCs can outperform non-capacitive MFCs in terms of charge storage and charge recovery[1]. In this project, we use granular activated carbon (AC) as the electrode material in bio-anodes because it is a cost-effective way of creating high electrode surface area and can easily be implemented in an anaerobic fluidized bed for wastewater treatment[2]. Our goal was to determine capacitance or, in other words, electrical charge storage of single AC granules by analysing their charge-discharge behaviour. The principle of charge storage relies on the formation of electrical double layer (EDL), a very thin layer with opposed positive and negative charges in the electrode-electrolyte interface. By repeating charge-discharge cycles, we continuously form and release the EDL and can, in this way, quantify the amount of electrons stored in AC granules after certain time period and under specific conditions. In addition, our aim was to determine the contribution of biofilm formation around AC particles to their initial capacitance (i.e. prior bacterial growth). To study this, we perform same charge-discharge cycles distinguishing between faradaic current (produced from continuous acetate oxidation by bacteria) and capacitive current (produced from electrons stored in the granule). We did these experiments at different development stages of biofilm and with capacitive (AC) and non-capacitive (graphite) granules. Capacitance of AC granules was 1000 times higher than graphite granules due to their large porosity. When biofilm was grown, the overall capacitance of graphite granules increased while that one of AC granules decreased. A combined effect of electron storage inside the carbon granules and the biofilm, together with the blockage of electrode pores due to biofilm growth, determined the final capacitance of these granules. From this study we conclude that the choice of electrode material and the extent of bacterial growth are parameters of great importance to take into account when optimizing charge storage and charge recovery of bio-anodes.

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Flow cell for simultaneous detection of electroactive bacteria with EIS and CLSM

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Understanding the attachment of electroactive bacteria to electrode surfaces and their subsequent biofilm formation is one of the major challenges for the establishment of bioelectrochemial systems (BES). Monitoring techniques for electroactive biofilm formation given in literature range from classical electrochemical techniques to different optical and spectroscopical methods. In this work, the simultaneous application of two powerful methods of analysis - electrochemical impedance spectroscopy (EIS) coupled with confocal laser scanning microscopy (CLSM), is presented.
A custom-built and membrane separated flow cell provides a reproducible flow at the substratum surface and makes macro- as well as microscopic online observation of biofilm growth feasible. Cyclic voltammetry and EIS were done abiotically for electrochemical reactor characterization. Moreover, in simultaneous abiotic EIS and CLSM measurements, no influence of the imaging process on the EIS measurement or vice versa was found. Biotic experiments were performed to demonstrate the practical application of the flow cell for: (a) electrochemical monitoring of microbial activity and adhesion via chronoamperometry as well as EIS with (b) simultaneous imaging of the adherent biomass to the transparent electrode via CLSM. Therefore, the flow cell was run in Microbial Fuel Cell mode cultivating an engineered *Shewanella oneidensis* MR-1 producing the fluorescent protein eGFP. Results show a typical current curve of a MFC complemented by confocal time series images demonstrating adhesion.

Reduction of Greenhouse gas emissions in a sedimentary-microbial fuel cell

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The Ph.D project aims at designing a sedimentary microbial fuel cell (SMFC) to promote self-purification process (biodegradation of organic matter) and reduce greenhouse gas (GHG) and toxic gas emissions in river and sewers (e.g. CH_4, H_2S). It has been setup to meet the goals of reducing GHG emissions (Strategy Europe 2020) and achieving a "good status" for surface waters (Water Framework Directive). Electrochemical reduction of GHG with MFC has been little studied so far and must be considered as a novel application of SMFC. It is interesting to notice that in 2011, Jung and Regan* managed to reduce CH_4 by roughly 7,7 % between MFC's with R ext of 970Ω and R ext of 150Ω starting from aerobic sludge fed with a glucose medium. Three years later, Ueno and Katijima* poised an anodic potential of +300mV vs Ag/AgCl in a doped sediment wih a potentiostat and supressed methane by approximately 36% from control.
A preliminary experiment will be conducted to identify the circuit closing process (fixed R ext or potentiostat) leading to the greatest GHG/ H_2S reduction from sediment of the experimental river "Chaudanne", site of Grézieu-la-Varenne, France. Based on previous results, a new experiment will be conducted in same conditions with a full set of analyses beside gas analysis (N_2, N_20, CO_2, CH_4, O_2, H_2S): isotopic analysis on gases and sediment (δ13C, δ15N), elemental analysis on sediment (%C, %N, %H, %S (w/w), proteomic and biomolecular analyses on sediment (Enzyme quantification of nitrate-reductase, microbial and functional abundance and diversity). Finally, Denitrification, nitrification, methanogenesis, methanotrophy, sulfate oxidation & reduction activities in sediment involved in the GHG will complete microbiological analysis. They will enable to apprehend the biogeochemical functioning of the sediment linked to GHG emissions and the way it evolves depending on the anodic redox potential. By optimizing it, we are targeting a reduction in GHG (particularly CH_4) and H_2S of 50%.

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Exoelectrogenic activity of hydrocarbonoclastic strains and toluene degradation in bioelectrochemical systems using a pure culture of *Cupriavidus metallidurans* CH34

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Bioelectrochemical systems (BESs) have proven to be a useful tool for bioremediation and have been applied to achieve the oxidation of organic compounds (*e.g.* hydrocarbons) at the anode. Microbial metabolism can be stimulated in a BES when overpotential is applied, increasing the rate of pollutant degradation. The aim of this work was to test the exoelectrogenic capacity of five hydrocarbonoclastic strains of the genera *Cupriavidus* and *Pseudomonas* and to determine if the applications of overpotential stimulates microbial metabolism, analyzing current density and substrate consumption. Exoelectrogenic activity was studied with succinate as sole carbon source. Three of the five strains (*Cupriavidus metallidurans* CH34, *Pseudomonas* sp. DN34 and *Pseudomonas* sp. DN36) showed exoelectrogenic activity. Comparing the current densities obtained with the three exoelectrogenic strains, *Pseudomonas* sp. DN36 reached a maximum of 1.00 mA/m^2, followed by *Cupriavidus metallidurans* CH34 (0.65 mA/m^2) and *Pseudomonas* sp. DN34 (0.23 mA/m^2). *C. metallidurans* CH34, *Pseudomonas* sp. DN34 and *Pseudomonas* sp. DN36 reached a 91.74%, 89.38% and 35.28% of succinate removal respectively in 42 h. The application of overpotentials between the anode and the cathode lead to an increase in substrate consumption, turbidity and current density production.
Although whole genome sequencing reveal that *C. metallidurans* CH34 lacks in the upper pathway of toluene degradation in anaerobic conditions, *C. metallidurans* CH34 significantly reduced (from 100 to 42 ppm in 27 days) the concentration of toluene under denitrifying conditions, while the abiotic control maintained toluene concentration around 100 ppm.
A Microbial Fuel Cell (MFC) and a Microbial Electrolysis Cell (MEC) were set with a pure culture of *C. metallidurans* CH34 and with toluene as sole carbon source. In the MEC, overpotential (+800 mV) was applied between the anode and the cathode. When *C. metallidurans* CH34 was inoculated in a MFC containing toluene as sole carbon source, current densities reached 0.80 mA/m^2. Conversely, in the abiotic control no current was detected. Toluene concentration in the control remained stable (38 ppm) while in inoculated MFC toluene decreased by 36.6% in 10 days.
In the biotic MEC, current densities increased from 21 to 30 mA/m^2 in 20 days, while in the control, current densities remained constant at 10 mA/m^2. After the initial adsorption of toluene on the anodes of both inoculated and sterile control MECs, 63.3% of the initial toluene in the biotic MEC was removed, while it remained stable in the abiotic reactor. The current density increased when toluene was further spiked in the reactors. These data showed that *C. metallidurans* CH34 was able to degrade toluene under anaerobic conditions and to transfer electrons to a solid electrode.

Acid mine drainage treatment with microorganisms coming from a low-pH operated microbial fuel cell

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Acid Mine Drainage (AMD) is a widespread environmental problem, characterized by low pH and high concentrations of sulfate and heavy metals. Since conventional AMD treatment technologies have several limitations, we have been assessing the utilization of Microbial Fuel Cells (MFC) technology as an alternative for AMD treatment systems. In order to find microorganisms which may achieve the double purpose of electricity generation and AMD treatment, we chose a site located in Northern Chile, reported as affected by AMD (Leiva et al. 2014), to take inoculums for MFC reactors.

MFCs were operated in batch mode using a synthetic AMD water with pyruvate as electron donor at pH 3.8, obtaining a maximum power density of ~10 mW m^{-2}. Electric performance showed two different groups of reactors. Independently of electricity generation, we observed increases of ~4 pH units after two-days batch cycles, in all reactors.

Batch and continuous-flow experiments showed promising results in AMD synthetic water neutralization. We also tested the ability to increase pH of different microbial communities (from cathode, anode and reactor walls), by separate. All of these communities showed similar pH increases, suggesting that water neutralization may be associated to a synergic action of the entire consortium coming from the MFC. Furthermore, since neutralization may facilitate precipitation of metals, we also are performing experiments including iron and arsenic, in order to study treatment possibilities for more realistic AMD synthetic waters.

This study is an initial effort for the development of sustainable AMD treatment systems. Although MFC operation did not result in a very high energy generation, it may have an important role in selection of microbial communities able to remediate AMD waters.

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On the effect of anodic biofilm enrichment by applying selected electro-active microbes in microbial fuel cells

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Bioelectrochemical systems, such as microbial fuel cells (MFCs) or microbial electrolysis cells (MECs) are considered as relatively new alternatives on the field of renewable energy-producing systems. MFCs play an important role on wastewater treatment and biosensorics, as well. The operation of these bioelectrochemical cells is based on the biochemical activity of so-called exoelectrogenic bacteria which are able to form an anodic biofilm and catalyze the electron transfer between the microbes and the electrode. In consequence, the initial cell deposition and exoelectrogenic biofilm growth may have an impact on the polarization behaviour of the anode.
In this work the strategy of bioaugmentation has been tested for enhancing the efficiency of bioelectricity production. The mixed consortia derived from the effluent of a biogas plant has been enriched applying different electro-active bacteria (e. g. *Shewanella*). Substrate degradation and electrode polarization measurements have been carried out for characterizing the biofilm enrichment efficiency and for monitoring the biofilm formation.

