General Meeting of the International Society for Microbial Electrochemistry and Technology

Lisbon 3rd - 6th October 2017
Campus de Campolide, Universidade NOVA de Lisboa

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COMMITTEES

SCIENTIFIC COMMITTEE

Annemiek ter Heijne, Wageningen University, The Netherlands
Ashley Franks, La Troube University, Australia
Bruce Logan, Penn State University, USA
Caroline Ajo-Franklin, Berkeley University, USA
Cees Buisman, Wetsus, The Netherlands
Cesar Torres, Arizona State University, USA
Falk Harnisch, Leipzig University, Germany
Federico Aulenta, La Sapienza Rome University, Italy
Jeff Gralnick, Minnesota University, USA
Korneel Rabaey, Ghent University, Belgium
Largus Angenent, Cornell University, US Naval Research Lab, USA
Madalena Alves, Minho University, Portugal
Miriam Rosenbaum, Aachen University, Germany
Sarah Strycharz-Glaven, US Naval Research Lab, USA
Stefano Freguia, Brisbane University, Australia
Taeho Lee, Pusan University, Korea
Tian Zhang, DTU/Wuhan University of Technology, Denmark
Xia Huang, Tsinghua University, China

ORGANIZING COMMITTEE

Ricardo Louro, ITQB-NOVA
Catarina Paquete, ITQB-NOVA
Carlos Salgueiro, FCT-NOVA

Ana Silva, ITQB-NOVA
Ana Fernandes, FCT-NOVA
Inês Trindade, ITQB-NOVA
Joana Dantas, FCT-NOVA
Leonor Morgado, FCT-NOVA
WELCOME MESSAGE

Dear colleagues,

It is with great pleasure that we welcome you to the 6th General Conference of the International Society for Microbial Electrochemistry and Technology. It was for us a great honour and an exciting challenge to organize and host this conference in Lisbon.

We hope that you make of ISMET6 an opportunity to disseminate and celebrate the latest advances in the multidisciplinary field of Microbial Electrochemistry and Technology. We have gone out of our way to stimulate the active participation of students and young researchers. We encourage the more senior colleagues to share their knowledge and experience with the numerous students that are attending as poster or oral presenters. They are the future of this field that is clearly gaining maturity, and you will find ample opportunities to engage these students in formal and informal occasions during the meeting.

The commitment of the global ISMET community to making this conference a success can be appreciated by the geographic and thematic diversity of the applications received. This supports our confidence that advances in Microbial Electrochemistry and Technology that will emerge from this conference will have a global impact, given their relevance for numerous contemporary challenges to be met and overcome towards establishing a sustainable society that fulfils the legitimate aspirations of future generations.

We hope that you enjoy your stay in Lisbon and have a fruitful meeting.

Catarina M. Paquete, Carlos A. Salgueiro, Ricardo O. Louro
Conference chairs
SPONSORS

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ERT (www.ert.pt)
Frontiers in Energy Research (journal.frontiersin.org/journal/energy-research)
LaborSpirit (laborspirit.com)
Palmsens (www.palmsens.com)

SUPPORTERS

Centro de Tecnologia Química e Biológica
Faculdade de Ciências e Tecnologia, Universidade NOVA de Lisboa
Fundação para a Ciência e a Tecnologia
Instituto de Tecnologia Química e Biológica, Universidade NOVA de Lisboa
NZYtech
Sociedade Portuguesa de Química
Sociedade Portuguesa de Microbiologia
UCIBIO-Requimte
VWR
GENERAL INFORMATION

Venue

The meeting will take place at Campus de Campolide of Universidade NOVA de Lisboa in the heart of Lisbon.

Universidade NOVA de Lisboa
Campus de Campolide
1099-085 Lisboa

Useful contacts

National Emergency number: 112

Campolide Police (PSP): +351 21 385 8817

Universidade NOVA de Lisboa: +351 213 715 600

Teletaxis: +351 218 111 100
Retálios: +351 21 811 9000
Rádio Taxis Lisboa: +351 219 362 113
Facilities

Registration will open at 14h on Tuesday October 3rd. Registration includes all the sessions, abstract book, lunch and coffee breaks from 4th to 6th of October, welcome reception, poster sessions and dinner on the October 5th.

Sessions will be held in Rooms 1 and 2.

Free WiFi is available in the building: eduroam and AuditorioUNL.

Poster Sessions

Poster sessions will take place on the main hall and posters will be up for the duration of the meeting. Odd numbers will present their posters on Wednesday October 4th 18h20-19h30. Even number posters will present their posters on Thursday October 5th 13h10-15h.

Awards

The organizers and the members of the scientific committee will be voting to select the best oral and best poster presentation. The result will be announced in the closing session.

Group Photo

On Thursday October 5th at 14h45 we will meet outside in the staircase for a Group photo.
Meals

Lunches and coffee-breaks will be held in the main hall.

Social Program

The Welcome Reception will be held in the main hall.

The conference dinner will be on the 5th of October in Quinta do Lumarinho in Sintra. The participants will take the bus at 18h from the conference venue and will do a city tour in Lisbon before heading to Sintra.

Quinta do Lumarinho
R. Maestro Alferes Álvaro Augusto de Sousa 29,
2715 Montelavar, Sintra
# Meeting of the International Society for Microbial Electrochemistry and Technology, Lisbon 2017

<table>
<thead>
<tr>
<th>October 3rd</th>
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<tbody>
<tr>
<td><strong>09h00-10h00</strong></td>
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<tr>
<td>Workshop registration</td>
<td>Plenary</td>
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<td><strong>10h00-13h00</strong></td>
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<tr>
<td>Pre-ISMET Workshop at ITQB</td>
<td>Session I</td>
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<td><strong>10h00-10h20</strong></td>
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<td>A. Okamoto A. Sydow</td>
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<td>M. Ferreira I. Vassilev</td>
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<td>K. Katuri V. Agostino</td>
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<td>U. Schröder X. Li</td>
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<td>P. Liu S. Xiao</td>
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<td>J. Deutzmann L. Jourdin</td>
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<td>M. TerAvest R. Blasco-Gómez</td>
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<td>Lunch at ITQB</td>
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<td>Session IV</td>
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**Session I**: Fundamentals of the extracellular electron transfer processes  
**Session II**: Microbial Electrochemical Technologies: from fundamental to applied research  
**Session III**: Electrochemical cell design and electrode materials  
**Session IV**: Microbial fuel cell applications
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<th>Session VIII</th>
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<td>Tian Zhang</td>
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<td>10h00-10h20</td>
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<td>R. Hegner</td>
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<td>C. Engel</td>
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<td>A. Goglio</td>
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<td>M. Tucci</td>
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<td>A. Bergel</td>
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<td>R. Veerubhotia</td>
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<td>F. Barrière</td>
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<td>A. Sacco</td>
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<td>I. Vargas</td>
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<td>F. Kracke</td>
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<td>11h30-11h50</td>
<td>X. Zhang</td>
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<td>T. Sleutels</td>
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<td>P. Chong</td>
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<td>Y. Asensio</td>
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<td>12h50-13h10</td>
<td>C. Cruz Viggi</td>
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<tr>
<td>13h10-14h45</td>
<td>Lunch &amp; Poster session</td>
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<tr>
<td>14h45-15h00</td>
<td>Group Photo</td>
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<tr>
<td>15h00-15h40</td>
<td>Plenary</td>
<td>A. Fernandes</td>
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<td>15h40-16h00</td>
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<td>D. Phillips</td>
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<td>16h00-16h20</td>
<td>A. Spormann</td>
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<td>16h20-16h50</td>
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<td>T. Arinda</td>
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<td>L. Tender</td>
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<td>17h30-17h50</td>
<td>F. Zhao</td>
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<td>18h00-19h30</td>
<td>Lisbon visit by bus</td>
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<td>19h30-23h00</td>
<td>Dinner in Sintra</td>
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SCIENTIFIC PROGRAM

Tuesday 3rd October

9h00  Workshop registration
10h00  Pre-ISMET Workshop at ITQB
13h00  Lunch at ITQB
14h00  ISMET Registration
16h00  WELCOME SESSION

PLENARY (Room 1)

Chair: Ricardo Louro

16h20  Gemma Reguera  “Microbes to power!”

Department of Microbiology and Molecular Genetics, Michigan State University, USA

17h00  Welcome Reception

Wednesday 4th October

PLENARY (Room 1)

Chair: Jeffrey Gralnick

09h00  Stefano Freguia  “Ion exchange membranes turn METs into productive factories”

Advance Water Management Centre, The University of Queensland, Australia

SELECTED ORAL COMMUNICATIONS

SESSION I (Room 1)  SESSION V (Room 2)

Chair: Jeffrey Gralnick  Chair: Tian Zhang

Fernanda Jimenez Otero  Nikolaos Xafenias
(U. Minnesota, USA)  (Chalmers U. Tech., Sweden)

“Enhancing current production through genetic manipulation”  “Electrified fermentations enhance anaerobic lysine production by Corynebacterium glutamicum”
<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker 1</th>
<th>Speaker 2</th>
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</thead>
<tbody>
<tr>
<td>10h00</td>
<td>Akihiro Okamoto (Nat. Inst. Mat. Sci., Japan)</td>
<td>Anne Sydow (DECHHEMA, Germany)</td>
</tr>
<tr>
<td></td>
<td>“Critical Molecular Structure in Bound Flavin Cofactor to Enhance the Rate of Extracellular Electron Transport”</td>
<td>“Expanding the product spectrum of microbial electrosynthesis – Engineering of Cupriavidus necator to produce the sesquiterpenoid α-humulene”</td>
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<tr>
<td>10h20</td>
<td>Marisa Ferreira (UCIBIO-Requimte, Portugal)</td>
<td>Igor Vassilev (U. Queensland, Australia)</td>
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<tr>
<td></td>
<td>“Molecular interactions between Geobacter sulfurreducens triheme cytochromes and the electron acceptor Fe(III) citrate studied by NMR”</td>
<td>“Microbial Electrosynthesis: Microbes turning CO₂ into volatile fatty acids and the associated alcohols”</td>
</tr>
<tr>
<td>10h40</td>
<td>Krishna Katuri (KAUST, Saudi Arabia)</td>
<td>Valeria Agostino (I. I. Tecnologia, Italy)</td>
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<tr>
<td></td>
<td>“Electromicrobiology insights of D. acetoxidens through genomic and transcriptome analysis”</td>
<td>“Characterization of new cathodically active microorganism Desulfo sporosinus orientis: simultaneous sulfate reduction and acetate production”</td>
</tr>
<tr>
<td>11h00</td>
<td>COFFEE BREAK</td>
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### SELECTED ORAL COMMUNICATIONS

#### SESSION I (Room 1)

**Chair:** Alfred Spormann

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
</tr>
</thead>
<tbody>
<tr>
<td>11h30</td>
<td>Pan-Pan Liu (Tsinghua Univ., China)</td>
<td>“Accelerating interior electron transfer of electroactive biofilm by magnetite for high performance microbial fuel cell”</td>
</tr>
<tr>
<td>11h50</td>
<td>Estelle Lebègue (U. Rennes, France)</td>
<td>“Charge Transfer Properties of Membrane Proteins Probed at Modified Electrodes”</td>
</tr>
<tr>
<td>12h10</td>
<td>Joerg Deutzmann (Stanford Univ., USA)</td>
<td>“Identification of Enzymes Mediating Cathodic Electron Uptake in Methanococcus maripaludis”</td>
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</table>

#### SESSION V (Room 2)

**Chair:** Frédéric Barrière

<table>
<thead>
<tr>
<th>Time</th>
<th>Speaker</th>
<th>Title</th>
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<tbody>
<tr>
<td>11h30</td>
<td>Xiaohu Li (Tech. Univ. Denmark, Denmark)</td>
<td>“Salinity-gradient energy driven microbial electrosynthesis of value-added chemicals from CO₂ reduction”</td>
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<tr>
<td>11h50</td>
<td>Shuai Xiao (Chongqing Univ., China)</td>
<td>“Enhanced methane production in a microbial electrosynthesis system using a bipolar membrane”</td>
</tr>
<tr>
<td>12h10</td>
<td>Ludovic Jourdin (Wagen. U. &amp; R., Netherlands)</td>
<td>“Continuous caproate (C₆) production from CO₂ by microbial electrosynthesis: making feed additive precursor with electricity”</td>
</tr>
</tbody>
</table>
12h30 Simone Schmitz  
(RWTH Aachen Univ., Germany)  
“P. aeruginosa’s phenazine-based 
electroactivity is tightly regulate”

12h50 Uwe Schröder  
(Tech. Univ. Braun., Germany)  
“Excited bacteria: In situ 
autofluorescence 
spectroelectrochemistry for the study of 
extracellular electron transfer”

12h30 Kristof Verbeeck  
(Ghent Univ., Belgium)  
“Increased concentration and current 
efficiency for in situ extraction of acetic acid in 
microbial electrosynthesis from CO₂”

12h50 Ramiro Blasco-Gómez  
(U. Girona, Spain)  
“From flying blind to a better understanding of 
the bioelectrochemical transformation of CO₂ 
into valuable chemicals”

13h00 LUNCH

SELECTED ORAL COMMUNICATIONS

SESSION VI (Room 1)  
Chair: Taeho Lee

14h30 Clément Flayac  
(INRA, France)  
“Microbial anodic consortia fed with 
fermentable substrates in microbial 
electrolysis cells: the significance of ecological 
interactions”

14h50 Hong Liu  
(Oregon State Univ., USA)  
“Predicting Microbial Fuel Cell Biofilm 
Communities and Bioreactor Performance 
using Artificial Neural Networks”

15h10 Nils Risgaard-Petersen  
(Aarhus Univ., Denmark)  
“Electrostatic potential measurements as 
proxies for cable bacteria activity – potentials 
and resistances”

15h30 Jo Philips  
(Ghent Univ., Belgium)  
“Isolation and characterization of novel 
electrotrophic microorganisms using metallic 
iron as sole electron donor”

SESSION II (Room 2)  
Chair: Gemma Reguera

14h30 Sebastian Beblawy  
(Karlsruhe Inst. T., Germany)  
“Electrode-assisted acetoin production in a 
metabolically engineered Escherichia coli 
strain”

14h50 Caroline Ajo-Franklin  
(I. Lawr. Berk. N. Lab., USA)  
“Playing favorites: Molecularly-precise 
bioelectrocatalysis and biosensing”

15h10 Robert Keith Brown  
(TU Braunschweig, Germany)  
“Selection, conditioning and controlling - 
Improving the performance of 
electrochemically active microorganisms 
treating real wastewater”

15h30 Alaa Ragab  
(KAUST, Saudi Arabia)  
“Electrode-assisted methanogenesis in a 
microbial electrolysis cell: Examining the effect 
of set potential on the microbial activity and 
functional response”
### 15h50

<table>
<thead>
<tr>
<th>Roman Moscoviz</th>
<th>Matthew Yates</th>
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<tbody>
<tr>
<td>(INRA, France)</td>
<td>(Naval Res. Lab. USA)</td>
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<tr>
<td>“Interspecies electron transfer mediated parasitism: co-culture of <em>Geobacter sulfurreducens</em> and <em>Clostridium pasteurianum</em>”</td>
<td>“Microbial electrochemical energy storage and recovery in a combined electrotrophic and electrogentic biofilm”</td>
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### 16h10

**COFFEE BREAK**

### 16h40

#### SELECTED ORAL COMMUNICATIONS

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<th>SESSION VI (Room 1)</th>
<th>SESSION VII (Room 2)</th>
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<td><strong>Chair:</strong> Sarah Glaven</td>
<td><strong>Chair:</strong> Carlos Salgueiro</td>
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<td><strong>Brian Eddie</strong></td>
<td><strong>Dario Rangel Shaw</strong></td>
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<td>(Naval Res. Lab. USA)</td>
<td>(KAUST, Saudi Arabia)</td>
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<tr>
<td>“Probing the Functions of Biocathode Microbial Community Members with Inhibitors, Transcriptomics, and Genetic Manipulation”</td>
<td>“Are anammox bacteria electrochemically active?”</td>
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<tr>
<td><strong>Alexiane Godain</strong></td>
<td><strong>Márcia Santos</strong></td>
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<td>(Univ. Lyon, France)</td>
<td>(Univ. Porto, Portugal)</td>
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<td>“Evolution of anodic biofilm formation under shear stress conditions”</td>
<td>“Microbially charged redox flow battery: coupling a bioelectrochemical cell with a redox flow battery”</td>
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<td><strong>Taeho Lee</strong></td>
<td><strong>Johannes Jermakka</strong></td>
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<td>(Pusan Nat. Univ., Korea)</td>
<td>(Tampere U. Tec., Finland)</td>
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<tr>
<td>“Microbial populations and putative functions for complete ammonia removal in single-chambered microbial nitrogen-removal cells (MNCs)”</td>
<td>“Chemical free nitrogen capture from urine by precipitation as ammonium bicarbonate: overcoming limits of precipitation”</td>
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### 17h00

#### PLENARY (Room 1)

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<td>“Bioelectrorefineries: Joining the forces of microbiology and electrochemistry for production of chemicals and fuels”</td>
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<td>(Helmholtz Centre for Environmental Research GmbH - UFZ, Germany)</td>
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### 17h20

**POSTER SESSION (with Wine and Beer)**
Thursday 5th October

PLENARY (Room 1)

Chair: Korneel Rabaey

09h00  Tian Zhang  “From the cathode to the microbe: understanding and optimizing microbial electrosynthesis”
School of Chemistry, Wuhan University of Technology, China
Technical University of Denmark, Denmark

SELECTED ORAL COMMUNICATIONS

SESSION IV (Room 1)

Chair: Korneel Rabaey

9h40  Matteo Tucci  (U. Studi Milano, Italy)
“Wastewater treatment plant field application of a real time MFC-based BOD sensor”

10h00  Ramya Veerubhotla  (Indian Inst. Tec. K., India)
“Bioelectricity generation using a miniature biophotovoltaic device”

10h20  Adriano Sacco  (Ist. Ital. Tecnol., Italy)
“Pre-colonization of anodic electrodes in seawater sediment for Single Chamber Floating Microbial Fuel Cells application”

10h40  Meriah Arias-Thode  (NWSCP, USA)
“Benthic Microbial Fuel Cell Provides Power to a pH Meter for almost One Year”

SESSION VIII (Room 2)

Chair: Falk Harnisch

Andrea Goglio  (U. Studi Milano, Italy)
“Treating wastewater while recovering nutrients: electrochemical biofilters coupled to innovative biochar-based cylindrical cathodes”

10h00  Yi Zuo  (Chevron, USA)
“Bioelectrochemical Technology for Enhanced Remediation of Petroleum Hydrocarbon-contaminated soil”

10h20  Lukasz Szydlowski  (Okinawa I. S. T., Japan)
“Mature electrogenic community is able to drive efficient denitrification regardless of applied potential”

10h40  Sara Tejedor-Sanz  (IMDEA Water, Spain)
“Fluidized electrodes as electron acceptors or electron donors: strategies and applications”

11h00  COFFEE BREAK
SELECTED ORAL COMMUNICATIONS

SESSION II (Room 1)

Chair: Luciana Peixoto

11h30

Xu Zhang
(Ghent Univ., Belgium)

“Periodical charge/discharge can enhance the characteristic of electroactive biofilms”

11h50

Tom Sleutels
(Wetsus, Netherlands)

“Optical Coherence Tomography and Electrochemical Impedance Spectroscopy as non-invasive tools for biomass quantification”

12h10

Poehere Chong
(Univ. Toulouse, France)

“Microbial 3D porous anodes: what are the limitations to the progression of multispecies biofilms at the scale of a single pore?”

12h30

Yeray Asensio
(FCC Aqualia, Spain)

“Combination of electrochemical technologies as a paradigmatic change in industrial wastewater treatment plants”

12h50

Carolina Cruz Viggi
(IRSA-CNR, Italy)

“Enhancing the electrocatalytic activity of microbial bioanodes with conductive magnetite nanoparticles”

SESSION V (Room 2)

Chair: Abraham Esteve-Núñez

11h30

Zhiyong (Jason) Ren
(U. Colorado Boulder, USA)

“Metagenomic Insights and System Scale up for Bioelectrochemical Petroleum Hydrocarbon Remediation”

11h50

Orianna Bretschger
(Aquam LLC, USA)

“Field-tested 110L microbial fuel cell for continuous treatment of swine waste”

12h10

Alae Elabed
(USMBA, Morocco)

“Modified anode material to enhance electrochemical and tannery wastewater treatment performance in bioelectrochemical systems”

12h30

Eileen Yu
(Newcastle Univ., UK)

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

PLENARY (Room 1)

Chair: Uwe Schröder

15h00

Jeffrey Gralnick
“Extracellular Electron Transfer: The Flavin Strikes Back”
BioTechnology Institute, University of Minnesota-Twin Cities, USA
## SELECTED ORAL COMMUNICATIONS

### SESSION I (Room 1)

**Chair:** Uwe Schröder  
Ana Fernandes  
(UCIBIO-Requimte, Portugal)

“Living Capacitors: functional characterization of a novel cytochrome acting as a nanowire”

**Alfred Spormann**  
(Stanford Univ., USA)

TBA

### SESSION II (Room 2)

**Chair:** Madalena Alves  
Daniel Phillips  
(Naval Res. Lab., USA)

“Optimizing electric reporter strains by in situ visualization of induced fluorescence in Marinobacter biofilms”

Tutut Arinda  
(Karlsruhe Inst. T., Germany)

“A Recyclable Bioelectrocatalyst Enhancing Electricity Generation in Bioelectrochemical Reactor”

### 16h00 COFFEE BREAK

### SELECTED ORAL COMMUNICATIONS

### SESSION I (Room 1)

**Chair:** Caroline Ajo-Franklin  
Leonard Tender  
(Naval Res. Lab., USA)

“Contemplating Microbial Electrochemistry at Very Large and Very Small Scales”

**Michaela TerAvest**  
(Michigan St. Univ., USA)

“Important role of sodium-pumping NADH dehydrogenase in Shewanella oneidensis”

**Feng Zhao**  
(Chinese Acad. Sci., China)

“The key role of extracellular polymeric substances on extracellular electron transfer”

### 18h00 LISBON VISIT BY BUS

### 20h00 DINNER AT SINTRA
### Friday 6th October

#### PLENARY (Room 1)

**Chair:** Bruce Logan

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<tr>
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<td>Madalena Alves</td>
<td>“Scale-up and other challenges of Bioelectrochemical Systems”</td>
<td>Centre of Biological Engineering, University of Minho, Portugal</td>
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#### SELECTED ORAL COMMUNICATIONS

### SESSION VII (Room 1)

**Chair:** Bruce Logan

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<tr>
<td>09h40</td>
<td>Richard Hegner</td>
<td>“Eu tenho dois amores - Coupling selective electrochemical CO₂ reduction to formate with microbial biosynthesis “</td>
<td>Helm. C. Envir. Res., Germany</td>
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<tr>
<td>10h00</td>
<td>Christina Engel</td>
<td>“Morphological analysis of electrochemically active biofilms of Geobacter sulfurreducens and Shewanella oneidensis”</td>
<td>TU Braunschweig, Germany</td>
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<tr>
<td>10h20</td>
<td>Frédéric Barrière</td>
<td>“Microbial bioanodes as sensors for ionic liquids toxicity and anti-biofouling properties”</td>
<td>U. Rennes, France</td>
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<tr>
<td>10h40</td>
<td>Ignacio Vargas</td>
<td>“Study of The Electrochemical Capacity of A New Chemolithoautotrophic Arsenic Oxidizing Bacteria Ancylobacter Sp.Ts-1”</td>
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### SESSION III (Room 2)

**Chair:** Stefano Freguia

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<td>09h40</td>
<td>Qing Du</td>
<td>“In-situ Visualization of Mixed Electroactive Biofilm Growth using an Integrated System”</td>
<td>Nankai Univ., China</td>
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<tr>
<td>10h00</td>
<td>Alain Bergel</td>
<td>“Experimental and theoretical analysis of rate-limiting steps in microbial fuel cells”</td>
<td>CNRS, France</td>
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<td>10h20</td>
<td>Annemiek ter Heijne</td>
<td>“High Rate Methane Production at Low Overpotential on Granular Activated Carbon Biocathodes”</td>
<td>Wagenin. U., Netherlands</td>
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<tr>
<td>10h40</td>
<td>Frauke Kracke</td>
<td>“Novel Transition Metal Cathodes for Sustained Microbial Electrosynthesis”</td>
<td>Stanford U., USA</td>
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#### 11h00 COFFEE BREAK
## SELECTED ORAL COMMUNICATIONS

### SESSION VII (Room 1)

**Chair:** Lars Angenen

**Juan Anaya**  
(Univ. Gren.-Alpes, France)  
“Neodymium recovery: from ionic liquids to bioelectrochemical systems”

**Albert Guisasola**  
(U. Aut. Barcelona, Spain)  
“Opportunities of BES for sulfate wastewater treatment: potential sulfur recovery”

**Lina Bird**  
(Naval Res. Lab., USA)  
“The effect of the Mtr pathway on current production in *Marinobacter CP1*”

**Andrea Schievano**  
(U. Stud. Milano., Italy)  
“A new applicative frontier for microbial fuel cells: bioelectrochemical fertilizers”

### SESSION III (Room 2)

**Chair:** Michaela TerAvest

**Stefania Marzorati**  
(U. Stud. Milano., Italy)  
“Ligno-cellulosic Materials in Low-cost Microbial Fuel Cells Architectures for Nutrients Recovery”

**Bruce Logan**  
(Penn State Univ., USA)  
“Addressing Ohmic Resistance and the Challenges of Evaluating Very Large Cathodes”

**Melanie Pierra**  
(Ghent Univ., Belgium)  
“Impact of sub-microscale surface roughness on the colonization, the current production and the settlement of mixed culture anodic biofilms”

**Pierre Champigneux**  
(CNRS, France)  
“Rough vs Micro-structured surfaces: which impacts on *Geobacter sulfurreducens* bioanode performances”

### Schedule

- **12h50**  | PRIZE AWARDS and CLOSING SESSION (Room 1)
- **13h30**  | LUNCH and FAREWELL
- **14h30**  | VISIT TO SINTRA
PLENARY SPEAKERS
Microbes to power!

Gemma Reguera

Department of Microbiology and Molecular Genetics; 567 Wilson Rd., Rm. 6190; Biomedical and Physical Science building; Michigan State University; MI 48824, USA

Email: reguera@msu.edu

In Nature, highly efficient and diverse consortia of microbes process organic matter, such as agricultural and human wastes, while generating energy for growth and recycling carbon and other vital elements. Driving these reactions are exoelectrogenic organisms, such as those in the Geobacter genus, that extract electrons from the chemical substrates and transfer them to solid-phase electron acceptors (e.g., iron and manganese oxide minerals). This natural process can be mimicked in electrochemical devices that provide a poised anode electrode as an electron sink to drive the growth of an electrochemically active biofilm. In this lecture, I will describe efforts by my research team to gain mechanistic understanding of bioanode catalysis and exploit the natural properties of the electronic biofilm components in biotechnology. I will also describe approaches by my group to engineer bioanodes for synergistic interactions with fermentative bacteria and to implement anodic electro-fermentations at industrial scales. The advantages of these electro-fermentation platforms to harness energy from a wide range of complex organic wastes and to reduce processing costs during the recovery of fermentation products will be discussed.
Plenary Speakers

Ion exchange membranes turn METs into productive factories

S. Freguia\textsuperscript{1}, G. Pozo\textsuperscript{1}, J. Jermakka\textsuperscript{1,2}, I. Vassilev\textsuperscript{1}, M. Zhou\textsuperscript{3}, B. Virdis\textsuperscript{1}, P. Ledezma\textsuperscript{1}, J. Keller\textsuperscript{1}

\textsuperscript{1}Advanced Water Management Centre, The University of Queensland, Brisbane, QLD 4072 Australia
\textsuperscript{2}Department of Chemistry and Bioengineering, Tampere University of Technology, Tampere, Finland
\textsuperscript{3}Qingdao Institute of Bioenergy and Bioprocess Technology, Chinese Academy of Sciences, China

Email: s.freguia@uq.edu.au

Nearly 20 years after their first appearance in the science scene, microbial fuel cells (MFCs) have not yet broken the barriers of commercialization, for the simple reason that they are no match to conventional technologies such as the activated sludge process. Intrinsic limitations including the low conductivity and buffering capacity of typical wastewaters have kept the current densities of MFCs well below the levels at which the technology would be economically comparable to the activated sludge or anaerobic digestion for wastewater treatment. As a consequence, research has moved on to find other microbial electrochemical technologies (METs) with higher potential for application and commercialization, based on the understanding that METs can be viable only if they (i) generate added value in the form of products, or (ii) successfully target problematic waste streams which are not easily treated with conventional technology.

The emphasis of this talk is the essential role that ion exchange membranes play in these high-potential METs, allowing for the selective transport of ions which can thereby be removed from the waste streams and subsequently concentrated into a product stream. Three examples of technologies developed at the Advanced Water Management Centre are given:

1. The Ugold process: removal and recovery of N, P, K from source-separated urine, where cation and anion exchange membranes enable the separation and recovery of these nutrients in a clean and reusable stream.
2. The Sulfred process: removal of sulfate from acid mine drainage in a permanent way with elemental sulfur and end-product. This process addresses a problematic waste stream which currently sees no straight-forward option for treatment. This elegant process relies on an anion exchange membrane for selective sulfate removal from mining waste streams.
3. Production of butyrate and caproate from carbon dioxide by microbial electrosynthesis. These higher volatile fatty acids are separated from their broth through a system of anion exchange membranes, ultimately leading to pure product streams.

The message of this talk is that the path towards commercialization of METs will not only require research on electrode materials, microbiology and reactor design, but also research in the area of membranes, as each individual process requires ad hoc membrane characteristics for optimal performance.
Bioelectro refineries: Joining the forces of microbiology and electrochemistry for production of chemicals and fuels

Falk Harnisch\textsuperscript{1}

\textsuperscript{1}Department Environmental Microbiology, Helmholtz Centre for Environmental Research GmbH - UFZ, Permoserstraße 15, 04318 Leipzig, Germany

falk.harnisch@ufz.de

Technologies that allow the preservation of scarce fossil resources will pave the way towards resource security. The two main factors that contribute to a sustainable future industry are the source of electric energy and the carbon feedstock. First, the electrical power production based on renewable resources, such as wind and solar energy, is promoted. Second, renewable feedstocks and waste streams are considered as valuable precursors for the production of commodities and fuels. Building a bridge between both fields means linking the conversion of electric energy – especially from local peak productions – to chemical energy carriers and commodities. Here, we show that this bridge can be build by a bioelectrorefinery. A bioelectrorefinery can be defined as a facility which integrates biomass conversion processes and equipment to produce fuels, power, and chemicals from biomass, and which also exploits the combination of microbial and electrochemical conversions. Thereby the combination of electrochemistry and microbiology can be realized by primary MET, secondary MET or hybrid systems, i.e. with the electrochemical and microbial process being functionally disconnected\textsuperscript{1}.

In this presentation the general concept of a bioelectrorefinery will be introduced and exemplified. Among others the production of drop-in fuel from biomass using the combination of reactor microbiomes, pertraction and Kolbe-electrolysis will be discussed\textsuperscript{2}.

\textsuperscript{1}U. Schröder, F. Harnisch, L.T. Angenent Energy Environ. Sci., 2015, 8, 513

\textsuperscript{2}Urban et al., \textit{submitted}
From the cathode to the microbe: understanding and optimizing microbial electrosynthesis

Tian Zhang

1School of Chemistry, Chemical Engineering and Life Science, Wuhan University of Technology, Wuhan 430070, PR China
2The Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kgs. Lyngby, Denmark

Storage of electricity from intermittent renewable energy is a major challenge in the development of a healthy sustainable energy industry. One promising approach named microbial electrosynthesis is to convert electricity into biofuels with microbes employing a cathode as sole source of electrons. With this method, electrical energy is amassed by living cells into the chemical bonds of compounds that can be stored and used whenever it is necessary. When compared with abiotic strategies, the main advantage of microbial electrosynthesis comes from the metabolic versatility of biocatalysts, which enables the synthesis of a wide range of longer carbon chain molecules. For this reason, using a pure culture as catalyst becomes an interesting option because it can be genetically engineered for the high-rate production of specific targets. Although multiple groups around the world have been pursuing the development of microbial electrosynthesis in the last decade, productivity and energetic efficiency must still be improved significantly before competing with more well-established approaches such as the Sabatier process. In priority, this requires a better understanding of microbial electrosynthesis system and more specifically of the nature of electron transfer between the cathode and the microbe. Furthermore, a major axis in the development of microbial electrosynthesis should include an important effort for the optimization of the reactor’s core, which comprises the cathode and the microbial catalyst. Here, I will detail the recent work made in my lab to try to understand how electrons are transferred from the cathode of microbial electrosynthesis reactor to the microbes at different potentials. I will also report on our effort to design and fabricate low-cost, robust and high-performance cathode as well as optimal microbial catalysts.
Extracellular Electron Transfer: The Flavin Strikes Back

Jeffrey A. Gralnick

BioTechnology Institute and Department of Plant and Microbial Biology, University of Minnesota – Twin Cities, St. Paul, MN, 55110, USA

Email: gralnick@umn.edu

Dissimilatory metal reducing bacteria couple growth and persistence to reduction of extracellular substrates that are often insoluble, such as oxide minerals and poised electrodes. Electrons enter the cytoplasmic membrane via reduction of lipid-soluble electron carriers, exit the membrane via the activity of quinone oxidoreductases, and travel across the periplasm via periplasmic electron carriers arriving at porin-cytochrome complexes in the outer membrane. The best understood system for extracellular electron transfer is from *Shewanella oneidensis* where the Mtr respiratory pathway is responsible for electron flux to a variety of extracellular substrates, including minerals and electrodes. In *S. oneidensis* the Mtr pathway consists of CymA in the cytoplasmic membrane, FccA and CctA in the periplasm and MtrCAB in the outer membrane. *S. oneidensis* also secretes flavins, which are reduced by the MtrCAB complex to facilitate electron transfer to insoluble substrates via electron shuttling and may have a role in directly influencing the activity of this complex via binding interactions. Here I will describe our work to characterize the Mtr pathway in *Aeromonas hydrophila*, an aquatic facultative anaerobe that has previously been found in microbial fuel cell communities. *A. hydrophila* neither secretes flavins nor is able to catalyze the reduction of oxidized flavin, yet when the *mtrCAB* genes from *A. hydrophila* are expressed in a *S. oneidensis* strain missing *mtrCAB*, they restore both metal and flavin reduction. The inability of *A. hydrophila* to catalyze the reduction of flavin is due to the differences in cytoplasmic and periplasmic respiratory components, which we identify in *A. hydrophila* and reconstruct in *S. oneidensis*. 
Scale-up and other challenges of Bioelectrochemical Systems

Madalena Alves

Centre of Biological Engineering, University of Minho, Braga, Portugal

Email: madalena.alves@deb.uminho.pt

Scientific interest in Bioelectrochemical Systems (BES) is increasing exponentially, as assessed by the number of published papers after 2000. Microbial Fuel Cells and Microbial Electrolysis Cells are interesting research devices but real and full scale applications are still challenging. How far these systems can go beyond the controlled and simulated lab environment?