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Integration of microbial electrolysis cells and anaerobic digesters: impact on the stability of the digestion process

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Both anaerobic digestion (AD) and bioelectrochemical systems (BES) have a great potential as a technology for energy recovery from waste streams. Moreover, their mutual integration can help to overcome some of the hurdles associated to both technologies. In this study we explore the benefits of integrating a microbial electrolysis cell (MEC) within an anaerobic digester where the digestion process is prone to inhibition due to high VFAs production. Electrogenic populations were pre-adapted to reactor conditions by two parallel processes: pre-grown in acetate, as this is an easily degradable carbon source for them, and pre-grown in glucose, as this was the substrate for AD in the tests. The results revealed that the presence of the MEC helps to reduce significantly the concentrations of VFAs during the first 75 hours of the AD process, suggesting that electrogenic consortia allowed for VFAs consumption and for a higher stability of the system as a consequence. This translates into a higher specific CH_4 production. Nevertheless, after 75 hours of operation the digestion process led to a complete stoppage in all cases. Interestingly, these results are independent of the applied voltage: when the MEC was operated in open circuit conditions VFAs concentration was still significantly reduced compared to digester with no MEC inside. However, V application optimized this response. In reactors with acetate-adapted microorganisms VFAs concentrations maintained low for longer, while electrogenic performance was improved with glucose-adapted microorganisms, suggesting that a mixed adaptation process would be advisable. In conclusion, the presence of MEC electrodes inside the anaerobic digester helped to delay the build-up of VFAs inside the reactor thus delaying the stoppage of the process. These results highlight the great potential of MEC to help to overcome the hurdles derived from excessive VFAs production in AD.

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Determination of Electrochemical Accessible Surface Area (ECSA)

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The surface area of solid materials plays a major role in multiphase processes. In electrochemical experiments a normalization of the unit area of the electrode is needed for a better comparison of experimental data and results between different researchers and laboratories. Based on the need to increase performance, researchers seek to develop electrodes with a high surface area. This applies to bioelectrochemical systems (BES) as well as for many other electrochemical applications and devices. Unfortunately the characterization of these electrode surface areas is not straightforward and uniform. A few methods have been investigated to estimate the ECSA of electrodes. Since most of them require extraction of the electrode from the system or alter the electrode-electrolyte-interface in the process, these methods are not employable to biofilm-electrodes. Such electrodes have to be measured in situ and without any damage to the biofilm.
Electrochemical techniques for the evaluation of the ECSA of several materials with different roughnesses and geometrical surface areas are presented and discussed in this study.

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Fuel cell for chemical and microbial wastewaters treatment

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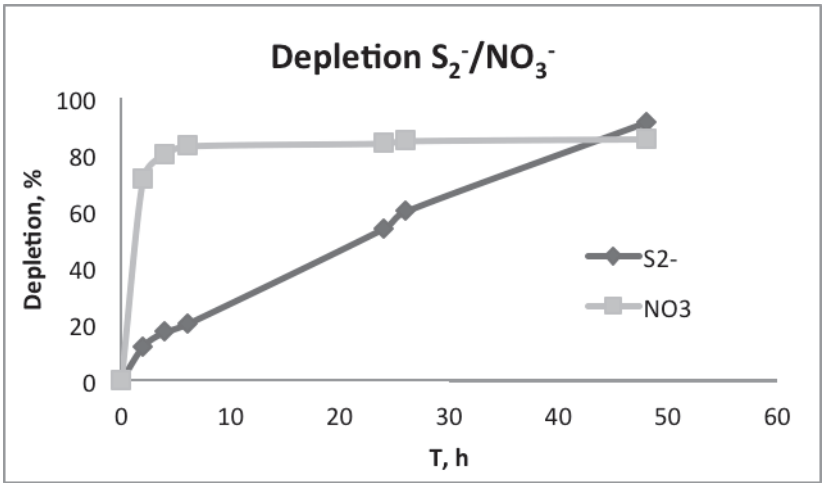
Purification of sulfite- and nitrate-contaminated wastewaters is a complex and expensive technological process. In our previous works we have proposed an effective and cheap method for simultaneous removal of sulfates and nitrates from municipal, agricultural and industrial wastewaters by oxidation of the sulfides to sulfates and reduction of the nitrates to nitrogen in electrochemical way. This is done in a fuel cell of our own design and it allows electrical energy to be harvested and used.

Pseudomonas denitrificans performs complete denitrification in the cathode compartment using the electrons supplied by oxidation of sulfide ions on the anode.)The use of immobilized culture enhance10 times the process efficiency compared to this conducted with free cells. Activated carbon was chosen as a carrier for cell immobilization due to its advantages as adsorbent and its electroconductivity. Moreover, it is a catalyst for the process and an electrode with well developed operating surface.

Differeent initial concentrations were studied- stoichiometric ratio, excess of sulfide ions or excess of nitrate ions. Excession of nitrate ions leads to better results for both electricity harvest and sulfide and nitrate dipletion. The higher tested concentrations were 500 mg/L sulfide ions and 1000 mg/L nitrate ions. The fuel cell operating with 100Ω external resistance reached 3.30 mA current Nitrate and sulfide ions depletion is more than 80 % for 48 hours (fig.1).



The "bioelectrochemical groundwater circulation well": a scalable reactor configuration for in situ treatment of contaminated groundwater

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In both Europe and USA, groundwater contamination by petroleum hydrocarbons is a widespread problem which poses serious environmental and health concerns. In situ remediation techniques are becoming more and more popular for the treatment of this type of contaminants, being typically cheaper and quicker than pump and treat systems. Among these, aerobic bioremediation holds a great potential for its general sustainability in terms of environmental impact and costs. On the other hand, inefficient delivery and distribution of oxygen in the subsurface, aquifer clogging caused by air bubbles and excessive biomass growth are factors which, at present, challenge the application of aerobic bioremediation systems.
Bioelectrochemical systems (BESs) are devices in which microorganisms catalyse redox reactions through electrodes interactions. The discovery that carbon-based electrodes can be used by a wide diversity of microorganisms as terminal electron acceptors during the anaerobic oxidation of a variety of organic substrates has raised the possibility that they could also be employed in situ to accelerate the oxidation of environmental contaminants, such as petroleum hydrocarbons in soils and groundwater. In principle, key advantages of BES over conventional in situ bioremediation systems include the following: (i.) electrodes can serve as a low-cost, low-maintenance, continuous sink for electrons; (ii.) the kinetics of contaminants oxidation can be monitored and controlled by simply manipulating the electric current or the applied potential; (iii.) since hydrocarbons tend to adsorb on carbon-based electrodes the BES approach allows co-localizing the contaminants, the electron acceptor and the degrading microorganisms, with these latter typically forming a biofilm on the electrode surface. In spite of that, field scale applications of BES are presently limited by the lack of scalable system configurations, which could allow sustaining high degradation rates while minimizing voltage (and accordingly energy) losses. Here, we designed a novel concept of BES, which combines the Groundwater Circulation Well (GCW) technology with an electrode-driven hydrocarbons oxidation system. This Bioelectrochemical Groundwater Circulation Well (BGCW) recirculates in situ the contaminated groundwater several times through a well equipped with a granular graphite bioanode and a concentrical stainless-steel cathode, before it is discharged downgradient. A laboratory-scale prototype of the BGCW has been realized and operated under a range of conditions (e.g., applied anode potential, recirculation rate, groundwater hydraulic retention time) with phenol as model contaminant. Preliminary results indicate that this novel reactor configuration holds great promise for in situ treatment of a range of oxidizable contaminants.

Microbial fuel cell for Cr(VI) reduction and simultaneous bio-electricity production using an abiotic cathode

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A possible method for Cr(VI) reduction using a microbial fuel cell (MFC) has recently been proposed, where Cr(VI) was used as an electron acceptor in the cathode to generate electricity[1]. In the present study, Cr(VI) reduction and electricity generation using synthetic wastewater as a medium (based on 5.5 gdCOD / L glucose) and synthetic Cr(VI)- containing wastewater (200 mg / L) as a catholyte were investigated. All experiments were conducted in fed-batch mode, using a dual chamber (H-type) MFC (anode and cathode electrodes: carbon paper 4 cm x 2.7 cm, Nafion 117 PEM: total apparent surface area 7.1 cm^2). The performance was evaluated in terms of Cr(VI) removal efficiency and power production. In particular, this study examined the effect of the initial catholyte pH (2-4) on the performance of the MFC, the catholytes' pH adjustment (using 30% H2SO4) at pH 2 during cycle operation as well as the effect of the catholytes' conductivity (no addition of KCl) on Cr(VI) removal efficiency and power generation. The best electrochemical performance (Pmax 58 mW/m^2, Rint 1.1 kΩ) and Cr(VI) removal efficiency (60%) were achieved at pH 2. However, the performance decreased by 91% (Pmax 5.2 mW/m^2, Rint 4.6 kΩ) and 51.5% (Cr(VI) removal efficiency 8.5%) respectively as pH was raised to 3. No power production and Cr(VI) removal were observed at pH 4. These results indicate that higher pH values increase the cathode overpotential, thus inhibiting Cr(VI) reduction. Moreover, pH adjustment at 2 during cycle operation did not enhance the performance of the cell (Pmax 51 mW/m^2, Rint 1.6 kΩ, Cr(VI) removal efficiency 56%). On the contrary, an increase in the internal resistance of the cell and back migration of protons from the catholyte to the anolyte were observed (initial anolyte pH 7, final anolyte pH 5). Additionally, when KCl was not added in the catholyte at pH 2, the conductivity decreased from 12.4 mS/cm to 5.57 mS/cm resulting in a decrease of the Cr(VI) reduction rate and in the electrochemical performance of the MFC (Pmax 8.2 mW/m^2, Rint 3.1 kΩ). This result highlighted the importance of conductivity for Cr(VI) reduction. Different issues need to be addressed in order to improve MFC performance while treating Cr (VI) containing wastewaters. The use of abiotic or biotic cathodes as well as the effect of the presence or absence of organic carbon in the catholyte are some of them [1, 2].

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Pilot scale Microbial Electrolysis Cell for nitrogen and carbon removal with simultaneous energy production

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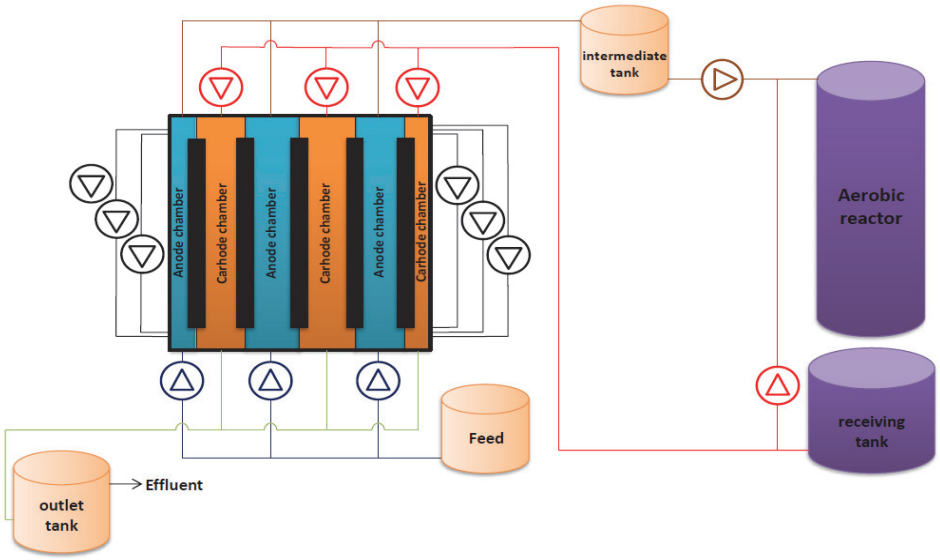
Bioelectrochemical systems (BES) can perform combined carbon and nitrogen removal with simultaneous energy production, thus creating new opportunities for BES as an integral wastewater treatment.
The main objective of this work wasto develop a BES for centrate treatment. A pilot plant with a total volume of 150 L has been designed, built and commissioned. The BES reactor consisted of 5 independent bioelectrochemical units, each unit comprising two electrodes (one anode and one cathode) and was provided with stainless steel current collectors.
The units were arranged to provide three anodic chambers and three cathodic chambers. The five units were hydraulically and electrically connected in parallel. Both the anodic and cathodic electrodes were made of a carbon felt with a surface area of 0.5 m^2.
This plant was capable of achieving a 80% (average value) elimination rate of organic carbon in the anode while the nitrogen removal rate was 40% at the exit of the cathode. Hydraulic retention time and applied voltage were set at 1 day and 1 V respectively throughout the whole operating period. The temperature was maintained at 16 ± 1.5 °C.
In this study, the feasibility of using a pilot-scale MEC for simultaneous carbon and nitrogen removal was tested. There were some problems and the reactor needs to be improved with respect to the hydrogen production, stream recirculation and simplifying the pumping circuit.



Cathodic Reduction of Proton by *Shewanella oneidensis* and Its Extracellular Electron Transfer Mechanism

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Hydrogen production with bioelectrochemical system is an economical, green and energy efficient technology to replace traditional fermentation process. The biocathodes, without precious metal as catalysts, for H_2 production have attracted much attention. However, the cathodic extracellular electron transfer (EET) mechanisms for the hydrogen evolution reaction (HER) are still lack of knowledge. It has been reported that bacteria functioning as biocatalysts in cathodic HER must carry hydrogenase, such as *Geobacter sulfurreducens* and *Desulfovibrio vulgaris* Heighdenbrogh. *Shewanella oneidensis*, commonly recognized as bioanodic bacteria possessing the direct and indirect electron transfer, also contains hydrogenase and has hydrogen respiration capabilities. Therefore, it deserves to find out whether *Shewanella* can accept electrons from electrodes and how it works in cathodic EET. In this research, a bioelectrode, formed with *S. oneidensis* MR-1 biofilm growing on a carbon felt, was poised at the potential of -1.0 V *vs.* standard calomel electrode (SCE) in a three electrode system. Electrochemical characteristics were studied by using linear sweep voltammetry (LSV) and differential pulse voltammetry (DPV). Results show that the current density reached stable at 0.22 A/m^2 in 300 h of acclimation in an electrolyte of defined medium (DM), while a control reactor generated a negligible current. However, the electrolyte replacement by the fresh DM decreased the current density by 65%, which could not be recovered in 5 h unless the original supernatant refilled back into the reactor. Correspondingly, a similar variation in LSV profile occurred, indicating that self-secreted redox components produced by *Shewanella* were present in the original supernatant but not existed in fresh DM and they could mediate the EET from the electrode to MR-1 cells for HER. Furthermore, the DPV result also demonstrates that two redox components were detected at the potential of -0.49 V & -0.64 V *vs*. SCE, which may participate in cathodic EET of MR-1. However, flavins, which commonly appear in bioanode EETs, were exempted by using 2-dimensional fluorescence spectrum. These redox components that enhanced the cathodic EET of *Shewanella* for HER warrant further identification.

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Direct electron transfer by *S. cerevisiae* in a lab-scale microbial fuel cell

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Microbial Fuel Cells are bio-electrochemical devices able to directly convert organic substrates into electricity.
Yeasts as catalyst in MFCs are attracting attention for their impressive growth capacity and not specificity of the substrate.
However, these microbes needs an exhaustive study on the electron transfer mechanism from the microbes to the electrode.
Our findings showed that cells of common baker's yeast such as Saccharomyces cerevisiae could be immobilized by inclusion techniques in cellulose acetate membrane on the surface of a graphite electrode, without damaging the cells through the process. Here, immobilized cells were able to grown and be adsorbed on an external electrode showing a huge potential to generate electrons. The presence of an electron mediator, such as methylene blue (MB), was able to increase the MFC performances, however our findings suggested that methylene blue, once reduced, was entrapped into the cells, enhancing the electron transfer on the graphite electrode, probably by discharging the accumulated electrons on a trans-plasma membrane protein.
Our findings suggested that a common protein on the cell wall of Saccharomyces cerevisiae: the Ferric Reductase, was able to mediate the electron transfer to the electrode, also in absence of an exogenous mediator. The inhibition of the protein with Carbonyl cyanide m-chlorophenyl hydrazone (CCCP) shows both a drop in the reductive capacity of the microorganism and a decrease in the electrons transfer to an external electrode.

Bioremediation of crude-oil-contaminated estuarine sediments from River Tyne (UK) by a microbial electrochemical snorkel

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Oil spill disasters are a worldwide problem and current technologies do not satisfactorily address the issue. Chemical dispersants are frequently employed as a first response option to an oil spill. While this approach makes the oil spill less visible, dispersants and dispersed oil under the ocean surface are hazardous for marine life. Particularly, once the dispersed oil reaches the sediments it tends to persist there for a very long time due to the prevailing anoxic conditions which drastically limit the occurrence of oxidative biodegradation processes.

To overcome this major drawback, a novel bioelectrochemical system, the "Oil-spill Snorkel", has been proposed to bioremediate oil contaminated sediments (Cruz Viggi et al., 2015). The "Oil-Spill snorkel" aims to accelerate hydrocarbons biodegradation by creating an electrochemical connection between the anaerobic sediment and the overlaying aerobic water. The snorkel, takes advantage of the capability of certain bacteria, such as Geobacter spp. and Shewanella spp., to anaerobically oxidize hydrocarbons with a carbon-based electrode, deployed in the sediment, serving as respiratory electron acceptor. The electrons travel from the bottom part of the snorkel (anode) to the upper part of the snorkel (cathode) where they reduce oxygen to water.

Here, we assessed the influence of the bioelectrochemical snorkel on hydrocarbons degradation and other key metabolic activities in contaminated estuarine sediments from the River Tyne (UK). To this aim, sacrificial microcosms were constructed with oil contaminated sediment. Five different treatment conditions were setup: live microcosms containing 3 graphite rods (S, the snorkels), the biotic controls without graphite rods (C) and the abiotic (autoclaved) controls (B) which contained 3 graphite rods; furthermore two additional no-oil controls were setup, namely treatment SNO which contained the sediment and the graphite rods and CNO which contained the sediment without the graphite rods. Results will be presented in terms of oil hydrocarbons biodegradation, sulfate reduction, and microbial community composition.

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Exploring the role of anode and cathode bacterial communities on soil organic matter turnover in a two-chamber microbial fuel cell

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Microbial Fuel Cells (MFCs) are devices that use 'electroactive' bacteria (EAB) to directly generate current through catalytic oxidation of organic matter (OM) under anaerobic conditions. Despite research on biological reactions occurring at the MFC's electrodes have long been carried out in the last decade, our knowledge on bacterial consortia associated to each electrode is still poorly understood. Furthermore, although native soils are known to be a versatile source of EAB, their role in soil processes is still completely neglected. In this work, a constant voltage (1.7V) was supplied to a two-chamber MFC filled up with a top-soil over three weeks in order to simultaneously select an electron-donor EAB community on the anode and an electron-acceptor EAB community on the cathode. After three weeks, a Next Generation Sequencing (NGS) approach was used to characterize the bacterial community associated to each electrode and compared with a control chamber not supplied with any voltage. Results highlighted that the voltage shaped the soil bacterial communities, providing a selection of different bacterial groups preferentially associated to the anode (*Betaproteobacteria*, *Bacilli* and *Clostridia*) and to the cathode (*Actinobacteria* and *Alphaproteobacteria*). Furthermore, a significant increase of CO_2 emissions was detected in the cathode chamber compared to the anode, suggesting an enhanced mineralization of the soil OM. These results confirmed that EAB naturally present within a top-soil display different electrical properties and, moreover, that MFCs could represent a powerful tool for exploring the mineralization and humification processes of the soil OM.

Efficiency of acidic environment for biocathode design

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Microbial fuel cells are devices that use microorganisms as catalysts to drive or accelerate electrochemical reactions at the anode and/or the cathode. While oxygen reduction at the cathode is optimal at low pH, the bioanode reactions are efficient at neutral or at higher pH. Many bacterial species were shown to respire anodes, however only very few species are able to catalyze oxygen reduction at the cathode.
To build biocathodes, we screened almost 50 strains from different species and genera. Our data showed that organotrophic bacteria are not good candidates for biocathode design, whereas, chemolitotrophic bacteria revealed good performances at the cathode, such as *Acidihtiobacillus ferrooxidans*, which produced up to -1 $A.m^{-2}$.
We focused thus our research on diverse acidic environments. We used samples of phosphogypsum from Tunisia with a pH about 4.0 and acidic mine rocks from Morocco with pH about 2.5. Using these phosphogypsum and mine rocks samples as inoculum of MFC delivered 0.6 and 0.5 $A.m^{-2}$ respectively. In Both cases, these biocathodes were useful in recovering different metals such as Ag, Fe, Cu from sediments on the electrodes. Altogether, our data indicate that biocathodes build from heavy metals contaminated acidic environments are efficient for current production and bioremediation process.

Microbial fuel cells: bioconversion of pollution in Berre lagoon to electricity

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Fossil fuels shortage and pollution increase cause a serious threat to global warming and human beings. To remedy this problem, renewable energies represent a great alternative and provide nowadays a significant interest. In this purpose, a microbial fuel cell (MFC) is a device that can convert organic matter into electrical energy by microbial catabolism at the anode and the reduction of a terminal electron acceptor at the cathode. Actually, microorganisms catalysis occur through formation of an electroactive biofilm.

Berre lagoon is a coastal lagoon bordering the Mediterranean Sea in southern France. This lagoon is surrounded by numerous oil industries that have discharged their wastes over the 40 past years. We selected two sites in Berre lagoon: Jai beach and Bolmon pond contaminated by heavy metals and PAHs. We used samples from these sites to inoculate fuel cells and to identify electroactive microbial diversity and bioremediation impact combined to electricity production.

Our work shows that electroactive biofilms can form from these sites and deliver current densities up to 0.8 $A.m^2$ in 15 days. Confocal imaging of the electrodes revealed relatively dense biofilms. Characterization of the microbial community structure by metagenomic approaches illustrates an interesting microbial diversity.