In my talk, scale-up considerations of BES will be revised and some examples discussed, as for example the pilot scale BES developed in the FP7 Value from Urine project.

Some questions will be addressed such as:

What are the main advantages of these systems as compared with traditional wastewater treatment processes?
How these systems can be used for efficient resource recovery?
How they can be coupled with energy storage devices as for example redox flow batteries?

Finally I will present some fundamental aspects of carbon materials-bacteria/archaea interactions of relevance in BES and beyond.
ORAL COMMUNICATIONS
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| OC I – 2 | Critical Molecular Structure in Bound Flavin Cofactor to Enhance the Rate of Extracellular Electron Transport  
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| OC I – 3 | Molecular interactions between Geobacter sulfurreducens triheme cytochromes and the electron acceptor Fe(II) citrate studied by NMR  
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| OC I – 7 | Identification of Enzymes Mediating Cathodic Electron Uptake in Methanococcus maripaludis  
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| OC I – 9 | Important role of sodium-pumping NADH dehydrogenase in Shewanella oneidensis  
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| OC I – 10 | Living Capacitors: functional characterization of a novel cytochrome acting as a nanowire  
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| OC I – 14 | Charge Transfer Properties of Membrane Proteins Probed at Modified Electrodes  
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OC II – 2  Playing favorites: Molecularly-precise bioelectrocatalysis and biosensing  
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OC II – 3  Selection, conditioning and controlling - Improving the performance of electrochemically active microorganisms treating real wastewater  
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OC II – 6  Periodical charge/discharge can enhance the characteristic of electroactive biofilms  
Xu Zhang, Antonin Prévot, Ricardo Louro, Catarina Paquete, Korneel Rabaey

OC II – 7  Optical Coherence Tomography and Electrochemical Impedance Spectroscopy as noninvasive tools for biomass quantification  
Tom Sleutels, Sam Molenaar, Joao Pereira, Cees Buisman, Annemiek ter Heijne

OC II – 8  Microbial 3D porous anodes: what are the limitations to the progression of multispecies biofilms at the scale of a single pore?  
Poehere Chong, Benjamin Erable, Alain Bergel

OC II – 9  Combination of electrochemical technologies as a paradigmatic change in industrial wastewater treatment plants  
Yeray Asensio, Sara Tejedor, Patricia Fernández, Juan M. Ortiz, Victor Monsalvo, Juan F. Ciriza, Frank Rogalla, Abraham Esteve-Núñez

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OC II – 11  Optimizing electric reporter strains by *in situ* visualization of induced fluorescence in *Marinobacter* biofilms  
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OC III – 2 Innovative Microbial Electrolysis Cell configuration with a two-sided cathode to enhance biogas upgrading and nitrogen recovery
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OC III – 3 Conversion of goose feature waste to biochar as anode for enhancement of microbial extracellular electron transfer
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OC III – 4 In-situ Visualization of Mixed Electroactive Biofilm Growth using an Integrated System
Qing Du and Xin Wang

OC III – 5 Experimental and theoretical analysis of rate-limiting steps in microbial fuel cells
A. Bergel, M. Oliot, A. Mosdale, L. Etcheverry, M.-L. Délia

OC III – 6 High Rate Methane Production at Low Overpotential on Granular Activated Carbon Biocathodes
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OC III – 7 Novel Transition Metal Cathodes for Sustained Microbial Electrosynthesis
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OC III – 8 Ligno-cellulosic Materials in Low-cost Microbial Fuel Cells Architectures for Nutrients Recovery
Stefania Marzorati, Andrea Goglio, Laura Rago, Pierangela Cristiani, Andrea Schievano

OC III – 9 Addressing Ohmic Resistance and the Challenges of Evaluating Very Large Cathodes
Bruce E. Logan, Emily Zikmund, Jaewook Myung, David Jones, Kyoung-Yeol Kim, and Wulin Yang

OC III – 10 Impact of sub-microscale surface roughness on the colonization, the current production and the settlement of mixed culture anodic biofilms
Melanie Pierra, Mehdi Golozar, Xu Zhang, Antonin Prévotau, Michael de Volder, Dominiek Reynaerts, Korneel Rabaey

OC III – 11 Rough vs Micro-structured surfaces: which impacts on Geobacter sulfurreducens bioanode performances
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Ian Head

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Abdullah Al-Mamun, Mahad Said Baawain
Session I
Fundamentals of the extracellular electron transfer processes
Enhancing current production through genetic manipulation

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We recently constructed mutant strains of \textit{Geobacter sulfurreducens} altered in key electron transfer or regulatory proteins that produce 140\% to 180\% greater current density compared to wild type. Increased current density could be due to a range of factors from cell-level changes in performance, to whole-biofilm modifications like thickness or conductivity. Respiration and maximum growth rates, maximum current densities, and conductivity via double potential step chronoamperometry were measured for wild type and mutant biofilms using electrodes poised at +0.24 V vs SHE. Biofilms were also sectioned and imaged using electron microscopy to determine changes in biofilm thickness and cell density. Biofilms incubated in the presence of nitrogen and carbon stable isotopes were analyzed using NanoSIMS to localize metabolically active zones. Finally, differences in genome-wide expression of exponentially growing biofilms were analyzed using RNA-seq. Our results reveal that \textit{G. sulfurreducens} mutants reached higher current densities through higher per-cell respiration rates, which support faster growth rates, and a denser packing of cells close to the electrode. Surprisingly, NanoSIMS revealed that the zone of cells growing within the biofilms remained within 10 microns from the electrode surface, similar to wild-type biofilms. The combination of imaging, physiological and transcriptomics tools determined that current-producing bacteria are capable of producing more current per cell and, in combination with higher biofilm density, can produce a final current density almost twofold greater than wild type. However, there still remain cells in these biofilms that are not contributing to current production. Overcoming the limitation of the extent of metabolically active zones remains a major challenge.
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The iron-reducing bacterium \textit{Shewanella oneidensis} MR-1 has an ability to transport respiratory electrons generated from cell inside to extracellular solid substrates via electron transfer integral c-type cytochrome complex, located at outer membrane (OM c-Cyts). This interfacial electron transport between OM c-Cyts and solid substrates is termed extracellular electron transport (EET). In recent years, we found that the rate of EET is largely enhanced by self-secreted flavin molecules associated with the formation of semiquinone (Sq) state as a binding redox cofactor in the OM c-Cyts\textsuperscript{[1]}. However, the more negative redox potential of bound flavin Sq than the hemes in OM c-Cyts is energetically unfavorable for the kinetics of EET. In order to clarify the mechanisms in acceleration of EET by flavin, we, hereby, replaced flavin cofactor to other redox molecules and compare the kinetics of EET\textsuperscript{[2]}. Our data showed that only the heterocyclic compounds containing nitrogen atom at 5-position in isoalloxazine ring (N(5)) largely enhance the current production of \textit{S. oneidensis} MR-1 to the similar extent with the bound flavin, indicating that N(5) plays an important role for the stabilization of flavin as Sq cofactor in OM c-Cyts. Assuming the redox cofactors receive electrons from the heme reaction center in OM c-Cyts, the cofactors with more positive redox potential are expected to be more favorable for the acceleration of EET. However, the current production correlated not with the redox potential but with the pK\textsubscript{a} at N(5) in cofactor calculated by a quantum chemical approach. Because higher pK\textsubscript{a} represents stronger proton acceptability in N(5), this correlation suggests that the protonation reaction at N(5) in flavin associates and limits the rate of EET. We will further discuss about the rate-determining step of proton transport coupled with the redox reaction of the bound flavin cofactor in OM c-Cyts, with dataset for solvent kinetic isotope effect and partial deletion of OM c-Cyts complex.

References
Molecular interactions between *Geobacter sulfurreducens* triheme cytochromes and the electron acceptor Fe(III) citrate studied by NMR

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Proteomic and genetic studies have identified a family of five triheme cytochromes (PpcA-E) that are essential in the iron respiratory pathways of *Geobacter sulfurreducens*. These include the reduction of Fe(III) soluble chelated forms or Fe(III) oxides, which can be used as terminal acceptors by *G. sulfurreducens* [1]. The relevance of these cytochromes in the respiratory pathways of soluble or insoluble forms of iron is quite distinct. In fact, while PpcD had higher abundance in the Fe(III) oxides supplanted *G. sulfurreducens* cultures, PpcA, PpcB and PpcE were important in Fe(III) citrate supplanted cultures [2]. Based on these observations we probed the molecular interactions between these cytochromes and Fe(III) citrate by NMR spectroscopy. NMR spectra were recorded for natural abundance and $^{15}$N-enriched PpcA, PpcB or PpcE samples at increasing concentrations of Fe(III) citrate. The addition of this molecule caused pronounced perturbations on the line width of the protein NMR signals, which were used to map the interaction region between each cytochrome and Fe(III) citrate. The perturbations on the NMR signals corresponding to the backbone NH and heme substituents showed that complex interfaces consist of a well-defined patch, which surrounds the more solvent-exposed heme IV methyl groups in each cytochrome [3]. Overall, this study provides for the first time a clear illustration of the formation of an electron transfer complex between Fe(III) citrate and *G. sulfurreducens* triheme cytochromes shown to be crucial in this respiratory pathway.

References

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Electromicrobiology insights of *D. acetexigens* through genomic and transcriptome analysis

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*Desulfuromonas acetexigens* strain 2873 is a novel electricigen capable of extracellular electron transfer (EET) to the anode producing high peak current densities >9 A/m2 in a very short growth period (20-30 h after inoculation) under potential induced growth (0.1 V vs. NHE) and with acetate as the electron donor. Cyclic voltammetry analysis revealed that three distinct membrane bound redox proteins were responsible for direct electron transfer to the anode. However, our understanding of how *D. acetexigens* strain 2873 transfers electrons to the anode is lacking, and genomic and transcriptomic approaches could help elucidate its EET mechanism(s). Genome sequencing, assembly and annotation revealed the presence of 3,376 protein coding sequences (CDSs) in *D. acetexigens* genome, of which 1,483 are shared with *Geobacter sulfurreducens* PCA genome. Cytochrome annotation revealed the presence of 39 putative multiheme c-type cytochromes including the outer membrane (OM) c-type cytochromes, OmcS and OmcE, which have been reported to play a key role in current generation in *G. sulfurreducens*. Differential expressed genes were identified between *D. acetexigens* biofilm cells growing on the anode as the electron acceptor versus *D. acetexigens* cells growing on fumarate as the electron acceptor. In addition, genes encoding for the OM c-type cytochromes, such as OmcE, OmcN_1, OmcN_2, OmcO and OmcT appear to be more expressed than OmcS and OmcZ under anode-respiring condition. Interestingly, gene encoding for OmcE cytochromes was expressed more in early biofilm, whereas gene encoding OmcZ cytochromes was more expressed in mature biofilms. Taken together, these results reveal the importance of OM c-type cytochromes for EET in *D. acetexigens*. The expression of OmcE and OmcS (for optimum current production) and OmcZ (for long range current generation) suggest that the interaction of *D. acetexigens* with the anode resembles that of *G. sulfurreducens*. The effect of several growth conditions on the physiology of *D. acetexigens* were also investigated in this study. The insights obtained from this study are useful to fine tune the electromicrobiology of *D. acetexigens* to further improve its anodic reaction kinetics.
Excited bacteria: In situ autofluorescence spectroelectrochemistry for the study of extracellular electron transfer

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Microbial extracellular electron transfer, EET, requires a highly efficient intra- and intermolecular alignment of all electron transfer components. The role of the individual components of this EET chain is currently under intensive discussion. Especially supramolecular structures like multiheme complexes ¹-³ and electronically conductive pili ⁴ are considered as the main components responsible for EET. Despite recent research progress, major aspects of the individual electron transfer steps within and across the involved electron transfer components are still unexplored.

C-type cytochromes of *G. sulfurreducens* have been reported to possess autofluorescence properties that are dependent on the redox state of the cytochrome ⁵. In this study, we utilized these autofluorescence properties for the development of an in situ fluorescence spectroelectrochemical (spectrofluoroelectrochemical) approach in order to gain a deeper understanding of the EET processes of *G. sulfurreducens* biofilms. We show that this spectroelectrochemical approach has the potential to provide further insights in the understanding of molecular mechanisms of microbial EET.

Literature:
Accelerating interior electron transfer of electroactive biofilm by magnetite for high performance microbial fuel cell

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Inefficient extracellular electron transfer between electroactive bacteria and electrode leads to low current production by microbial fuel cell (MFC) and brings a major barrier for MFC’s practical applications. In this study, magnetite was sprinkled in electroactive biofilm at various stages of growth with the aid of magnet field. The performed electroactive biofilm and magnetite were either intermingled interiorly or in surface contact, which assign to interior-modified biofilm and surface-modified biofilm. Results from the double potential step chronoamperometry tests showed that electroactive biofilm with magnetite interior-modified delivered higher electron transfer efficiency, which was improved by 12% and 37% compared with that of biofilm with magnetite surface-modified and without magnetite modified respectively. Thus the output power density of MFC (724 mW/m²) with interior-modified biofilm was greatly increased by 129% and 154% compared with that of MFC with surface-modified biofilm(604mW/m²) and the control (473 mW/m²). Electrochemical impedance and cyclic voltammetry also revealed the outstanding electrochemical properties of biofilm with magnetite modified interiorly. SEM images showed that the magnetite evenly distributed inside the interior-modified biofilm and penetrated the electroactive biofilm, which would facilitate extracellular electron transfer across electroactive biofilms. This study extended our understanding of the electron transfer process in the electroactive biofilm and provided an effective method to improve performance of the bioelectrochemical system.
Identification of Enzymes Mediating Cathodic Electron Uptake in *Methanococcus maripaludis*

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Electromethanogenesis is an emerging technology to convert electricity and CO₂ to methane for storage and transport in the natural gas grid. The methanogen *Methanococcus maripaludis* has been studied as a model organism for this process. In *M. maripaludis*, electron uptake proceeds indirectly via enzyme-mediated synthesis of soluble intermediates, such as formate or H₂.

In this study, we determined the importance of one specific multi-enzyme complex from *M. maripaludis*, the heterodisulfide reductase supercomplex (Hdr-SC), on enzyme-mediated formation of H₂ and formate from cathodic electrons. The Hdr-SC consists of a hydrogenase (Vhu), a formate dehydrogenase (Fdh), and a heterodisulfide reductase (Hdr). Physiologically, the Hdr-SC couples in methanogenesis the oxidation of formate and hydrogen to the reduction of a heterodisulfide and a ferredoxin. Thus, the Hdr-SC offers a potential interfaces for electron transfer with an electrode and an internal wiring to supply electrons to a hydrogenase or formate dehydrogenase for H₂ or formate formation, respectively. A His-tagged Hdr-SC was purified via Ni²⁺-affinity chromatography and subsequently assayed for H₂ and formate formation in electrochemical reactors at -800 mV vs. Ag/AgCl and in Fe(0)-corrosion essays.

In Fe(0) corrosion essays, the purified Hdr-SC mainly reduced CO₂ to formate, while the Hdr-SC depleted cell extract showed mainly H₂ formation activity. In electrochemical reactors, purified Hdr-SC catalyzed formate formation at high selectivity and a coulombic efficiency of 90% for several days. Our results also indicated that only Hdr-SC-bound formate dehydrogenase exhibits high electrosynthetic activity.
P. aeruginosa`s phenazine-based electroactivity is tightly regulated

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Understanding the mechanisms underlying extracellular electron transfer in mixed bacterial cultures of bioelectrochemical systems (BES) is of utmost importance to improve performance. P. aeruginosa exhibits electron transfer mediated by phenazines. In a synergistic interaction with Enterobacter aerogenes increased current production was observed (1). E. aerogenes used the phenazines for electron discharge and P. aeruginosa utilized 2,3-butanediol produced by E. aerogenes, which in turn stimulated phenazine production. Thereby, phenazine synthesis is tightly regulated, e.g. by quorum sensing. This work may help to understand the regulation patterns of P. aeruginosa`s phenazine production and its effect on co-culture performance.

To analyze the tight regulation of the phenazines, genes in the biosynthesis pathway of P. aeruginosa were deleted and different combination of knockouts were tested. The mutants were studied on two different carbon sources: glucose and 2,3-butanediol. Phenazine production levels were analyzed and transcription levels of the phenazines genes were studied via qPCR. Finally, the mutants were examined electrochemically in a potentiostatically-controlled BES as pure and as co-cultures with E. aerogenes.

Our findings suggest a complex cross-regulation between the two redundant phenazine operons resulting in altered phenazine productions (Fig.1). Furthermore, an influence of catabolite repression is indicated due to differences between the two carbon sources. In BES, the effect of the altered phenazine pathway is directly translated in current generation.

**Important role of sodium-pumping NADH dehydrogenase in *Shewanella oneidensis***

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Electrodes are powerful tools for optimizing engineered metabolic pathways by creating redox balance between available substrates and desired products. Ideally, an electrode could be used to balance and fine-tune the oxidation state of the intracellular NAD(P)H pool at will. However, a better understanding of the connection between the electrode and intracellular reducing equivalents is necessary. For example, in *Shewanella oneidensis* MR-1 little is known about how the intracellular NADH/NAD⁺ pool is connected to the electrode through NADH dehydrogenases. To address this knowledge gap, we assessed growth rates, membrane potential, and metabolism of knockout mutants for each of the four NADH dehydrogenases in *S. oneidensis* MR-1. We observed that a sodium-translocating NADH dehydrogenase is the major complex contributing to membrane potential in *S. oneidensis* MR-1 under a variety of growth conditions. The proton-pumping NADH dehydrogenase had a more limited role and was most important in acidic conditions. This finding is in contrast with prior assumptions that the proton-translocating NADH dehydrogenase was most important in *S. oneidensis* MR-1. Our continuing work focuses on understanding how proton- and sodium-motive forces contribute differentially to motility and ATP generation. In the future, we will use our discoveries about the role of NADH dehydrogenases to optimize engineered electrofermentation pathways.
Living Capacitors: functional characterization of a novel cytochrome acting as a nanowire

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Multiheme cytochromes are vital for extracellular electron transfer (EET) in Geobacter bacteria. In EET, electrons are transferred from the interior of the cells to the extracellular environment (1). This ability constitutes the basis for the development of diverse biotechnological tools, such as microbial fuel cells that harvest electricity from waste organic matter and bioremediation of polluted environments (2). Geobacter sulfurreducens (Gs), one of the most studied microorganism capable of EET, encodes proteins that may act as electron-storage devices or capacitors that allow the bacterium to produce energy in short periods of extracellular electron acceptors deprivation and subsist under hostile environmental conditions (3).

In this work, the functional properties of the dodecaheme cytochrome GSU1996 from Gs were investigated for the first time. The cytochrome has a linear modular structure with four triheme domains connected by flexible peptide linkers (4). The functional characterization was achieved using an innovative approach, where the thermodynamic and kinetic properties were firstly determined for the triheme domains, followed by the hexaheme fragments in a puzzle based methodology (5). This approach allowed us to elucidate the ET mechanisms and how GSU1996 functions as a nanowire. Furthermore, interaction studies performed by nuclear magnetic resonance led to the identification of GSU1996 potential physiological redox partners, making it possible to predict new paths of EET in Gs (6). The knowledge acquired with this work is pioneer and sets the ground to improve bioelectrochemical devices based in these organisms.

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(1) Santos, TC et al. Dalton Trans. 2015, 44(20), 9335–44.

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TBA

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Contemplating Microbial Electrochemistry at Very Large and Very Small Scales

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Research in the MEG (Microbial-Electrochemistry Group) at the Naval Research Laboratory is proceeding full steam ahead at many scales. At one end, we are performing field demonstrations of 2-m diameter x 1000 kg oceanographic moorings equipped with BMFCs (benthic microbial fuel cells) to persistently power oceanographic sensors using marine sediment organic matter as the fuel and oxygen in overlying water as the oxidants. At the other end we are using CRRM (confocal resonance Raman Microscopy) to image a cytochrome redox gradient across a single \textit{Geobacter sulfurreducens} cell adhered to a mineral surface. In between, we continue to advance the study of extracellular electron transport (EET) through various biofilms: studying the effects of deletions in the case of \textit{G. sulfurreducens} and the role of phenazines in the case of \textit{Pseudomonas aeruginosa} just to name two; and explore the use of SPRI (surface plasmon resonance imaging), to visualize variations in current density across a single electrode surface, as a tool to study biofilm growth and redox state of the biofilm/electrode interface. In today’s talk I will describe our latest results in which we advance understanding of the mechanisms underpinning biofilm EET down to the molecular level.
The key role of extracellular polymeric substances on extracellular electron transfer

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Microorganisms exploit extracellular electron transfer (EET) in growth and information exchange with external environments or with other cells. Every microbial cell is surrounded by extracellular polymeric substances (EPS). Understanding the roles of 3D EPS in EET is essential in microbiology and microbial exploitation for mineral bio-respirations, pollutant conversion and bioenergy production. Here we have addressed these challenges by comparing pure and EPS depleted samples of three representative electrochemically active strains viz. Gram-negative Shewanella oneidensis MR-1, Gram positive Bacillus sp. WS-XY1 and yeast Pichia stipites using technology from electrochemistry, spectroscopy, atomic force microscopy and microbiology. Voltammetry discloses redox signals from cytochromes and flavins in intact MR-1 cells, while stronger signals from cytochromes and additional signals from both flavins and cytochromes are found after EPS depletion. Flow cytometry and fluorescence microscopy substantiated by N-acetylglucosamine and electron transport system activity data showed less than 1.5% cell damage after EPS extraction. The electrochemical differences between normal and EPS depleted cells therefore originate from electrochemical species in cell walls and EPS. Combination of all the data with electron transfer analysis suggests that electron “hopping” is the most likely molecular mechanism for electrochemical electron transfer through EPS.
Charge Transfer Properties of Membrane Proteins Probed at Modified Electrodes

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Extracellular electron and proton transfers are known to occur in electroactive bacteria but the fundamental understanding of these processes is still in its infancy and precludes the optimization of microbial bioelectrochemical technologies.¹ To determine the charge transfer properties (electron and/or proton transfer) of outer-membrane proteins of electroactive bacteria, we present here an efficient and versatile electrochemical platform based on artificial lipid layers supported on modified carbon electrode. This original approach consists in functionalizing a carbon electrode with pH-responsive electrophores and a biomimetic lipid membrane through which the properties of membrane proteins are probed (see scheme below). As a proof of concept, we discuss the detection of the intrinsic electroactivity of the model redox protein cytochrome c probed directly at a lipid-modified electrode concomitant with the detection of a pH-responsive redox probe (quinone units).² The goal of this project is the design of a modular lipid-modified electrode platform for detecting electron and/or ion transfer of outer-membrane proteins of electroactive bacteria.

Session II
Microbial Electrochemical Technologies: from fundamental to applied research
Electrode-assisted acetoin production in a metabolically engineered *Escherichia coli* strain

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In this study we describe the metabolic engineering of *Escherichia coli* for the anaerobic fermentation of glucose to acetoin. Acetoin has well-established applications in industrial food production and was suggested to be a platform chemical for a bio-based economy. However, the biotechnological production is often hampered by the simultaneous formation of several end products in the absence of an electron acceptor. Moreover, typical production strains are often potentially pathogenic. The goal of this study was to overcome these limitations by establishing an electrode-assisted fermentation process in *E. coli*. Here, the surplus of electrons released in the production process is transferred to an electrode as anoxic and non-depletable electron acceptor. In a first step, the central metabolism of *E. coli* was steered towards the production of pyruvate from glucose by deletion of genes encoding for enzymes of central reactions of the anaerobic carbon metabolism (ΔfrdA ΔadhE ΔldhA Δpta Δack). Thereafter, the genes for the acetolactate synthase (*alsS*) and the acetolactate decarboxylase (*alsD*) were expressed in this strain from a plasmid. Addition of nitrate as electron acceptor lead to an anaerobic acetoin production with a yield of up to 0.9 mol acetoin per mol of glucose consumed (90% of the theoretical maximum). In a second step, the electron acceptor nitrate was replaced by a carbon electrode. This interaction necessitated the further expression of *c*-type cytochromes from *Shewanella oneidensis* and the addition of the soluble redox-shuttle methylene blue. Fermentation with the non-depletable electron acceptor led to an acetoin formation with a yield of 79% of the theoretical maximum (0.79 mol acetoin per mol glucose). Electrode-assisted fermentations are a new strategy to produce substances of biotechnological value that are more oxidized than the substrates. At this early stage, we see promising results regarding carbon and electron recovery. Further strain development and improved fermentation conditions might lead to an increased anaerobic metabolic turnover rate.

Playing favorites: Molecularly-precise bioelectrocatalysis and biosensing

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Since both microorganisms and devices use electrons as information and energy carriers, interfacing living cells with electrodes in microbial electrochemical systems offers the opportunity to control key biological processes electronically. While a variety of small molecules, polymers and nanostructures are able to inject electrons into living cells, none of these approaches can target a specific redox pool, which leads to cellular toxicity and off-target effects. Here we explore whether an electron transfer pathway, the Mtr pathway of \textit{Shewanella oneidensis}, can act as a module to deliver electrons to specific redox pools and trigger specific biological processes in a non-native host, \textit{Escherichia coli}. Our work shows that Mtr pathway can deliver cathodic electrons specifically to menaquinone-linked reductases, reveals this electron flux is modulated by competing electron flows into the menaquinone pool, and demonstrates stoichiometric electrically-driven biosynthesis. More broadly, our work describes the Mtr pathway as modular component that delivers electrons to specific intracellular carriers, opening the door to using electricity as a direct feedstock, a control on metabolic rate, and a regulator of gene expression in microbial electrochemical systems.

\textbf{Figure 1.} Schematic showing engineered microorganisms that use the Mtr pathway to electronically interface with inorganic materials.
Selection, conditioning and controlling - Improving the performance of electrochemically active microorganisms treating real wastewater

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Microbial electrochemical technologies (MET) such as microbial fuel cells and microbial electrolysis cells are part of a developing and widely diversified microbial electrochemical technology platform [1]. All of these technologies utilize electrochemically active microorganisms (EAM) to catalyze one or both of the reduction as well as oxidation half-reactions at the anode and/or cathode. EAMs at the anode convert e.g. organic carbon in a wastewater stream into a current flow as part of their metabolism. This presentation will present two methods for achieving sustained and enhanced electrocatalytic biofilm turnover rates [2] and their consequences for real wastewater treatment [3]. The first method discusses the modification and use of a tailored culture medium for a sophisticated biofilm selection and conditioning procedure [4]. The second method deals with the controlling of bioelectrochemical reactions in the EAM biofilm via the applied potential at the bioanode [5]. Both methods are characterized from a fundamental perspective as well as by their effectiveness for a real application. Finally, a modelling approach will be used to demonstrate how these methods effect both the scaling-up and the treatment capacity as well as the associated energetic considerations for METs being fed real wastewater.

Literature:
Electrode-assisted methanogenesis in a microbial electrolysis cell: Examining the effect of set potential on the microbial activity and functional response

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Electrode-assisted methanogenesis in microbial electrolysis cells (MECs) involves the reduction of CO₂ to methane by methanogens at the biocathode. While studies have demonstrated the effect of set potential on reactor performance, little is known regarding the functional response of methanogenic biofilms in MECs. The objective of this study was to understand the functional dynamics of a methanogenic community in a MEC as a function of set cathode potential using a metagenomic and metatranscriptomic approach. We hypothesize that differences in hydrogen evolution and electron availability due to different set potentials will affect the gene expression of methanogenic biofilms and, consequently, current and product formation. Methanogenic biocathodes were enriched for three months in duplicate single-chamber MECs, and then transferred to two dual-chambered MECs for the set cathode potential experiments with 20% CO₂ in the headspace and no additional carbon sources. The MECs were operated for 48 hours at each set cathode potential (-1.0 V, -0.8 V and -0.6 V vs Ag/AgCl) with samples taken before and after each change. Current densities were about 0.3 – 0.4 mA/cm² at -1.0 V, and close to 0 mA/cm² at less negative potentials in both reactors. However, the current density increased in one of the reactors to about 0.3 mA/cm² after operating for 15 hours at -0.6 V, with a corresponding increase in methane and hydrogen production. Metagenomic analyses revealed that the enriched biocathodes were dominated by Methanobacterium spp. (M. oryzae, M. kangiense, M. petrolearium and M. beijingense), with relative abundances that varied with set potential. Metatranscriptomic analyses are currently underway to quantify the dynamic responses of key genes associated with electron uptake, hydrogen production/utilization and carbon dioxide fixation.
Microbial electrochemical energy storage and recovery in a combined electrotrophic and electrogenic biofilm

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Electroactive biofilms, used as biocatalysts in bioelectrochemical systems (BESs), are usually enriched for either electrogens (the electrode is the electron acceptor) or electrotrophs (the electrode is the electron donor). Here, we enriched a non-photosynthetic bifunctional electroactive biofilm capable of both electrogenic and electrotrophic processes. By operating the electrode sequentially as an anode (0.0 V\text{SHE}) and a cathode (−0.4 V\text{SHE}), we enriched a single community capable of potential-dependent generation of electrical energy and production of chemical energy with a maximum current density of ±1.4 ± 0.4 A/m² and a coulombic efficiency of ~97%. Cyclic voltammograms exhibited a sigmoidal shape and square wave voltammograms exhibited reversible peaks at −0.15 and −0.05 V\text{SHE}, suggesting surface-bound redox mediators facilitated electron transport at the biofilm/electrode interface. Hydrogen, carbon monoxide, and methane, but no VFAs, were detected in reactors, despite no regular addition of a carbon source. Cells and cell clusters were spread across the electrode surface, as seen by confocal laser scanning microscopy. Further analysis of the system, including a microbial community analysis, is currently underway. These results indicate that a non-photosynthetic electroactive community is capable of producing high current densities using vastly different potential-dependent metabolic processes on a single electrode, furthering the potential relevance of BESs as alternative biotechnologies in energy storage and conversion applications.
Periodical charge/discharge can enhance the characteristic of electroactive biofilms

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Electroactive bacteria can use electrodes as terminal electron acceptors while oxidizing organic substrates. Electroactive biofilms (EABs) have been considered promising for several potential applications such as microbial fuel cells, microbial electrolysis cells or microbial biosensors. However, low current densities still limit their practicability and numerous questions remain on the parameters impacting their characteristics and performance. EABs are able to store electrons in the absence of external electron acceptors ("charge" process in open circuit). Once the microbial anode is polarized again, accumulated charges can be released and it produces an additional transient current ("discharge" process). This process has already been studied solely for short-term and on already mature EABs. In particular, the effect of periodical "disconnections" on the characteristics of EABs has not been explored.

Here we applied periodical charge/discharge (i.e. OCV/\textendash 0.1 V vs. Ag/AgCl) operations during the full growth of EABs on glassy carbon electrodes (i.e. starting from inoculation). We investigated the impact of the frequency of the signal on current generation, charge storage capacity, heme content, redox conduction and biofilm morphology.

When compared with a continuous polarization, the shortest half-periods of charge/discharge (\textlesssim 10 s) enhanced current production, increased the content of redox cofactors (and hemes) in the EABs, and improved the redox conduction. Oppositely, longer half-periods (\textgtrsim 60 s) inhibited the growth and electroactivity of the EABs. Control EABs formed under continuous polarization were flat, while EABs formed under intermittent operations presented torus-shaped structures on their outer-layer. The results indicated that periodical charge/discharge can regulate the formation and electroactivity of EABs. In addition to the fundamental relevance, this may provide opportunities for future applications.
Optical Coherence Tomography and Electrochemical Impedance Spectroscopy as noninvasive tools for biomass quantification

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To create an efficient bio-anode, the aim is to transfer as much of the available energy from the substrate to the eventual product. A dominant parameter in this conversion is the biomass density available on the electrode surface and its efficiency to extract electrons from substrate and donate these electrons to the anode. The amount of biomass changes with time and is dependent on the conversion of substrate and the electrode potential.

Here, we studied the use of Optical Coherence Tomography (OCT) and Electrochemical Impedance Spectroscopy (EIS), as a non-invasive tool for the in-situ visualization and determination of anodic biofilm thickness and structure. OCT is a visualization technique that gives a 3D image and volume of the biofilm while EIS gives the capacitance of the biofilm which reflects the biomass density. A flowthrough Microbial fuel Cell was modified to include a transparent fluoride tin oxide (FTO) coated glass electrode as anode. OCT and EIS analyses, throughout different development stages of the anodic biofilm, were performed. After OCT and EIS analyses, the biomass was harvested from the electrode and quantified by COD, TNb, and weight.