Applying synthetic biology as a tool to understand simultaneous bioenergy production and biodegradation process

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Many approaches have been employed to increase the understanding and consequently the performance of Microbial Fuel Cells in terms of power production and biodegradation, one approach is the use of synthetic biology which fundamentally relies on pre-characterized parts and sub-systems. The simultaneous bioenergy and biodegradation of waste water has been the focus of work in the recent years. Here, we used recombinant *Escherichia coli* K-12 with MtrA, MtrC and MtrCAB inserts to study the specific involvement of each gene in bioenergy production and biodegradation of congo red using a double chamber microbial fuel cell. The obtained results indicated that MtrC was the key gene required for energy production where the current reached 400 mV for 4000 min and power density that reached 59 mW/m^2. On the other hand, *E. coli* with MtrCAB insert showed the highest decolorization which reached 62 % in 2160 min under microbial fuel cells conditions. The residual chemical oxygen demand (COD) indicated that MtrCAB recombinant strain utilized the least substrate as compared to the other tested strains. Exploring metabolic pathways is a means to shed the light on optimal strategies to maximize the performance of microbial fuel cells, the metabolites obtained at the end of the process indicate that butyric acid was the predominant metabolite among all strains. The production of flavin by each recombinant strain at the end of the MFC process indicate that although MtrA secreted the highest levels, nevertheless, it showed low performance on both bioenergy and decolorization. The adherence to the electrode was also examined using confocal scanning laser microscopy (CSLM) to study the biofilm formation for each strain. This study helps us improve our understanding of the dual bioenergy/decolorization process taking place in MFCs in order to maximize the outcome.

A novel microbial electrochemical sensor for on-line monitoring of anaerobic digestion processes

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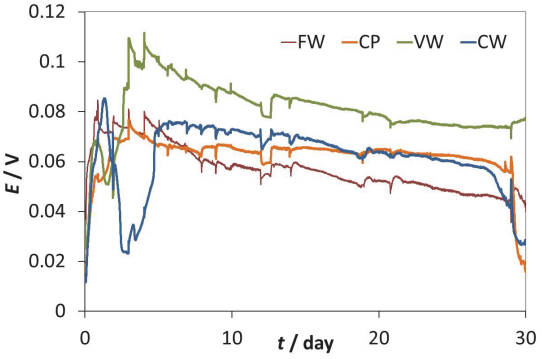
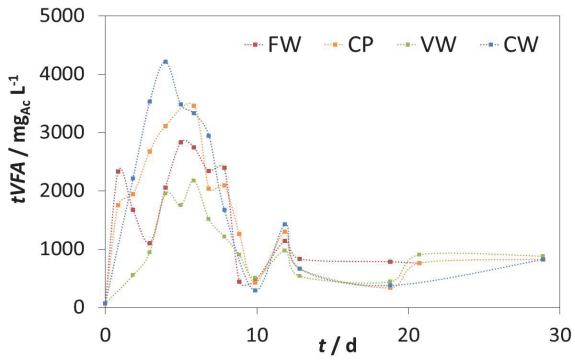
In Microbial Electrochemical Sensors (MES), either anodic or cathodic electro-active microbial populations can produce current/voltage signals, in proportional extent to bioavailable organics (Chang et al., 2005). Current/voltage trends can be used as ideal continuous monitor of specific parameters (e.g. COD, BOD, etc.) in biological systems (Modin and Wilén, 2012), such as bioreactors, wastewater treatment plants, anaerobic digesters, landfill bio-cells, compost piles, etc. Here we present an innovative type of MES, studied for the continuous monitoring of microbial activity in anaerobic digesters (AD). A preliminary experiment was performed on 4 lab-scale anaerobic digesters (120 mL working volume), fed in a batch cycle with 4 types of waste materials: cheese whey (CW), mixed vegetable waste (VW), citrus pulp (CP), fishery waste (FW). Open-air cathodes (carbon cloth + carbon powder/PTFE) of about 3 cm^2 were placed on one side of the digester. The anode (rolled carbon cloth, 15 cm^2) was completely submerged in the biodigester, at a distance of 2 cm from cathode surface. Voltage trends were proportional to the increase in total volatile fatty acids (tVFA) concentrations observed in the AD(see figure), due to the acidogenic phases (days 1-3). For CW, a peak of tVFA over 5 g_{Acetic} L^{-1} (day 4), caused a sudden drop of voltage, that recovered after approximately 24 h. After day 5, all voltage trends decreased progressively, proportionally to the concentration of soluble COD. In ADs, VFA concentrations are normally measured by operators, because peaks of VFA concentration are typically the primary cause of methanogenic activity inhibitions. Here we found that, within certain ranges, voltage/current trends of a MES can be used as a direct measure of tVFAs. Additionally, sudden drops in current generation can be used as hints of possible incoming inhibition of the whole microbial consortium in the bulk AD. In the next future, other tests will be carried out to understand the sensibility of electroactive anodic microbes to single VFAs and to peaks of concentrations. Similarly, current trends might be correlated to biogas production and composition (H_2, H_2S, CH_4).





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Layer-by-layer Glucose microsensor: application in MFC

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Two families of enzymes are commonly used for glucose biosensor construction: glucose dehydrogenases (GDH) and glucose oxidases (GOx). Glucose oxidases are defined as oxidoreductases enzymes that can utilize oxygen as external electron acceptor while Glucose dehydrogenases are not able to utilize oxygen and they utilize other natural or artificial electron acceptors. Glucose oxidase (GOx) is widespread applied for the construction of glucose biosensors as presents higher stability compared to GDH, high selectivity for glucose and lower price (Ferri et al., 2011; Heller and Feldman, 2008).
Second-generation glucose biosensors based on glucose oxidase are oxygen dependent, with negative effects on the sensor reading and decreased efficiencies. For application of the glucose biosensors to the study of bioelectrochemical systems, such as bio fuel cell, where oxygen is most likely expected to be present, the oxygen dependence of the device is of critical interest. Herein, we describe a simple method to obtain an oxygen insensitive biosensor based on thickness control using the layer-by-layer self-assembled deposition with a poly(allyl)amine osmium redox mediator. The correlation between film thickness and biosensor response is discussed. Additional encapsulation of the biosensor with nafion® membrane allow to protect the enzyme molecules and the application of the biosensor in real wastewater. The performances loss of the biosensor over 24 hours was determined, remarking the role of the nafion® protective layer. Finally the sensor was applied to operating MFCs fed with glucose. The obtained results suggest that the biosensor can be successfully applied to perform analysis in MFC systems.

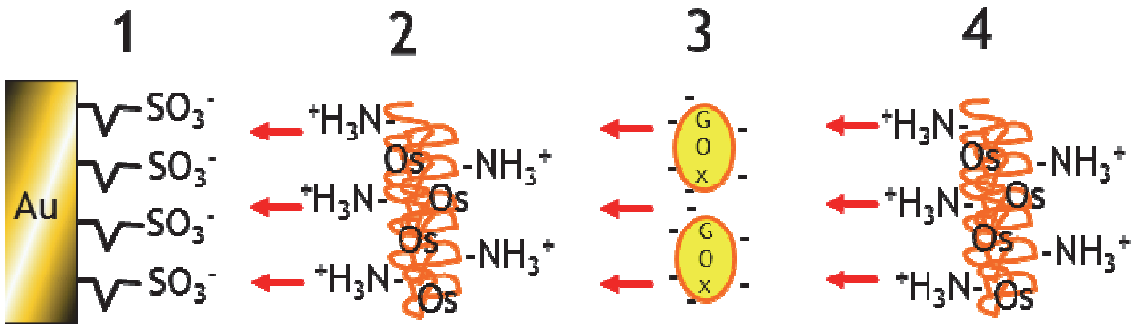

Figure 1. Schematic of self-assembled Layer-by-layer Glucose microsensor

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Sustainable wastewater treatment coupled to energy recovery with microbial electrochemical technologies: the WE-MET project

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In Mediterranean countries, around 1% of municipal electricity consumption is attributed to wastewater treatment (WWT) plants. Thereof, the main share is owed to the aeration of the activated sludge tanks. Recent studies have experimentally demonstrated that the energy content of influent municipal wastewaters is typically over 10 times greater than the energy required to run the plants. This clearly demonstrates that the energy content of raw wastewater is substantial and should accordingly be regarded as a valuable energy resource rather than a waste to simply dispose of. If the energy that is contained in wastewater is harnessed (even only partially), it could help the water industries become self-sufficient in energy or even net-providers. In this context, the WE-MET project, funded under the EU-FP7 ERANETMED scheme, will devise the use of Microbial Electrochemical Technologies (MET), possibly integrated with other technologies, as a groundbreaking approach to recover energy trapped in wastewater while simultaneously cleaning up the wastewater. Overall, using MET will therefore offer a net environmental benefit from wastewater treatment and an economic and environmental upside of using a waste stream for high value energy recovery.
To reach these ambitious objectives, the WE-MET Project brings together a multidisciplinary team of scientists from Universities and Research Institutions, as well as industrial partners (i.e., a SME with expertise in industrial engineering). The WE-MET's pathway to impact combines both fundamental science and up-scaling activities, in order to facilitate the development of technologies which are technically effective and sustainable and also to reach out end-users and stakeholders. This will be possible also with the help and support of an influential Advisory Board composed of policy making institutions (Ministries of environment), professionals (wastewater treatment companies) as well as international representatives of scientific associations.

New floating MFCs for energy harvesting. A cell design overview

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Microbial Fuel Cells are a very promising technology for energy harvesting in remote sensing devices. Several attempts were conducted in the past with sedimentary MFCs, with promising results. The challenge is now to scale up cells and operate them in remote real environments where there is the necessity of monitoring water parameters. Here, a simple design of floating MFCs was studied, suitable for energy harvesting in both aerobic and anaerobic waters. Several sets of cells were tested, using cheap materials, like plastic fruit boxes or panels, using a polystyrene frame or wood as floater. Untreated carbon cloth was used in both electrodes. The electrodes were insulated by felt and clay. In case of anoxic water, the anode faced the water and the cathode faced the air, while in case of aerated water the cathode faced the water and the anode was buried in a box of soil, arranged as a "floating garden" fluctuating on the surface of the water body (Fig. 1). To test this new architecture of energy harvesters, some cells were connected with electronic devices, such as LED lights (500Ω) and buzzers (1000Ω), with power management system. Difference of potential measure and Power curves were performed to evaluate the productivity of these cells. Stacks of MFCs were also tested in Milano Nosedo aerated wastewater.
The cells with dimensions 50 x 30 cm were the most effective among other tested dimensions (with average power and current densities around 15 mW/m^2 and 750 mA/m^2, respectively), in experiment conducted in anoxic wastewater [Martinucci et al. 2015). The experimentation in natural water bodies (OD>3 mg_{O2}/l) was conducted in city lakes of Milan from February to October 2015, for dissemination events at the International EXPO2015 in Milan (average power and current densities of around 5 mW/m^2 and 100 mA/m^2, respectively).

Fig. 1. Three MFCs in floating garden with grown plants at Orto Botanico pool in Milan

E. Martinucci, F. Pizza, E. Guerrini, A. Colombo, S.P.M. Trasatti, A. Lazzarini Barnabei, A. Liberale, P. Cristiani Energy balance and microbial fuel cells experimentation at wastewater treatment plant Milano-Nosedo. Int. J. of Hydrogen Energy 40 (42) 9 (2015), 14683–14689

Flavocytochrome at the microbe-mineral interface of Shewanella oneidensis under mineral respiring conditions

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Shewanella oneidensis must respond to fluctuations in oxygen and nutrients at the oxic / anoxic interface in river and lake sediments. To achieve this Shewanella species have evolved complex systems to utilise a wide range of terminal electron acceptors. S. oneidensis is a facultative anaerobe which is well known for its ability to respire on solid minerals in the extracellular environment, minerals such as manganese oxides and iron oxides as well as radionuclide compounds. To do this the bacteria must transport electrons from their inner membrane, across the periplasm and outer membrane to these terminal acceptors. This process is facilitated by the MtrCAB complex, a complex made up of two decaheme cytochromes (MtrA and MtrC) associated with a β-barrel porin (MtrB), which spans the outer membrane.