The obtained amount of biomass after harvesting, was compared with the OCT and EIS measurement to fit its correlation. Analysis of the results show that OCT and EIS measurements can be used as a proxy for the amount of biomass. Therefore, both OCT and EIS can be used to measure in-situ biomass development and determine the effect of substrate loading and anode potential. From this, the growth kinetics and yield of electrogenic biomass can be determined under different conditions, which are important parameters for future reactor design and operation.
Microbial 3D porous anodes: what are the limitations to the progression of multispecies biofilms at the scale of a single pore?

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Pore size heavily impacts both the substrate and the microorganisms transport in 3D porous electrodes, leading to major limitations on microbial biofilm colonization and, thus more widely, on bioelectrochemical performance of 3D anodes. If the pores are too thin, clogging effect appears in few days, erasing the advantage of the 3D configuration and limiting the geometry to a 2D electrode. Our work aims to determine the range of optimal pore size allowing a homogeneous and complete colonization of the porosity of anodes. This range of pore size is essential for dimensioning innovative porous anodes.

A first experimental set-up was designed for studying the bacterial colonization profile inside the pore depth (10 cm length, variable diameter from 1 to 5 mm). A second device was used to evaluate the accessibility of electroactive bacteria to the bottom of pores gradually deeper (2mm diameter, variable length from 5 to 24 mm). All tests were conducted under fixed potential (-0.2V vs SCE), 20 mM of acetate in a leachate of garden compost. The effect of pore size and pore length on the current was analyzed via chronoamperometries. Microbial colonization were examined by LIVE/DEAD epifluorescence microscopy and statistical analysis of biofilm biovolumes.

All tests were duplicated for different operation times 5, 10 and 30 days. The single pore electrode always achieved a maximum current 70% to 85% lower than the planar anode, showing clearly a limitation of the current generation at a simple millimeter-scale pore. Concerning the different length of pores (5 to 24 mm), the start-up time needed to detect current occurred practically in the same time as the control. A specific mechanism of active mobility of electroactive bacteria is consequently suspected (and is being demonstrated).

This work has been carried out within the framework of the Bioelec project ANR 13 BIME 006.
Combination of electrochemical technologies as a paradigmatic change in industrial wastewater treatment plants

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The treatment of industrial wastewaters, is a promising niche of application of Microbial Electrochemical Technologies (METs) due to the high levels of organic matter and nutrients, and their biodegradability. METs have shown to stimulate organic matter degradation through supplying to electroactive microorganisms an anode as final electron acceptor and at the same time, to produce value-added products as hydrogen. However, METs are not efficient for both removing organic matter and nutrients at a single step, being necessary the integration of a complementary technology to achieve a full wastewater treatment.

With this background, an integration of electrochemical technologies has been proposed for treating real brewery wastewaters. The activities are developed under the LIFE-ANSWER Project (ENV/ES/000591), which aims to demonstrate the technical and economic feasibility of electrocoagulation and METs in medium to small industry wastewater treatment plant. A pre-pilot electro-coagulation (EC) unit, which has been extensively studied as a highly-efficient nutrient removal method, has been designed for N and P removal using recycled aluminum pellets as sacrificial anode. This EC step is expected to remove up to 96% of total suspended solids (TSS) and 98% of the total nutrients of the industrial effluent. Secondly, the effluent of the EC step is fed into a Microbial Electrochemical Fluidized Bioelectrochemical Reactor (ME-FBR) of 400 L, in which most of the soluble organic matter is removed with a polarized conductive bed of fluidized carbon acting as electron acceptor for electroactive bacteria. At the cathodes of both electrochemical reactors hydrogen is produced, which could be used for real applications, as biofuel or cogeneration to decrease the operational costs of industrial wastewater treatment plants (WWTP).
Enhancing the electrocatalytic activity of microbial bioanodes with conductive magnetite nanoparticles

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In recent years, sustainable treatment and utilization of wastewater are receiving considerable attention due to the growing shortage of freshwater resources, depletion of fossil fuel, and environmental pollution. Microbial Electrochemical Technologies are a groundbreaking approach to recover energy while simultaneously cleaning up the wastewater.

The main objective of this study was to verify the possibility to improve/stabilize the anaerobic oxidation of organic substrates, at the anode of a bioelectrochemical reactor, by means of the addition of electrically conductive magnetite (nano)particles (NP) capable to facilitate/accelerate extracellular electron transfer processes.

Bioelectrochemical experiments were carried out in H-type cells, equipped with a 3-electrode setup. The cells were operated in a fill and draw mode, with the graphite anode potentiostatically controlled at -100 mV vs. SHE. The anode compartment of two identical cells was inoculated with a methanogenic sludge and spiked with either a mixture of butyrate, propionate, and acetate (to a final concentration of approximately 300 mg COD/L) or the OECD synthetic sewage. One of the two cells was supplemented with a suspension of magnetite NP, whereas the other one served as a control.

Interestingly, magnetite NP accelerated the oxidation of organic substrates, particularly propionate and favoured electric current generation over competitive methane production. The higher rates of oxidation, in the cell supplemented with magnetite NP, were mirrored by a higher electric current generation and a higher cumulative electric charge transferred to the electrode. Cyclic voltammetry (CV) helped out identifying the impact of NP on putative electron transfer mechanisms. Over repeated fill and draw cycles, the positive effect of nanoparticles tended to diminish, most likely due to passivation effects, as suggested by X-Ray Photoelectron Spectroscopy (XPS).

Taken as a whole, these preliminary findings suggest that a potential exists for improving the efficacy of microbial electrochemical technologies for wastewater treatment, through the addition of conductive NP. Further work is currently in progress to gain a deeper understanding on the impact of magnetite on the involved electron transfer mechanisms and on the microbial composition of the bioanode.

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Optimizing electric reporter strains by *in situ* visualization of induced fluorescence in *Marinobacter* biofilms

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*Marinobacter* sp. CP1 is an abundant member of the Biocathode-MCL (*Marinobacter-Chromatiaceae-Labrenzia*) consortium and is capable of generating both cathodic and anodic current when grown separately as a biofilm on an electrode. In order to establish *Marinobacter* sp. CP1 as a viable and ideal chassis for synthetic biology applications in marine environments, we are characterizing a range of inducible promoters as genetic sensors in a biofilm. Microbial genetic sensors are typically optimized under conditions that are not representative of the environment in which they will be deployed. As such, there is a need to develop platforms for more rapid screening of promoter constructs and other genetic elements during use on an electrode. *Marinobacter* sp. CP1 was engineered with plasmids containing a yellow fluorescent protein (YFP) reporter under control of a number of sensors for small molecules including 2,4-diacetylphloroglucinol (DAPG), naringenin, choline, vanillic acid, cumate, and tetracyclin. The *Marinobacter* sp. CP1 strains were grown as a maturing biofilm in electrochemical flow cells while taking confocal images over time. In this system, we were able to detect inducible YFP expression *in situ* and record videos of the fluorescence output while visualizing the growing biofilm. This method allows spatiotemporal expression data of biofilm growth conditions and will be used to examine expression induction by putative potential sensing proteins, visualize real-time voltage response pathway activation, and correlate gene expression with extracellular electron transport.
A Recyclable Bioelectrocatalyst Enhancing Electricity Generation in Bioelectrochemical Reactor

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In this study, a recyclable bioelectrocatalyst was constructed via the coupling of paramagnetic beads with riboflavin. Three different linker molecules characterized by short, medium and long chain length were placed between the bead and the riboflavin molecule. These beads were successfully applied in bioelectrochemical reactors (BER) and lead to an increase of Shewanella oneidensis catalyzed exoelectrogenic electron transfer. The result of the experiments demonstrated that riboflavin beads with the short linker (C4) showed the best performance in BER in terms of current production and recovery efficiencies. The current density of BER with C4 riboflavin beads was 7.5 μA/cm², which was more than 100% higher compared to BER without beads (3.1 μA/cm²). The addition of medium (C11) linker riboflavin beads also increased the current density by 50% (4.6 μA/cm²) while the addition of the long (C40) linker beads lead to current densities (3.1 μA/cm²) comparable to control experiments in which the addition of beads was omitted. In following experiments, we investigated the possibility to recover the beads and the stability of the riboflavin content. The bead number and riboflavin recovery efficiencies were high for all the beads (63-90 %), allowing for an efficient recycling process from the BER. The recovery efficiencies of the C4 riboflavin beads were the highest among the three different tested variants. We could recover 90% of the beads as well as 91% of the initial riboflavin content.

Increasing current densities in BER with added beads can be explained with the higher cell number on the electrode surface. Cell quantification via qPCR was performed to monitor the total cell number in the planktonic phase in the electrolyte and in the electrode biofilm. The number of attached cells correlated positively with current production. The attached cell number in C4 riboflavin beads was 3.4 times higher (1.57x10¹¹ cells) than in control reactors without beads (4.57x10¹⁰ cells). The increase of 50% current density in C11 riboflavin beads could be correlated with 2.4 times more cells (1.1x10¹¹ cells) on the electrode surface. Addition of the C40 riboflavin beads did not lead to increased current production and the number of attached cells was lower (3.1x10¹⁰ cells) than in the control experiments. Although it seems obvious that higher biofilm cell densities were the reason for higher current densities, it is not clear how the beads triggered increased biofilm growth and whether only the increase in cell mass or also the increase in biofilm conductivity was causing the effect. Therefore, we currently investigate the system further via electrochemical impedance spectroscopy.
Session III
Electrochemical cell design and electrode materials
Artificial living biocomposites to mimic and surpass electroactive biofilms

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A living material was formed by self-assembly of \textit{Shewanella oneidensis} MR-1 with carbon nanotubes in the presence of c-type cytochrome from \textit{Desulfovibrio} and \textit{Desulfuromonas} genus and from bovine heart, with the goal to mimic electroactive biofilms [1]. The role of cytochromes on self-assembly, cell viability and extracellular electron transfer was studied for formate oxidation and fumarate reduction. Scanning electron microscopy and dynamic light scattering experiments highlighted the role of cytochrome on the self-assembly of bacteria-carbon nanotube aggregates within only 2 hours in solution. The deposition of these aggregates on glassy carbon surfaces led to a homogenous composite film in which the bacteria were embedded in a carbon nanotube network. A comparable cell density of 1 cell \(\mu m^{-2}\) was achieved in the presence or in the absence of cytochrome \(c\), but some proteins allowed maintaining a higher bacterial viability [2]. Further optimizations demonstrated the strength of the concept, leading to cathodic current density higher than 10 A m\(^{-2}\) in the presence of 50 mM fumarate.

Innovative Microbial Electrolysis Cell configuration with a two-sided cathode to enhance biogas upgrading and nitrogen recovery

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An innovative microbial electrolysis cell (MEC) configuration with three chambers has been developed to enhance biogas upgrading and nitrogen recovery from both the gaseous and liquid effluents of the anaerobic digestion. The three-chamber configuration was set up with a two-sided cathode and an anode placed in the center of the cell (Fig.1). A cation and an anion exchange membranes (CEM and AEM, respectively) were used to separate the anode from the two cathode chambers in order to ensure the electroneutrality of the cell and to promote the migration of ammonium and bicarbonate ions. The bio-anode anaerobically oxidized the COD and transferred the reducing power to the two-sided cathode, where the methanogenic inoculum reduced CO₂ into CH₄. A gas mixture composed by N₂ and CO₂ (70/30 v/v) simulating a biogas in terms of CO₂, was continuously fed through both cathodic chambers where the CO₂ was removed through both biotic (methane production) and abiotic (CO₂ sorption as bicarbonate) mechanisms. Notably, with respect to another explored three-chamber configuration (consisting of an accumulation chamber between the anode and cathode chambers), the present configuration allowed to double both methane generation (59 vs 105 meq/Ld) and CO₂ removal (2.0 vs 3.8 gCO₂/Ld). Also, the two-sided cathode configuration permitted the ammonium recovery by its accumulation and spill from the cathode chamber separated with the CEM from the anode due to the daily removal of the catholyte. Whereas, in the cathode chamber separated with the AEM from the anode the concentration of ammonium remained similar to that in the anodic chamber. Overall, the doubling of the cathodic surface allowed for the doubling of the cathodic performances, i.e. methane generation and CO₂ removal, without affecting the MEC energy consumption which was similar (2.9 vs 2.6 kWh/Nm³CO₂removed) to that detected in the three-chamber configuration equipped with an accumulation chamber between the anode and the cathode.

FIGURE 1: Schematic representation of the two-sided cathode MEC
Conversion of goose feature waste to biochar as anode for enhancement of microbial extracellular electron transfer

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Poultry feature is abundant, inexpensive and renewable. It will produce huge economic benefits if poultry feature can be converted into valuable materials. In this article, we provide an easy-to-operate method to convert goose feather solid waste into biochar as anode to enhance the microbial extracellular electron transfer (EET) in bioelectrochemical systems. Pyrolysis of 1 g of waste goose feather and 3g KOH at 650 °C for 3 h in N₂ atmosphere leads to the formation of about 20 mg goose-feather biochar (GFC). An anode with excellent performance was produced by electrophoretic deposition, in which carbon paper was used as the base material. The hollow porous structure and good hydrophobic of the anode increased the bacterial loading capacity. The microscale coating of GFC favored EET due to the enhanced interaction between the bacteria and the anode as well as the good conductivity of GFC. By using a single chamber electrochemical system equipped with the fabricated anode, a current density of 0.05 A/m² was achieved, which was nearly an order of magnitude compared to the control group. This study introduces a potential method for the utilization of the poultry feature solid waste and the fabrication of high performance anodes from abundant, inexpensive and renewable abandoned materials in the environment.
In-situ Visualization of Mixed Electroactive Biofilm Growth using an Integrated System

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Monitoring the forming process of microbial film and studying the interactions between biofilm and the natural or anthropogenic environment, are not only important for understanding the formation and succession of biofilms, but also can provide theoretical guidance for the application of microbes and microbial diversity maintenance. Development of integrated, multidisciplinary platforms capable of providing in-situ real-time observation of the biofilm-forming microorganisms can assist this aim. Bioelectrochemical systems (BESs), which utilize the biofilms formed on the electrode under controlled conditions as catalysts for energy harvest from wastewater, is ideal for monitoring the activity of biofilms. However, although several electro- and spectroelectrochemical techniques have been utilized for biofilm analysis, most of these techniques require expensive setups and are not suitable for routine online diagnostics. Here, we fabricated a novel electrochemical reactor with excellent sealing and nonopaque bottom, and then integrated with an ordinary microscope and a potentiostat for simultaneous optical and electrical recording of the biofilms in the operating BES. Fluorescence and bright field images of the morphology of microbial film on the working electrode, and also real-time videos are outputted to the computer. Current and voltage signals were meanwhile recorded by the potentiostat. As an application example, this experiment system captured the impact of the relative area and distance between the working electrode and the counter electrode on the biofilm formation and succession processes. Primarily, we found the growth of electroactive biofilm strictly follows the electric field. Such system can serve as a powerful platform for microbiology and microbial ecology research.

Fig. 1 Images detected by a microscope-potentiostat integrated system
Experimental and theoretical analysis of rate-limiting steps in microbial fuel cells

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Power density provided by microbial fuel cells (MFCs) equipped with air-breathing cathodes does generally not exceed 1 or 2 W/m\textsuperscript{2} with respect to the cathode surface area [1]. The record has recently been raised to 4.7 W/m\textsuperscript{2} [2]. Several rate-limiting steps have been identified such as poor efficiency of the oxygen-reduction catalysts at neutral pH, low ionic conductivity of the electrolyte, local acidification of the bioanode, oxygen crossover, local alkalinizing of the cathode that favours fouling, cathode biofouling, etc. The major rate-limiting causes will be described and analysed on the basis of a literature review coupled with theory of ion transport in electrolytes [3]. Based on this analysis, a new separator-electrode assembly was designed, including a 3-dimensional anode and an air-cathode that can be replaced during operation [4]. Electrode kinetics were monitored by voltammetry during a 1-month operation period with 6 MFCs. Among the different separators that were checked, the highest power density of 6.4 W/m\textsuperscript{2} was obtained by using a simple, large-mesh, plastic grid [5]. The microbial communities identified by 16S metagenomics showed that oxygen crossover did not affect the bioanode, although the separator did not mitigate it. Replacing the air-cathodes allowed 5 W/m\textsuperscript{2} to be still produced after 1-month operation. The theoretical analysis explained that so high performance was reached because the large-mesh grid allowed free mass transfer of the buffering species between the anode and the cathode, which presented pH balance as the main key for future progress.

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High Rate Methane Production at Low Overpotential on Granular Activated Carbon Biocathodes

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Methane-producing bioelectrochemical systems (BESs) are a biological power-to-gas technology, in which methanogens generate methane from electricity at the cathode. The aim of this study was to achieve high methane production rates at low energy input (low cathode overpotential). We used a cathode material with high specific surface area: granular activated carbon (GAC), and compared its performance to graphite granules (GG), which have low specific surface area. Four reactors (two GAC and two GG cathodes) were operated at constant current of 5, 10, and 35 A/m². Granules were packed tightly in the flow channel to ensure good contact between granules and current collectors, resulting in a resistance over the granule bed of <2 Ω for all cells.

All reactors achieved methane production rates of 60 L CH₄/m²cat/d at 35 A/m²cat. Methane production of GAC biocathodes occurred at -0.5 V vs Ag/AgCl, close to thermodynamic equilibrium, whereas GG biocathodes produced methane at -0.9 V vs Ag/AgCl (Fig. 1). When methanogens were inhibited with 2-BES, the cathodic current of GAC, when controlled at -0.5 V cathode potential, decreased to zero, pointing out a direct pathway of methane generation for GAC. This effect was not found for GG. When current was controlled instead of cathode potential, 2-BES addition resulted in hydrogen and acetate formation for both materials. 16S rRNA gene analysis for all biocathodes showed that Methanobacterium was the dominant methanogen, and there was no clear link between community and biocathode performance. Total energy efficiency was around 20% for all reactors. These results show that GAC is an attractive electrode material for methane producing cathodes resulting in high rates and low overpotentials.

Fig. 1 Methane production occurred at less negative cathode potentials for GAC than for GG.
Novel Transition Metal Cathodes for Sustained Microbial Electrosynthesis

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Microbial electrosynthesis holds the promise of providing a technological platform for storage of renewable electrical energy and production of CO\textsubscript{2}-neutral chemicals from CO\textsubscript{2} and electric energy. However, the process currently remains primarily limited by low microbial electron uptake rates from a cathode. This issue can be circumvented by uncoupling the electrochemical reaction from the microbial synthetic reaction. The use of a purely inorganic cathode for the hydrogen evolution reaction (HER) offers high current densities and efficiencies. However, a common drawback of effective metal electrodes, such as platinum, is their decreasing performance in CO\textsubscript{2}-rich environments.

Here, we report the use of novel, inexpensive transition metal cathodes that exhibit stable HER performance in the presence of CO\textsubscript{2}. In particular, CoP and MoS\textsubscript{2} cathodes show constant hydrogen production for at least 100 hours. This performance remains stable in the presence of microbial growth medium, which indicates a low sensitivity of the cathode materials towards inactivation by microbial media components. Further, the electrodes are highly biocompatible, showing no negative effects when in direct contact with microbes. By using a pure culture of methanogenic Archaea, stable methane production from CO\textsubscript{2} and catalytically produced hydrogen could be achieved, while no H2 accumulation in the reactor headspace was observed. When used in combination with homoacetogenic microbes, the product spectrum is broadened further to soluble organics chemicals such as organic acids and alcohols. Therefore, these novel cathode materials enable a promising route for viable bioelectrochemical systems for energy storage applications as well as synthesis for chemicals and fuels.
**Ligno-cellulosic Materials in Low-cost Microbial Fuel Cells Architectures for Nutrients Recovery**

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Low-cost architectures could give competitive advantage in scaling-up microbial fuel cells (MFCs). In this work we propose novel solutions for cylindrical MFC design, based on Giant Cane (*Arundo Donax L.*), available as agricultural residues. Giant Cane is naturally cylindrical and porous and can be used (as it is) as separator or (pyrolized) as cathode to develop low-cost architecture for MFCs.

In a first experiment Giant Cane was used as tubular separator for air-cathode MFCs, with electrodes positioned inside (cathode) and outside (anode) the cane. These systems yielded 40 mW m$^{-2}$, relatively low if compared to state-of-the-art MFCs, oriented to electricity harvesting. However, the generated electric field was enough to sustain electro-osmotic ions mobility and high pH at the cathode interface. Over 70 days of operation, deposition phenomena of valuable elements (Na, Ca, Mg, Mn, K) were observed in the separator and on cathodic surface [1].

In a second experiment, 900°C pyrolized Giant Cane was set as the cathode. The robustness in the MFC was conveyed directly from the carbonaceous cylinder, surrounded by some felt, as to set a space between electrodes. A BET analysis on the pyrolized Giant Cane reported a specific surface area of 114 ± 4 m$^2$g$^{-1}$ and a porosity mainly centered on the micropores range. The MFC module, equipped with the pyrolized cylindrical cathode, showed significative performances, yielding up to 100-200 mW m$^{-2}$. Again, deposition of macro and micro nutrients was observed in the cathode, as demonstrated by ICP-MS, ionic chromatography, SEM and 3D X-Ray Tomography. A microbiological analysis was conducted to investigate the anodic and biocathodic microbial communities.

These types of MFCs were completely built by biogenic and low-cost materials and at the end of their life, they could be entirely re-utilized as fertilizers in agricultural soils.

Addressing Ohmic Resistance and the Challenges of Evaluating Very Large Cathodes

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Scaling up microbial fuel cells (MFCs) for energy recovery and wastewater treatment has many inherent challenges, but the two that have proven to be the most difficult to address are low solution conductivity and manufacturing of large cathodes. High ohmic resistance, or the low conductivity of a wastewater, has been understood (but underappreciated) with respect to limits on power production. In this presentation, we provide examples on how the need to have open flow channels (to prevent reactor clogging with solids) and the challenge of placing the electrodes closely limits power densities in these systems due to ohmic resistance compared to more optimal laboratory conditions. For example, using acetate and 50 mM phosphate buffer solution with a conductivity of 7.3 mS/cm could at most produce a maximum power density of ~6 W/m2 with brush anodes and a 1 cm electrode spacing. However, in a low conductivity solution (1 mS/cm) such as domestic wastewater, this falls to a maximum of 1.5 W/m2, with electrode overpotentials decreasing this to 0.8 W/m2 using the best materials available. In practice, we have obtained only half this maximum power in larger reactors with wastewater. Manufacturing large cathodes capable of producing power can be challenging due not only to the size of the cathodes but the requirements for water retention and high electrical conductivity. We are currently building a ~1000 L reactor that will contain 98 cathodes each 0.68 m2 in projected area. To anticipate the performance of these larger cathodes, we have built a series of scaled electrochemical test cells for examining water leakage and current generation with increasing cathode size. The results of these tests will be shown along with the modular reactor design that will be tested for wastewater treatment in the coming months at a site in Pennsylvania, USA.
Impact of sub-microscale surface roughness on the colonization, the current production and the settlement of mixed culture anodic biofilms

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In anodic biofilms, electrodes act not only as electron acceptors, but also as physical support for an electroactive biofilm. The production of a functional microorganism-electrode interface is essential to achieve an efficient bioelectrochemical system. This interface can be modified by changing the chemical or topographical features of the electrode surface. In this study, the surface roughness of glassy carbon electrodes was successfully modified at the submicron level using micro electrodischarge machining, while preserving the surface chemistry of the glassy carbon. After inoculation, all microbial electrodes showed similar startup-time, maximum current density, biofilm conductivity and biomass produced per projected surface area. However, increasing cavities dimensions were observed on the biofilm surface as a function of surface roughness (from 7.4 ± 0.4 \textmu m to 15.5±0.5 \textmu m for the surface roughness of 5.1 ± 0.1 nm to 681.6 ± 127.8 nm, respectively). These results indicate that the surface roughness at a sub-microscale does not influence the attachment or current production of mixed-culture anodic biofilms. However, this work highlights the emergence of microscale cavities on the biofilm surface with increasing sub-microscale roughness.

![Figure](image)

\textit{Figure:} (a) Biomass occupation of the top 20 \textmu m of the biofilm (b) Surface roughness measurements and roughness topography using white light interferometry. (c) Examples of confocal laser scanning microscopy images of biofilms. The images show the top 2 cross sections of the biofilms.
Rough vs Micro-structured surfaces: which impacts on *Geobacter sulfurreducens* bioanode performances

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In the field of bioanodes, surface topography may impact both bacterial adhesion and biofilm efficiency but the effects of electrode profile are still to be determined. This study aims at assessing the influence of the surface topography using different roughnesses and well-controlled surface patterns. Electrode surfaces are created on ultraflat gold substrata (Ra of 0.8 nm) by deposition of gold from nanometer size (3 nm) to micro-porous surfaces (from 68 to 200 nm) presenting 10μm-wide holes. Gold electrodes (3nm) are also designed with micro-pillars of 500 μm height and 100 μm side with different spacing from 100 to 200 μm. Surface areas are electrochemically characterized and bioanodes are polarized at 0.1 V/SCE and inoculated with *Geobacter sulfurreducens* and 10 mM acetate. The bioanodes are characterized by cyclic voltammetry (catalytic and non-turnover), and the biofilm coverage ratios are determined by epifluorescent microscopy.

Flat and nano-rough surfaces produce unequal current between 0.2 and 2 A/m² which are not influenced by roughness variation and the current and the biofilm coverage ratio are proportional. However increasing roughness from 68 nm to 191 nm lead to increased current from 5 to 13 A/m² showing the direct impact of large asperities on biofilm productivity. The micro-pillar structured electrodes lead to higher current than nano-rough gold reaching 6 to 8 A/m². The current improvement correlate with the increased surface area. On biofilm coverage, whereas the nano-rough or micro-porous electrodes show erratic colonization, pillars ensure a global and uniform colonization. By combining the positive effects of roughness and micro-structuration, surface topography amelioration could significantly increase microbial anode performance.

This work was part of the Koropokkuru project (ANR-14-CE05-0004).
Session IV
Microbial fuel cell applications
Wastewater treatment plant field application of a real time MFC-based BOD sensor

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In order to monitor the wastewater treatment process, the most widely used test is BOD5. Nevertheless, this method is not effective for process control and real-time monitoring where rapid feedback is needed. In fact, there are some analytical and operational limitations, such as questionable accuracy and reproducibility, labour intensity and time consumption [1].

Microbial Fuel Cells (MFCs) are attracting increasing attention as a tool for on-line BOD monitoring, as the current generated from a mediator-less microbial fuel cell (MFC) is proportional to the concentration of fuel used [2]. However only few field tests were conducted [3].

A simple and low-cost MFC-based BOD sensor is successfully tested in a wastewater treatment plant, in comparison with an on-line Total Organic Carbon (TOC) instrumentation. The structure of the sensor consisted in a floating frame in polystyrene, holding identical carbon cloth (SAATI P10) plane electrodes separated by a clay layer and an insulating felt in polypropylene. The submerged anode (13x10 cm) and the air cathode (16x16 cm) were connected to a circuit with 100Ω of external load. A laboratory test was conducted in order to calibrate the sensor: a set of cells constructed with this configuration were placed in a tank using acetate as the carbon source and row wastewater as both inoculum and electrolyte. The Total Organic Carbon (TOC) and Total Inorganic Carbon (TIC) were continuously monitored with Sievers 820 Portable Total Organic Carbon Analyzer and compared to the current production of the cells. The cells showed a linear relationship up to 45 ppm TOC. These cells were then placed in the denitrification tank of the wastewater treatment plant of Carimate (MI) and the current production was measured and compared with the load of organic carbon.

References
**Bioelectricity generation using a miniature biophotovoltaic device**

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Biophotovoltaic devices enable conversion of light energy into electrical energy by employing photosynthetic entities such as cyanobacteria. They have a unique potential to generate electricity from the energy derived by photosynthesis during day, and from endogenous respiration of the accumulated carbon sources at night. Hence, they are self-sustainable and they do not require an organic source of carbon for energy. Miniaturization can significantly reduce the start-up times and allow high throughput studies to optimize various parameters involved in the power generation. In this work, we present a double chambered, miniature biophotovoltaic device of 300 μL deploying stainless steel electrodes and an anion exchange membrane as separator. To harness the light energy, *Anabaena* sp.PCC 7120 was used as the anode biocatalyst. The device generated an open circuit voltage of 370 mV under illumination without the addition of any mediator. A maximum power of 0.16 W/m³ and a current density of 7.85 mA/m² was obtained when a light intensity of 100 μmol m⁻² s⁻¹ was used. Further, the bioelectricity generation was proportional to the concentration of the biocatalyst added. This indicates that electrogenesis of cyanobacteria in the device solely contributed to power generation. These systems can find application as portable bio-solar cells generating power from solar energy besides sequestering carbon-dioxide.
Pre-colonization of anodic electrodes in seawater sediment for Single Chamber Floating Microbial Fuel Cells application

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In order to power remote environmental sensors and/or data transmission devices, Sedimentary Microbial Fuel Cells (SMFCs) have proven to be a useful technology, representing a continuous source of renewable and sustainable energy [1]. SMFC functioning is based on a buried anode and an air cathode, which is arranged at a certain distance from the anode, over the surface of soil (for terrestrial applications) or seawater (for marine applications). This configuration induces large ohmic losses and represents one of the main limits for the development and application of this technology. In order to overcome this limitation, a new architecture, named Floating Microbial Fuel Cells (FMFCs) has been proposed [2].

In the present work, the performance of Single Chamber FMFCs (scFMFC) in marine environment was investigated. To develop a biofilm in equilibrium with this specific environment and to reduce the start-up time of the scFMFCs, a method for anodic in situ colonization was exploited. After 50 days of pre-colonization, carbon felt-based anodic electrodes were used to build different replicas of scFMFCs; the scFMCCs overall performance were evaluated over a period of one month of operation in the Mediterranean Sea. The study demonstrated a very short start-up time equal to 3/5 days, as well as a high stability of the scFMCCs even in real, uncontrolled environment. The easy preparation procedure of the anodic electrodes together with the simple architecture of the scFMFC suggested the use of these devices as power supply for environmental sensors in hostile environment such as open sea application.

References

Fig.1. Picture of the housing for scFMFCs used in the marine experiments.
Benthic Microbial Fuel Cell Provides Power to a pH Meter for almost One Year

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The Navy has a requirement for monitoring environmental information in marine environments. Different sensors often monitor and report environmental parameters and potential activities such as animal movements, ships, or personnel. However, power must be provided to these sensors. One promising enabling technology that has been shown to provide long-term, low power production are benthic microbial fuel cells (BMFC). BMFCs generate energy by coupling bioanodes and biocathodes. Recent studies have demonstrated success for usage of BMFCs in powering small instruments and other devices on the seafloor over limited periods of time. However, knowledge of long-term power production is lacking. This paper will demonstrate our potential to use a BMFC to power a pH meter for one year—even though the BMFC was not fully buried.

Sediment was collected from two working sites in Italy on July 21, 2016 at two locations within 10 meters of each other using cores scooped along the bottom. Three 0.5 m³ (geographical area) and one 1 m³ BMFC systems were deployed at the two sediment collection sites. The Big Grid was developed to provide power to a battery that in turn, powered a pH meter.

Laboratory data from the small grids site outperformed the Big Grid site, with peak performances of ~30 mW/m². In-situ data from the Small Grids demonstrated that two of three smaller systems provided steady state power production for 150 days and continued to produce power even though the cathodes were sinking and by 200 days, were laying on the ground. Regardless, they provided power for 100 more days until fully covered by sediment. More importantly, the Big Grid, even though *not ever fully buried*, managed to produce power for ~350 days and powered a pH meter for most of that time.

Data will be presented on both field systems demonstrating that BMFCs are a viable means to power a small sensor.
Session V
Microbial electrochemical synthesis
Electrified fermentations enhance anaerobic lysine production by *Corynebacterium glutamicum*

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It has been suggested that microbial fermentation rates and yields can be enhanced by application of external redox power in the form of electrical energy. We tested this hypothesis on lysine fermentation by *Corynebacterium glutamicum* ZW04 which we exposed to different conditions regarding the anaerobic gas environment (with N₂ or CO₂), applied electrode potential (cathodic -1.25 V vs. Ag/AgCl, open circuit, anodic +0.45 V vs. Ag/AgCl), and the presence of a redox mediator (anthraquinone-2-sulfonate; AQ2S). The gas environment was found to play a major role, with CO₂ leading to more than double the lysine concentrations and yields when compared to N₂. Electrode potentials also played a major role, with reductive conditions under CO₂ doubling the lysine titers and yields. This was not the case under N₂ and reductive conditions though, indicating a synergetic effect under a CO₂ gas environment. Addition of AQ2S in the presence of CO₂ and reductive conditions led to additional doubling of the titers, although the yields were not altered considerably. Our study demonstrates that electrochemically assisted fermentations can significantly improve the yields and titers of lysine production compared to conventional fermentations, paving the way for bioprocess improvements based on the microbial use of raw electricity.

**Figure.** Lysine production under reductive (-1.25 V), open circuit, and oxidative (+0.45 V) electrode conditions with CO₂ (a), N₂ (b), CO₂ and AQ2S (c). Major metabolic pathways and the importance of CO₂ and regeneration of the redox factors are shown in (d).
Expanding the product spectrum of microbial electrosynthesis – Engineering of *Cupriavidus necator* to produce the sesquiterpenoid α-humulene

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In microbial electrosynthesis, carbon dioxide is fixed by using energy supplied by electrodes. Besides building up biomass, so far mainly low value organic acids and biofuels are produced. Genetic tools are limited for microorganisms used in the field of microbial electrosynthesis. To date, heterologous gene expression for the production of high-value products from carbon dioxide is rare. *Cupriavidus necator* is one of the exceptions, because it is genetically accessible and able to fix carbon dioxide with electrochemically produced hydrogen.