Flavin molecules have long been implicated in the process of moving electrons from the surface exposed MtrC to solid mineral terminal electron acceptors. However the exact mechanism of this is still debated. Recent advances in our understanding of the structure of MtrC has shown flavin molecules form a tight association upon reduction of the disulfide bond in domain 3 of the protein [1]. This suggests the reduction of the disulfide bond is necessary to achieve a physiologically relevant flow of electrons, capable of sustaining growth.

Alongside this discovery, we have shown this disulfide bond could be acting as an oxygen sensitive switch. When the cysteine residues that form the disulfide bond are substituted for alanine cells struggle to grow under aerobic conditions, suggesting a toxicity to oxygen. Further investigation highlights increases in levels of reactive oxygen species (ROS) such as hydrogen peroxide as a potential cause for this, generated by oxygen interacting with the more reactive flavocytochrome. From this data we propose that the MtrC flavocytochrome to be the dominant mineral reductase under environmental conditions.

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Rapid and accurate assay for biological oxygen demand via hydrodynamic chronoamperometry

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Biological oxygen demand (BOD) is usually monitored via microbial O_2 consumption measured by pressure evolution in BOD test bottles. The measure is time-consuming (5 days, mostly because of low O_2 solubility), of limited accuracy, and the setup relatively expensive [1]. An electrochemical alternative has been proposed where O_2 is replace by a much more soluble electron acceptor (e.g. ferricyanide), implying a dramatic rise in organics consumption rate and therefore much faster BOD determination (only ~ 1 h to 6 h incubation) [2-3]. The electrochemical techniques used (cyclic voltammetry and/or amperometry in stagnant solution) required time-consuming pre-steps of sampling and centrifugation to remove the solid fraction which could impede the analyte diffusion toward the microelectrodes and/or foul its surface.
Here we show that using a rotating disc electrode (RDE) to perform hydrodynamic measurements allows *in situ*, rapid BOD determination without the need of any pre-step in synthetic and real wastewater samples. Furthermore, the RDE-based method presented a substantially better sensitivity and smaller detection limit over a broad range of BOD values.

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Experimental evaluation of organic carbon and ammonium reduction through nitrite accumulation in microbial fuel cells (MFCs)

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Food industries and zootechnical wastewaters are characterized by high organic carbon and ammonium pollutants (identified by C/N ratio). In case of unbalanced concentration of this pollutants (lower value of C/N), ammonium content becomes a problem for biological technologies such anaerobic digestion (AD). In this work the simultaneous reduction of organic carbon and ammonium compounds was investigated in H-type Microbial Fuel Cells (MFCs), and was evaluated the possibility to reduce ammonium content in line with a Short-Cut Biological Nitrogen Removal (SBNR) through nitrite accumulation.
30 days cycles experiments were performed at different operating conditions (C/N between 1 and 0.35) using sodium acetate and glucose as carbon source, and ammonium sulfate and ammonic chloride as ammonium source. Anaerobic sludge was used as a source of microorganisms. NH_4^+, NO_2^- and NO_3^-, pH, Total Organic Carbon (TOC) and open circuit voltage (OCV) were monitored along time. In addition, the electric performances of the MFCs were evaluated by linear sweep voltammetry.
Results showed that ammonium reduction (60% –80%) in MFCs caused nitrite accumulation, followed by their reduction (40%–80%), once stopped aeration of cathodic chamber and by adding organic carbon and an additional microorganisms source. Excellent values in terms of carbon removal (up to 78% during nitrite accumulation and a further 50% during nitrite reduction) were also obtained. As regards the electrochemical performances of the cell, OCV increased at increasing of organic content while ammonium content increased internal resistance of the system.

Improving hydrogen mediated microbial electrochemical reduction of CO_2 by separating the two steps of the process

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In the context of CO_2 conversion to fuels and chemicals, the association of electrochemistry with microbial catalysis has opened up promising new routes to reduce CO2 to acetate and other multi-carbon compounds. Some of these electro-microbial processes are based on two consecutive steps: first the electrochemical production of hydrogen by water electrolysis and, second, the reduction of CO2 by microbial species that use the hydrogen produced. For the demonstration, experiments of microbial electrochemical reduction of CO2 were carried out under two different applied potentials, -0.36 V and -0.66 V vs. SHE, using a biological sludge as the inoculum. Both potentials were thermodynamically appropriate for converting CO2 to acetate but only -0.66 V enabled hydrogen evolution. No acetate production was observed at -0.36 V, while up to 244 +/-20 mg L-1 acetate was produced at -0.66 V vs. SHE. The same microbial inoculum implemented in gas–liquid contactors with H2 and CO2 gas supply led to acetate production of 2500 mg L-1. When a salt marsh sediment was used as the inoculum, no reduction was observed in the electrochemical reactors, while supplying H2 + CO2 gas led to formate and then acetate production. Finally, pure cultures of *Sporomusa ovata* grown under H2 and CO2 gas feeding showed acetate production of up to 2904 mg L-1, higher than those reported so far in the literature for *Sporomusa ovata* implemented in bioelectrochemical processes. Unexpected ethanol production of up to 1411 mg L-1 was also observed. All these experimental data confirm that hydrogen produced on the cathode by water electrolysis is an essential mediator in the microbial electrochemical reduction of CO2. In consequence, the implementation of homoacetogenic microorganisms with direct CO2 and hydrogen gas supply should now be considered as a worthwhile strategy for CO2 conversion. Hydrogen can be produced under optimal conditions by conventional electrolysis processes preferentially powered with renewable energies or by microbial electrolysis cells fed with wastes or effluents, and then used to drive CO2 microbial conversion in an H2–CO2 gas–liquid bioreactor.

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A Novel Electrically Conductive and Porous Hollow Fiber Cathode design for Recycling CO_2 to CH_4 through Electromethanogenesis

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CO_2 capture and recycle to one of the most interesting fuel sources i.e. CH_4, through electromethanogenesis is an attractive approach, not only due to its high energy density but also for addressing one of the global challenges facing human society today, i.e. energy security. Electrotrophic methanogens grow as a biofilm on the cathode using it as an electron donor for reducing CO_2 to CH_4. One method to deliver CO_2 to methanogens in the cathode chamber is through bubbling. However, this approach has some limitations due to the uneven distribution of CO_2 in the cathode chamber resulting in losses of CO_2 and hence reduced efficiency of conversion. To improve process efficiency, we developed a novel cathode design that is made of electrically conductive and porous hollow fiber (ECPHF) membranes. The ECPHF played the dual role, as the cathode and for facilitating direct delivery of CO_2 to methanogens through the pores in the membrane. We hypothesize that this approach will minimize incomplete CO_2 utilization or losses. Control reactors were operated in parallel where CO_2 was delivered to the cathode chamber through hollow fiber membranes positioned 4 cm away from the cathode. Experiments were conducted in two-chambered glass reactors and operated under -1.0 V vs. Ag/AgCl applied potential. The results revealed that the new cathode design achieved higher CO_2 conversion efficiencies to CH_4 (Coulombic efficiency of 118.04 %) compared to the control reactor (Coulombic efficiency of 3.18 %). Scanning electron microscopy imaging revealed a well-colonized biofilm around the ECPHF cathodes after 60 days of operation. In addition to improving process efficiency, the novel ECPHF cathode design provides high packing density, which is required for scaling this technology for practical applications.

Multi-stage microbial fuel cell-based biosensor for biochemical oxygen demand and toxicity detection

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There is a global need to establish state-of-the-art monitoring techniques for water quality to ensure that standards are maintained with increasing domestic and industrial water usage. An important parameter used in water quality assessment is Biochemical Oxygen Demand (BOD), a measurement of the oxygen consumed by microorganisms in the oxidation of biodegradable organic material. BOD measurements have been correlated with the output from Microbial Fuel Cells (MFCs), which are a potential solution for online monitoring of wastewaters. Previously studied MFC sensors have had a limited amperometric range of approximately 250 mg/l BOD_5 (Di Lorenzo *et al.*, 2009), which is often attributed to substrate saturation of the anode biofilm. In this work, a proof-of-concept configuration of multi-stage MFCs connected hydraulically in series was tested extensively to eliminate the saturation effect and extend the sensing range. The summation current generated by a three-stage array was calibrated against BOD_5 for different glucose-glutamic acid concentrations in artificial wastewater. A linear response was obtained up to approximately 710 mg/l BOD_5 with $R^2 > 99\%$ and average standard deviation < 9%. The array range was 3 times greater than obtained by the first MFC operating individually. The effect of toxicant presence (4-nitrophenol) on the multi-stage sensor response was also studied. Toxic and low BOD events could be differentiated using a MFC sensor (both result in decreasing current). Toxic events decreased current from reactors in series in the order 1>2>3, whereas decreasing BOD resulted in current decrease in the order 3>2>1 due to toxicant and substrate concentration gradient through the hydraulic array of MFCs. This modular mode of operation permits high-load BOD wastewaters to be measured online without dilution and an explicit differentiation between toxic and low BOD events based on the ordered response of MFCs.

A new sensor development project in collaboration with WH Partnership Ltd and University of South Wales is building upon the proof-of-concept configuration described using an optimised, tubular MFC design (Kim *et al.*, 2010) and industrial control system.

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A biochemical model for Microbial Fuel Cells treating wastewater

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Microbial Fuel Cells (MFCs) can treat wastewater with direct electricity recovery. Electro-active bacteria (X_a) oxidise the organic content of wastewater and transfer the electrons to the anode of the MFC. However, the anode chamber often shows a more complex microbiome including heterotrophic bacteria (X_h) and methanogenic archaea (X_m). The first cut down complex organic molecules and provide easily biodegradable compounds to X_a and X_m. While X_a produce electricity, X_m generate CH_4 reducing MFCs Coulombic efficiency. In order to describe these microbial interactions, a previously developed MFC model[1] was integrated with the ASM2d model[2]. The resulting model was calibrated with laboratory data gathered from a MFC treating swine manure[3]. Model application will be useful for future MFCs scaling-up and ICS tools development.