Here we present the production of α-humulene by a genetically modified *C. necator* strain. In the last decade, the monocyclic sesquiterpenoid is in the focus of research due to its therapeutic uses. α-Humulene is a powerful anti-inflammatory and analgesic agent with additional anti-bacterial and anti-cancer properties.

The facultative chemolithoautotrophic bacterium *C. necator* produces the C15 compound after heterologous expression of the respective terpene synthase gene. Water electrolysis provides the strain with electron donor hydrogen and electron acceptor oxygen, respectively. By using the indirect extracellular electron transfer approximate 10 mg/L α-humulene, corresponding with 5 mg per gram cell dry weight, is produced. Productivity is increased by the introduction of a heterologous mevalonate pathway, which intensifies the precursor flux to the humulene synthase. For the first time, chemolithoautotrophic and electroautotrophic terpene production is presented.

Principle of α-humulene production with *C. necator*. 
Microbial Electrosynthesis:
Microbes turning CO$_2$ into volatile fatty acids and the associated alcohols

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The industrial production of many chemicals is often not a sustainable process, as it is based on the use of non-renewable resources. Therefore, research into finding sustainable alternative production processes is critical.

Our research focuses on microbial-electrochemical conversion of the greenhouse gas CO$_2$ into industrially relevant chemicals using the emerging biotechnology: Microbial Electrosynthesis (MES). We supply a microbial community with electricity to provide the microorganisms with the reducing-power needed to transform CO$_2$ into valuable products (biofuels and important building blocks for chemical industry).

We operated a bioelectrochemical system for about one year under a selective environment to establish a microbial consortium demonstrating excellent properties being capable of turning CO$_2$ into volatile fatty acids and the associated alcohols (Figure 1). Metagenomics results show a remarkably dominance of one species (>60%) in the well-adopted mixed culture. This unique species has hardly been described in the literature, but could potentially be a future candidate for MES.

In conclusion, we demonstrate a well-adapted microbial community synthesizing valuable products from CO$_2$ and electricity. Thereby, we show the great potential of MES being a sustainable future-technology.

Figure 1: Microbial electrosynthesis of VFAs and alcohols from CO$_2$ by the well-adapted microbial community.
Characterization of new cathodically active microorganism *Desulfosporosinus orientis*: simultaneous sulfate reduction and acetate production

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Electrotrophs are microorganisms able to perform different electroreduction reactions by accepting electrons from the cathode. The high diversity of electrotrophs resides on the wide range of electron acceptors that can be coupled with cathodic electron uptake (Lovley, 2011). This work was focused on the characterization of the new cathodically active microorganism *Desulfosporosinus orientis* (Rodrigues and Rosenbaum, 2014) in a H-type BES reactor. *D. orientis* is an anaerobic gram-negative, sulfate-reducing and acetogenic bacterium, able to grow chemoautotrophically on H\textsubscript{2} and CO\textsubscript{2}.

After systematic optimization of *D. orientis* growth medium, BES experiments were carried out under two different applied potentials: a start-up condition of -900 mV vs Ag/AgCl, followed by an electrocatalytic phase of -550 mV vs Ag/AgCl. Both potentials were thermodynamically appropriate for converting CO\textsubscript{2} to acetate and for sulfate reduction, but only -900 mV allowed hydrogen evolution. The start-up phase was characterized by high current consumption, high planktonic biomass growth, a maximum acetate and formate production rate of 0.6 ± 0.1 mM day\textsuperscript{-1} and 88.4 ± 8.4 μM day\textsuperscript{-1}, respectively, and a maximum sulfate reduction rate of 5.4 ± 0.8 mM day\textsuperscript{-1}. On the contrary, the -550 mV phase exhibited constant levels of acetate and sulfate, with a decrease in optical density. These results pointed to a H\textsubscript{2}-mediated electron uptake capability of *D. orientis* in these experimental conditions. At the end of the test, 94 ± 13% of the total charge delivered by the current was assigned to acetate and formate production, sulfate reduction and planktonic and biofilm biomass production, with the highest electron recovery reflected in sulfate reduction (74 ± 10 %). These preliminary results highlighted the great potential of *D. orientis* as biocatalyst in wastewater sulfate removal application, with simultaneous acetate production.

Salinity-gradient energy driven microbial electrosynthesis of value-added chemicals from CO₂ reduction

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Direct conversion of CO₂ to value-added chemicals and biofuels has emerged as an attractive strategy to address the energy and environmental concerns caused by the over-reliance on fossil fuels and CO₂ emission. Microbial electrosynthesis (MES) technology has been demonstrated as one promising method to convert CO₂ into value-added chemicals under applied potentials from power grids. To reduce the energy consumption, here we report an innovative MES system named microbial reverse-electrodialysis electrosynthesis cell (MREEC) system (see Figure 1), which utilizing the electrical potential generated from the exoelectrogens and salinity-gradient energy to drive CO₂ fixation to acetate and ethanol. The optimal acetate and ethanol production was observed at high and low concentration solutions flow rate of 1.0 mL min⁻¹, CO₂ flow rate of 1100 mL d⁻¹, cathode potential of 0.690 ± 0.025 V (vs Ag/AgCl). The maximum acetate and ethanol accumulated concentration of 9.87 and 2.11 mM were obtained at corresponding production rate of 206.25 and 43.75 mmol m⁻² d⁻¹, respectively. In the MREEC, the cathode potential as the key parameter for the CO₂ fixation to acetate and ethanol production, which can be controlled by the salinity ratios and flow rate of high and low concentration solutions. This work for the first time proved the potential of MREEC system for CO₂ fixation to acetate and ethanol. The approach demonstrated here can also be applied to produce other value-added chemicals, thus opening the possibility of salinity-gradient energy-driven bioconversion of CO₂ to a variety of value-added chemicals.

Figure 1. Schematic illustration of the MREEC. LC: low concentration NaCl solution; AEM: anion exchange membrane; CEM: cation exchange membrane; HC: high concentration NaCl solution.
Enhanced methane production in a microbial electrosynthesis system using a bipolar membrane

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Microbial electrosynthesis is a promising strategy for the microbial conversion of carbon dioxide to carbon-containing products (i.e., carbon-containing fuels, organic commodities). In this system, microorganisms attached on the cathode act as biocatalysts to drive the reduction of carbon dioxide. Notably, the pH value of catholyte in the system tended to increase with the operating time, reducing the activity of the microorganisms and hence the performance of the systems. In this study, we constructed a microbial electrosynthesis system with a bipolar membrane (BPM), which could dissociate H\textsubscript{2}O into H\textsuperscript{+} and OH\textsuperscript{–} in situ to maintain the pH value of catholyte, as the separator for carbon dioxide conversion to methane. Compared to the systems with anion or cation exchange membranes, the proposed system using BPM showed a remarked improvement of current output and methane production rate at a constant cathodic potential of −0.7 V vs. Ag/AgCl. The electrochemical analyses including cyclic voltammetry (CV) and electrochemical impedance spectroscopy (EIS) were also conducted to investigate biocathodes activity in different systems. This study provide a potential approach to increase the methane production rate and biocathode activities in microbial electrosynthesis by maintaining the pH value of catholyte.
Continuous caproate (C6) production from CO₂ by microbial electrosynthesis: making feed additive precursor with electricity

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A challenge for microbial electrosynthesis from CO₂ is to produce longer carbon-chain, higher value, organic compounds, than acetate. Here, we report on a microbial electrosynthesis process achieving elongation of CO₂ (sole carbon source) to acetate (C2), n-butyrate (n-C4), and n-caproate (n-C6), using electricity as electron donor. Continuous and long-term (>365 days) chain elongation was demonstrated. Though performance enhancement was out of the scope of this study, relatively high production rates were achieved, while no alcohols, lactate, nor any other organics that could have acted as intermediate electron donor for chain elongation, were detected in our reactors. To the best of the authors’ knowledge, this is the first demonstration of C6 production from CO₂, with electricity as sole electron source.

Carbon felt was used as cathode electrode material, in optimized forced flow-through reactor systems (duplicate). Electron uptake rates showed to be significantly enhanced by continuous supply of nutrients, reaching high current densities up to 10 kA m⁻³ electrode (i.e. 120 A m⁻² projected surface area). This corresponds to the highest current density recorded on carbon felt, a relatively cheap electrode material. Furthermore, hydraulic retention time showed to select for product spectrum and selectivity. CO₂ was first converted to acetate. After reaching an acetate concentration threshold (2-4 g L⁻¹), nC4 production was initiated. Similarly, nC6 production started at nC4 concentration above 1-2 g L⁻¹. Maximum production rates of 12 g L⁻¹ day⁻¹ C2, 5.9 g L⁻¹ day⁻¹ nC4, and 1.5 g L⁻¹ day⁻¹ nC6 were achieved, at high electron recoveries of 70-100%. A thick, uniform, and highly electroactive biofilm was formed throughout the unmodified carbon felts (see images). This work represents a step forward to practical implementation of the technology.
Increased concentration and current efficiency for in situ extraction of acetic acid in microbial electrosynthesis from CO₂

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Microbial electrosynthesis (MES) has emerged as a promising bioreactor technology for the production of organics from CO₂ 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 CO₂ 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). Furthermore, 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 (Gildemyn et al, 2016). Although it has been shown that the electricity-driven extraction of acetate over an AEM allows pure product recovery at higher efficiency, the current use for acetate extraction is low (e.g., 5 %) and the maximum acetic acid concentration seems to be limited by the water transport across the AEM (14 g L⁻¹). In this study we demonstrated that coupling a MES reactor (with AEM) to a bubble column can increase the hydrogen uptake efficiency, and thus the production rates. By injection of additional hydrogen gas (produced via water electrolysis) the charge balancing efficiency by acetate can be increased up to 35 %, thus making more efficiently use of the intrinsic ability to extract anions. We showed that the use of external H₂ for CO₂ conversion can lower the power input for bioproduction and increase the product concentration in the extraction liquid (up to 37 g L⁻¹). Furthermore, the extraction of acetic acid across an AEM prevents the pH decrease associated with H₂/CO₂ fermentations, thus avoiding base consumption.
From flying blind to a better understanding of the bioelectrochemical transformation of CO₂ into valuable chemicals

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The bioelectrochemical transformation of CO₂ into chemicals has recently attracted the scientific community. Short chain fatty acids and alcohols define the current portfolio of bioelectrochemical production. Even though its technology readiness level (TRL) of 3-4, it is has been recently postulated as a promising approach to transform CO₂ into carbon-neutral commercially. This contribution reviews the recent advances of our research group on microbial electrosynthesis of C2-C4 platform chemicals using CO₂ as the sole carbon source. Batlle-Vilanova et al.[1] found that low pH and high hydrogen partial pressure are key operational factors to trigger the production of acetate and ethanol, which were subsequently used by the microbes to produce butyrate as end-product through chain elongation. In addition, the use of an inoculum able to catalyze hydrogen production/consumption reactions is crucial for achieving the desired final product. In this sense, further insights on the lately found compartmentalization of the processes among the biocathodic communities will lighten the preponderance of hydrogen-mediated over direct electron transfer processes in bioelectrochemical production [2,3]. Recently, we considered enriched cultures of a new solventogenic strain I-19 (100% similar to Eubacterium limosum and Butyribacterium methylotrophicum according to 16S rRNA gene similarity) as a promising candidate to drive bioelectrochemical ethanol production. Initial tests with I-19 conducted in serum bottles fed with H₂:CO₂ showed ethanol production, confirming its suitability as inoculum. However, tests carried out in bioelectrochemical systems enriched with the I-19 have not met the expected production yet, suggesting culture maturity and feeding frequency as unconsidered factors for the bioelectrochemical production of alcohols.

Session VI
Microbial ecology
Microbial anodic consortia fed with fermentable substrates in microbial electrolysis cells: the significance of ecological interactions

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The microbial community structure of anodic biofilms plays a key role in bioelectrochemical systems (BESs). Bacteria composing these biofilms confer different capabilities to the system to convert organic matter into electrical current, using the anode as sole electron sink. As complex ecosystems are generally used as inoculum, many bacterial species having interconnected ecological interactions are present which makes more complex the elucidation of their exact role in these systems. Thus, the aim of the present study was to identify the key bacteria for the conversion of single short chain acids into electrical current.

Here, two-compartment lab-scale microbial electrolysis cells were inoculated with the same activated sludge. Batch tests were operated in quadruplicate, at the same time, and were fed with four different substrates: acetate, lactate, butyrate and propionate, at 80 mMe⁻ equivalents. Biofilm and planktonic communities were analyzed at the end of the batch operation.

Mainly, the anodic biofilms were dominated by the Geobacter genus (62.41% of the total sequences) known to be electroactive. At the species level, Geobacter sulfurreducens dominated in presence of lactate and acetate, while other species such as Geobacter toluenoxydans and Geobacter pelophilus were present when fed with butyrate and propionate. These results indicate for the first time a specificity within the Geobacter genus compared to the electron donor, suggesting in one hand that a competitive process occurred for electrode colonization and in the other hand that syntrophic interactions are necessary for the complete oxidation of substrates such as propionate and butyrate.

All together, these results provide a new insight into the ecological relationships within electroactive biofilms and suggest eco-engineering perspectives to improve the performances of BESs.
Predicting Microbial Fuel Cell Biofilm Communities and Bioreactor Performance using Artificial Neural Networks

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The complex interactions that occur in mixed-species bioelectrochemical reactors, like microbial fuel cells (MFCs), make accurate predictions of performance outcomes under untested conditions difficult. While direct correlations between any individual waste stream characteristic or microbial community structure and reactor performance have not been able to be directly established, the increase in sequencing data and readily available computational power enables the development of alternate approaches. In the current study, 33 MFCs were evaluated under a range of conditions including 11 separate substrates types including 3 wastewaters. Artificial Neural Networks (ANNs) and machine learning algorithms were used to establish mathematical relationships between wastewater/solution characteristics, biofilm communities, and reactor performance. ANN models that incorporated biotic interaction predictions predicted reactor performance outcomes more accurately than those that did not. The average percent error of power density predictions was 16.01 ± 4.35%, while the average percent error of coulombic efficiency and COD removal rate predictions were 1.77 ± 0.57% and 4.07 ± 1.06%, respectively. Predictions of power density improved to within 5.76 ± 3.16% percent error through classifying taxonomic data at the family versus class level. Results suggest that the development of ANNs is a workable approach to predict performance of engineered biological systems.
Electrostatic potential measurements as proxies for cable bacteria activity – potentials and resistances

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Cable bacteria are multicellular filamentous bacteria that play an important role in sediment biogeochemistry by connecting sulfide oxidation in anoxic sediment layers with oxygen reduction in the oxic sediment surface layers, using the filament itself as an electron conductor. The charge transport by the electron current in the filaments results in electrostatic forces which by virtue of the electroneutrality principle drive an equally-sized ionic counter-current in the sediment porewater. The resulting electric potential distribution reflects the cable bacteria activity as well as any spatial variations in sediment resistivity, and the distribution of activity can easily be calculated under the assumption of horizontal homogeneity as in typical laboratory systems. However, many natural cable bacteria systems are not horizontally homogeneous, rendering a simple 1D modelling approach invalid. Further complications to take into account in many situations are presence of additional electric fields with other physical basis. Of primary concern is the phenomenon of diffusion potentials, caused by concentration gradients of ions with different mobilities. Such concentration gradients may build up over time by electro-migration due to the electric fields caused the cable bacteria themselves, or they may be due to changes in the composition of the overlying water. Here we present experimental data that illustrates the extent of these complications. Furthermore, we discuss approaches to handle them through experimental design and modelling.
Isolation and characterization of novel electrotrophic microorganisms using metallic iron as sole electron donor

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Some microorganisms are capable of using metallic iron as electron donor. This capacity leads to severe corrosion to metallic structures, but can also have interesting biotechnological applications. Microbial electrosynthesis (MES) depends on homoacetogens capable of deriving electrons from a cathode to reduce carbon dioxide to organic molecules. Both metallic iron and a cathode are solid state electron donors, therefore, the use of metallic iron as an electron donor for enrichments and isolations seems a promising strategy to obtain novel high-performing strains for microbial electrosynthesis. The goal of this work was to isolate acetogenic strains using metallic iron as electron donor from environmental rust samples. The rust layer of metallic waste present in a local river was scraped off and used to set up enrichments. Metallic iron particles were added as sole electron donor and methanogenic growth was inhibited. Isolation was performed using agar plates containing iron powder, while agar plates with an organic substrate were used for further purification. Most isolates were strongly related (99%) to Acetobacterium malicum, while also a Sporomusa and Shewanella strain were obtained. The new strains enhance Fe(0) corrosion at least five times in comparison to abiotic corrosion. Consequently, these strains must have a mechanism for direct electron uptake or the ability to enhance abiotic hydrogen evolution. Insights into the extracellular electron uptake mechanisms, as well as their capacity for microbial electrosynthesis will be presented. The study of these Fe(0) oxidizing strains will lead to new insights into biocorrosion and extracellular electron uptake mechanisms and will contribute to the improvement of biofilm based microbial electrosynthesis.
Interspecies electron transfer mediated parasitism: co-culture of *Geobacter sulfurreducens* and *Clostridium pasteurianum*

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In the present work, interspecies electron transfer (IET) between fermentative and electro-active species was investigated using *Geobacter sulfurreducens* and *Clostridium pasteurianum* as model microorganisms in co-culture experiments\(^1\). In a fermentation medium containing glycerol and acetate as substrates, *G. sulfurreducens* was able to grow using *C. pasteurianum* as sole electron acceptor. As a result, *C. pasteurianum* fermentation pattern was significantly altered compared to pure culture controls. Indeed, 1,3-propanediol and butyrate production were both improved at the expense of butanol and ethanol. Interestingly, *C. pasteurianum* growth yield decreased by almost 40% in co-cultures despite a theoretical ATP production (i.e. calculated from metabolic patterns) being 8.4% higher in co-culture compared to *C. pasteurianum* pure culture. To better understand such drop in biomass yield, energetics of IET between electro-active and fermentative species was theoretically explored using a thermodynamical model. Although this approach could predict an IET-mediated parasitism when fermentative species acts as electron-accepting microorganism, it failed to explain the drastic biomass yield decrease observed experimentally, thus suggesting that more complex biological regulations were implicated.

While fundamental principles are still to be elucidated, IET-mediated parasitism could be a promising way to optimize carbon recovery during fermentations by both reducing biomass production and improving metabolite yields of value-added compounds such as 1,3-propanediol.

Probing the Functions of Biocathode Microbial Community Members with Inhibitors, Transcriptomics, and Genetic Manipulation

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Biocathode MCL (Marinobacter-Chromatiaceae-Labrenzia) is a microbial community that contains at least one uncultivated electroautotroph. It has been extensively investigated by our lab as a model for microbial electroosynthesis at a high electrode potential (310 mV vs. SHE). Our investigations have focused on understanding the physiology of the electroautotrophic bacterium “Candidatus Tenderia electrophaga”, including the use of metabolic inhibitors to selectively inhibit key components of the hypothesized electron transport pathways. In addition, we have been exploring the role of other dominant MCL constituents and their role in cathodic current and potential for genetic manipulation to engineer electrode associated microbial consortia. Marinobacter sp. CP1 has proven to be amenable to cultivation and genetic manipulation and has displayed an ability to perform extracellular electron transport. Marinobacter sp. CP1 has an interesting phenotype when growing in the presence of an electrode poised at 310 mV vs. SHE. Extracellular electron transfer between the electrode and the cells begins as increasing cathodic current and then shifts to anodic, presumably after the oxygen in the medium is exhausted. Transcriptomics of wild-type Marinobacter sp. CP1 and mutants growing in an electrode environment will help to identify the molecular mechanisms of this transition from cathodic to anodic current on the working electrode. This work will help lay the foundations of a synthetic biology chassis for marine biofilm environments.
Evolution of anodic biofilm formation under shear stress conditions

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In natural environments and in industrial reactors, microbial biofilms are structured by hydrodynamic forces. However, few papers on Microbial Fuel Cells (MFCs) study the impact of hydrodynamic force on MFC performances. Pham et al. showed that a higher shear stress increased the electricity production but without studying the biofilm structure [1]. We hypothesize that shear stress plays a major role in the biofilm formation and the selection of ElectroActive Bacteria (EAB) on the anode. Pure culture work published to-date show that shear stress influences bacterial adhesion and bacterial density of mature biofilm. However, few studies show the impact of shear stress on bacterial community selection either during the bacterial adhesion step or biofilm growth. The objective of this study was to test whether the bacterial and physical anodic biofilm structure formed under shear stress condition during bacterial adhesion step to mature biofilm was driven by the shear stress. Firstly, MFC reactors with a shear stress chamber that created specific shear stress on the anode were designed and fabricated. Then, the evolution of the biofilm formation under different shear stress conditions (1, 10 and 50 mPa) were compare. The control was a classical MFC without shear stress. The microbiological structure was studied by 16S RNA gene (rrs) sequencing. The physical structure was studied by fluorescence microscopy and the electrical performance was measured regularly. This project improved our understanding of the chronology of the anodic colonization by different functional bacterial groups and the role of a key parameter, the shear stress, in the MFC engineering.

Microbial populations and putative functions for complete ammonia removal in single-chambered microbial nitrogen-removal cells (MNCs)

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The flat-panel air-cathode microbial nitrogen-removal cell (FA-MNC) successfully treated domestic wastewater without any aeration and satisfied effluent quality standards of chemical oxygen demand (COD) and total nitrogen (TN) under a short hydraulic retention time of 2.5 h. The removal efficiencies of COD and TN increased up to 85% and 94% for 8 months of operation, respectively. The effluent COD and TN concentrations were 20.7 ± 2.5 mg/L and 1.7 ± 0.1 mg/L, respectively. Analysis of Microbial community based on 16S rDNA sequences using Illumina MiSeq revealed that there are a variety of bacterial populations involved in various metabolisms such as nitrification, denitrification, fermentation, sulfate reduction, and iron reduction on biofilms at anode and cathode of the FA-MNC system. Based on the frequency of OTUs (16S rDNA sequences) detected in the microbial communities, four mechanisms for removing ammonia in the single-chambered FA-MNCs have been proposed: (1) the oxidation of ammonia (*Nitrosomonadaceae*) and nitrite (*Nitrospiraceae*) using oxygen penetrated through the air-cathode, (2) heterotrophic denitrification (*Rhodocyclaceae*) at both the anode and cathode, (3) anaerobic ammonium oxidation (anammox; Candidatus Brocadia) in the anaerobic zone between anode and cathode, and (4) autotrophic denitrification (*Thiobacillus*) at the anode. The study shows that the FA-MNC configuration, two separator electrode assemblies and a large ratio of electrode surface area to reactor volume, can lead simultaneous nitritation, anammox and denitrification. The FA-MNC system will be a cost-effective domestic wastewater treatment process because it eliminates the needs for aeration for nitrification and external carbon sources for heterotrophic denitrification required in conventional biological nitrogen removal processes.
Session VII
Novel applications of microbial electrochemical systems
Are anammox bacteria electrochemically active?

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To date, nitrogen removal by anaerobic ammonium oxidation (anammox) is the most cost-effective process for the treatment of ammonium in wastewater. The application of anammox process appears to be a pre-requisite for achieving energy neutral/positive wastewater treatment. In anammox process, ammonium is converted to nitrogen gas using nitrite as the electron acceptor. The supply of electron acceptor (i.e. nitrite) required by anammox bacteria is obtained from the interaction with different types of microorganisms such as ammonia oxidizing archaea/bacteria, denitrifiers, anaerobic methane oxidizing archaea and commamox bacteria. In three-electrode electrochemical systems, electrochemically active bacteria use the anode as the electron acceptor by applying a potential. A recent review showed that there is no specific ecological niche for electrochemically active microorganisms (Koch and Harnisch, 2016). Thus, this study was motivated by the question: are anammox bacteria electrochemically active? To address this question, we tested eight different potentials in a three-electrode electrochemical system inoculated with highly enriched (>99%) anammox culture. The reactor was operated for more than two months without the presence of any electron acceptor other than the electrode. Biotic and abiotic controls, inhibition of nitrifiers and metagenomics analysis revealed that anammox bacteria were responsible for the oxidation of ammonium and current generation without any production of nitrite or nitrate. To our knowledge, this is the first report showing the ability of anammox bacteria to use a solid-state electron acceptor and behave as an electrochemically active microorganism. Also, this study showed that there is no need for partial nitrification and/or the requirement of nitrite for anammox process. The implication of this study is that it opens the possibility of simultaneous removal of organics and nitrogen at the anode of bioelectrochemical systems.
Microbially charged redox flow battery: coupling a bioelectrochemical cell with a redox flow battery

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Redox flow batteries (RFB) are electrochemical systems applied in the conversion and storage of chemical energy in electricity. Redox chemical species (in soluble form) are the main responsible for the energy storage. Quinones are electroactive molecules applied in RFB because of their chemical and physical properties.

The aim of this work is to develop an innovative technology to generate and storage the energy resultant from Bioelectrochemical system (BES). The strategy outlined was coupling a BES with a RFB that present potential to combine bioenergy production and storage in a microbially charged redox flow battery.

Firstly, a BES system was studied with \textit{Geobacter sulfurreducens} as biocatalyst to convert a quinone (2,6-anthraquinone) in its respective reduced form, acetate being the carbon source used. The BES presented current intensities around 500 mA.m\textsuperscript{-2} and power densities around 2 Wm\textsuperscript{-2}. The reduction was assessed visually by a typical colour change (from yellow to dark red) and by cyclic voltammetry. Simultaneously, as a control, the 2,6-anthraquinone was electrochemically reduced applying and controlling the cathode potential where the reduction was also observed by colour change and by cyclic voltammetry.

In an RFB, the quinone bioreduced in the BES and electrochemically reduced in the electrochemical cell were studied using potassium hexacyanoferrate as the second redox chemical species for discharging/charging cycles in the RFB as the proof of concept of the microbially charged redox flow battery. The study was performed in a 25 cm\textsuperscript{2} single redox flow cell (RF) with a constant current density of 0.2 mA.cm\textsuperscript{-2} where coulombic efficiency, voltage efficiency and energy efficiency were observed for 10 cycles.
Chemical free nitrogen capture from urine by precipitation as ammonium bicarbonate: overcoming limits of precipitation

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Chemical free nitrogen capture as solid from source-separated urine can be achieved through bio-electro-concentration and subsequent cooling, producing a crystalline ammonium bicarbonate solid (Ledezma et al. 2017). Capture is limited by the high solubility of ammonium bicarbonate and large competing ion concentrations (Thompson Brewster et al. In Press) and maximum 17 % N capture was recorded in a series of electro-concentration experiments. To enable higher capture rates, different electro-concentration setups were investigated. A solids retention system concentrates urine into a cold trap precipitating ammonium bicarbonate and feeds the liquid supernatant back to the reactor for further concentration, allowing for increased 23 % N retention in solid with same electric energy consumption. Applying three solids retention reactors in series for the same feed with a single cold trap reduced N recovery (12 %) as competing ion concentrations started to dominate, inhibiting precipitation. To overcome competing ion effects, a double reactor setup was proposed (Figure 1) in which high pH effluent from cathode is circulated to a waste anode where predominantly Na and K are removed (nitrogen appearing as inert NH₃), and low pH effluent from anode is circulated to a waste cathode where predominantly chloride and phosphate are removed (inorganic carbon appearing as inert CO₂).

Figure 1 Waste salt removal for enhanced solid ammonium bicarbonate capture from urine. In the waste salt reactor, the high or low pH renders ammonium and bicarbonate inert to electromotive force at anode and cathode, respectively.


Eu tenho dois amores - Coupling selective electrochemical CO₂ reduction to formate with microbial biosynthesis

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Fluctuating and decentralized production of electricity from renewables triggers the search for respective electric energy storage and utilization. At the same time a sustainable bioeconomy calls for the exploitation of CO₂ as feedstock. Secondary microbial electrochemical technologies (MET) can tackle both challenges since electrochemical CO₂ reduction can be coupled with microbial biosynthesis (see Figure). Exploiting only electrochemical CO₂-reduction does not easily lead to high-value products composed of more than one carbon atom, as the formation of C-C bonds is not favorable. However, this limitation can be overcome by coupling of electrochemical CO₂ reduction to formate (HCOO⁻) with its subsequent conversion by microbial biosynthesis. As this interfacing creates special challenges, the electrochemical CO₂ reduction is investigated under biocompatible conditions using indium as model electrocatalyst. A reproducible electrodeposition procedure of indium on graphite backbone allowed a systematic study of formate production from CO₂. In this system coulombic efficiencies (CE) and formate production rates ($r_{formate}$) of up to 91.7 ± 3.5 % and 0.060 ± 0.006 mmol/formate h⁻¹ cm⁻² (mean ± CI), respectively, were achieved. Further, the effect of components used in microbial media, i.e. yeast extract, trace elements and phosphate salts, on the electrode performance is addressed. At the same time a screening for microorganisms capable of metabolizing formate to value added products was conducted. Therefore, microbiomes (open mixed cultures) as well as pure cultures are tested with different formate feeding strategies. In this study it is demonstrated that the integration of electrochemical CO₂ reduction in secondary METs can become technologically relevant and selected model processes will be shown.
Morphological analysis of electrochemically active biofilms of *Geobacter sulfurreducens* and *Shewanella oneidensis*

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The analysis of electrochemically active biofilms is of great importance for bioelectrochemical applications. Among others, two electrochemically active bacteria have gained special interest in this field of study, namely *Geobacter sulfurreducens* and *Shewanella oneidensis*. Electrochemical cultivations were performed in a half-cell setup containing lactate and acetate as electron and carbon source, while a graphite anode (0.2 V\(_{\text{Ag/AgCl}}\)) served as the sole electron acceptor. The stated organisms were cultivated in respective pure cultures and in a defined mixed culture. Biofilms formed on the anodes were analysed by confocal laser scanning microscopy in terms of their thickness and surface morphology.

In *S. oneidensis* pure cultures, only poor biofilms and low current production was observed. *G. sulfurreducens* cultivations yielded good biofilms and accordingly the generated current densities were about ten times higher as with *S. oneidensis*. To investigate beneficial interactions between the two species, *S. oneidensis* and *G. sulfurreducens* were also cultivated in a defined mixed culture. Here, even higher current densities compared to the *G. sulfurreducens* pure culture could be observed and thicker biofilms with a different surface morphology formed on the anodes.

Further investigation of the interactions of the two organisms in the system is proposed for future experiments to discover if *S. oneidensis* is incorporated in the mixed culture biofilm. These investigations include the distribution of the bacteria within the biofilm and the cultivation medium by cytometric analysis.

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Microbial bioanodes as sensors for ionic liquids toxicity and anti-biofouling properties

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Bioanodes were developed from a wastewater inoculum in the presence of acetate, the absence of oxygen and a connection to a chemical cathode using ferricyanide as the oxidant, in a H-shape, membrane-separated, two compartments fuel cell design. The fully developed catalytic bioanodes were used to test the effect of adding different ionic liquids such as 1-Butyl-3-methylimidazolium acetate (BMIM Ac) at different concentrations into the anolyte. The catalytic oxidation of acetate by the bioanodes provides a straightforward mean of measuring the effect (toxicity) of the ionic liquid on the electroactive biofilms. Further studies confirming this effect include the (failed) recovery of the catalytic activity of the biofilm in a clean anolyte and the (impeded) development of bioanodes in the presence of the ionic liquid. The effect of the ionic liquid on the wastewater and on the membrane permeability has also been investigated. Finally the grafting of the imidazolium onto the electrode surface has shown that it prevents the development of a biofilm.

Decrease of acetate oxidation catalytic current upon BMIM Ac addition monitored by recurrent cyclic voltammograms

Reference:
Study of The Electrochemical Capacity of a New Chemolithoautotrophic Arsenic Oxidizing Bacteria *Ancylobacter* Sp. Ts-1

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The study of electrochemically active microorganisms (EAM) has revealed the metabolic versatility of chemolithoautotrophic microorganisms in biocathodes, being principally oxidizers of Fe$^{2+}$ and H$_2$. To our knowledge, chemolithoautotrophic arsenite oxidizers (CAOs) have not been reported as EAM. This research studies the electrochemical activity of *Ancylobacter TS-1*, a new CAO isolated from a hydrothermal source with natural arsenic attenuation in northern Chile. The electrochemical capacity of *TS-1* was evidenced by (i) linear sweep voltammetry (LSV) tests, revealing a peak of cathodic current dependent on biomass concentration at -500 mV (vs. Ag/AgCl, at pH 7.2); and by (ii) chronoamperometry, showing an increase in the cathodic current over time at this observed potential. Bacterial growth observed by scanning electron microscopy on electrodes tested in the chronoamperometry suggests the development of a *TS-1* biofilm that is using the cathode as an only electron donor. This paper expands the knowledge of electrochemically active bacteria associated with metals and metalloids. In particular, our results support the hypothesis that bioelectrochemical systems could be a potential tool of bioaugmentation for strengthening the biological arsenite oxidation in contaminated natural environments.
Neodymium recovery: from ionic liquids to bioelectrochemical systems

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Neodymium is considered as a scarce metal and has become essential for manufacturing permanent magnets, extensively used for green technologies such as wind turbines or electrical vehicles. Therefore, it is crucial to recover it from secondary resources to balance its supply and demand. Electrodeposition processes have taken a particular interest to separate neodymium from others metals due to its selectivity and low environmental impact. The choice of the electrochemical configuration is a real challenge since the standard potential of this lanthanide is highly negative (around -2.5 V vs. ENH). Room temperature ionic liquids (RTIL) appear as promising electrolytes thanks to their wide electrochemical window. However, an unexpected degradation of the RTIL ions appears during the electrodeposition process due to the uncontrolled oxidation reaction occurring at the anode electrode. Microbial electrolysis cells offer an innovative alternative since the metal reduction is coupled to the oxidation of organic compounds in a two chambers reactor (Figure 1). Galvanodynamic and galvanostatic tests were employed to reduce Neodymium in a butyl-methylpyrrolidinium-bis-trifluoromethanesulfonyl imide ([BMPyr][Tf\textsubscript{2}N]) solution. Acetate oxidation took place in an aqueous solution and was catalyzed by electrogenic bacteria. Graphite electrodes were employed in both chambers and a microporous membrane (MP) was used to separate them. A study of the electrodeposition kinetic and its limitations is proposed. Deposits were characterized by scanning electron microscopy and energy-dispersive X-ray spectroscopy. Interactions between the ionic liquids and the aqueous solution through the membrane are also discussed.