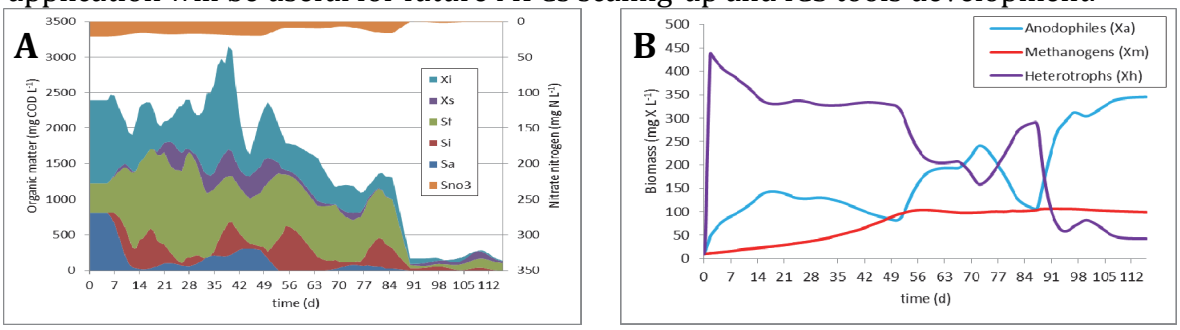

Figure 6 – MFC influent characterization (**A**) and microbial populations dynamics (**B**) over time.

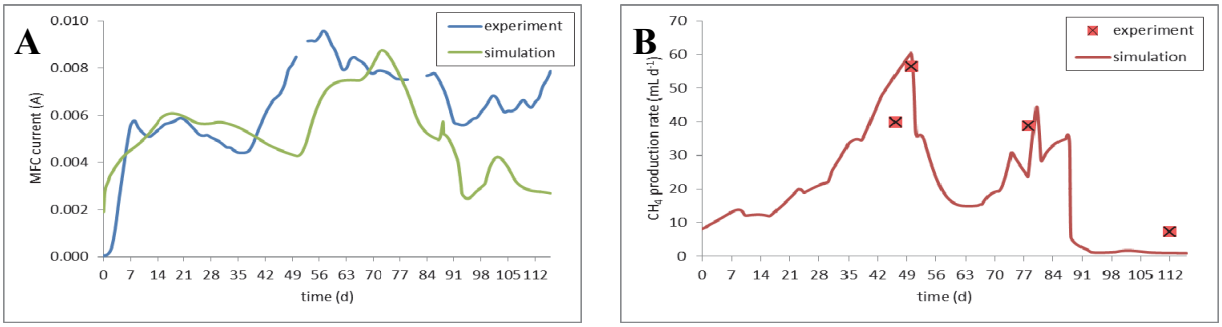

Figure 7 – Experimental and simulated evolution of MFC current (**A**) and CH_4 (**B**) generation over time.

[1] Pinto, R.P., Srinivasan, B., Manuel, M.F., Tartakovsky, B. (2010), A two-population bio-electrochemical model of a microbial fuel cell. Bioresour. Technol. 101, 5256–5265.

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Two phase anaerobic digestion effluents as feedstocks to bioelectromethanogenesis sustenance

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In a microbial electrolysis cell (MEC), it is possible to conduct the two main reactions of anaerobic digestion (AD) in two physically separated chambers, by coupling COD oxidation into CO_2 (in the bio-anode) to the CO_2 removal and reduction into methane (in the bio-cathode), thanks to the transfer of reducing power by the electrical and ionic current. Moreover, AD and MEC can be integrated, by using the MEC to upgrade methane content of the AD biogas while also using residual COD from AD anaerobic digestate, so improving the overall energy efficiency and the quality of the products of conventional AD (Villano et al 2013). However, this approach has not been tested with real substrates yet and concerns also exist on possible fouling and poisoning effects on ionic membrane and/or electrodic material.

Here, a continuous-flow 2-chamber MEC was operated under anodic potentiostatic control (at 0.2 vs SHE), to compare its performance by feeding the bio-anode with synthetic vs real substrates; both an anaerobic digestate (from methanogenic stage) and an acidogenic fermentate (from preliminary acidogenic stage) were tested and compared with a synthetic substrate mixture (as described in Zeppilli et al 2014). The MEC was equipped with a proton exchange membrane (PEM) and both electrodic beds made by graphite granules. The cathode chamber was fed by a continuous sparging of a gas mixture of N_2/CO_2 (70/30 *v/v* to simulate biogas), whereas a concentrated liquid stream was spilled to counterbalance osmotic water flow across PEM.

The MEC performed poorly (23 ± 4 mA) when fed by the anaerobic digestate because its residual COD resulted to be poorly available for anodic oxidation, whereas the mixture of both first and second stage AD effluents gave slightly better performance than the synthetic mixture(60 ± 4 mA *vs* 50 ± 1 mA, respectively). The latter evidence was not only due to high VFA-content but also to high ammonia concentration. Being ammonia higher than in the synthetic mixture, the percentage of ionic current transported across the PEM by the ammonium instead of the proton was increased from 2 to 20 %. This eventually increased the net generation of the alkalinity in the cathodic chamber and thus bicarbonate concentration in the cathodic spill. Overall, by using the VFA-rich and ammonia-rich mixture of both real effluents, a nitrogen removal rate of 228 mg/Ld was obtained while an average CO_2 removal of 3.4 g/Ld was observed in the cathode.

Fouling phenomena were observed to decrease the MEC performance, likely due to the high content of suspended solids in both real substrates (in spite of preliminary filtration at around 0.2 mm cut off). However, adverse fouling effects were easily recovered by periodic backwashing of the bio-anode.

Villano M, Scardala S, Aulenta F, Majone M (2013) Carbon and nitrogen removal and enhanced methane production in a microbial electrolysis cell. Bioresource Technology 130:366-37

Zeppilli M, Villano M., Aulenta F., Lampis S., Vallini G., Majone M. (2014) Effect of the anode feeding composition on the performance of a continuous-flow methane-producing microbial electrolysis cell. Environ Sci Pollut Res. doi:10.1007/s11356-014-3158-3

Thioalkalivibrio nitratireducens, a sulfur-oxidizing bacterium able of electrolithoautotrophic growth to design biocathodes.

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Over these few last years, several works have outlined the autotrophic growth of microorganisms on polarized electrodes (thus forming biocathodes) used as sole electron donor. Ishii *et al*. (2015), dealing with *Acidithiobacillus ferrooxydans*, coined the term "electrolithoautotrophy", to describe this ability[1].

Chemolithoautotrophic bacteria are able to grow on mineral minimum media by using inorganic electron donor (e.g. sulphur, hydrogen, CO_2, *etc.*) and carbon source (carbonates and/or carbon dioxide). Some, for instance *Acidithiobacillus ferrooxydans*, have been shown to use electrodes as their sole electron donor to form biocathodes. Hence, substituting the cathode to the usual inorganic electron donor of chemolithoautotrophic bacteria may lead to the discovery of new electrolithoautotrophic bacteria.

In this study, we demonstrate the electrolithoautotrophy of *Thioalkalivibrio nitratireducens*[2], a chemolithoautotrophic alkaliphilic bacterium from Gammaproteobacteria. This bacterium grows on carbonates as sole carbon donor while oxidizing inorganic sulphur compounds and reducing oxygen (aerobic conditions) or nitrate (anaerobic conditions). When a carbon cloth cathode was provided as the sole electron donor, *T. nitratireducens* was able to grow electrolithoautotrophically and to catalytically reduce oxygen. The carbon cloth was fully colonized within a month by a tight and strained biofilm, which were characteristics of high saline conditions. The oxygen-reducing biocathode was characterized by chronoamperometry and cyclic voltammetry, providing down to 150 mA m^{-2} at -0.3 V/Ag/AgCl and pH 10. A similar work is now undergoing to analyse the behaviour of the strain under anaerobic conditions, with nitrate as final electron acceptor.

[1] Ishii T., Kawaishi S., Nakagawa H., Hashimoto K., Nakamura R. (2015) Frontiers in Microbiology 6, 994
[2] Sorokin D. Y., Tourova T. P., Sjollema K. A., Kuenen J. G. (2003) International Journal of Systematic and Evolutionary microbiology 53, 1779-1783

How the uncompensated resistance and double layer capacitance can influence your polarization data

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Introduction

The need of a best practice for characterizing bioelectrochemical systems has often been emphasized (Harnisch & Rabaey 2012). However, effects like the uncompensated resistance and capacitive currents are not taken into account in most publications. This study presents and evaluates different methods to quantify the uncompensated resistance (R_u) and double layer capacitance and shows their effect on polarization data.

Experimental

The R_u is the electrolyte resistance between working electrode and reference electrode and directly falsifies the measured potential due to Ohms law (IR-drop). It is dependent on cell geometry, spatial arrangement, and electrolyte conductivity. Furthermore the way of determining it can be of significant importance. The R_u in two typical cell geometries was analysed with different methods (Current Interrupt, Electrochemical Impedance Spectroscopy, Luggin capillary, and COMSOL simulation) and compared. Depending on the electrode's double layer capacitance and the voltage or current sweep rates, capacitive current can superimpose faradaic current. To correct for this, the double layer capacitance has been determined using cyclic voltammetry.

Results and Conclusion

In Fig. 1A polarization curves of *G. sulfurreducens* on an activated carbon cloth anode (CTex13, MAST Carbon) are shown. Due to CTex's high capacitance of $12 \cdot 10^3$ Fm^{-2}, the capacitive contribution to the overall current is high when using a typical sweep rate of 1 mVs^{-1}. Evaluated at 0.1 V vs. SCE, the current density is overestimated by 170% due to the non-faradaic (abiotic) capacitive current. Overestimation is negligible at a sweep rate of 0.1 mVs^{-1}. Regarding the determination of R_u, COMSOL simulation is shown to be a more reliable and easy to handle method compared to CI and EIS. Fig. 1B illustrates the potential shift in a two compartment MFC due to an R_u of 132 Ohm (simulated). As can be seen, even when using a synthetic medium as anolyte with comparably high conductivity (3.85 $mScm^{-1}$) the error in potential measurement is significant. In summary, our study underlines the importance of considering the effect of capacitive currents and uncompensated resistances when recording bioelectrochemical polarization curves.