\textbf{Figure 1. Microbial electrolysis cell for Neodymium recovery in an ionic liquid catholyte.}
Opportunities of BES for sulfate wastewater treatment: potential sulfur recovery

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We present a new application of BES for the treatment of high-strength sulfate wastewaters (usually lacking of electron donor) and the potential recovery of sulfate as elemental sulfur without adding any external electron donor. The process lays on the simultaneous occurrence in a biocathode of: i) autotrophic sulfate reduction to sulfide and ii) partial oxidation of sulfide to elemental sulfur (Figure 1).

The controlled oxygen diffusion from the anode (resulting from anodic water electrolysis) to the cathode leads to oxygen limiting conditions in the cathode that enables the coexistence of sulfate reducing bacteria (SRB) and sulfide oxidizing bacteria (SOB). The biocathode of the proof of concept experiment could remove sulfate with hydrogen as sole electron donor at a maximum sulfate reduction rate up to 388 mg S-SO\text{4}^{2-} L^{-1} d^{-1} despite the low applied potential (-0.8 V vs. SHE in the cathode). Moreover, we present systematic studies of the most influencing parameters in view of successful sulfur recovery. For example, we will show how a cation-exchange membrane reduces sulfate and sulfide adsorption as well as diffusion from the cathode to the anode. Among the different pHs tested (5.5, 7 and 8.5) in a long-term operation (at cathode potential of -0.8 V vs. SHE), pH = 7 was the optimal for sulfate removal, achieving reduction rates around 150 mg S-SO\text{4}^{2-} L^{-1} d^{-1}. A microbiological study showed that the abundance of SRB at pH = 7 was higher (67 %, mainly Desulfovibrio sp.) than that at pH = 5.5 (42 %, mainly Desulfovibrio sp. also) and at pH = 8.5 (60 %, mainly Desulfonatronum sp.). The cathode potential was studied from -0.7 to -1.2 V vs. SHE achieving sulfate removal rates higher than 700 mg S-SO\text{4}^{2-} L^{-1} d^{-1} at cathode potentials from -1.0 to -1.2 V vs. SHE. Also, the highest cathodic recovery and the highest sulfur species imbalance were observed at a cathode potential of -1.0 V vs. SHE, which indicated a higher elemental sulfur production. Finally, we will discuss the scalability of this process in view of its application at industrial level.

Figure 1 – Schematic representation of the proposed methodology
The effect of the Mtr pathway on current production in *Marinobacter CP1*

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A number of microorganisms are able to donate electrons to solid substrates using a range of mechanisms. Of these, the pathway of *Shewanella oneidensis* is one of the best understood, and consists of a set of multi-heme cytochromes and membrane proteins (known collectively as the Mtr pathway) that form a conduit for electrons from the inner membrane to the cell exterior, at which point they can flow to a solid electron sink. Our understanding of the proteins involved in this pathway has allowed researchers to successfully export the electron transfer system to *E. coli* for use in synthetic biology. In order to investigate the modularity of the Mtr pathway in environmentally relevant gram negative Proteobacteria, we have expressed the pathway in *Marinobacter CP1*, a marine organism with a demonstrated ability to form electroactive biofilms on carbon electrodes. We show that wild type *Marinobacter CP1* produces limited current on a positively poised electrode, and examine the effects of the components of the Mtr pathway, singly and together in inducible systems that sense a number of small molecules, on current production. This opens up the possibility of using the Mtr pathway in combination with distributed marine sensing devices alongside existing benthic microbial fuel cell technology and advances our basic understanding of extracellular electron transfer in *Marinobacter*. 
A new applicative frontier for microbial fuel cells: bioelectrochemical fertilizers

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Here we present a complete shift in the way to look at microbial fuel cells (MFCs), definitely beyond energy-harvesting and with a new goal: recovering carbon and nutrients from wastewater and fabricating bio-based renewable fertilizers.

For nearly two decades, MFCs have been extensively studied for their potential use in wastewater treatment and for power harvesting from waste organic matter. To date, even if technological materials are used (e.g. carbon-fibers, graphite, stainless steel, titanium) to harvest electricity, the best power densities ever achieved (around 10 W m\(^{-3}\)reactor) are not high enough [1]. Even avoiding the use of precious metals as cathodic catalysts and of selective membranes or gas diffusion layers, the overall MFC architecture is surely too expensive to think about applications were high numbers of MFC modules must be stacked, to reach electrode surface area in the orders of 10\(^3\) – 10\(^4\) m\(^2\) m\(^{-3}\) of reactor volume [2]. Despite possible future improvements in MFC systems performances, another constraint would impede their application in wastewater treatment: over long-term operations (more than 60 days) clogging and biofouling, due to organic materials as well as inorganic salts deposition on electrodes surfaces, strongly invalidate cell performances [3].

In our view, electrodes and separators should be fabricated using low-cost, biogenic and biocompatible materials. Examples currently under study in our laboratories are: a) ligno-cellulosic biomass and b) inert matrixes (e.g. clay, terracotta, etc.) as porous separators (porosity 1 – 500 nm); c) pyrolised biomass (e.g bio-based charcoal) of different geometries and properties as electrodes (surface area in the range 10\(^2\) – 10\(^3\) m\(^2\) g\(^{-1}\)). In lab-scale experiments, electrical power generation resulted in the range of 10 – 10\(^2\) mW m\(^{-3}\) of reactor volume. This power should be thought as a driver of electro-osmotic forces to move ions from the water phase to the electrodes surfaces. Inorganic salts precipitation as well as biofouling and clogging due to organic-matter deposition, instead of being avoided, should be favored and maximized. In this perspective, both inorganic and organic forms of the main macronutrients (N, P, K, Mg, Mn, Ca, Co, etc.), as well as consistent amounts of inorganic carbon are sequestered from wastewater on separators and electrodes. Once these MFC modules are saturated (typically after 40-80 days), they can be substituted with new ones and recycled as organic-mineral fertilizers for agricultural application.

On this basis, we propose a new platform of research and development: the ‘bioelectrochemical fertilizers’.

Session VIII
Water treatment and bioremediation
Treating wastewater while recovering nutrients: electrochemical biofilters coupled to innovative biochar-based cylindrical cathodes

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METLand results from integrating Microbial Electrochemical Technologies (MET) with the constructed wetland concept. This new generation of electrochemical biofilters outperform classical fixed-bed systems by substituting the inert bed (generally gravel) with electroconductive materials. These systems were validated for oxidizing BOD, COD and ammonium (Aguirre-Sierra et al., 2016).

Here, we present a new version of METlands, that aims not only at oxidizing organic loading, but also at recovering soluble minerals from the liquid phase. To do this, we propose a new type of cylindrical cathodes built by pyrolysis of low-cost lignocellulosic materials such as Giant Canes (Arundo Donax L.). These conductive cylinders are also hollow and porous. A BET analysis on the pyrolised Giant Cane reported a specific surface area of $114 \pm 4 \, \text{m}^2\text{g}^{-1}$ and a porosity mainly falling into the micropores range (< 2nm).

Anodes (conductive granules) and cathodes (pyrolised Giant Cane) were connected across external loads. The tests were repeated in subsequent batch tests using organic-rich wastewater with 10-15 g COD/L. The use of these devices helps in maximizing air-water interface into the fixed bed of the METLand, implement the snorkeling effect to oxygen. Also, electro-osmotic forces generated by the MFC-like system, contribute to cation transport towards the cylindrical cathode. Simultaneously, the high cathodic pH (10-12) caused by oxygen reduction lead to inorganic salts precipitation on cathodes. Along 60 days, consistent amounts of the main macronutrients (N, P, K, Fe, Mn, Ca, Mg) were removed from the wastewaters and deposited on the cathodic surface. After their life-course, these cathodes can be replaced and entirely re-utilized to amend agricultural soils or to produce bio-based fertilizers.

Bioelectrochemical Technology for Enhanced Remediation of Petroleum Hydrocarbon-contaminated soil

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For soils impacted with petroleum hydrocarbons, in-situ remedial technologies are often limited by the depletion of electron acceptors (e.g. O₂) in the subsurface environment. The Bioelectrochemical System (BECS) technology is a new process co-developed by Chevron with academic and industrial partners. This technology offers an inexhaustible electron acceptor source through its unique tubular configuration of electrodes and conductive circuit, to initiate, stimulate, and enhance in-situ microbial remediation of petroleum hydrocarbons. BECS has the potential to reduce remediation costs and carbon footprint by avoiding soil excavation and eliminating energy and chemical consumption. BECS has been successfully demonstrated in bench tests for treating diesel and crude oil impacted soil. A pilot field test was recently conducted at a former storage and transfer terminal site, where soil and groundwater are impacted by petroleum hydrocarbons. Four soil BECS units and one groundwater BECS unit were installed in November 2016. Since installation, all five systems have been continuously generating recordable electricity as a result of hydrocarbon degradation. Five geophysical sensors were installed to allow non-intrusive geophysical monitoring of soil electrical resistivity changes. Real time CO₂ sensors were also installed along with soil vapor probes to monitor subsurface CO₂ evolution and potential vapor during bioremediation process. Preliminary groundwater sampling results indicate that BECSs have started to enhance the degradation of petroleum hydrocarbons in the proximity.
Mature electrogenic community is able to drive efficient denitrification regardless of applied potential

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Denitrification is a redox-active process, therefore nitrogen-containing waste can be treated using Microbial Fuel Cells (MFCs). Various factors, including potential and oxygen gradient, have been reported to affect this process. In our study, we inoculated four, 2-chambered MFCs, using a mature electrogenic community obtained from a 6-month operating, acetate-fed MFC that was preconditioned for the presence of nitrate at 500 mg/L (8 mM) concentration for 1 week. We observed a removal rate of 0.53-1.2 mM N/h under the wide range of applied potentials, from -0.6 to 0.2 V. As no ammonia was found in the counter electrode chamber, nitrate removal can be attributed to denitrification. Microbial community analysis revealed the presence of aerobic denitrifier, Microvirgula aerodenitrificans, as well as the nitrate-reducing Acidovorax spp. Our current attempts are to demonstrate the feasibility of this process using pilot scale MFC operating with nitrate-containing swine waste.
Fluidized electrodes as electron acceptors or electron donors: strategies and applications

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Microbial electrochemical fluidized bed reactors (ME-FBR) represent a novel bioelectrochemical design in which a fluid-like 3D electrode with large specific surface stimulates the biodegradation of substrates by microbial electrogenesis. This design avoids some of the problems of using static biofilm-based electrodes like the slow mass transfer between solution and microorganisms, the low active surface area of the electrode and the pH changes produced at the vicinity of the electrode. The performance and versatility of ME-FBRs for treating effluents was tested either with the bed serving as electron donor or as electron source for the electroactive community.

When a ME-FBR was operated with a fluidized anode, electroactive bacteria could couple the oxidation of volatile fatty acids to the fluidized particles respiration. In fact, acetate and propionate consumption rates were 13-fold and 2.4-fold higher, respectively, when the fluidized anode was polarized (0.2 V) than when no current could flow through the system. The ME-FBR showed as well to be an effective technology for removing most of the organic matter from a brewery wastewater (efficiencies up to 95%), being the system assayed at different organic loads, hydraulic retention times and working electrode potentials. Fluorescence \textit{in situ} hybridization (FISH) analysis revealed a natural \textit{Geobacter}-wired stratification of microbial communities on the fluidized anodic particles during the treatment of the brewery effluent.

The capacity of fluidized electrodes for acting as biocathodes was also tested. A fluidized electrode, previously acting as anode, was polarized so that it could serve as electron source for the electroactive community in order to reduce nitrate. This naturally selected mixed culture could indeed perform denitrification using a fluidized cathode as electron donor, and, additionally, produce hydrogen. Our results show the versatility of using fluidized electrodes for wastewater treatment purposes due to its capacity for bioelectrochemically removing organic matter, nutrients (N) and hydrogen production.
Metagenomic Insights and System Scale up For Bioelectrochemical Petroleum Hydrocarbon Remediation

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Petroleum hydrocarbon contamination in soil and groundwater is a widespread environmental problem. Since 2013 we have developed tubular bioelectrochemical systems (BES) to efficiently degrade hydrocarbons in situ and generate electrical current for non-intrusive monitoring. We investigated system performance in different conditions, developed scaled reactors for field implementation, characterized microbial community distribution, and integrated system monitoring with geophysical probes. This presentation will summarize our progress in reactor development and scale up and highlight the newest findings regarding the unique microbial community structure developed during BES soil remediation. We found BES has a better performance for sandy soil remediation than clayey soil due to improved hydrocarbon transfer in sandy soil texture. While anaerobic degradation of hydrocarbons on the electrode served as an electron acceptor is previously thought to be the dominant process to explain BESs’ enhanced degradation function, metagenomics analysis of microbial communities revealed the unexpectedly widespread presence of soil aerobic taxa and genes (enzymes), most of which were involved in converting hydrophobic hydrocarbons into hydrophilic daughter products, which were subsequently oxidized by anaerobic electroactive bacteria (EAB) grown on the electrode (Fig. 1). Sandy soil texture is more beneficial to facilitate these interactions between different microbiomes than clay soil texture. These findings reveal that the BES may create a unique environment for microbial consortia to cooperatively convert recalcitrant petroleum substrates into electrical current that facilitates subsurface hydrocarbon remediation.

(A) Sandy soil BESs  (B) Clay soil BESs

Fig. 1. Phylogenetic molecular ecological networks (pMENs) of microbial communities (soils and anode biofilms) in the (A) sandy soil and (B) clay soil BESs.
Field-tested 110L microbial fuel cell for continuous treatment of swine waste

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Microbial fuel cells (MFCs) hold the promise for energy-neutral wastewater treatment; however, commercial applications have so-far been limited by system costs and scalability. To address this hurdle, several research groups have begun wastewater treatment pilot demonstrations. Here we present results from a MFC system piloted at a hog farm to continuously treat swine waste at a scale of 150 gallons per day (0.6 m\textsuperscript{3}/day) for nearly one year. The MFC system was comprised of twelve MFCs operated in hydraulic series. Each MFC was a single-chamber box-type reactor with a 7.9L volume. The reactors were constructed with brush anodes and gas diffusion cathodes. The overall volume of the system was 110L. The system was shaded, but otherwise completely exposed to the environment throughout operation.

The system was inoculated with a mixture of stock swine waste solution, lagoon sediment, and 30mM carbonate buffer, pH 7.5 and operated in a batch mode for the first 30 days with recirculation of the solution at a flow rate of 1.9 L/min. After 30 days, the operation was switched from batch to continuous flow mode (0.4 Lpm) and the reactors were fed a continuous supply of swine waste chemical oxygen demand (COD) of approximately 1000 mg-COD/L.

The organic removal rate of the system varied during operation with results between 1-5 kg-COD/m\textsuperscript{3}/d, which is comparable to the removal rates demonstrated by small scale MFC treating swine manure and current aerobic treatment technologies. The reactors had consistent power output in the range of 84-105 mW/m\textsuperscript{2} at 339-379 mA/m\textsuperscript{2}. The normalized energy recovery (NER) of the MFC system operated at 47Ω was 0.8 kWh/kg COD, which is comparable to the NER of anaerobic digestion treatment plant with energy recovery from methane. Overall, these data suggest that MFC systems can be practically scaled for energy-neutral wastewater treatment.
Modified anode material to enhance electrochemical and tannery wastewater treatment performance in bioelectrochemical systems

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Microbial fuel cell (MFC) is an innovative technology for renewable energy source and wastewater treatment from biodegradable materials. As far as the anode materials are one of key factor to increase current generation and treatment in BES, A carbon anode materials should promote bacterial attachment and facilitate electron transfer. The developments of cost-effective anode, operating at acidiphic pH and efficiency electron transfer are a crucial challenge for the practical application of MEC technology. In this study, carbon felt was modified by bentoite and zeolite clay. The newly developed electrodes were applied as anodes in bioreactor electrochemical systems, inoculated with tannery wastewater and active sludge from a municipal wastewater treatment plant. The microbial anodes were formed under chronoamperometry (-0.2 V/ECS) and cyclic voltammetries (CVs) were recorded at different times in turnover (in the presence of substrate) and non-turnover (after substrate depletion) conditions, in order to characterize the catalytic kinetics and the biofilm redox systems, respectively. The performance of the modified and non modified were discussed on the basis current generated and capacity of wastewater treatment, which were correlated with the biofilm structure imaged by scanning electron microscope and epifluorescence microscope. The obtained values of current densities was the highest compared with those reported in the literature using real wastewater in bioelectrochemical systems, which reveals the potentials for practical application of the developed electrode materials.
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. BES fed spent lees at the cathode were tested for copper removal and deposition using different operation modes, cathode materials and anode feeds. For the best-performing BES operated with a fixed voltage input of 0.5 V from a power supply (MEC mode), a synthetic anode feed, and a graphite plate cathode, 9.7 mg of copper metal was deposited onto the cathode over 7 days from a 640 ml catholyte. The copper concentration in solution fell from 19.2 to 0.3 ppm, with the removal rate described by a first-order rate equation (k = 0.87 day⁻¹). When the BES were operated in MEC mode, copper metal deposition was observed on the cathode, and copper was removed from solution. However, when the BES were operated with a low external resistance (MFC mode), copper removal from solution was observed, but the main form of copper deposited was cuprite (Cu₂O). 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.
Optimization of bio-cathode based microbial fuel cell suitable for practical application of wastewater treatment and electricity generation

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Microbial fuel cell is an emerging technology for producing direct electricity from wastewater. The use of metallic and chemical coated cathodes has made the bio-electrochemical system (BES) costly and unsustainable for practical application. That’s why, the abiotic cathode has been replaced by an autotrophic denitrifying bio-cathode to make the BES feasible and sustainable. Here, it was disclosed the performance of autotrophic nitrogen removal from nitrate to nitrogen gas in the bio-cathode of an MFC while utilizing anodic electron transfer from the oxidation of organics in wastewater. The oxidation of complex and insoluble organics like cellulose or lignin present in wastewater was quite difficult, which is effectively reducing the electron transfer efficiency. In this study, an anaerobic digestion (AD) for pre-treatment of wastewater was introduced before feeding it in MFC with the aim of evaluating the feasibility of this combination in terms of power generation and organics removal. The maximum power generation with this set-up using pre-treated wastewater as anodic influent was 7.05 W·m⁻³ net cathodic compartment (NCC), with a current of 45.88 A·m⁻³ NCC. The maximum autotrophic denitrification achieved in this bio-cathode based BES was 125.7±6 g NO₃⁻-N·m⁻³ NCC·d⁻¹. By using an AD pre-treatment of wastewater, the removal of organics in the anode chamber and nitrogen in cathode chamber was approximately 2.2 and 1.4 times higher respectively, comparing to the same system without using AD pretreatment. These results showed the potential of using AD as pretreatment for a subsequent treatment of wastewater by a bio-cathode based MFC system and the prospects of electrical energy recovery and organics/nitrogen removal for practical application.
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Session I
Fundamentals of the extracellular electron transfer processes
Elucidating the role of native electron transport chain in Extracellular electron transport in Microbial electrochemical systems

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Microbial electrochemical systems (MES) can function as renewable and sustainable option for converting organic waste to bio-energy and various bio-based products. In MES, transport of electrons from bacteria to an external electrode is the key to its working, but the mechanism by which bacteria exocellulary transport electrons is not well understood. In this study, a single chambered MES using fluorine coated tin oxide (FTO) plate as anode and stainless steel 316 mesh as cathode was used to understand the role of NADH dehydrogenase II (ndh2) (from Bacillus subtilis) in exoelectron transport. To understand this, ndh2 gene coding electron transport protein NADH dehydrogenase II was over-expressed in E. coli and its electrogenic profile was studied. Expression construct was created in pET23a and the cloned into E. coli BL21 strain. Chronoamperometry, cyclic voltammetry and iron reduction assays using various iron forms were used to confirm the extracellular electron transport. Expressed protein was affinity purified and western blotting was used to confirm the localization of protein into membrane. Bio-physical techniques were used to understand the structure of the protein and was further studied by using protein film voltammetry. Cell free culture filtrate was analyzed using HPLC to understand the influence of NADH DH II on metabolic flux. The final outcome of the study will give the details about the role of NADH DH II in MES and its effect on other metabolic pathways.
Mechanisms of Methanogenic Electron Uptake from Electrodes

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Metabolic flexibility in the Methanosarcinales (cytochrome-containing methanogens) has recently been proposed to include “direct” electron transfer interactions (or hydrogen independent electron transfer) within syntrophic communities. We set out to test the potential for “direct” electron uptake using electrochemical techniques in Methanosarcina barkeri—an important model system that is genetically tractable, and utilizes a wide range of substrates for methanogenesis. M. barkeri has also been shown to receive electrons from Geobacter metallireducens when grown in consortia, making it an ideal model organism for studying the potential for hydrogen independent electron-uptake. Using cathodes as electron donors, we have confirmed the ability of M. barkeri to enhance cathodic current which we can link to quantitative increases in methane production. The nature of this electron uptake includes recently proposed mechanisms whereby extracellular enzymes (e.g. hydrogenase) can facilitate cathodic electron uptake generating soluble methanogenic substrates (e.g. hydrogen). However, we developed methods for minimizing the effects of extracellular enzymes, that still demonstrate cathodic activity coupled to methane production in M. barkeri. Additionally, a hydrogenase deletion mutant behaves similarly to wild-type on cathodes. Electrochemical tests have confirmed similarity in cathodic electron uptake between wild-type and mutant experiments pointing a novel and extracellular enzyme-free mode of electron uptake. Overall these data suggest that multiple modes of electron transfer from electrodes (i.e., hydrogenase, and non-hydrogenase mediated) can function in M. barkeri. Our current work is focusing on resolving the different mechanisms at play, as well as understanding their biophysical basis.
Understanding the maturation of c-type cytochromes: key players in energy production in METs

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Cytochromes-c are essential proteins for living organisms across all domains of life. These proteins are ubiquitous and play key roles in important biological processes, from electron transfer to apoptosis. Furthermore, these proteins have been shown to be key players in the process of energy generation in METs. Given the importance of this class of proteins, the process that leads to their formation in nature is equally important. The covalent attachment of heme and subsequent folding of the protein requires a dedicated maturation machinery. Despite the recognized biological importance and biotechnological applications of c-type cytochromes, the maturation process is far from fully understood. Several maturation systems have been described, including the Cytochrome-c maturation (Ccm) System I. This system is present in most Gram-negative bacteria, including the genus Geobacter and Shewanella. These are especially important as model electroactive bacteria and have been widely used for the understanding and operation of METs.

The aim of this work is to characterize the cytochrome-c maturation system I (Ccm system). Using NMR spectroscopy, we aim to characterize protein interactions and recognition mechanisms between the substrates (heme and apocytochrome-c) and components of System I to understand the functioning of this system towards its optimization.

Preliminary data show evidence of folding upon interaction of the apocytochrome-c with the system’s protein CcmI with residues in the apocytochrome-c showing significant alterations in their chemical shift. Evidence also points towards the occurrence of two distinct binding processes.
CRP coordinately regulates D-lactate oxidation and extracellular electron transfer in *Shewanella oneidensis* MR-1

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[Background]
*Shewanella oneidensis* MR-1 is known as a model organism for studying current-generating mechanisms in bioelectrochemical systems (BES). Studies have demonstrated that MR-1 transfers electrons released during carbon catabolism to electrodes via the extracellular electron transfer (EET) pathway, and that this strain utilizes cyclic AMP (cAMP) receptor protein (CRP) as a transcriptional activator for EET-related genes (i.e., *mtrCAB*). However, little is known about how MR-1 regulates expression of carbon catabolic genes. Here, we investigated transcriptional mechanisms for the *dld* gene encoding respiratory D-lactate dehydrogenase in order to elucidate regulatory mechanisms for carbon catabolic pathways in MR-1.

[Result]
We found that a *crp*-deletion mutant of MR-1 showed impaired growth on D-lactate. *In vivo* transcription and *in vitro* electrophoretic mobility shift assays revealed that CRP directly activates the transcription of *dld*. These results indicate that CRP coordinately regulates expression of carbon catabolic and EET pathways in MR-1, suggesting that intracellular cAMP concentration is a key factor determining the catabolic and EET activity of this strain. To test this hypothesis, we constructed a mutant overexpressing an adenylate cyclase gene responsible for cAMP synthesis (*cyaC*), and evaluated its ability to generate current in BES. The result revealed that the *cyaC*-overexpressing strain generated higher current than the wild-type strain, suggesting that modification of cAMP/CRP regulatory system is useful for enhancing current generation by MR-1.
Methionine induces a signaling cascade that promotes current generation by *Shewanella oneidensis* MR-1

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*Shewanella oneidensis* MR-1 is a facultative anaerobe that can respire using a variety of electron acceptors, including metal oxides and electrodes in bioelectrochemical systems (BES). Although MR-1 is an extensively studied model organism for understanding the mechanisms of current generation in BES, information is still not sufficient as to how MR-1 regulates catabolic and respiratory pathways involved in current generation. Here we report that methionine serves as a signaling molecule regulating expression of catabolic and anaerobic respiratory genes in MR-1. When MR-1 was grown in BES supplemented with 10 mM lactate and 0.13 mM methionine, it generated 1.5-fold higher current as compared to the control without methionine (Fig. 1). Transcriptome analyses revealed that the addition of methionine up-regulated the expression of many catabolic and anaerobic respiratory genes, including *lldEFG* (L-lactate dehydrogenase), *pflB* (pyruvate-formate lyase), *fccA* (fumarate reductase), and *mtrCAB* (metal/electrode reductase), while it down-regulated the expression of genes involved in methionine synthesis, including *metE* (methionine synthase) and *metR* (LysR-type transcriptional regulator). In vivo transcription and in vitro electrophoretic mobility shift assays revealed that MetR serves as a negative regulator for these catabolic and respiratory genes. These findings demonstrate that the intracellular concentration of methionine is an important factor determining the catabolic activity of MR-1, representing a novel mechanism for global regulation of catabolic pathways in bacteria.

![Graph](image)

**Fig. 1. Current generation by MR-1 in the presence (black line) or absence (gray line) of methionine.**

Current was measured in an electrochemical cell with a working electrode poised at +0.2 V (vs. Ag/AgCl).
Geobacter activity in electrode biofilms is spatially dependent

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*Geobacter sulfurreducens* grown with acetate as sole electron donor and a suitably poised electrode as the electron acceptor can form biofilms 10’s of cells thick. Extracellular electron transfer (EET) is supported by the production of a conductive extracellular matrix composed of multiheme cytochromes, polysaccharides, and pili proteins. While this system has been key for understanding many aspects of microbial fuel cell behavior, fundamental questions about the EET process remain, specifically with regard to how microscale gradients of electron donor, pH, and redox potential affect the metabolic activity of cells at different positions in the biofilm.

Here we directly image the spatial anabolic activity patterns of *G. sulfurreducens* cells in electroactive biofilms. Biofilms were grown on graphite electrodes poised at +240mV until steady-state current density was reached, at which point isotopically labeled substrates were introduced including $^{15}$N-$\text{NH}_4^+$, $^{13}$C-acetate, and D$_2$O, to quantify cellular anabolic activity. After six hours of incubation biofilms were chemically fixed and imbedded in resin. Thin sections of biofilms were analyzed for stable isotope ratios with nanometer-scale secondary ion mass spectrometer (NanoSIMS). Sub-cellular resolution isotope ratio maps revealed that the greatest activity occurs at the biofilm-electrode interface, and this activity drops off with distance from the electrode, implying a penalty exits with increasing distance to the terminal electron acceptor. Additional experiments varying the electrode potential, growth time, labeling duration, and acetate concentration yielded similar overall patterns of cellular activity, with slight variations. These observations help understand the controls on cellular activity within *G. sulfurreducens* biofilm and will help constrain future models.

![Isotope maps of $^{14}$N ($^{14}$N$^{13}$C) and $^{15}$N ($^{15}$N$^{12}$C) of Geobacter biofilms grown on a graphite electrode (e) and imbedded in resin (r). $^{15}$N incorporation reflects greater anabolic activity and is higher at the electrode surface and steadily decreases with distance from the electrode.](image-url)
Insights on a key protein for bioelectricity production in *Geobacter sulfurreducens*

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*G. sulfurreducens* is the best studied *Geobacteraceae* bacterium. This family of microorganisms produces energy and are important agents in biogeochemical cycles where Fe(III) and Mn(IV) reductions are essential [1]. Analysis of the *G. sulfurreducens* genome revealed that more than one hundred genes encode for c-type cytochromes [2]. Some of these proteins are involved in the extracellular electron transfer process, that allow the electron transfer to the cell exterior [3]. OmcF is an outer-membrane associated cytochrome required for the transcription of genes that are involved in the electricity production [4]. OmcF has one heme group, with a his and met axial coordination, and has 9.4 kDa [5]. Although it is known that OmcF is associated with the outer-membrane, it is unknown the orientation of its soluble part in relation to the periplasm and the cell exterior. To elucidate this point, backbone assignment of OmcF, in the oxidized state, was attained. NMR interaction studies were carried out between OmcF and PpcA, a periplasmatic triheme cytochrome.

The soluble part of OmcF has high structural similarity with a *c*<sub>6</sub> cytochrome from the algae *Monoraphidium braunii* (cyt *c*<sub>6</sub>) [4]. Despite their similarity, they differ significantly in their formal reduction potential (*E''*): +180mV and +358mV for OmcF and the cyt *c*<sub>6</sub> at pH 7, respectively [6]. Electrochemical studies showed that the formal potential of OmcF is strongly dependent on pH. Kinetic and thermodynamic parameters were also obtained for the protein. OmcF mutants were constructed in order to understand which amino acids are responsible for the redox-Bohr effect and which ones may cause the difference in *E''* between the two proteins. Mutants *E''* was attained through electrochemical procedures and accessed if the values reached the ones from cyt *c*<sub>6</sub>.

**References**


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NMR interaction studies between cytochromes MacA and PpcA from *Geobacter sulfurreducens*

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*Geobacter sulfurreducens* is a dissimilatory metal reducing bacterium with notable properties and significance in biotechnological applications that rely on extracellular electron transfer (EET). Interestingly, the analysis of its genome sequence revealed that this bacterium encodes 111 cytochromes *c*, 73 of which are multiheme cytochromes [1]. MacA is a diheme cytochrome *c* associated to the inner membrane of *G. sulfurreducens*. Previous genetic experiments revealed that the absence of the gene encoding for MacA affected the extracellular reduction of Fe(III) and U(VI) oxides [2]. Additionally, MacA is more abundant during growth with Fe(III) oxides *versus* Fe(III) citrate [3]. These results suggested the involvement of this protein in the *G. sulfurreducens* EET process. However, more recent studies showed an indirect involvement of MacA in this process, indicating that the inhibition of Fe(III) reduction when *macA* is deleted is the result of a negative impact on the transcription of *omcB*, that encodes for an outer membrane cytochrome [4]. Recently, biochemical studies revealed that MacA as peroxidase activity and that it is also able to exchange electrons with the periplasmic triheme cytochrome PpcA [5]. In this work, NMR chemical shift perturbation experiments were used to map the interface interacting region and to measure the binding affinity between the two proteins.

References

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What electrifies *Pseudomonas aeruginosa*? - A comparative RNA-sequencing study

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In natural microbial communities, *Pseudomonas aeruginosa* was found to be a thriving member in bioelectrochemical systems (BES). The organism can interact with an anode through its soluble redox active phenazines. Hence, these mediators might enable an entire microbial community to access an electrode as an alternative electron acceptor. An earlier study showed that this interaction is strain and carbon source dependent [1]. The study showed that the *P. aeruginosa* strains PA14 and KRP1 showed the highest phenazine and current production, depending if 2,3-butanediol or glucose were used as carbon source, respectively. The natural occurring KRP1 isolate was the superior current producer. To reveal the genetic and regulatory basis of this dependency and to gain new insights into the extracellular electron transfer processes, a large RNA-sequencing study with these two strains and carbon sources was performed. Besides a global investigation for altered gene expressions, we focused our analysis on genes related to the quorum sensing network.

Furthermore, RNA-libraries were created focusing on the 5′-transcriptional start sites of the two strains. It is hereby possible to identify the exact starting position of a gene transcript. This is crucial for identification of the regulatory regions and elements flanking these sites. To complement the study, small-RNA enriched libraries of PA14 and KRP1, grown with the different carbon sources, were investigated, since small-RNAs are known to act as regulatory elements, altering the gene expression of their respective target genes.

Biocorrosion and bioelectrosynthesis by *Methanococcus maripaludis* Mic1c10

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In anaerobic environments, corrosion of iron (Fe$^0$) is induced or accelerated by the metabolic activities of various microbes causing economic and environmental damage. Biocorrosion was intensely studied in sulfate-reducing bacteria (SRB) and much less in other microorganisms.

In this study, we investigated the mechanism of biocorrosion in a methanogen - *Methanococcus maripaludis* Mic1c10, recently isolated from an oil facility. Mic1c10 was proposed to corrode iron via an indirect mechanism of electron uptake (1). However, in this study we discovered that Mic1c10 required direct contact to initiate corrosion.

First indication was that abiotically produced hydrogen [H$_2$] was insufficient to explain the amount of CH$_4$ produced. To examine whether Mic1c10 required direct contact or a shuttling compound to facilitate Fe$^0$ corrosion, ZVI was encapsulated within microporous alginate hydrogel, which prevents the contact between microbial cells and Fe$^0$. If cells did not need direct contact, encapsulated Fe$^0$ would not maintain methanogenesis, whereas if cells required direct contact, encapsulated Fe$^0$ would abolish/diminish the growth of the methanogen. For the first 20 days of incubation Mic1c10 diminished methanogenesis to a $5^{th}$ if grown without direct contact to Fe$^0$.

The capacity to take electrons directly from a solid surface was evaluated with indium-doped tin oxide electrodes poised at -600 mV (vs. Ag/AgCl sat. KCl). Current uptake was only observed with live cells of Mic1c10, but not with heat-killed cells or spent-medium filtrate. Our results suggest that direct contact is necessary for initiation of the biocorrosion process by Mic1c10.

Reference:
Electrochemical profiling of electrode oxidizing marine bacteria

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Examined here are Gammaproteobacteria species belonging to the Idiomarina and Marinobacter genera. These bacteria were identified in marine sediment that was enriched for cathode colonizing bacteria. The isolation of several new electrode oxidizing bacteria was accomplished by taking the electrode enrichments and allowing isolates to grow in several different lithotroph favoring conditions. Idiomarina sp. FeNA was isolated in conditions favoring use of elemental iron as the electron donor whereas Marinobacter sp. FeSN1 was isolated in iron sulfide medium. To provide a strong foundation for electrochemical measurements, basic physiological requirements and antibiotic resistance were quantified. Electrode colonization and growth was assessed with chronoamperometry and cyclic voltammetry alongside confocal electron microscopy. Despite being isolated from the same environment in the same manner, these two bacteria display very different phenotypes during electrode oxidation. For instance, the midpoint potential of Idiomarina sp. FeNA is 90 mV more positive than Marinobacter sp. FeSN1 and both are inconsistent with the oxidation/reduction of hydrogen and are more consistent with a mechanism that involves direct electrode contact. Genomes were sequenced and include a minimal number of c-type cytochromes, of the cytochromes with known function, none are known to function during metal or electrode oxidizing/reducing respiration. These strains present particular challenges but add to the understanding of electron flow in marine environments.
Rational engineering of *Geobacter sulfurreducens* electron transfer components: a foundation for building improved *Geobacter*-based bioelectrochemical technologies

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Multiheme cytochromes have been implicated in *Geobacter sulfurreducens* extracellular electron transfer (EET) [1,2]. These proteins are potential targets to improve EET and enhance bioremediation and electrical current production by *G. sulfurreducens*. However, the functional characterization of multiheme cytochromes is particularly complex due to the co-existence of several microstates in solution. Over the last decade, new strategies have been developed to characterize multiheme redox proteins [3]. These strategies were used to reveal the functional mechanism of *G. sulfurreducens* multiheme cytochromes. In previous studies, we characterized a family of five periplasmic triheme cytochromes (PpcA-E) that is implicated in electron transfer between the oxidative reactions of metabolism in the cytoplasm and the reduction of extracellular terminal electron acceptors at the cell’s outer surface [4]. The results suggested that PpcA can couple e⁻/H⁺ transfer, a property that might contribute to the proton electrochemical gradient across the cytoplasmic membrane. The structural and functional properties of PpcA were characterized in detail and used for rational design of a family of 23 single site PpcA mutants [5]. In this work, we summarize the functional characterization of the native and mutant proteins. Mutants that retain the mechanistic features of PpcA and adopt preferential e⁻/H⁺ transfer pathways at lower reduction potential values were selected for in vivo studies as candidates to increase the EET rate of *G. sulfurreducens*.

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Biochemical characterization of the extracellular metal reductase of the thermophilic Gram-positive bacterium *Thermincola potens* JR

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Extracellular electron transfer is the key biochemical process that allows the development of microbial electrochemical technologies (MET). Studies on several microbial fuel cells revealed that Gram-positive bacteria are capable to generate higher current than the representative Gram-negative electroactive bacteria *Shewanella oneidensis* MR1 and *Geobacter sulfurreducens* PCA. Despite the differences in the cell envelope, Gram-positive bacteria were found to perform direct extracellular electron transfer to solid electron acceptors using multiheme c-type cytochromes. This discovery was a breakthrough in the field of MET.

In this work cloning, expression and purification of the surface exposed nine heme cytochrome of the electroactive thermophilic Gram-positive bacterium *Thermincola potens* JR was achieved using a previously developed heterologous expression system. This protein has spectroscopic features typical of c-type cytochromes, and was shown to react with soluble redox shuttles such as FMN and riboflavin. The EPR spectrum reveals more variety in the heme coordination than the previously observed for Gram-negative outer-membrane cytochromes. Interestingly, the potential range where this protein is redox active at 25°C is wider than observed for already characterized outer-membrane cytochromes. Overall, the data obtained in this work points to an adaptation of the multiheme cytochromes as key players in extracellular electron transfer in these phylogenetically distant organisms. Moreover, this work is pioneer on the exploration of the role of multiheme cytochromes in extracellular electron transfer on Gram-positive bacteria, broadening the view of this important process for the development of MET.

References
Session II
Microbial Electrochemical Technologies: from fundamental to applied research
Subminimal Inhibitory Concentration (Sub-MIC) of Antibiotic Induces Electroactive Biofilm Formation

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Electroactive biofilm (EAB) oriented from mixed inoculum is attractive due to its unique nature of direct extracellular electron transfer and potential use in pollution control. Here we for the first time demonstrated a chemical that can be used for EAB regulation (both inhibition and promotion). Tobramycin, an antibiotic previously demonstrated to inhibit the activity of EAB, is confirmed as an agonist at subminimal inhibitory concentration (sub-MIC) during EAB formation. At tobramycin concentrations of 0.05 (1/80 MIC) and 0.1 mg/L (1/40 MIC), the time needed to reach 3 A/m2 was shorter, and the limiting current densities increased by 17 % than the control. The enhanced EAB activity was primarily attributed to the 50 % increase of biomass density from 289±21 to 434±12 μg protein/cm2 and the increased biofilm thickness from 28±1 to 37±0.5 μm. Geobacter families in microbial community was selectively increased from 76 % to 82 %, and its population was estimated to increase by 1.63 times. The accelerated growth was further confirmed using the model strain of G. sulfurreducens PCA. Transcriptomic analysis revealed that 0.05 mg/L tobramycin led to a significant upregulation of genes related to cytochromes and type IV pilus, a possible mechanism for the current enhancement. These findings extend our knowledge on regulating EAB formation by antibiotics and selective enrichment of Geobacter from a mixed culture, with a broader implication on the potential impact of trace antibiotics to dissimilatory metal reduction process.

Figure 1 The stimulation mechanism of sub-MIC tobramycin on electroactive biofilm.
Selection of efficient H₂ producing bacteria during glucose electrofermentation

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H₂ can be produced by dark fermentation, a technology extensively studied. However, only a low number of bioprocess controllers are available to maintain fermentation patterns in mixed cultures. In this context electrofermentation is a powerful and innovative controller of fermentation systems. This is an emerging technology that combines dark fermentation and polarized electrodes, with the aim of redirecting metabolic patterns to interest products. This study aims to evaluate the influence of the presence of polarized electrodes in the medium bulk on metabolic pathways and H₂ production with a special focus on how the bacterial populations are affected. For that, and to know the effect of a polarized electrode on a mixed culture during glucose electrofermentation, we applied four different voltages on the working electrode in batch fermentation tests (triplicates).

During electrofermentation, two metabolic behaviors for H₂ production were observed. The first led to a higher production of H₂ and butyrate. The second led to lower H₂ production along with ethanol and lactate or acetate as main metabolites. In all cases H₂ production was higher than in traditional fermentation (without polarized electrodes). These changes in metabolic pathways were statistically related to the selection of Clostridium, Escherichia and Klebsiella genera; all of them being very well-known as H₂-producers. Our results showed a shift in the metabolic patterns towards the production of metabolites more reduced due to the presence of polarized electrode. Current was not significant to explain these changes. Although a clear effect was evidenced independent of the voltage used, more research is required to better understand the mechanisms of interactions behind the microbial population selection in presence of a polarized electrode.
Identification of Sequential Multi-layered Biofilm Formation of *Shewanella oneidensis* MR-1 in Microbial Electrochemical System and its Relevance with Electron Transfer System

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Electrochemically active bacteria (EABs) form biofilms on the electrodes of microbial electrochemical systems (MESs) and transfer the electrons through the inherent electron transfer system (ETS). Recently, several different types of ETSs have been observed including direct and indirect ways. For instance, *Shewanella oneidensis* MR-1 has an ETS in which the cytochrome of the outer membrane contacts the electrodes and transfers electrons. It also has an ETS that transfers electrons from a relatively long distance without direct contact with the electrodes by using mediating chemicals such as flavins. Therefore, the biofilm of *S. oneidensis* MR-1 has been expected to be in a multi-layered form other than single layered. However, the sequential biofilm formation process has not yet been validated based on scientific analysis. Thus, in this study, biofilm morphology was confirmed based on electrochemical and morphological analyses. In addition, the development of biofilm over time and the trend of ETSs were comparativey analyzed through differential pulse voltammogram. As a result, it was verified that the biofilm of *S. oneidensis* MR-1 is in a multi-layered form. Moreover, it was discussed that flavin-mediated ETS predominate as the biofilm grows. Study on the development of biofilm is expected to be important implications for understanding ETSs of EAB and further performance of MESs.
Graphene oxide electrodeposition enhances start-up and selective enrichment of exoelectrogens in bioelectrochemical systems

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This study presents a single-step graphene oxide (GO) electroreduction procedure for the modification of graphite electrodes to be used in bioelectrochemical systems (BESs). It evaluates the impact of the anodic electrodeposition of GO on the start-up process and on the development of microbial communities on the anode of a BES. Electrochemical abiotic characterization of the graphene modified electrodes (erGO electrodes) by means of CVs (Figure 1A) revealed that the deposited graphene enhanced the heterogeneous electron transfer between the solution and the solid electrode. When the electrodes were transferred to a microbial electrolysis cell (MEC), the erGO electrode allowed to reduce both the start-up and current stabilisation periods (compared to the unmodified electrode). Interestingly, after three months of operation, both cells displayed a nearly similar behaviour in terms of current production, revealing that the performance-enhancing role of graphene can be circumscribed to the start-up process. Microbial analysis (high throughput sequencing for 16S rRNA gene) showed that electrochemically reduced GO facilitates the formation of a robust biofilm and also acts as a selective agent toward certain exoelectrogenic bacteria as Geobacter (Figure 1B). Overall, and although there is still some controversy on the effects of graphene on BESs performance, this study supports the beneficial role of graphene especially on biofilm formation.

Figure 1. A) Cyclic voltammetry performed at a scan rate of 20 mV·s⁻¹, and B) taxonomic assignment of high throughput sequencing data from eubacterial communities.
Semi-pilot scale MEC for pig-slurry treatment and resources recovery. Limiting the impact of high nitrogen concentration

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Biological treatment of pig slurry becomes problematic due to its inherent high ammonium content. When directly fed to the anode of a bioelectrochemical systems (BESs) it usually results in a significant deterioration of its performance or even a total process failure (Mahmoud et al., 2017). Our research aims at limiting the impact that ammonium has on the performance of a microbial electrolysis cell (MEC) during pig slurry treatment by using a two-fold strategy. On the one hand, nitrogen concentration in the fed was gradually increased from 500 to 3000 mg-TN L⁻¹, as the proportion of slurry in the feed increases (from 40 to 80% (Figure 1A)) to favor the acclimation of microbial communities. On the other hand, nitrogen was allowed to migrate to the cathodic chamber through a cation exchange membrane where it was recovered for its subsequent use in struvite production. This study was performed in two semi-pilot double-chamber MECs (each having a total volume of 16 L (Figure 1B) and designated as MEC-1 and MEC2), and using phosphate buffer as catholyte.

The results show that current density increases gradually as the proportion of pig-slurry in the feed increases (Figure 1A) and that nitrogen can be removed from the anode and recovered at the cathode at a maximum rate of 325 mg-NT L⁻¹ reactor d⁻¹ (57 % efficiency), which indicates the suitability of this approach for successfully feeding pig slurry to a MEC. Moreover, a significant amount of hydrogen was recovered in the cathode (it was produced at a rate of 0.2 L H₂ L⁻¹ reactor d⁻¹), all of which would help to improve the technoeconomical feasibility of this technology.

Figure 1. A) Current density (A/m²) at the different stages of the process (designated according to the percentage of slurry in the feed (40, 50, 60, 70 or 80%)) in MEC-1 and MEC-2. B) Reactor set-up.

42 on EIS: how substrate concentration and biofilm integrity influence EIS spectra of electroactive biofilms

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Primary microbial electrochemical technologies rely on the exploitation of electroactive microorganisms, often in form of biofilms, as catalytic elements. The use of electroactive biofilms in industrial applications creates the need of in situ diagnostics and function control that is, condition monitoring. Condition monitoring is a special challenge, when the biofilms are difficult to access, e.g. when acting as receptor of a biosensor or in a MFC stack. Electrochemical impedance spectroscopy (EIS) seems to be promising for condition monitoring, as it exploits the existing electrochemical connection. We report on the principle suitability of EIS as tool for condition monitoring. EIS measurements were conducted at 0.2 V vs. Ag/AgCl on biofilm electrodes using fully intact matured biofilms facing different acetate concentrations (0, 2, 3, 4, 10 mmol L⁻¹) as well as at 2 mmol L⁻¹ acetate under successive removal of the biofilm (100, 94, 47, 0 % biofilm).

We demonstrate that differentiating different acetate concentrations can hardly be achieved, whereas the total absence of acetate had a distinct influence on the EIS spectra. Surprisingly, also the successive removal of biofilm had only marginal influence on the results. Only the total biofilm removal showed distinct effects on the EIS spectra. Combining the results from both experiments showed nearly the same effects, making it impossible to differentiate between the absence of biofilm or acetate. We discuss that such a binary behaviour enables the application of electroactive biofilms for system control purposes.
Influence of glycerol as electron donor on electrochemical parameters and microbial composition

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Glycerol is a promising electron donor for microbial electrolysis cells (MECs). It is the main by-product of biodiesel production and can get converted to valuable platform chemicals for polymer industry. Our experimental design included the generation of secondary and tertiary biofilms by transferring pre-conditioned bacteria from primary biofilms to new MECs. Secondary and tertiary biofilms showed more reproducible current profiles, higher maximal current densities and higher Coulombic efficiencies (CEs) compared to primary biofilms. Maximal current densities of up to 0.69 mA/cm² and CEs of up to 54 % were obtained with glycerol as electron donor.

However, little is known about the detailed microbial composition of glycerol fed biofilms derived from wastewater and the influence of glycerol on the microbial community. It was shown that acetate fed biofilms are mostly dominated by Gram-negative Geobacter species [1]. We used partial 16S rRNA sequencing for glycerol fed anodic biofilms and revealed a Geobacter sp. domination of up to 45 % throughout primary, secondary and tertiary biofilms. Furthermore, highly diverse bacteria are represented in planktonic cells. Thus, potential partners for a defined cocultivation with Geobacter sp. were identified. Future attempts will be focused on establishing the defined cocultivation as bioelectrochemical model system.

Influence of mediators and reactor systems on electrochemical acetone-butanoolethanol fermentation

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For the combination of electrochemical techniques with microbial fermentations, it is essential not only to understand the interactions between the microorganisms and the electrode but also to identify side reactions between the fermentation medium and the electrode. In our study, we investigate both reaction types using the electro-fermentation with Clostridium acetobutylicum. The bacterium is well-known for its acetone-butanol-ethanol (ABE) fermentation process which has already been carried out industrially in the beginning of the 20th century. Lately, it has gained new attention because butanol has become an important bulk chemical as well as a promising candidate as a sustainable biofuel.

Electro-fermentations are carried out in H-shaped reactors, as well as conventional bioreactors, using carbon fabrics or graphite rod electrodes in a three-electrode arrangement. The difference of reactors separated by proton-selective membranes and non-separated systems is studied concerning the butanol production as well as the side reactions. When a potential of -600 mV is applied at the working electrode of a separated system, an increase in pH from the initial 6.8 to 10.5 in cell-free fermentation medium can be observed. This increase in pH, which is not visible in a non-separated system, seems to be advantageous for the process because limiting the pH drop is essential for efficient solvent production during ABE fermentation. Additionally, it is observed that with applied potential a secretion of flavins with concentrations of up to 50 μM as endogenous mediator is triggered and subsequently final butanol concentration is doubled. A similar increase in butanol production is also obtained by using 250 μM neutral red as exogenous mediator.
Mediator-free Enzymatic Electrosynthesis of Formate from Carbon Dioxide by an Archaeal Heterodisulfide Reductase Supercomplex

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Cathodic reduction of carbon dioxide is an attractive means for converting transiently available electric energy into storable chemical energy. This reaction is catalyzed by various catalysts of which enzymes appear attractive for bioelectrochemical systems as they have evolved to be highly active and stable under conditions that support cell growth. The physical adsorption and the electrical connection of enzymes to bioelectroreactor cathodes represent engineering challenges, which are commonly addressed by addition of soluble mediators and chemical crosslinking. Such measures, however, increase the complexity and costs of the bioelectrochemical system and potentially suffer from a loss of enzymatic activity through chemical modification. We describe here an archaeal enzymatic complex that spontaneously interacts with bioelectroreactor electrodes and performs highly selective formate electrosynthesis in the absence of an added mediator. The electrosynthesis of formate was demonstrated on iron granules and in an electroreactor system. In the latter context, continuous formate electrosynthesis during a five-day-period yielded product concentrations of up to 13.5 mM at a coulombic efficiency of ~90% with hydrogen as a minor product (below 10%). Electrons were supplied to the enzymatic complex at a potential of ~800 mV (vs. Ag/AgCl) through a graphite rod working electrode.
Reflections from Lizland: progress on the road to application

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Lizland is a place where the temperature is between 4-15 °C, flush water is clean and not highly conductive, and everyone excretes excreta – not acetate. It is a place where the putative uses of BES lie, yet it sits far away from the place where much laboratory research is done. While the research community has produced many valuable insights into BES operation, we have looked at the differences between simple controlled systems and those which reflect reality. We have developed a new methodology and established a statistical link between COD and energy content in wastewater, enabling us to quantify the energy in domestic wastewater in the basis of a simple COD measurement. We have established that although sequencing reveals there may be around 7500 \textit{Geobacter} per ml of wastewater, there are only 17 electrogens per ml capable of initiating the start-up of and BES, this drops to 0.6 per ml in wastewater. We have established that feeding a reactor acetate leads to a very different start-up, and produces a different, and surprisingly more diverse community of microbes. We have used these insights and lessons learned from previous pilot work to redesign and upscale our pilot systems, and have ran a pilot reactor with 1m\textsuperscript{2} anodes occasionally achieving European Urban Wastewater Treatment Directive (1991) consent. There is still a long way to go: we have not yet broken into energy neutral or positive treatment. Experiments with new materials and radical new designs tested through sound mathematical modelling prior to actual building are helping to pave the way along this road.
Plant Microbial Fuel Cell: technology assessment from lab to commercial application

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The Plant-Microbial Fuel Cell (PMFC) emerged from a lab-scale proof-of-principle to a commercially applied technology. Since the proof-of-principles in 2008, research and development supported the realization of tubular systems generating electricity and powering LEDs. As such, the PMFC is one of the first applied microbial electrochemical technology word-wide. The unique selling point ‘living plants generate electricity’ attracts clients that have interest in being affiliated with this new technology. With this study we present a technology assessment of the plant-microbial fuel cell. As such, the performances of all PMFC studies were compared showing that completely biocatalysed \textit{Spartina anglica} systems were best; while tubular-designs were most advanced for application. The potential power output is wetland dependent and systems must be designed specifically for each application. The economic feasibility shows that PMFC can compete with small battery applications; though further optimization is needed to economically compete with other renewable electricity sources. The additional and potential values of the PMFC including plant growth promotion, electricity storage, coastal area protection, green image, nature restoration, education & water cleaning, all can contribute to future applications. To further exploit electricity generation from wetlands, a better understanding of the working principles is needed as well as scale-up studies to evaluate the prospective applications while taking the environmental and societal impact into account.

Figure 1. Tubular Plant-MFCs from lab to commercial application\textsuperscript{13}. 
Low-energy drinking water production using microbial desalination cells: assessment of desalination efficiency and water production at different initial salinity

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This work shows the feasibility of microbial desalination cell (MDC, area=100 cm²) for complete brackish water and seawater desalination using synthetic wastewater as energy source. Two objectives have been simultaneously achieved by the optimization of this system: i) treatment of wastewater and ii) production of drinking water from brackish and sea water. The performance of the MDC is mainly affected by the organic matter concentration in the wastewater as well as the salinity of the saline stream. Organic matter present in wastewater was effectively removed (90%) at a rate in the range of 124-480 g COD/m³ day (depending on the initial salinity), with desalination efficiency of 77-94%.

Figure 1. Salinity evolution for desalination experiments with different initial salinity (i.e. NaCl concentration)

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Session III
Electrochemical cell design and electrode materials
New polysiloxanes-based ceramic anode materials for Microbial Fuel Cells (MFCs)

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Microbial Fuel Cells (MFCs) represent a technology that has the potential to address simultaneously environmental pollution and energy demand. This project is focusing on the development of new anode materials based on polysiloxanes as starting material that allow the generation of conducting materials, which have a higher chemical stability than pure carbon materials, high specific surface areas, adjustable porosity and surface characteristics as well as low process costs[1]. Of main interest is the optimized interaction of the biofilm by the adjustment of porosity and improvement of electrochemical properties that may increase the overall performance. By combination of the inorganic-organic polymer with conducting carbon fillers, metal particles and varying pyrolysis conditions, electrode materials can be produced with conductivities in the range of 0.04 - 0.4 S/cm. In means of hydrophilicity, the surface characteristics can also be adjusted during pyrolysis, as well as micro-meso-macroporosity[2]. Performance tests of the developed anode material were carried out in a MFC with an aqueous cathode design using a clayware cylinder as anode chamber[3], as shown in Figure 1, with a synthetic wastewater containing chemical oxygen demand (COD) concentration of 2000 mg/l during 2 days batch feed. The performance was compared to commercially available carbon felt and it showed a two-fold increase in power density and normalized energy recovery and demonstrated COD removal efficiency about 85%. Thus, the synthesized electrode material is found to be promising for MFC application.

Figure 1. MFC design and macrostructure of the anode material synthesized.

References
Functionalized Polysiloxane Derived Ceramic Membranes for Microbial Fuel Cells (MFCs)

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Stepping towards ceramic membranes may be an alternative choice for replacing high priced polymeric membranes, to make the Microbial Fuel cell (MFC) a feasible technology for near future [1]. Recently, polymer derived ceramic materials have attracted much attention because of their simple fabrication and processing technique, providing extraordinary thermal, chemical and mechanical properties. Additionally, it allows the tailoring of the pore sizes from the meso to the macropore range as well as the possibility to adjust the surface characteristics from hydrophobic to hydrophilic [2]. Herein, we develop novel polysiloxane derived ceramic (PDC) functionalized with proton conducting fillers like montmorillonite and phosphomolybdenic acid as a proton conducting ceramic membranes by using simple pressing and pyrolysis techniques. The variation of pore size, porosity, surface characteristics like hydrophilic-hydrophobic behavior is investigated for PDC and functionalized PDC with respect to pyrolysis temperature. Additionally, the results of physical characterization of membranes such as proton and oxygen diffusion, mass transfer coefficient and cation transport number have been discussed. The performance of functionalized PDC membrane pyrolyzed at 400 °C achieved up to 80 % of power density and normalized energy recovery (NER) as compared to commercial Nafion membrane along with demonstrating 85 % of chemical oxygen demand (COD) removal efficiency.

Figure 1. Schematic view of membrane and as prepared ceramic membrane

Reference
Assessment of anode and air-cathode materials for BES reactors

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Anode and cathode electrode materials have an important influence on the performance of Bioelectrochemical Systems (BES). In the present work, 18 anode materials were assessed, including unidirectional, bidirectional and nonwoven carbon fibers and felts, activated with different thermal and chemical treatments. In terms of cathode materials, more than 15 materials were characterized to evaluate their performance as air-cathode electrodes.

On the one hand, the performances of anode materials were evaluated by monitoring the inoculation process kinetics. In order to allow both a fast inoculation and counter electrode stability, a tailor-made inoculation process was developed using 150 mL air-cathode MFC. The process consists in polarizing the anode at sequential and decreasing potentials from +100 mV to -150 mV Vs. SHE. Biofilm growth has been continuously evaluated through electrochemical techniques. Results demonstrate that carbon fibers and carbon felts are suitable as anode materials and their thermal activation improve the inoculation kinetics. These materials could be efficiently inoculated in less than 20 days, achieving current outputs beyond 0.15 mA cm⁻². Besides, anode open circuit potential (OCP) ranged around -350 mV Vs. SHE. Finally, cyclic voltammetries (CV) showed oxidation peaks, indicating the formation of an electroactive-biofilm on the anode surface, which was further confirmed by SEM images.

On the other hand, the oxygen reaction reduction (ORR) is one of the main bottlenecks of air-cathode electrodes. The ORR kinetics is affected by both cathode material and applied catalyst. In this study, air cathodes based on carbon nanofibers (CNFs), acting as a support matrix for metal nanoparticles (Co and Fe) that act as catalysts, have been developed. Air cathode materials were fabricated using electrospinning technique followed by a thermal treatment. ORR kinetics of different materials were characterized electrochemically in an abiotic cell by OCP and CV techniques. Results indicate that Fe doped CNFs have better ORR performance than Co doped CNFs. Also, the concentration of metal and the thermal treatment are important parameters to improve cathode activity. Achieved results can lead to an overall better design of BES reactors, an improvement of power generation (in case of MFCs) and a general reduction of internal resistance (in case of MECs or MES).

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Reactor design for Electromethanogenesis

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Methane could be a suitable replacement of fossil oil, which is about to be depleted during the next decades. Therefore, new routes for the production of fuels, e.g. methane have to be developed. Currently, several research groups are investigating electromethanogenesis, a process in which methanogens convert CO₂ to methane, using electrons from a cathode. Although the mechanism of the electron transfer is not completely elucidated, the investigations are on the move to industrial application. It has been shown that the electrode material, the methanogenic consortium and also the anodic reaction can affect the process of electromethanogenesis. Another critical aspect is the design of the reactor. Most of the used systems in laboratory scale are not transferable to industrial size. The combination of electrochemistry and biotechnology leads to several challenges in terms of reactor design. The surface limited electron transfer from the cathode (2-dimensional) has to be integrated into a 3-dimensional reactor, hosting also sensors for process monitoring. At the same time, the gas transfer has to be sufficient to supply the microorganisms with CO₂. Apart from that, the electrode chambers need to be separated to prevent the contact of anodically produced oxygen with the oxygen-sensitive microorganisms. It is crucial to optimize both, CO₂ supply and electron supply to gain a scalable process. In this poster, we present a newly developed, scalable reactor system designed for electromethanogenesis.
Electrochemical silver removal and recovery from nanowires in fuel cell

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The increasing use of precious metals (gold, silver, etc.) in nanomaterials used in high-tech products can have an adverse impact on the environment because at all stages of a nanoproduct life cycle, these metals may be released into the environment and come in contact with the biosphere. Alongside current efforts to understand the environmental fate and eco-toxicological behavior of metallic nanomaterials, there is a need to design safer forms of nanotechnology and to develop efficient, innovating and economically viable recycling processes.

We are focusing on silver nanowires (AgNW). Their extreme aspect ratios, with diameters in nanometers and lengths in micrometers, bears specific asbestos-like toxicity, motivating the development of a method for demetallizing AgNW enabled devices directly, before end-of-life disposal. Our objective is to develop a “green” methodology, able to eliminate the environmental and toxicological risk of these materials entering an e-waste stream and at the same time allowing the recovery of nano Ag for further reuse, a solution that combines sustainability and risk reduction.

The Ag/Ag⁺ standard potential predicts that silver is readily oxidized to Ag⁺ when oxygen is reduced. Here we propose an electrochemical cell equipped with an air cathode for oxidant reduction, and an AgNW thin film as anode, where silver is oxidized.

In the cell prototype, the electric potential proves to be positive over a wide pH range, indicating favorable conditions for spontaneous Ag oxidation. However, optimization is needed for the reaction to proceed at measurable speed. We are presenting results on Ag oxidation in the fuel cell under improved conditions where cell potential is high enough to generate electric current and where electrode passivation is avoided. This control should lead us to a method for Ag⁺ recovery and possible valorization.
Carbon black modified stainless steel for high-performance bioelectrode in microbial electrochemical system

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The popularly used bulk carbonaceous materials have comparatively low specific conductivities, which may not represent a critical problem in small, lab-scale of microbial fuel cell (MFC), but can be a major obstacle for upscaling. The use of metal materials, such as stainless steel (SS), as electrodes or current collectors can decrease the electrode resistance considerably. However, the direct use of SS as anode material is so far relatively rare, most likely because the Cr component in the SS would inhibit the microbial activity and lead to a low microbial bioelectrocatalytic activity.

In this presentation, surface modification of SS mesh (SSM) was conducted to improve the microbial bioelectrocatalysis. The modified SS mesh electrode could generate a high current density of up to 1.91 mA cm\textsuperscript{-2}, is nearly three orders of magnitude higher than the untreated SSM electrode (0.0025 mA cm\textsuperscript{-2}).\textsuperscript{1} Moreover, the modification layer was firmly attached to SSM surface and have a good corrosion-resistant. It could deliver the equal current density after removing the established biofilms and shows good stability under the oxidizing potential of + 0.2 V (vs. Ag/AgCl) which was close to the real situation in the MFC. Moreover, 3D-CB/SSM electrode could be prepared by simple folding and delivered a much higher current density of 10 mA cm\textsuperscript{-2}, which is comparable to the highest reported level from the layered corrugated carbon. This modified SSM would be an ideal high-performance bioelectrode for upscaling applications of MFC.

![Figure](image)

Figure. (A)modified SSM and (B) and (C) modified SSM covered with thick biofilm
On the relationship of energy generation and ionic liquid-based membrane properties in biocatalytic electrochemical systems

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In this study, the dependency of energy recovery on separator characteristics applied in MFCs was sought by testing supported ionic liquid membranes (SILMs) – prepared with [hmim][PF₆] and [bmim][NTf₂] ionic liquids (ILs) – comparatively with well-known proton exchange (Nafion N115) and microfiltration (PVDF) counterparts. Crucial membrane features such as O₂ and substrate (acetate fed as sole carbon source in a synthetic wastewater) crossover were assessed and as a result, mass transfer as well as diffusivity coefficients of these compounds (kO₂, kA, DO₂, DA, respectively) were determined. The experiments showed that SILM-operated MFCs could work in a reliable way and among them, the [bmim][NTf₂]-based one produced higher specific energy yield (YS = 9.78 kJ g⁻¹CODin m⁻²) than the Nafion-MFC (YS = 8.25 kJ g⁻¹CODin m⁻²) used as an important reference. This outcome was found to be associated with the membrane-cross oxygen shuttle properties of the membranes (kO₂ = 1.25 cm s⁻¹ and 1.31 cm s⁻¹, respectively).

As for the two SILMs, significant differences in terms of the energy yield, mass transfer and diffusion coefficients were noted, however, it has appeared from cell polarization measurements that the internal resistances of the SILM-MFCs were nearly the same. Hence, the evaluation of the MFC’s power production was complemented by measuring the dielectric traits of ILs that can be related with the ion conductivity of these materials. It was concluded that the [hmim][PF₆] had an order of magnitude lower conductivity (σ = 21.8 mS m⁻¹) than [bmim][NTf₂] (σ = 283.5 mS m⁻¹).

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Properties of biomineralized electrode materials

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Bacteria promote the precipitation of a diversity of Fe mineral phases through biomineralization, both in the environment and in laboratory cultures. We explored electrochemical properties of Fe-bearing biominerals produced in lab cultures of two different strains: FeOOH was precipitated in the cell wall of Acidovorax sp. strain BoFeN1 upon microbially-mediated Fe(II) oxidation1, whereas the surface of S. pasteurii served as a template for FePO₄·xH₂O nucleation and growth2. Both strains became encrusted by a thin layer of Fe-bearing minerals leading to hollow mineralized shells (bacteriomorphs, Fig. 1A).

We used these bacteriomorphs as positive electrode materials in lithium batteries vs. Li(0) to investigate their electrochemical properties. The specific texture of the bacteriomorphs enhanced both ionic conductivity and electrical percolation. In contrast, organic matter content limited electronic conduction. Noteworthy, a heat treatment restored electroactivity, as evidenced down to the sub-micrometer scale by Scanning Transmission X-ray Microscopy (STXM, Fig. 1B-C). Given the ubiquity of Fe-bearing minerals in the environment and the diversity of biomineralizing bacteria, these results call for a deeper investigation of biomineral electroactivity in the environment and for electrochemical applications such as microbial fuel cells.

FIGURE 1: SEM image of Fe-phosphate bacteriomorphs (A) and STXM study of their electroactivity when used as electrode material vs. Li⁺/Li (B-C).