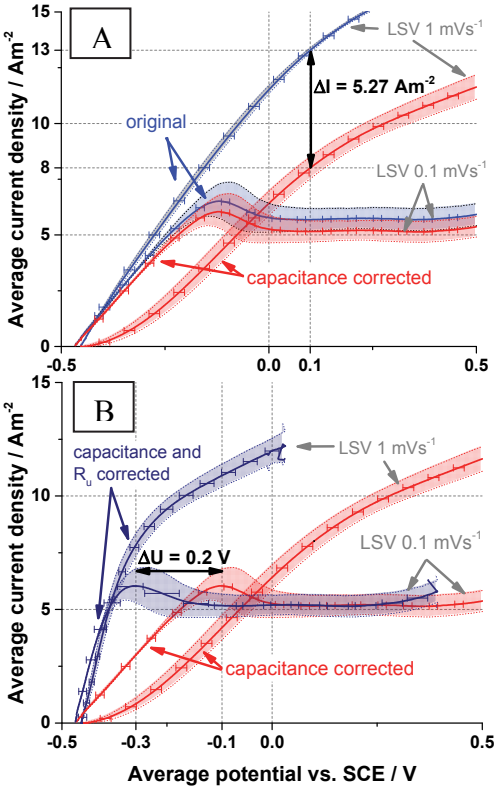

Fig. 1: Triplicate curves obtained in same system: activated carbon cloth (CTex13) characterized with *G. sulfurreducens* in a continuous system (HRT=24h)

Harnisch, Falk; Rabaey, Korneel (2012) The Diversity of Techniques to Study Electrochemically Active Biofilms Highlights the Need for Standardization. *Chemsuschem* 5 (6): 1027–1038

Kinetic parameter estimation of microbial electrolysis cells fed with acetate for hydrogen production

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Hydrogen (H_2) is an alternative energy carrier that has been extensively explored in recent decades. H_2 is considered as an excellent substitute for fossil fuels, since its combustion produces only water vapor instead of greenhouse gases. Additionally, it has an energy yield 2.75 times higher (122 kJ/g) than hydrocarbons.
Microbial Electrolysis is a promising emerging technology, which combines sustainable H_2 production and organic material degradation (Rozendal et al, 2006). In recent years, several studies have been carried out with the main objective to optimize Microbial Electrolysis operational variables (organic load, applied voltage, temperature, inoculum, and pH) in order to obtain higher yields of hydrogen production(Escapa et al., 2016). One possibility to enhance this technology, in order to be industrial scale feasible is to develop mathematical models that help us to understand the physical, chemical and biological processes, involved in the reactor.
The aim of this work was to estimate the kinetics parameters of the anodic electroactive biofilm growth and the relative current density obtained; showing the dependence of the initial organic load and the applied voltage as main operation factors. In order to develop a simple predictive model for H_2 production based on easy identifiable variables, for future applications in process control and optimization, acetate was used as model carbon source in two chambers reactors operating in batch, using sediment as inoculum.
A modified Monod equation, which integrates the Nernst term for the balance of electrons in the system, was developed. This equation adequately models the correlation between the applied potential and the current density generated by the electroactive biofilm.
The results proved that the microbial kinetic rate controls the current generated; however some effects due to the substrate diffusion trough the biofilm and/or variation in the conductivity were observed.

Rozendal R.A., Hamelers H.V.M., Euverink G.J.W., Metz S.J. (2006), Buisman CJN. Principle and perspectives of hydrogen production through biocatalyzed electrolysis. Int *J Hydrogen Energy*; 31: 1632-1640.

Escapa A., Mateos R., Martínez E.J., Blanes J., (2016). Microbial electrolysis cells: An emerging technology for wastewater treatment and energy recovery. From laboratory to pilot plant and beyond. *Renew. Sustain. Energy Rev*. 55, 942–956.

Visible-light driven H_2 production by *Shewanella oneidensis* MR-1

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New technologies which harness solar energy for the sustainable production of fuel are needed to combat the detrimental environmental effects of accessing finite fossil reserves. Solar-H_2 generation can be achieved through artificial photosynthesis using devices with synthetic light absorbers and electrocatalysts, but there are also opportunities to use *hydrogenase*-containing living organisms for this purpose. The use of bacteria may be beneficial due to the self-renewing nature of the system.

Cyanobacteria and algae use light-harvesting protein complexes to absorb visible-light and generate excited electronic states. Charge separation then occurs and solar energy is conserved in the reducing power of electrons. However, many organisms are unable to absorb visible-light naturally. One way to impart this ability is through genetic engineering but it is also possible to combine the microorganism with exogenous reagents which absorb light [1].

Shewanella oneidensis MR-1 (MR-1) is a model bacterium for trans-outermembrane electron transfer. It is a facultative anaerobe capable of producing two periplasmic *hydrogenases* and respiring on numerous electron acceptors including solid iron and manganese oxides. Electron transfer to insoluble minerals is achieved via a series of well-characterised heme-containing proteins which act as a conduit between the inside and the outside of the cell [2]. One such conduit is the MtrCAB complex which spans the otherwise electrically non-conductive outer membrane.

In this project we established a set of growth conditions which favour the production of active *hydrogenase*s within MR-1. We then combined the bacterium with a range of light-harvesting reagents (e.g. eosin Y), a sacrificial electron donor (triethanolamine) and an appropriate mediator (methyl viologen) to assess photo-initiated electron transfer across the bacterial outer membrane to *hydrogenases* in the periplasm. The reduction of protons to hydrogen has been quantified using a Clark-type electrode and gas chromatography. In the future, we hope to use this system as a platform for more complex chemical transformations within the bacterium. For example, the reduction of CO_2 to formate could be achieved by targeting *formate dehydrogenase* enzymes.

[1] Park, J. *et al.* (2015) Cofactor-free light-driven whole-cell cytochrome P450 catalysis. *Angew. Chem. Int. Ed.*, **54:969-973**

[2] Breuer, M. *et al.* (2015) Multi-haem cytochromes in *Shewanella oneidensis* MR-1: structures, functions and opportunities. *J. R. Soc. Interface*, **12:1-27**

Lifes-CO_2R: Liquid Fuel and BioEnergy Supply from CO_2 Reduction

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As the world population increases, breakthrough technologies tackling both fuel supply and carbon emission challenges are needed. The use of CO_2 from, or captured in industrial processes, as a direct feedstock for chemical fuel production, are crucial for reducing green house gas emission and for sustainable fuel production with the existing resources. The aim of this project is to develop a breakthrough technology based upon integrated low cost bio-electrochemical processes to convert CO_2 into liquid fuels for transportation, energy storage, heating and other applications. CO_2 is firstly electrochemically reduced to formate using electric energy generated by Bioelectrochemical Systems (BES) from wastes and other renewable sources. Formate then goes through a biotransformation SimCell reactor with microorganisms specialised in converting formate to medium chain alkanes using a Synthetic biology approach. The proposed technology will be developed around existing wastewater treatment facilities from utilising the carbon source in wastewater and CO_2, thus minimising the requirement to transport materials.
Therefore, the three major research challenges of this project are:
1. How to maximise the power output and energy from wastewater and other waste using BES?
2. How to achieve CO_2 conversion to medium chain alkanes through reduction to formate in Microbial Electrolysis Cells (MEC), and then use SimCells for large scale production?
3. Can we develop a viable, integrated, efficient and economic system combining bio-electrochemical and biological processes for sustainable liquid fuel production?
A multidisciplinary team of engineers and scientists will develop this innovative technology in this project. The consortium consists of five UK universities, including: Oxford, Sheffield, Surrey, U. South Wales and Newcastle, and several industrial collaborators as well as international partners from leading research institutions (Pennsylvania State University, IIT Delhi, Ghent University and Dong Hua University). Rigorous life cycle assessment (LCA) will identify the optimum pathways for liquid biofuel production. We will also examine policies on low carbon fuel production and explore the ways to influence low carbon fuel policies. Through the development of this ground breaking technology, a positive impact to the UK's target for reducing CO_2 emissions and increasing the use of renewable energy will be achieved.

Biohydrogen production from residual glycerol coupled with microbial fuel cell treatment of the spent medium

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Hydrogen is a promising energy carrier for portable applications using fuel cells. Currently hydrogen production is expensive and not sustainable. An alternative is biohydrogen production using organic wastes as a feedstock. Residual glycerol is being produced in large amounts in the biodiesel industry; it contains impurities that required costly and energy intensive purification steps. Therefore, residual glycerol may be useful as substrate for biohydrogen production. In this study, an *Enterobacter* sp. strain was used for hydrogen production from residual glycerol. It was evaluated the influence on the hydrogen production rate of glycerol concentration (10 – 40 g L^{-1}), type of nitrogen source (tryptone vs. ammonium chloride), and amount of oxygen in the gas the phase. Since besides hydrogen Enterobacter sp. also produces organic acids as fermentation end products, it was evaluated the treatment of the spent medium with microbial fuel cells (MFC). A two-chamber MFC with carbon felt electrodes and a proton exchange membrane was used to treat in the anode the spent medium from hydrogen fermentation using *Bacillus* sp. or *Shewanella putrefaciens*; in the cathode was used a solution of ferricyanide. The results indicate that *Enterobacter* sp. can produce hydrogen in minimal salt medium using ammonium chloride as substitute of tryptone, and higher hydrogen production rates were obtained in microarobic conditions instead of anaerobic conditions. The highest hydrogen production (37 mmol L^{-1}) was obtained with 20 g L^{-1} of crude glycerol in microaerobic conditions. Treatment of the autoclaved spent medium with a pure culture of *Shewanella putrefaciens* in a two-chamber MFC, allowed 329 mV and with *Bacillus* sp. 317 mV. Thefore in the global process the crude glycerol waste from the biodiesel industry was utilized to produce hydrogen and the waste medium was further utilized to produce electricity.

Graphite-coated stainless steel felt as a high-performance anode for bioelectrochemical systems

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Scale up of bioelectrochemical systems (BESs) requires highly conductive, biocompatible and stable electrodes. Here we present graphite-coated stainless steel felt (C-SS felt) as a high-performance and scalable anode. The electrode is created by generating a carbon layer on stainless steel felt (SS felt) via a multi-step deposition process involving α-D-glucose impregnation, caramelization, and pyrolysis. Physicochemical characterizations of the surface elucidate that a thin (20 ± 5 μm) and homogenous layer of polycrystalline graphitic carbon was obtained on SS felt surface after modification. The carbon coating significantly increases the biocompatibility, enabling robust electroactive biofilm formation. The C-SS felt electrodes reach current densities (jmax) of 3.65 ± 0.14 mA/cm^2 within 7 days of operation, which is 11 times higher than plain SS felt electrodes (0.30 ± 0.04 mA/cm^2). Graphite coating not only generates a thin (20μm) carbon layer on SS felt surface but also breaks down the passive layer on the SS felt surface. Remove of passive layer reduces the corrosion resistance of SS felt but improves the electron transfer between carbon layer and the bulk SS substrate. The excellent biocompatibility, high specific surface area, high conductivity, good mechanical strength, and low cost make C-SS felt a promising electrode for BESs.

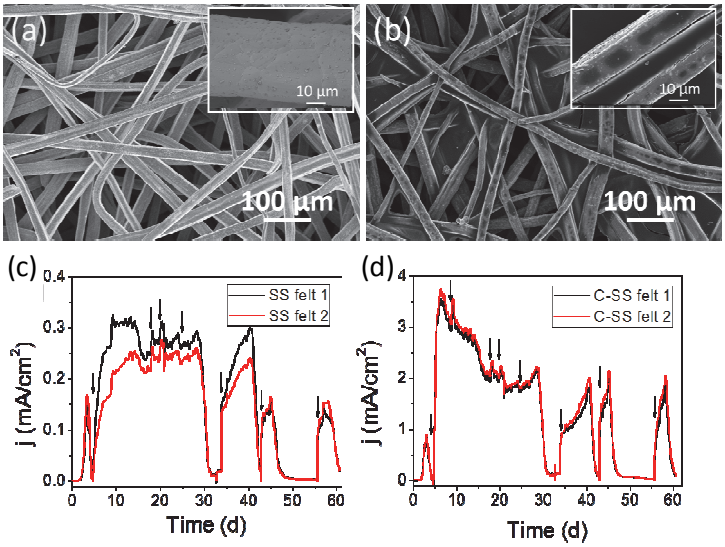

Figure 1: Representative FESEM images of SS felt (a) and C-SS felt (b); bioelectrocatalytic current generation for SS felt (c) and C-SS felt (d).