Electrochemical characterization of graphite-zeolite electrodes for their use as bioanodes in microbial fuel cells

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A Microbial Fuel Cell (MFC) can be defined as a technology that uses microorganisms to convert the chemical energy present in a substrate to electrical energy. The performance of MFC is heavily influenced by the electrodes materials they are made from. Specifically, the anode surface plays a significant role in promoting and maintaining biocatalytic activity. Surfaces can be modified to become favourable habitats for biofilms which are capable of enhancing electron transfer from bacteria to anode surface. Generally, the achievement of more bacterial adhesion enables the generation of more power with minimum loss. For this reason, the work studied a novel electrode material to be used as anode in dual chamber MFC. The anode material is based on graphite (Gr) modified with a type of zeolite namely Faujasite Y (ZY). The anodic electrode was prepared with a mixture graphite-zeolite doped with iron supported on glassy carbon serigraphied electrode (GC-GrZYFe). By scanning electron microscopy (SEM) was evaluated the bacterial adhesion on the anode and the morphological characteristics of the bacteria on the biofilms. Furthermore, we used cyclic voltammetry (CV), electrochemical impedance spectroscopy (EIS) and chronoaeramperometry (CA) to investigate the microbial electron transfer and biofilm formation. SEM images show the growth of a heterogeneous biofilm on anode after 7 days operation and the presence of bacteria with rod shape was observed. Moreover, the catalytic activity of bioanodes were characterized trough CV under turnover and non-turnover conditions. The results show a couple of redox peaks in absence of substrate. A sigmoidal shape voltammetry was observed that to confirm the capability of anodic biofilms to acts as a bioelectrocatalyst under acetate-oxidizing conditions.
Stainless steel-based bioanodes for applications in Bioelectrochemical Systems

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The majority of studies focusing on bioelectrochemical systems (BES) report the utilization of carbon-based electrodes. However, in order to overcome the limitations related to the scale-up of Microbial Fuel Cells (MFC) and Microbial Electro Synthesis (MES) cells technologies, the utilization of metal-based electrodes need to be considered due to their conductivity, cost and robustness. We investigated stainless steel fiber felts (SSFF) as bioanode material for BES applications. The unmodified SSFF was tested alongside with electrodes modified with reduced graphene oxide, manganese oxide (MnO₂) and flame-oxidation (FO). The performances of the metal-based electrodes as bioanodes were investigated in half-cells and MFCs in terms of stability, current and power densities produced in the different set-ups. In addition, the impact of the capacitance of the abiotic electrode materials on the performance of the bioanodes and MFCs was evaluated, especially to assess how these materials can increase the energy harvested compared to non-capacitive bioanodes. The results obtained showed that the stainless steel-based electrodes studied are competitive alternatives to the carbon-based electrodes. For example, current densities recorded in half-cells (figure below) were 1.96 and 2.26 mA/cm² for FO-MnO₂-SS and carbon cloth, respectively. The enhancement of performance from FO-MnO₂-SS is likely due to the high capacitance and biocompatibility of the material, showing that the corresponding bioanodes offer promising performances for renewable energy generation and storage in MFCs and MES cells systems and applications.

\[ \text{a) Galvanostatic charge-discharge curves of the FO-SS and FO-MnO₂-SS abiotic electrodes.} \]

\[ \text{b) Electrochemical performance of electrochemically active biofilms cultivated on different carbon- and stainless steel-based electrodes at -0.2 V vs Ag/AgCl} \]
Effect of different carbon materials on the performance of microbial electrolysis cells (MECs) operated on urine and their microbial composition

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Urine is rich in nitrogen and phosphorous and can considerably reduce domestic wastewater treatment requirements if collected separately. Source separated urine has been shown to be suitable for energy production and nutrients recovery in bioelectrochemical systems. However, there are still several challenges to overcome mainly related to organics conversion into electrical energy.

In this study, anode performance of three microbial electrolysis cells (MECs) fed with urine using different carbon anodes, Keynol (phenolic-based), C-Tex (cellulose-based) and PAN (polyacrylonitrile-based) was compared. Two strategies were used to supply energy to the MECs; cell potential control (1st assay) and anode potential control (2nd assay). In both assays, the C-Tex MEC outperformed MECs using Keynol and PAN. The C-Tex MEC with anode potential control at -0.300 V generated the highest current density of 904 mA m⁻², which was almost 3-fold higher than the MEC with Keynol, and 8-fold higher than the MEC with PAN. Analysis of anodes textural, chemical and electrochemical characteristics suggest that the higher external surface area of C-Tex enabled the higher current density generation compared to Keynol and PAN. The microbial composition on each anode and its correlation with the generated current was also investigated. No significant differences were observed in microbial diversity of the biofilm present in the studied anodes. Nonetheless, C-Tex had higher dominance of bacteria belonging to Lactobacillales and Enterobacterales suggesting its relation with higher current generation.
Engineering textile carbon anodes for industrial wastewater MFCs and other bioelectrochemical systems

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Microbial fuel cells (MFCs) are increasingly arousing industrial interest, especially regarding wastewater treatment applications. The demand for commercial products pushes worldwide efforts to upscale these bioelectrochemical systems, however, the lesson to be learned from first whole-system approaches is that the complex architecture of MFCs and the functionality of their individual components have to be understood in detail.

Accordingly, we focus on engineering one key building block in MFCs: A functional bioanode. For the electrode material, we will exploit the great potential of textile carbon fiber materials, as they can be custom tailored on fiber and fabric level as well as shaped into a 3D electrode configuration in the MFC reactor.

Two electroactive model organisms, *S. oneidensis* and *G. sulfurreducens*, are used to identify and investigate the relevant parameters that boost microbial current generation, e.g. the role of the carbon content of the fiber base, weaving parameters and electrical contacting to the circuit. The experimental selection of optimized electrode architectures is supported by the simulation of fluid dynamics and biochemical kinetics based on a model system with *G. sulfurreducens* and acetate as substrate.

Further, upscaling steps are realized with paper mill wastewater up to a final 20 L MFC prototype. The latter is currently being integrated into the industrial wastewater treatment plant.

This poster provides an overview on the engineering approach for textile carbon anodes including some preliminary results.
“ElektroPapier” – Developing paper-based electrodes and high performance ion exchange membranes to enable tailored modular microbial electrochemical – industrial – wastewater treatment

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An ecological and resource efficient wastewater treatment (WWT) is of crucial importance as part of a unified sustainable water management framework. Microbial electrochemical technologies (MET), such as microbial fuel cells (MFC) or microbial electrolysis cells (MEC), are potentially game-changing by combining WWT with product generation (electricity, chemicals, etc.). Industrial WWT represents an ideal field for developing/testing METs. The specific water characteristics and treatment requirements of certain industries allow for optimum conditions for introducing METs to full scale implementations. Upon successful completion, the dynamic industrial WWT market has the potential to speed up commercialization. The “ElektroPapier” project, funded by the German Federal Ministry of Education and Research (BMBF), aims to:

1. Create high performance ion exchange membranes to establish and maintain a low internal resistance of the MET. Existing exchange membranes must be optimized for the specific operation and targeted products of either MECs or MFCs.
2. Develop paper-based bioanodes, which can compete with other top-performing materials, while being able to be economically manufactured into effective 3D-architectures with high surface area to volume ratios.
3. Create a flexible modular treatment system by combining material development, reactor design and process controlling strategies. These modules can be tailored in terms of performance and costs to perspective customers.
The Influence of Electrode Surface Topography on the Performance of microbial electroactive biofilms in bioelectrochemical Systems

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Electrode resistance, mass transfer limitations and available surface area are major parameters determining the performance of electrochemically active biofilms, EAB, in bioelectrochemical systems. Particularly the impact of the electrode surface topography on EAB performance is poorly understood. Mostly, research efforts have been focused on random surface roughness approaches rather than on defined surface patterns or profiles on stable and non-porous materials. The goal of this study was thus to investigate the influence of defined surface topography on the EAB performance. The advantage of studying defined profiles is that contrary to chaotic roughness defined profiles allow to establish clear relations between surface parameters and EAB performance. Copper was chosen as a base electrode material because of its mechanical stability, non-porous nature, excellent conductivity and proven ability to yield a performance comparable to graphite – the most commonly used anode material in BES. Starting from a polished surface, wavelike profiles of different heights (ranging from 0 to 150 μm) were applied to solid copper electrodes leading also to different surface areas (both of which quantified by optical microscopy). Their influence on the EAB growth and performance was investigated by conducting bioelectrochemical experiments under controlled reactor and medium conditions. The initial observations based on bioelectrocatalytic oxidation current suggest that the applied profiles accelerate the start-up of current generation. However, on a longer term, they seem to be outcompeted by the control electrodes without any profiles in terms of maximum current generation.

Literature
Dynamic flow and the use of inexpensive nickel-added activated carbon cathodes to achieve cost-effective hydrogen production in microbial electrolysis cells

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The use of a platinum is not practical in microbial electrolysis cells (MECs), thus further work is needed to develop inexpensive cathodes. Activated carbon (AC) cathodes have been studied in microbial fuel cells (MFCs) and have shown good catalytic performances for oxygen reduction, but they have not yet been investigated in MECs for hydrogen evolution. Also, alternative cathodes in previous studies have been mostly examined under static (no catholyte recirculation) conditions, which can result in higher cathode overpotentials. In this study, AC cathodes with a poly(vinylidene fluoride) (PVDF) binder were tested in MECs under static and dynamic flow conditions of catholyte, and nickel-added AC cathode were also tested to see if nickel addition could improve catalytic abilities of the AC cathodes. Four different types of AC cathodes were fabricated with different amount of NiCl₂·6H₂O (0, 16, 31, and 62 mg) and a PVDF binder. The AC powder with and without Ni were spread onto the stainless steel mesh then immersed in DI water for 15 minutes to induce phase inversion. Abiotic electrochemical tests showed that Ni-added AC cathodes produced more positive potentials than plain AC. During MEC operation with static flow, maximum current densities of 6 A/m² were obtained by the MEC with Pt cathode (control), while ~5 A/m² of maximum current densities were produced with all AC cathodes. The hydrogen production rate was 0.31 ± 0.01 L-H₂/L-reactor/d by the MEC with a Pt cathode and comparable rates were achieved by Ni-added AC cathodes (0.31 ± 0.03 L/L/d). The plain AC cathode showed the lowest hydrogen production rate (0.20 ± 0.04 L/L/d), which indicated that the added nickel was critical for improving hydrogen evolution reactions of AC cathodes. Long-term tests are ongoing to examine the impact of flow and demonstrate the longevity of the Ni-added cathode in MECs.
The effect of oxygen traces on the current production of high performance electrospun anode materials for application with \textit{Shewanella oneidensis} MR-1

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\textit{Shewanella oneidensis} MR-1 is a well-studied electroactive model organism that can serve as chassis for production strains for, i.e., unbalanced fermentation (Flynn et al., \textit{mBio} 1 (2010), e00190-10). To minimize unwanted side reactions, which would decrease production yields, strict anaerobic conditions are required. Under such strict anaerobic conditions (also indicated by absence of flavin production and planktonic cell growth in the bioreactor), high performance electrospun anode materials can yield up to 205 $\mu$A/cm$^2$ at 200 mV vs. NHE, which is about 12 and 70 times higher than the commercial activated carbon cloth (C-Tex 13, MAST Carbon) and graphite felt (GFD 2, SGL Carbon), respectively. The average current production over the course of 2 weeks is up to 13-fold higher with respect to C-Tex 13 and about 150 times higher with respect to GFD 2. However, even traces of oxygen (<0.02% oxygen saturation) in a bioreactor, which can evolve from improper material of the N2 tubing (i.e. polyurethane), affect the current production drastically. With small traces of oxygen present, the currents increase for all tested materials, and with time the current production of the different materials converge. This is reflected in the average current production of the electrospun material being only 3-fold higher than with respect to C-Tex 13 and 6-fold higher with respect to GFD 2. In summary, our results highlight the importance of well controlled oxygen levels in order to accurately evaluate the performance of anode materials for \textit{Shewanella oneidensis} MR-1. Furthermore, we can demonstrate that under the strict anaerobic conditions required for high yield bioproduction electrospun anode materials exhibit an order of magnitude better performance compared to commercial carbon materials.
Air-cathode microbial fuel cells with Pt-Co coated CMI7000 membrane as the cathodic catalyst

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Electricity production in air-cathode microbial fuel cells is often limited by the slow reaction kinetics and high overpotential of oxygen reduction. The oxygen reduction efficiency can be improved with a catalyst, but materials with good catalytic efficiency (e.g. platinum) are often also expensive and would increase the MFC costs significantly. Therefore, there is a need for low-cost catalyst materials, which are also efficient and durable. Noble metals like platinum can be alloyed with non-precious transition metals without decreasing the oxygen reduction efficiency significantly. Cetinkaya et al. 2015 showed Pt-Ni coated (atomic ratio 1:1) membranes to provide higher power density from dairy wastewater than membranes coated with pure Pt.

In this study, bimetallic electrocatalysts of platinum and cobalt were synthesized using sodium borohydride reduction. In the electrochemical comparison of different Pt-Co molar ratios, the highest electrochemically active surface area normalized against the amount of platinum was obtained with ratio of 1:4. The performance of this 1:4 Pt-Co catalysts was then compared to Pt in xylose-fed air cathode MFCs with catalyst coated CMI7000 membranes. The maximum power density and current density obtained in the performance analysis were 28.3 mW/m² and 451 mA/m² for PtCo and 21.2 mW/m² and 407 mA/m² for Pt. Therefore, this study shows that alloy of platinum and cobalt is a promising cost-efficient alternative for the use of pure platinum in microbial fuel cells.

References:
Slip-Casting for ceramic membranes fabrication for Microbial Fuel Cell

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In recent years, Microbial fuel cells (MFC’s) have received a great attention as alternative clean energy technology due to its capacity for simultaneous electrical energy generation and wastewater treatment. However, number of technical challenges must be overcome before practical application of MFC: high cost of electrode material and cation exchange separators, unstable performance, etc. Separator is a critical MFC’s component and currently presents a number of problems: high price of commonly employed materials, high oxygen diffusion, ionic transfer limitations, etc. [1]. Recently, ceramics were proposed as alternative materials in a role of separator due to their low cost, large availability, good chemical resistance and environmental friendliness. Moreover, ceramic processing is a mature technology which makes more viable the scaling-up process. Thickness, porosity and ionic conductivity are the most determinative factors of these membranes and significantly influence the MFC efficiency [1]. Commonly reported values of thickness for ceramics membranes for MFC’s varied from mm to cm [2], being usually better the performance for low thickness membrane MFC’s. However to the best of our knowledge, application on thin-film ceramic membranes for MFC’s (hundreds of micra) has never been reported in literature. Furthermore, slip-casting is a low cost and versatile ceramic technique which is capable of producing complex shape and thin ceramic structures. Therefore, the present work aimed at developing low thickness ceramic membranes using slip-casting route and evaluating the performance of MFC’s assembled with these membranes. Obtained structures were characterized chemically and morphologically before MFC’s were fabricated. Preliminary results confirmed the suitability of slip-casting for MFC membranes fabrication.

Reference:
The “Bioelectrochemical-well” a novel reactor configuration for bioremediation of toluene-contaminated groundwater

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In recent years, microbial electrochemical technologies (MET) have attracted considerable attention for remediation applications, although their field application is often prevented by the lack of scalable system configurations. Recently, we developed a novel bioelectrochemical reactor configuration called “Bioelectrochemical-Well (BE-WELL)”, which can be installed directly within a groundwater well and that can be applied for in situ treatment of organic contaminants, such as petroleum hydrocarbons (Palma et al., 2017). In the present study, a laboratory-scale prototype of the BE-WELL has been set up and operated in a continuous-flow regime, under a range of operating conditions with toluene as the sole carbon and energy source. The performance of the bioelectrochemical reactor was analysed in terms of degradation rate and yield. Electrochemical techniques were used to characterize the catalytic behaviour of the reactor. Under optimal conditions (i.e., anode potential set at +0.2 V vs. SHE), the rate of toluene oxidation was 57 mg/L d, with a Coulombic efficiency exceeding 80%. GC-MS analysis revealed allowed identification of benzyl-succinate as a key metabolite of toluene degradation, thereby indicating that anaerobic contaminant oxidation proceeding via fumarate addition.

Session IV
Microbial fuel cell applications
TiO$_2$/Activated carbon photo cathode catalyst exposed to ultraviolet radiation enhance the efficacy of integrated microbial fuel cell-membrane bioreactor

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The world is now in the middle of a huge transition between fossil fuels to the renewable energy. Microbial fuel cell (MFC) technology, which uses microorganisms to transform chemical energy of organic compounds into electricity, is now considered to be one of the finest cutting edge technologies to eradicate future energy crisis of the world. Membrane bioreactor (MBR), a better alternative to the existing aerobic wastewater treatment technologies, when coupled with MFC offers advantage of enhancing the treatment efficiency along with bio-energy recovery. A comparative study had been done on the performance of integrated MFC-MBR technology incorporating TiO$_2$/Activated carbon (AC) as photo-cathode catalyst with or without the presence of ultraviolet spectrum (UV-A). Experimental results showed that the maximum power density and coulombic efficiency were nearly 2.1 and 1.6 times higher in case of MFC-MBR with TiO$_2$/AC/UV (494 mW/m$^2$ and 12.7 ± 0.7 %) than the control MFC-MBR (TiO$_2$/AC) without UV (238mW/m$^2$ and 8.3 ± 0.7 %), respectively, indicating the efficacy of TiO$_2$ (mixture of anatase and rutile)/AC nanopower as better cathode catalyst in the presence of UV-A spectrum. Moreover this integrated MFC-MBR systems offered more than 87 % and 96% of total kjeldahl nitrogen (TKN) and chemical oxygen demand (COD) removal from the synthetic wastewater having around 2 g/l of initial COD, respectively. This integrated MFC-MBR technology offers an immense potential of consequently generating high-quality treated effluent with acceptable quality to meet inland water disposal and for the onsite reuse for full-scale application with simultaneous advantage of green-energy recovery.
Microbial fuel cell sensor at open-circuit for real-time monitoring the nitrate in aquatic environment

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Microbial fuel cell (MFC) sensor has attracted increasing interests in recent years as it has been successfully applied to real-time monitor organic matters and toxins. Nitrate contaminations that widely exist in aquatic environment can also be detected by an MFC sensor. Previous study applied a close-circuit for MFC sensor to monitor the nitrate when the nitrate acts as an additional electron acceptor in heterotrophic denitrification process. But it turns out that the sensitivity of the sensor is so limited that the output electrical signal little changes when challenged by the nitrate. This study employed an open external circuit and greatly improved the sensitivity of MFC sensor when monitoring the nitrate at a concentration of 10^4~40 mg/L. Within the response time (30min), voltage drop across the two electrodes at open-circuit is much greater than that at close-circuit. A larger slope in the calibration curve at open-circuit (4.13mV/mg) was also observed, indicating an improved sensitivity. The influence of organic matter’s (NaAc) concentration on the sensor’s performance was evaluated by voltage drop ratio \( R = \frac{\Delta E_{anode}}{|E_{anode}|} \). And it was found that when NaAc’s concentration increased from 1mM to 5mM, voltage drop ratio at close-circuit decreased from 86.8% to nearly 0%. In contrast, it varied much less at open-circuit (from 47.75% to 26%).

![Diagram of MFC sensor at open-circuit and close-circuit](Image)
Carbon black graphite hybrid air-cathode for efficient hydrogen peroxide production in bioelectrochemical systems

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A novel carbon black and graphite hybrid air-cathodes made by rolling-press method were utilized in bioelectrochemical systems (BESs) for \( \text{H}_2\text{O}_2 \) production. The highest \( \text{H}_2\text{O}_2 \) yield of 11.9 mg L\(^{-1}\) h\(^{-1}\) cm\(^{-2}\) was obtained at the optimal mass ratio of carbon black and graphite (1:5), which is mainly due to the balance of specific area and catalytic activity in catalyst layer. Higher current efficiency (CE) was found in continuous system, which was benefit from the avoidance of \( \text{H}_2\text{O}_2 \) accumulation. In biological system, the highest \( \text{H}_2\text{O}_2 \) production rate of 3.29 mg L\(^{-1}\) h\(^{-1}\) was obtained with current density of 0.61 mA cm\(^{-2}\), which was 4.7% higher than that in abiotic system. Air diffusion has a dominant contribution (>90%) at a high current density (high \( \text{H}_2\text{O}_2 \) yield). This hybrid air-cathode combined the advantages of high \( \text{H}_2\text{O}_2 \) yield, high current density and no need of aeration, which make the synthesis of \( \text{H}_2\text{O}_2 \) more efficient and economical.
Long-term operation of a scaled-up air cathode MFC fed with acetate medium and partial anaerobic digestion effluent

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Microbial fuel cells (MFC), in particular air-cathode MFC technology, represent an appealing opportunity for the direct conversion of organic matter content of wastewater into electricity, with high current conversion efficiency, low GHG emission and low solids generation, compared to traditional wastewater treatment technologies. Although these advantages, the energy recovery achieved so far using domestic wastewater has been low (12 W m⁻³). Only a few scaled-up approaches are reported in the literature, in the range of 1 to 1000 L of reactor volume [1], and even fewer studies report long term operation results. In the present work, a 1.5 L air-cathode MFC, with flat plate configuration [2], was constructed. The cost of the air-cathode MFC reactor was evaluated: by using carbon based electrodes, a platinum-free air-cathode and a low-cost separator (2 €/m²), the total reactor could be built at a competitive price.

The MFC was operated for more than 2 years at room temperature, in continuous flow conditions, feeding it with 50 mM PBS containing 2.5 g L⁻¹ of sodium acetate. The electrical performance and the organic matter removal (expressed as COD) were regularly monitored and reached stable values over time. The MFC showed long-term stability without the need of high maintenance. The power density achieved was over 14.4 W m⁻³ (92 mW m⁻²), associated to COD removal efficiency between 70 and 85%. During these 2 years, the MFC has been occasionally operated with a liquid effluent coming from a hydrolytic-acidogenic anaerobic digester treating wastewater sludge. The MFC fed with this natural medium produced a power density up to 3.9 W m⁻³ (24.9 mW m⁻²), with COD removal ranging between 60 and 77 %. Taking into account the review from A. Janicek et al. [1] and only considering the continuous feeding approaches, with real wastewater streams and without any pH buffer addition, the power density reported in this study is among the highest ones, only surpassed by the study of L. Zhuang et al. [3] using a 10 L MFC fed with brewery effluents waste streams.

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References
A novel three-chamber stacked microbial fuel cell in transient circuit operation for enhancing nitrobenzen and mineralization

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Nitrobenzene (NB) is a toxic and recalcitrant compound that can be widely found in the wastewater of chemical plants. Microbial fuel cell (MFC) has been proven to be capable of removing NB in the cathode by reducing it to aniline (AN), a less toxic end product that can be easily mineralized. However, the present traditional dual-chamber MFC (DC-MFC) suffer from low organic removal efficiency and no further mineralization of AN. In this study, a novel three-chamber stacked microbial fuel cell (SMFC) which consisted of an anaerobic biocathode, an aerobic biocathode and an anode was constructed to treat NB-containing synthetic organic wastewater (Fig. 1A). During the continuous-flow period, the hydraulic retention time of each electrode chamber was set as 10 h. Due to the addition of aerobic biocathode, which significantly increased the electricity generation time of its adjacent anode, the anode of SMFC in constant circuit (CC-SMFC) had higher COD removal efficiency of 36% than the anode of DC-MFC (26%) (Fig. 1B). However, the anaerobic biocathode of CC-SMFC delivered a lower NB reduction and AN formation efficiencies of 42% and 67% than that of DC-MFC (50% and 76%) (Fig. 1B&C). This was because of the lower electric current flow from the anode to the anaerobic biocathode for NB reduction due to the electron competition of the aerobic biocathode in CC-SMFC. In order to balance the electron distribution between the two cathode, a transient circuit operation mode was implemented on another SMFC reactor (TC-SMFC) (Fig. 1A). TC-SMFC demonstrated to deliver larger amounts of electrons (97C) for NB reduction in its anaerobic biocathode at each operation cycle, thus leading to the highest NB reduction and AN formation efficiency of 58% and 84% among three MFC reactors (Fig. 1B&C). Due to the enhanced NB reduction and transformation performance in its anaerobic biocathode, TC-SMFC exhibited the highest total NB and COD removal efficiency of 92% and 87% (Fig. 1B) with the lowest final NB, AN and COD effluent concentration of 13, 8.7 and 113 mg/L. These results demonstrated an effective NB and COD mineralization strategy by employing this novel SMFC in transient circuit operation.

Fig. 1. Schematic of TC-SMFC (A) and the removal efficiencies of nitrobenzene and COD in each electrode (B) and the AN formation efficiencies in each anaerobic biocathode (C) of three different MFC.
Production of bulk chemicals by molecular-biological optimization of *Shewanella oneidensis* MR-1

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Due to the high ratio of catabolism versus anabolism, anoxic fermentation is the more preferred set-up for biotechnological production processes. However, by omitting aeration of the reactor the addition of alternative electron acceptors is necessary. During fermentation these electron acceptors accumulate in their reduced form within the fermentation broth and have to be removed expensively. Using electroactive organisms as biocatalysts that are capable of transferring their excessive electrons to non depletable electrodes as electron acceptors may overcome this problem. In this project the electroactive organism *Shewanella oneidensis* MR-1 is being investigated for such a bioelectrochemical production. The accessibility and the convertibility of its citrate cycle are being investigated for redirection of the metabolic flux to desired products. As a proof of concept study *S. oneidensis* is planned to be metabolically engineered to produce bulk chemicals. Furthermore, strategies for increasing the current production during electrochemical cultivation are planned to be tested in order to increase the overall flux und consequently the yield of the desired product. The produced current can additionally be used for running the whole fermentation process more efficiently.
Tungsten oxide as anode and cathode catalyst for improved power generation and wastewater treatment in a microbial fuel cell

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Microbial Fuel Cell (MFC) is a device that oxidizes the organic matter present in wastewater and simultaneously generates electricity from it. Theoretically, MFC can generate a maximum of 1.1 V but due to the various overpotential losses maximum voltage produced from it is very less. To reduce this overpotential loss use of catalysts is necessary. Such a novel catalyst WO₃ was synthesized and was used as both anode and cathode catalyst. WO₃ was prepared by hydrothermal method and 4 MFCs of anodic chamber volume 45 mL were fabricated to study the catalyzing effect of WO₃ on graphite felt electrodes. The performance of MFCs was elevated when WO₃ was used as a catalyst. Maximum power density was increased by 5 times when WO₃ was used as anode catalyst and by 4 times when used as cathode catalyst. Also, almost 6 times increment in maximum power production was observed when both the electrodes were coated with WO₃. It was comparable to the maximum power density obtained using platinum as cathode catalyst in MFC with similar operating conditions. Electrochemical analysis of WO₃ also proved that it can enhance the current density of the modified electrode owing to its electrochemical properties. WO₃ enhances interfacial transfer of electrons and thus biofilm growth on the anode and its high specific surface area increases cathodic reaction. Furthermore, COD removal of modified MFC was also observed to be higher, thus suggesting an overall enhancement in the performance of MFC by the use of WO₃.

Table 1 - Observed parameters of the MFCs used in the study

<table>
<thead>
<tr>
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<th>Anode</th>
<th>Cathode</th>
<th>Coulombic efficiency (%)</th>
<th>COD removal (%)</th>
<th>Operating voltage (mV)</th>
<th>Maximum power density (W/m³)</th>
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<tr>
<td>MFC-1</td>
<td>WO₃</td>
<td>No catalyst</td>
<td>21.9 ± 2.3</td>
<td>88 ± 3</td>
<td>121 ± 11</td>
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<tr>
<td>MFC-2</td>
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<td>WO₃</td>
<td>20.5 ± 2.6</td>
<td>86 ± 3</td>
<td>111 ± 09</td>
<td>3.11</td>
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<tr>
<td>MFC-3</td>
<td>WO₃</td>
<td>WO₃</td>
<td>23.1 ± 2.4</td>
<td>87 ± 2</td>
<td>126 ± 09</td>
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<tr>
<td>MFC-4</td>
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<td>22.5 ± 2.3</td>
<td>92 ± 2</td>
<td>130 ± 12</td>
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<tr>
<td>MFC-5 (Control)</td>
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<td>No catalyst</td>
<td>10.0 ± 1.3</td>
<td>85 ± 3</td>
<td>47 ± 05</td>
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Environmental Electroactive Biofilm as reusable sensing element for freshwater monitoring

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The development of innovative, cost effective and in-situ alarm systems for water toxicity assessment has become fundamental to overcome the decrement in world clean water resources.

In the present study the biosensing capability of a river sediment microbial consortium, in equilibrium with the natural composition of the environment to monitor, was tested towards different toxic types in a membrane-less Microbial Fuel Cell (MFC).

The semi-continuous operation mode of the MFC-based biosensors allowed obtaining high stability of non-toxic current profile, even in non-controlled temperature conditions. The environmental consortium showed a linear response towards Glutaraldehyde within the range of 5-1000 ppm. Moreover, a full recovery of bioanode’s activity, even after the highest concentration of Glutaraldehyde, was observed, allowing to test the same systems towards Nickel(II) (2, 20 and 60 mg L⁻¹) and Chromium(III) (2 and 20 mg L⁻¹). The heavy metals toxic tests resulted in different current response patterns indicating that, even if non-specific, these MFC-based biosensors could reveal some information about the modality of action of the single toxic substance used.

To effectively analyze the biosensors’ response, a novel algorithm, which identifies critical time points based on the analysis of the first derivative of the current, was proposed. This approach can be successfully exploited for long-term online monitoring applications, where energy efficiency is a critical concern, and, therefore, an improved communication protocol for data transmission is necessary.
A Study on Beverage-Based Microbial Fuel Cell for a Novel Portable Power Source

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Microbial fuel cell (MFC) provides not only an eco-friendly way of waste water treatment but also a sustainable power source. MFC systems have a great potential to be used as a portable or wearable power source, however, there have not been many attempts to apply the MFC in our daily lives. Recently, there have been a few researches on the wearable MFC’s based on the organic substance originated from human bodies such as urine or sweat, however, they depend on the user’s body condition and hard to employ back-up substrate for immediate re-energizing. For portable MFC, it is very important to investigate proper substrate which can be easily obtained, stored, carried, and utilized without hazard. This study proposes to use drinks such as juice, coffee, and milk as energizing substrate for portable MFC, which are abundant in our daily lives, safe to handle as well as contain a lot of organic substances such as carbohydrates and saccharides even in their residues. Experiments were carried out for the comparison of output characteristics driven by these beverages. Four identical MFC’s were connected to 3 kΩ resistive load and were initially inoculated with 400 ml of liquid vermicompost which contains many exoelectrogen species. After 13 hours, MFC_2, MFC_3, and MFC_4 were injected with 100 ml of orange juice, coffee, and milk, respectively. MFC_1 was injected with 100 ml of vermicompost as a reference. Then output voltage was observed for 11 hours as shown in Fig. 1. MFC_2 (orange juice) and MFC_3 (coffee) showed sudden decrease of power at the moment of injection but, gradually recovered afterwards. It seemed that lower pH level of juice and coffee affected the microbial activities. MFC_4 (milk) showed the best performance with rapid power increase and without abrupt change at the moment of injection. Milk is expected to make one of the strong candidates for substrate in portable MFC. Future study will be focused on portable implementation of the beverage-based MFC system, which is capable of accepting wide range of daily resources and robust to rapidly changing substrates and environments. (Research supported by RDA Korea under the Cooperative Research Program for Agriculture Science & Technology Development-PJ011751022017).

Fig. 1. Experimental setup and measured output characteristics of four MFC’s.
Control of voltage imbalance by hydraulic-serial connection of single-chamber MFCs with different anodic volumes

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MFC stacks connected in series or in parallel have been studied to overcome the low electricity generation of a single MFC. Electricity generation from stacked MFC can be affected by electric connection, hydraulic flow mode and operating conditions. Especially, energy loss occurs due to voltage imbalance of each MFC in MFC stack. In this study, three single chamber MFCs with identical electrode size and different anodic volume (S-MFC=19.2 mL, M-MFC=38.4 mL, and L-MFC=76.8 mL) were operated at 0.64 ml/min (Phase I). And three MFCs (flow mode: S-MFC→M-MFC→L-MFC) was hydraulically connected in series (phase II). Then, three MFCs (flow mode: L-MFC→M-MFC→S-MFC) was hydraulically connected in the opposite direction (Phase III). In phase I, each MFC showed different voltage generations due to different organic loading rates, although three MFCs were operated at same flow-rates. Voltage generation (0.5±0.05V) in M-MFC was similar to L-MFC (0.5±0.04V) in phase II. In phase III, L-MFC showed the highest voltage generation (0.8±0.08V), followed by M-MFC (0.3±0.04V) and S-MFC (0.1±0.03V). Hydraulic-serial connection of single-chamber MFCs with different anodic volumes would control voltage imbalance in MFC stack.

Fig. 1. Voltage generation in a single MFC and MFC stack according to hydraulic flow mode.
The influence of inoculum and substrate on power performance and the anode community in a microbial fuel cell

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Generating electricity while cleaning up wastewater gives microbial fuel cells (MFCs) a conceptual elegance to recover value from waste. But despite their potential and a huge improvement in the current density produced the bioanode is still a bottleneck for industrial use as scale up to larger than one liter leads to huge performance losses [1]. (Pham et al., 2009) In a bioelectrochemical system (BES) a resilient bioanode is crucial to recover resources from wastewaters on the cathode. To better understand the underlying principles, such as syntrophic behavior and electron transfer, of a wastewater fed bioanode we studied the influence of three different inocula (Tyne sediment, activated sludge and MFC enriched effluent) and three substrates (acetate media, OECD synthetic wastewater media and wastewater) on MFC performance and the anode community. Additionally biofilms grown on acetate media were switched to OECD and wastewater and the change in performance and anode community was studied. Results show that MFCs fed with acetate media show, as expected, the highest power performance and the largest peak current density was observed for acetate fed reactors using MFC enriched effluent as inoculum. The reactor switched from acetate to more complex substrate showed slightly better peak current densities than the reactors only fed the complex substrate. Cell counts of the anodic biofilm similarly showed the highest number of cells in the acetate fed reactors followed by the reactors switched to a more complex substrate and then the complex substrate alone. This indicated the power performance is dictated by substrate type probably due to competitive fermentation reactions taking place to break down the more complex substrate. Interestingly the CVs of the acetate fed reactors indicate the presence of G. sulfurreducens which we will confirm with sequencing.