Guo K., Hidalgo D., Tommasi T., Rabaey K. (2016) Pyrolytic carbon-coated stainless steel felt as a high-performance anode for bioelectrochemical systems, Bioresource Technology, In press.

Performance assessement of tubular microbial fuel cells (TMFCs) operated at low pH

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In recent years,, food waste fermentation has been considered eligible for Microbial Fuel Cells (MFCs) applications, (Nastro R.A. et al, 2015; Di Palma L. et al., 2015). In this work, the behavior of twelve ad-hoc designed single-chambered, air-cathode MFCs fed by composite food waste was investigated. All MFCs were provided with anodes realized by means of a carbon fiber brush, while the cathodes were realized through a graphite-based porous ceramic disk. Nafion membranes (117 Dupont) were used at the cathodic interface. MFCs Power Density (PD), Current Density (CD) and pH evolution were monitored during a 28-day period. The performances in terms of polarization curves and power production were assessed according to different operating conditions: feedstock dilution and salinity (in terms of NaCl concentration) and, finally, the effect of an initial potentiostatic growth. An ad-hoc acquisition data hardware was developed using the Arduino board MEGA 2560, (29), composed by a load array (for polarization curve acquisition) with 6 resistors, ranging from 10^6 to 10 Ω. The software for data acquisition was developed with LabVIEW Interface For Arduino, (LIFA) package. All TMFCs operated at low pH (2.5-3.5) as no pH amendment was carried out. Despite the harsh environmental conditions, our TMFCs showed a Power Density (PD) ranging from 20 to 55 mW/m^2kg_{waste} and a maximum CD of 20 mA/m^2kg_{waste} , referred to the cathodic surface. COD removal after a 28-day period was about 45%. The remarkably low pH values as well as the fouling of Nafion membrane very likely limited TMFC performances. However, a scale-up estimation of our reactors provides interesting values in terms of power production, compared to actual anaerobic digestion plants. These results encourage further studies to characterize the graphite-based porous ceramic cathodes and to optimize the global TMFC performances.

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Di Palma L., Geri A., Maccioni M., Paoletti C., Petroni G., Di Battista A., Varrone C., 2015, Experimental assessment of a process including microbial fuel cell for nitrogen removal from digestate of anaerobic treatment of livestock manure and agricultural wastes, Chemical Engineering Transactions, 43, 2239-2244, DOI: 10.3303/CET1543374.

Resource recovery with Extremophilic Microbial Electrochemical Systems

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Sulfide mineral processing often produces large quantities of wastewaters containing various inorganic sulfur compounds (sulfide, thiocyanate, thiosulfate and tetrathionate). If released untreated, these wastewaters can cause environmental damage such as acidification and corrosion, as well as being toxic to humans. Microbial electrochemical systems exploit the metabolism of microorganisms to generate electrical energy or a useful product. Extremophilic microorganisms grow in environments that are hostile to most forms of life and their utilization in microbial electrochemical systems has opened new possibilities to oxidize substrates and produce novel products under in situ conditions (Dopson et al., 2015). These include the conversion of the mentioned inorganic sulfur pollutions into energy or resources. In this project, we have investigated using acidophile-inoculated MFCs to harvest energy from acidic, tetrathionate and thiosulfate containing process water. The maximum cell voltage was 105±42mV (1000Ω external resistance). 16S rRNA gene sequencing of the microbial consortia resulted in sequences that aligned within the genera *Thermoplasma*, *Ferroplasma*, *Leptospirillum*, *Sulfobacillus* and *Acidithiobacillus* (Ni et al., 2016). By applying metagenomic approaches, we studied the phylogeny of the anode biomass and found evidence for metabolic processes such as inorganic carbon fixation, biofilm formation, and energy metabolism. Based on sulfur metabolism-related genes identified from the metagenome, we propose a novel pathway for tetrathionate degradation that has not been show from the mentioned species before. To treat low temperature thiocyanate containing process water, we operated MFCs under 8°C inoculated with psychrophilic microorganisms. Preliminary results showed that the current production is in response to the consumption of substrate thiocyanate. The maximum current density achieved was 12.8mA/m^2. The study of anode microbial consortia based on 16S rRNA gene sequencing is ongoing.

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Ni, G., et al. (2016). "Electricity generation from an inorganic sulfur compound containing mining wastewater by acidophilic microorganisms." Research in Microbiology.

Development of an anaerobic digester incorporating a Microbial Electrolysis Cell to enhance biogas production from sewage sludge

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Anaerobic digestion (AD) is a widespread technology to valorise sewage sludge from wastewater treatment plants while allowing energy recovery as biogas. However, biogas lacks quality for direct use, which makes necessary an up-grading process to achieve biomethane quality standard. Different technologies are being tested to decrease this reforming step cost. Among these, the use of Microbial Electrochemical Technologies (METs). Electroactive microorganisms' capacity to produce methane in a membrane-less Microbial Electrolysis Cells (MECs) was firstly reported in 2009 by Clauwaert and Verstraete. Other authors have reported the ability of different microorganisms to drive the conversion reduction of CO_2 to CH_4, known as electromethanogenesis. Most studies have focussed on studying the process in two-chamber configuration reactors (Villano *et al.*, 2010) aimed at the biogas reforming (Batlle-Vilanova *et al*., 2015). Recently, some authors have focussed on the use of MECs for enhancing biogas production (Moreno *et al.*, 2016).

In this context, we have developed novel anaerobic digester, at laboratory scale, to demonstrate the feasibility of incorporating a MEC to increase biogas production and quality. Different parameters have been considered for the design of the bioreactor for an easy subsequent scale-up. For this purpose, a single chamber configuration has been selected. The best performing electrode materials (a mixture of carbon fibers and stainless steel) to drive electromethanogenesis in the cathode, while avoiding water hydrolysis in the anode, have been studied. Different strategies to enrich an electromethanogenic population from anaerobic sludge in a single chamber bioreactor have been carried out, demonstrating the feasibility of the process. In a subsequent step, enriched biocathodes have been introduced to an operating AD reactor (1L), treating a mixture of sewage sludge to asses the biogas production and quality enhancement respect a control AD reactor.

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Effects of PEI, a membrane permeabilizer on genotoxic- and oxidative stress-sensitive Escherichia coli bioreporters

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Gram negative bacteria are intrinsically resistant to many compounds like antibiotics and high molar mass drugs due to the presence of an outer membrane permeability barrier. Reporter genes technology nowadays is used in different fields of science for various applications. One example of this, bacterial-based reporter genetic systems, are suitable for monitoring promoter strength and activity, as well as monitoring gene expression. They can also be used to study cellular protein localization. Each of these is affected by altered environmental conditions and their impacts on the bacterium. The *recA, sodA* and *aaeXAB* promoters, which control expression of repair proteins in response to DNA damage, oxidative stress response due to superoxide radical production and phenolic compounds, like ferulic acid, respectively, were made use of to construct bioreporter strains. These *E. coli* strains and their responses to various inducers were evaluated when cultured in the presence of PEI, a membrane permeabilizer. Tests with different forms of PEI found the branched form (BPEI) to be less toxic to the bacteria than the linear form (LPEI). Moreover, the BPEI also was beneficial as it led to better responses from the chemical inducers than LPEI. The improved responses with BPEI were more prominent with strain DS1 (*soda::lux*) than the other strains. With DS1, the relative fold induction (RFI), based upon the bioluminescent reporter proteins, was approximately 20-fold higher with BPEI addition with an approximately 8 fold increase in sensitivity. By comparison, the RFI for the other two strains was only about 2.5-fold. These results show that although the strains vary in their scope with regards to the stress being monitored, the responses from each were improved due to the membrane permeabilizing activity of BPEI.

Effect of sulphide scavenging on hydrocarbon biodegradation

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Bioremediation of oil contaminated marine sediments is a challenge due to the recalcitrant nature of hydrocarbons and the absence of thermodynamically favourable electron acceptors below the oxic zone. The anode of bioelectrochemical systems (BES) may serve as an exogenous electron acceptor and thus facilitate oil spill bioremediation by enrichment of hydrocarbon degrading microorganisms (Daghio et al., 2015; Viggi et al., 2015). Sulphur metabolism has been identified as a key player in oil spill bioelectro-remediation: oil-contaminated sediment bioreactors showed sulphate-reduction coupled to hydrocarbon degradation with resultant sulphide production oxidized on the anode and thus, enhanced biodegradation. To stimulate sulphide production and scavenging, a slow daily flow rate of 0.5 L artificial seawater was passed through the oil contaminated sediments and the effluent of 1.5 L/d was introduced in the anodic chamber of a BES (Fig.). Two control reactor set-ups; one in open circuit (OC) and one with autoclaved sediment show the effect of polarization and indigenous microbial populations on hydrocarbon degradation without the anode serving as electron acceptor. Sulphur species, i.e. sulphide, sulphate, thiosulphate and sulphite, alkanes, chemical oxygen demand, total organic carbon, microbial communities, current production in polarized anodes and cell voltage in OC conditions were monitored over time.

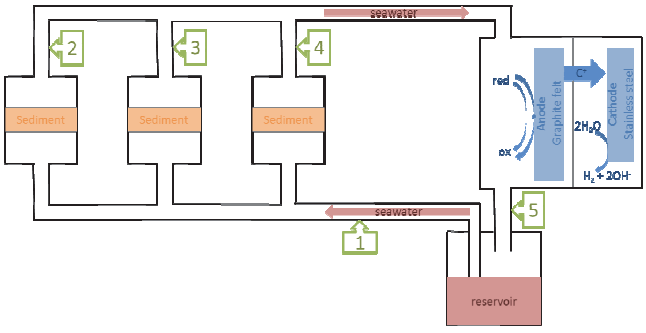

Figure: Scheme of the reactor set up

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Superacidic titania-loaded polymer membranes for microbial fuel cell applications

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Microbial fuel cells (MFCs) represent an appealing technology for the simultaneous electricity production and wastewater treatment. One of the issues, limiting the performance of such devices in terms of delivered current density, is the high internal resistance of the cell. This aspect is mainly related to the ion-exchange membrane separator and can be properly controlled by tuning the ion transport between anode and cathode. To this purpose, we here propose new nanocomposite membranes, formed by adding in-house synthesized sulphated titania (S-TiO_2) nanoparticles in Nafion. The synthesis and physical chemical characterizations of both the inorganic compound and hybrid membranes will be described. Different loading of S-TiO_2 in Nafion have been considered, in order to evaluate the effect of the additive on the polymer ion-conducting properties. It has been demonstrated that a critical filler concentration (i.e., 5 wt. %) exists, maximising ion-conductivity of the composite systems with respect to a bare Nafion membrane. Structural (XRD), morphological (TEM, AFM) and spectroscopical (FT-IR, Raman, ^{1}H-NMR) characterizations, as well as electrochemical tests for ion-conductivity, proved the occurrence of a peculiar mechanism for proton transport within the hybrid membranes, due to the acidic surface properties of the filler [1-3].

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