References
Winery wastewater treatment in a single chamber microbial fuel cell

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The microbial fuel cells (MFCs) combine the biological and the electrochemical processes to produce electrical energy.

In the past few years, studies have been reported regarding the applicability of MFCs in winery wastewater treatment, mostly based on the evaluation of the availability of activated sludge or the microorganisms that are known to spoilage the wine to perform the treatment.

Considering the complexity of the wastewater, the use of yeasts have particularly interest, once they are known for their higher ability of growing in higher sugar concentrations and harsh environments. The major drawback of using yeast in MFCs, relies on the fact that these microorganisms are eukaryotic, having the catabolic process occurring in the inner membrane of mitochondria and matrix, and therefore the electrons are not easy transferred to the outside of its structure.

The present work intends to evaluate the feasibility of using different microorganisms (bacteria and yeasts) in a single-chamber microbial fuel cell (SCMFC) to perform winery wastewater treatment and produce useful electrical energy, using a synthetic winery wastewater recipe adopted by Malandra et al.[1], which considers the complex composition of the winery wastewater. The SCMFC has an anode compartment with 1L of volume, a Nafion 212 membrane, a carbon fiber graphite brush as the anode electrode and a plain carbon paper, coated with 1 mg/cm² of platinum black as the cathode electrode. The MFC experiments were performed in sequential batch cycles. The SCMFC performance was evaluated through the polarization and power density curves, the wastewater treatment efficiency through the estimation of chemical oxygen demand removal rate and the biofilm formed on the anode electrode characteristics through its biomass dry weight and culturability, by counting the colony-forming units.

References

Development of an electrogenic bioreactor for wastewater treatment process

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Fluence corporation (former Emefcy) is developing the electrogenic bioreactor, a microbial fuel cell (MFC) based product for wastewater treatment. Such MFCs are considered a technology that will enable the treatment of wastewater coupled to production of electricity, which makes them more energy efficient and even energy positive, comparing to traditional methods such as activated sludge and anaerobic digestion. The technology is being studied for more than three decades but thus far no commercial product is available in the market.

The research performed at Emefcy has focused on achieving the highest performance possible using materials that will make the final product competitive. Therefore, an economic analysis in terms of organic load and flow rate was performed comparing to the other processes, and it was found out that the technology fits the best in treating wastewater containing high concentrations of organics in low flow rates. That analysis has considered the minimal performance required from the MFC in terms of COD removal and power output.

Following the analysis, we have designed a fuel cell using materials that will enable the development of a process of competitive price, such as carbon electrodes and non-precious metal based cathodic catalysis. The design of the cell has enabled us to reduce the resistance to minimum and achieve the performance considered minimal for the development of a full-scale reactor. The cell has generated a stable voltage of approx. 300mV and generated power output of approx. 1200mW/m² for several months, at coulombic efficiency of 35-50%. The cell has operated at COD loads of 3000-6000mg/L and has demonstrated removal of 70-90% of the COD.
Session V
Microbial electrochemical synthesis
Electrode-assisted bioproduction of chemicals using *Pseudomonas putida*

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*Pseudomonas putida* (*P. putida*) is a promising host for synthetic biology to produce chemicals but its obligate aerobic character significantly limits its application. In my PhD project, we demonstrated that the anoxic metabolism of *P. putida* could be exploited in a bioelectrochemical system (BES), and this process can be an efficient platform for microbial electrosynthesis of value-added chemicals. With the electron balance from BES, *P. putida* was able to survive and produce 2-keto-gluconate (2KGA) from glucose (yield of over 90%) under anoxic conditions. Soluble redox chemical is essential for the electron transfer, and the productivity increased with increasing redox potential of mediators, indicating this was a key factor affecting the anoxic production process. Quantitative analysis of the intracellular energy pools and redox status showed cells were able to generate energy but rebalance of redox co-factors were limited. Further investigations of cell behaviors were conducted by proteomics analysis.

In addition, metabolic engineering strategy was applied to facilitate the electrons transfer rate by overexpressing membrane-bound dehydrogenases of *P. putida*. RT-PCR revealed the up-regulation mechanism of gluconate dehydrogenase, and thus an optimal mutant was constructed accordingly. The resulted mutant presented significant improvements in electron transfer rate and specific sugar take rate (> 470%) and the productivity of 2KGA (> 640%), compared with wild type control. Overall, during my PhD, we proved the principle, for the first time, that redox power from BES could drive bioconversion of glucose for an obligate aerobe, and combining with metabolic engineering and system biology strategy, this electrode-assisted process could be a new robust platform for bioproduction of chemicals in the future.
Bioelectrochemical stimulation of methanogenic microbial communities in a seawater-based subsurface aquifer system

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To date, biogenic methane is standing in the spotlight as one of the sustainable energy sources, because of the microbial CO2 reduction to methane. Despite methane has already been largely explored in subsurface by commercial industries, a combined use of microbial methane production has not systematically accomplished yet.

Here, in order to utilize the methane-producing subsurface aquifer system for the sustainable carbon cycling, we applied a “bioelectrochemical” technique for the stimulation and acceleration of subsurface methanogenic microbial communities. The subsurface seawater-based aquifer sample was collected from a commercial gas-water-producing well in Chiba, Japan. An acetate-fed, single-chamber bioelectrochemical system was operated for 10 months where 600 mV of voltage was applied between anode and cathode, resulting in a successful conversion of CO2 to methane with electrical current consumption. The 16S rRNA-based community analyses showed that genera Methanocalculus and Methanobacterium were tightly associated with electromethanogenic cathode, while genus Geoalkalibacter was correlated with electrode-respiring anode. In addition, the metagenomics analysis revealed that the Geoalkalibacter genome coded numerous numbers of multi-heme c-type cytochromes (MH-cytCs), whereas methanogenic archaeal genomes contained many hydrogenases but lacked MH-cytCs (see figure). These results suggest that direct electron transfer via the redox-active proteins occurred on anode, and hydrogen-mediated electron transfer mainly functioned on cathode in this system.
Rational Design of Cathode Materials for Microbial Electrosynthesis

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Carbon di-oxide (CO₂) could be regarded as a resource rather than just a greenhouse gas which has to be remitted. Recycling of CO₂ into value added products, such as CH₄ or acetate, through microbial electrosynthesis (MES) is attractive from both an environmental and economic perspective. To improve biofilm formation and the rates of product generation, design of cathodes, which possess high specific surface area and enhance microbe-electrode electron transfer are needed. This study aimed to improve the CO₂ conversion efficiency in MES by developing a novel cathode design that is made of electrically conductive 3D porous hollow fibers (3D-PHFs). The 3D-PHFs played the dual role, as the cathode and for facilitating direct delivery of CO₂ to electrotrophs through the pores in the hollow fibers. The 3D-PHFs cathodes were further modified by electrophoretically depositing carbon nanotubes (CNTs), with superconductivity and high specific surface area, onto the surface to promote direct extracellular electron transfer[1]. A 32.2% increase in the specific surface area of 3D-PHF/CNT structures, as well as a reduction of cathode overpotential (~0.1V vs. Ag/AgCl), was observed after cathode surface modification. Pure culture of Sporomusa ovata, pre-enriched with H₂/CO₂, was used an inoculum source at the cathode. The cathode potential was set at -0.6V vs Ag/AgCl for all the reactors to facilitate CO₂ reduction to acetate through S. ovata electroautotrophic growth. An average acetate production rate of 233.24±35.51 mM/d/m² was achieved for 3D-PHF/CNT cathode provided with diluted CO₂ gas (20% CO₂:80% N₂) through passive route, compared to 152.37±3.56 mM/d/m² for 3D-PHF cathode. Electron recovery in the form of acetate was ~85% for the two cathode structures. These results demonstrate that modifying cathode morphology to enhance microbe-electrode interactions is an efficient approach to increase rates of carbon dioxide reduction in MES.

Microbial electrosynthesis of methane for biogas upgrading

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Microbial electrosynthesis (MES) provides a highly attractive perspective for the future generation of chemical products from electricity. Thereby electroactive microorganisms take up electrons from a cathode and convert CO₂ into chemical compounds. To establish this electron transfer several mechanisms as direct (DET), mediated (MET) or indirect electron transfer (IET) are known. Especially MES of methane, also called electromethanogenesis, offers the chance to store electrical energy or excess current as chemical energy (biofuel). A possible application of MES, we are working on at the moment, might be the upgrading of biogas in its methane content. Therefore a robust, electroactive biocatalyst is very important. So we screened several methanogenic strains from the orders Methanococcales, Methanosarcinales, Methanobacteriales and Methanomicrobiales for its hydrogenotrophic growth and its ability for MES of methane. One strain from the order Methanococcales, not described so far to take up electrons, produced methane by MES between potentials of -850 and -1000 mV (vs. Ag/AgCl). The coulombic efficiency was more than 80 %. Surprisingly gas analysis revealed unusual results dealing with a possible new mechanism of electron transfer in this strain. Additionally real-time qPCR analyses under MES conditions identified a gene/protein which might be responsible for the unusual electron transfer in this methanogenic strain. Finally we will give an outlook in the development of a prototype reactor for biogas upgrading with the MES technology.
Initial cathode potentials determine electron transfer behaviors of biocathodes catalyzing CO₂ reduction to CH₄

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Electromethanogenesis (EM) is a promising energy conversion system for carbon dioxide reduction to methane. The key component of EM is the biocathode, on which microorganisms (mainly methanogens) can accept electrons and reduce carbon dioxide to methane. There are mainly two pathways for electron transfer between the cathode and microorganisms, i.e., direct electron transfer and indirect electron transfer via H₂. To investigate the effects of applied potential during the startup time on the electron transfer pathway of biocathodes, we started up three biocathodes under three different constant potentials of −0.7 V, −0.8 V and −0.9 V vs Ag/AgCl, respectively. Carbon dioxide was used as the sole carbon source for all the biocathodes during the startup process. The results showed that the startup time of the biocathode cultivated at −0.7 V was longer than that of the biocathodes cultivated at applied at −0.8 V and −0.9 V. However, the cyclic voltammetry (CV) of the biocathodes started up at −0.7 V showed a typical sigmoidal shape with a midpoint potential of around −0.55 V, suggesting a direct electron transfer pathway. In contrast, no obvious sigmoidal shape of current was found in the CVs of the biocathodes cultivated at −0.8 V and −0.9 V, which only showed an remarked current due to the hydrogen evolution reaction at the potential ≤ −0.8 V. Finally, microbial community analyses were conducted to investigate the difference of microbial communities on the biocathodes.
CO₂-conversion to Acids and Alcohols by Microbial Electrosynthesis

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The national project CO2TRANSFER aims the synthesis of butanol by using CO₂ and electrons in a microbial electrosynthesis cell (MES). 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 CO₂ to butanol 24 electrons are needed. In the first project stage the best suited microorganisms for microbial electrosynthesis were identified. Bacteria such as Clostridium carboxidivorans as well as microorganisms such as Clostridium acetobutylicum, which are known for acetone-butanol-ethanol fermentation were tested for electron uptake. Figure 1 shows the first results with C. acetobutylicum in a MES with CO₂. Another approach is to produce butanol via an intermediate (acids) using co-cultures. Sporomusa ovata produced acetate via direct electron transfer, which was proved by Nevin [1] and Ganigué et al proved for the first time the bioelectrochemical production of butyrate from CO₂ as a sole carbon source [2].

Fig 1. Results of MES with C. acetobutylicum and synthetic medium (-900 mV vs Ag/AgCl)

The impact of externally added hydrogen gas on microbial electrosynthesis from CO₂

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Hydrogen is a key versatile biomolecule in microbial electrosynthesis (MES). It can be directly produced by electrolysis to be used as an intermediate, directly biosynthesize by electroactive microorganisms from protons and electrons, or externally added to drive other bioelectrochemical or biological reactions.

The aim of this study is to bring further understanding on how externally added hydrogen impacts product formation on MES. Two double-chamber microbial electrolysis cells were built in 500mL modified Schott-Duran bottles (Figure 1A). The cathode consisted of a 175 cm² carbon felt (+1V vs. Ag/AgCl) and a platinum wire was used as counter electrode. The cathode was inoculated according to the procedure detailed in Bajracharya et al. 2017, and following the acclimation period the biocathode was fed with a gas mixture containing 20% H₂ / 20% N₂ / 60% CO₂. After 2 weeks of operation hydrogen was removed from the feed (20% N₂ / 80% CO₂).

When the cell was fed with the hydrogen-containing mixture, acetate and ethanol concentrations (Figure 1B) grew steadily with time (composition ratio around 1:1 (w/w)). This behavior suggested that hydrogen was acting as a reducing agent driving direct production of ethanol, or even its production from acetate. However, when hydrogen was removed from the feed, ethanol concentration declined, while acetate concentration sharply increased showing CO₂-acetate selectivities near 100%. These results indicate how ethanol production is highly dependent on externally-added hydrogen, while the synthesis of acetate only requires the cathode as a source of electrons.

References

Microbial electrosynthesis of chiral alcohols by recombinant Escherichia coli whole-cell biocatalysts

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Biocatalytic routes towards valuable enantiopure chiral building blocks in pharmaceuticals and fine chemicals are promising alternatives to chemical synthesis. Microbial electrosynthesis as a novel production system might expand the scope of biocatalytic approaches and help to overcome current limitations.

Here, we present a proof of concept for microbial electrosynthesis for the enantioselective and NADPH-dependent conversion of acetophenone (AcPh) to 1-(R)-phenylethanol (PhEtOH) in recombinant E. coli whole-cells overexpressing the alcohol dehydrogenase from Lactobacillus brevis (LbADH). We use the engineered E. coli strain JG622 with electrogenetic capabilities for metal respiration allowing for both further genetic modification and easy catalyst preparation [1].

We were able to show that methyl viologen as mediator enables electron transfer from a cathode to the cofactor NADP⁺ for cofactor regeneration. The cofactor is subsequently consumed within the whole-cell for the reduction of AcPh to PhEtOH by LbADH. Thereby, we are able to produce 3 mM of PhEtOH within 142 h. The utilization of other mediators, variation of reaction conditions and further genetic modifications might shed light on rate limiting steps, which subsequently enable targeted process optimization. This will allow for the development of innovative biocatalytic reaction systems for the production of chiral building blocks by combining electrochemical and biotechnological methods.

Performance of biological microbial electrolysis cell with gas chamber for CO₂ diffusion and reduction

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As global greenhouse effect over the past decades is related to the unrestrained release of CO₂ into atmosphere, one of the solution is to bringing it back into terrestrial biosphere. Inorganic carbon in the form of CO₂ is a useful resource for microbial ecosystem. It is believe that microbial electrochemical system (MES) could provide a solution to this problem. The aims of this study is to investigate direct separation of CO₂ mixture and reduction of the CO₂ to value-added products in microbial electrochemical cells (MECs). Three-chamber MECs were used where anodic, cathodic and gas chambers were attaching side-by-side and separated by cation exchange membranes (Figure 1). The purpose of the gas chamber was to provide and contain CO₂ mixture for diffusion and reduction process. Electrochemically-active microbes obtained from bioanode of microbial fuel cell (MFC) was introduced into both chambers for fast enrichment. The anode was fed with acetate medium while the cathode was depended on the diffused CO₂ from gas chamber. Bioelectrodes was enriched after 10 days but only fully developed after 6 weeks with fixed voltage 0.3 V applied between the electrodes. Cyclic voltammograms revealed bioanode activity was vital to maintain the functionality of whole system. Biocathode was depended on bioanode to maintain its potential for CO₂ reduction. The MECs could generate maximum current up to 0.43 A/m² at fixed voltage 1.2 V before bioanode lost its oxidation ability. Electrochemical impedance spectroscopy showed internal resistance increased and mass transport of reactant to electrodes’ surface decreased as a result of thicker biofilm. The MECs reached steady state with constant performance after 8 weeks under 3 days per cycle mode. At the end of each cycle, almost 99% acetate was consumed at anode, while 142.6 mg/L propionate was produced at cathode corresponding to 24% of dissolved CO₂ in catholyte. It was also found that CO₂ diffusion slightly decreased due to the water permeation from cathode into gas chamber after weeks of operation.

Figure 1 Schematic of MEC setup in this study
Microbial Electroreduction of itaconic acid with *Clostridium ljungdahlii*

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In the last years, the idea of using electroactive microbial pure cultures to produce organic chemicals or even fuels from carbon dioxide gained increasing interest. This novel technology might be a suitable way to store electrical energy from renewable sources in the form of chemicals. The concept holds the promise to become a useful method for sustainable microbial reduction of precursor chemicals to target compounds within the TMFB Cluster of Excellence. *Clostridium ljungdahlii* is an especially attractive electrosynthesis host organism, since it is highly researched and developed also for other types of biotechnological productions such as syngas fermentation.

To avoid the use of non-renewable \(\text{H}_2\) as reductant our main goals are to understand the microbial electrophysiological processes, manipulate the important pathways to achieve an improved reaction behavior, and utilize them for new biotechnological applications.

In one part of this project we want to enhance the electroreduction activity of the well characterized cathodic biocatalyst *C. ljungdahlii* by controlled evolution. Therefore we constructed a mutator plasmid which gives us the possibility to perform a rapid whole cell evolution. This tool was then used to adapt the strain to process relevant factors.

Another part of this project deals with the genetic engineering of *C. ljungdahlii* to tailor this biocatalyst towards new reduction pathways from itaconic acid (IA) 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 IA are under investigation. One very promising way is the reduction with reductases that are ferredoxin-dependent or -independent.

With all this parts we want to generate an efficient biocatalyst that uses electrons from a cathode to produce biofuels from IA.
Evaluation of different inoculation strategies for electromethanogenesis reactors


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The microbial electrochemical conversion of carbon dioxide to methane (electromethanogenesis) represents an appealing technology to simultaneously achieve: (i) reduction of CO₂ emissions; (ii) generation of a valuable biofuel within a circular economy framework; (iii) storage of renewable energy excess into a chemically stable compound; and (iv) organic matter removal from domestic wastewater. Combining on-site the process of anaerobic digestion (AD) and a bioelectrochemical system (BES) is one of the proposed strategies to scale-up electromethanogenesis (named AD-BES). On the other hand, a similar process can be performed off-site to upgrade biogas (or any other CO₂ waste stream) to biomethane.¹² In all cases, an anodic oxidation must couple CO₂ reduction taking place at biocathode. Depending on reactor configuration and applied cell voltage, the counter reaction may be water or organic matter oxidation, each one with its own advantages/drawbacks (e.g. H₂O is a non-limiting electron donor, but its oxidation produces O₂ decreasing biogas quality). In view of promising future applications of electromethanogenesis, and the different adoptable approaches, we evaluated several scenarios at lab-scale, focusing on inoculation, start-up and operational monitoring. We operated:

a. 2 AD-BES reactors of 1 L volume, with unidirectional graphite fibers electrodes of 26 cm² (projected surface, p.s.), batch-fed with a CO₂ saturated mixture of AD sludge and mineral medium, at an organic loading rate of 8-24 g CODs m⁻³ d⁻¹. Their performances were evaluated in comparison with an AD-BES reactor with no energy input;

b. 2 dual-chamber BES reactors of 485 mL cathode chamber volume, with graphite felt electrodes of 170 cm² (p.s.) and Nafion membrane, batch inoculated with a CO₂ saturated mixture of AD sludge and mineral medium, and then fed with mineral medium at a continuous rate of 0.12 mL min⁻¹. The anode chamber was always operated in batch with 50 mM PBS;

c. 2 single-chamber BES reactors of 714 mL volume, with graphite felt electrodes of 170 cm² (p.s.), equally inoculated and fed at 0.12 mL min⁻¹ with synthetic medium containing 2.5 g L⁻¹ acetate.

The reactors of scenarios (a) and (b) were operated with a 3-electrodes chronoamperometric configuration, poising the cathode potential at -650 mV vs SHE. Both direct and H₂-mediated electromethanogenesis reactions were observed, reaching a biomethane production rate of 15-20 L CH₄ m⁻² cat d⁻¹, in line with literature maxima, while removing 27% of COD. On the other hand, reactors of scenario (c) were operated with a 2-electrodes configuration and applying an external voltage of 700 mV between anode and cathode. First results indicate that this inoculation strategy allows the fastest reactor start-up (both anode and cathode are simultaneously inoculated) and a high CH₄ production.

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Session VI
Microbial ecology
Isolation and characterization of microbes enriched under complex substrate condition in microbial fuel cells

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Microorganisms have the ability to degrade a wide range of compounds for their energy source. These microbes need some terminal electron acceptor to complete the oxidation process. In Microbial fuel cells (MFCs) it is the electrode that acts as terminal electron acceptor from bacteria. In this study we have used Oil seed cake as carbon source under MFC condition for enriching complex substrate utilizing bacteria, simultaneously producing electricity. Of different oil seed cake used, sesame oil seed cake has found to give higher power density with $365\pm 16\text{mW/m}^2$ in MFC. C: N ratio of this oil seed cake was done using CHNSO analyzer showing C: N ratio of 6:1. Enrichment process was carried out for over 120 days by regularly changing the media. Voltage was measured simultaneously maximum voltage of $650\pm 21\text{mV}$ was achieved within 24hrs. Enriched microbes were then grown anaerobically and isolated them into pure culture. Around 30 different bacterial species were isolated from the enriched electrode. Phenotypic studies have revealed the morphology of isolates. Genotypic studies were done by amplifying 16S r DNA region of the isolates which revealed wide range of bacteria belonging to different class and genus. Consistency and efficiency of each isolates were carried out in MFC. Colonization, biofilm formation and other type microbial interactions were clearly understood under SEM. This study has done to explore the microbes growing under complex substrate condition for bioelectricity generation which revealed different bacteria and their interaction during electron transfer in MFC.
Chemical or physical pretreatment for selectively enriched exoelectrogens on bio-anode

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Microbial fuel cells (MFC) utilize the exoelectrogens as the catalyst to transform chemical energy into electricity directly, so this process could generate renewable energy and carry out waste treatment at the same time. When electrodes formed thicker biofilm with the higher proportion of exoelectrogens, it generates higher electricity. Although the exoelectrogens is abundant in sludge or sediment, most of the electrodes contained little exoelectrogens after inoculation. Therefore, to enrich the exoelectrogens on the electrode is the critical technique for MFC development. In this study, we utilized paddy soil as inoculums and acetate as carbon sources in single chamber air cathode microbial fuel cell. While the current was stable, the anodes were treated with the chemical or physical process to diminish the non-exoelectrogens. 0.2mM 2-Bromoethanesulfonate (BES), or chloroform (CHCl₃) was added into MFC for the chemical pretreatment process. The biofilm was scratched from bio-electrode and dispersed in a new medium with a new electrode for the physical treatment process. Compared with the control system, the current could be postponed to 200 hrs for the scratch/dispersed and CHCl₃ addition process. On the contrary, BES process generated the lowest current. Although the scratch and dispersive method was tedious and need the skillful operation in an anaerobic glove box, the scratch/dispersed method got the highest current and coulombic efficiency after 4 batches (10 days). According to the phylogenetic analysis, the phylum of geobacteraceae accounts for 91% in scratch/dispersion process, 89% in the CHCl₃ process, 64% in BES process and 61% in control. It also reveals that the higher coulombic efficiency was contributed by the higher ratio of exoelectrogens.

<table>
<thead>
<tr>
<th>Methods</th>
<th>Columbic efficiency (%)</th>
<th>Highest current (mA)</th>
<th>Phylogenetic phylum – proteobacteria (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Scratched and dispersed</td>
<td>24.33</td>
<td>0.98</td>
<td>91</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>19.28</td>
<td>0.94</td>
<td>89</td>
</tr>
<tr>
<td>BES</td>
<td>20.34</td>
<td>0.88</td>
<td>64</td>
</tr>
<tr>
<td>Control</td>
<td>20.35</td>
<td>0.88</td>
<td>61</td>
</tr>
</tbody>
</table>
Exploring the molecular mechanisms of siderophore recycling in Shewanella

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The production of siderophores, small chelating compounds with high affinity for iron allows microorganisms to survive in iron/metal limiting environments. Despite the metabolic costs of their production, siderophores often serve as public goods for the bacterial community, being produced not only for their direct utilization but also as an altruistic behavior and hence scavenged by non-producing bacteria. The ability of producing and/or utilizing siderophores is an important survival and even a virulence trait illustrating the complexity between the organisms that make up the microbial community and their environment.

The siderophore pathway starts with the release of the siderophores in the apo-form which are later incorporated via specific receptors as ferric complexes. To be utilized, iron must be released from the ferrisiderophores and multiple routes have been proposed, including the hydrolysis of the siderophore by esterases and siderophore recycling. Siderophore recycling is the prevailing mechanism and it is mediated by siderophore interacting proteins (SIPs). Alltogether, two families can be distinguished: 1) a Siderophore Interacting Protein (SIP) family characterized by the presence of a FAD cofactor and the use of NADH or NADPH as the electron donors; and 2) a Ferric Siderophore Reductase FSR family, which includes an unusual [2Fe-2S] protein, Fhuf from E.coli that is able to reduce ferrioxamine E.

Here we describe the structural and biochemical characterization of two representative proteins from each family of SIPs from different species of Shewanella.
Hyperthermophilic Electrosynthetic Microbial Biodiversity from Deep Hydrothermal Vents

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In the 1980s was discovered in total darkness an unknown and luxuriant biocenosis, developed around vertical chimneys: the deep hydrothermal vents. Their observation have shown a microbial ecosystems occupied by highly diversified (hyper)thermophilic microbial populations which grow in dense biofilms. The primary production of organic matter would result from chemosynthetic microorganisms using the energy of reduced compounds (CH₄, H₂, H₂S) and metallic minerals from the hydrothermal fluid to create a complex food web. This raises the question of the importance of electro-active microorganisms in the development of biocenosis, especially since a recent study have shown the presence of a continuous electric current within hydrothermal vents structure.

In order to provide answers to this question, we undertook to develop and optimize a prototype of a bio-electrochemical reactor operating at high temperature (≈80°C) to study the extreme electro-active biodiversity resulting from hydrothermal vent. From an inoculum of a hydrothermal chimney (Lucky Strike, Atlantic), we carried out a targeted enrichment in cathodic condition using the electrode as an electron source, CO₂ as the sole carbon source and different electron acceptors. After molecular and microscopic analyses of the biofilm on electrode we have demonstrated the enrichment of Thermococcales, Aquificales, Archaeoglobales, Desulfurococcales and Thermales with nitrate as electron acceptor (tests with other electrons acceptors and on these isolated microbes are in progress). This enrichment is directly correlated with the production of organic compounds which may offer promising valorizations in biotechnology. Moreover, the growth of autotrophs and heterotrophs on an electrified solid support as the sole source of energy suggest the formation of a complex trophic network. This result gives a solid postulate of the importance of microorganisms called "electrolithoautotrophes" in the development of the biocenosis found in the hydrothermal sources.
Oxygen-Reducing Biocathodes: Relation to the Nitrogen Cycle and Characterization of Bacterial Communities

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Microbial fuel cells are able to generate electricity while treating wastewater. Dioxygen is very attractive for reduction reactions at the cathode of fuel cells because it is a strong oxidant freely available in large quantities. Microbial communities at oxygen biocathodes are relatively less documented in comparison to those at bioanodes. The diversity of communities involved in microbial biocathodes may explain the wide potential scale at which oxygen can be reduced (from +0.2 to -0.4 V vs. Ag/AgCl), as reported since the pioneering work of Bergel et al. The work presented here report the development of a reproducible and stable (> 8 months) microbial biocathode reducing oxygen by using an aerobic inoculum from a wastewater treatment plant (Beaurade, Rennes, France). The resulting oxygen biocathode is involved both in the oxygen reduction and in the nitrogen cycle as demonstrated by (i) electrochemical studies (see Figure left) (ii) chemical determination of nitrogenous species and (iii) metagenomics analysis of the biofilm bacterial community (see Figure right).

Metabolically active microbial populations in an anaerobic digestion-electromethanogenic microbial electrolysis cell integrated system

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A lab-scale AD, which was fed in continuous mode with pig slurry, was connected in series with the bioanode compartment of a two-chambered MEC. In turn, the MEC electromethanogenic biocathode (EB) was poised at -800 mV vs SHE and fed with CO2 to increase the methane production of the system. The diversity of total versus metabolically active microbial community was evaluated simultaneously at DNA and RNA level respectively. Microbial community assessment of AD biomass, bioanode and biocathode biofilms was performed by Illumina-based 16S sequencing (MiSeq) after a 222-day operation. Results revealed that 14% and 31% of the active eubacterial and archaeal operational taxonomical units (OTUs), respectively, were shared by the three communities (Figure 1). Interestingly, 12% and 6% of the active eubacteria and archaea were exclusive of the EB population. Active EB population were mainly dominated by the Methanobacteriaceae family, with predominant ribotypes belonging to the hydrogenotrophic Methanobrevibacter and Methanobacterium genera. Despite the connexion established with the recirculation loop between the AD and the bioanode compartment, both communities shared only 29% of metabolically active eubacterial population, and 47% among the archaeal active population. This study gives new insights on the diversity and interactions among the different metabolically active microbial communities of the AD-MEC-EB integrated system.

Figure 1. Overlap of the AD, bioanode and biocathode communities of eubacteria (a) and archaea (b).

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Implications and mitigation of methanogenesis in electroactive microbial communities: A comprehensive review

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Microbial electrochemical technologies (METs) rely on electroactive biofilms that often consist of diverse microbial communities. In contrast to pure cultures of electroactive bacteria, mixed cultures can offer several advantages such as greater resistance to environmental fluctuations and the ability to utilize a broader array of substrates. In many MET applications, however, competition for resources between electroactive bacteria and other microbes often leads to decreased columbic efficiencies and challenges in reactor operation. Methanogens are a group of archaea that have shown to be a prominent nuisance in many MET studies due their affinity for similar substrates and environmental conditions as electroactive bacteria.

The aim of the work is to evaluate the impact of methanogenesis in a variety of MET applications. An in depth analysis of the effect of methanogens on power production in microbial fuel cells (MFCs) and hydrogen generation by microbial electrolysis cells (MECs) will be presented. Furthermore, the implications of methanogenesis in emerging MET technologies such as microbial desalination cells (MDCs) and microbial electrosynthesis (MES) will be discussed. A detailed comparison of the various methods that have been used to mitigate methanogenesis in METs will also be provided. This will include an comparison of the effectiveness of chemical treatments such as 2-bromoethanesulphonate, sodium nitrate, and nitroethane as well as operational techniques such as variations in external resistance, drops in temperature, and the introduction of oxygen. The insight provided by this work will help further understanding of the microbial ecology in electroactive biofilms as well as deliver a useful compilation of mitigation techniques that will aid in future MET design and operation.
Transcriptomics of the cable bacterium *Candidatus* Electronema nielsenii

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Cable bacteria are filamentous members of the Desulfobulbaceae family capable of conducting electrons via long distance electron transport. They couple anodic sulfide oxidation and cathodic oxygen or nitrate reduction over centimeter distances in sediments. This process, named electrogenic sulfide oxidation (e-SOx) leads to the development of a suboxic layer, devoid of both sulfide and oxygen. Although supported by biogeochemical analyses, e-SOx as their means of energy metabolism remains paradoxical, as cable bacteria are phylogenetically affiliated with sulfate reducing bacteria. Accordingly, cable bacteria genomes reconstructed from both single filaments and metagenomes showed the complete sulfate reduction pathway and none of the canonical sulfide oxidation pathways. Reversed steps in the sulfate reduction pathway are therefore proposed to be part of the sulphide oxidation pathway. To test this hypothesis, and to evaluate potential differential gene expressions in the different biogeochemical zones, gene expression of the candidate species Electronema nielsenii via RNA-Seq is compared between sediment zones with either anodic sulfide oxidation, cathodic oxygen and nitrate reduction, or electron transmission only.
Novel survival strategy of sulfate-reducing bacteria using biogenic mineral and extracellular electron transfer mechanism

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A microorganism isolated from the surface of anode in a microbial fuel cell produced a black precipitate under anaerobic conditions. According to the nucleotide sequence of 16S rRNA gene, the microorganism was identified as Desulfovibrio sp. (named it strain HB). EDX, XRD, and SEM analyses revealed that the black precipitate was thin and rosette form and was identified as makinawite (FeS). Electrochemical analyses demonstrated that the biogenic makinawite produced by strain HB was a rechargeable like a capacitor; charge and discharge capacitances were 160 ± 35 mAh g⁻¹ and 50 ± 14 mAh g⁻¹. Strain HB converted lactate as electron donor to acetate under sulfate-reducing conditions, which is known as the incomplete oxidation. When strain HB was inoculated into a MFC supplied with lactate as sole electron donor, current production was observed and produced acetate was consumed. The current production was enhanced by addition of biogenic mackinawite into anode electrode. Electrochemical analyses demonstrated that extracellular electron transfer (EET) of strain HB was indirect transfer from cell to electrode or the biogenic mackinawite through mediators. These results suggested that strain HB changed metabolic pathway corresponding to electron acceptors, sulfate or solid conductors. We do not know how strain HB initiates the EET yet. However, strain HB uses biogenic mackinawite as an electron pool, suggesting the novel survival strategy of sulfate-reducing bacteria using biogenic conductive mineral and expressed EET mechanism.
Electrotrophic Activities of Iron-Oxidizers derived from Marine Hydrothermal Environments

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Electroautotrophy has been extensively studied in the interests of biocathodic fuel/chemical production. On the other hand, studies have indicated potential occurrence of this microbial activity not only in engineered systems, but also in natural, particularly in marine systems. For example, a recent study reported spontaneous electrical current generation through conductive mineral deposits around deep-sea hydrothermal vents. The study suggested that the mineral-seawater interface could harbor electrotrophic microbial activities involving electron uptake from the conductive mineral surface coupled with reduction of oxygen dissolved in the seawater (Yamamoto et al., 2017 ACIE). Based on such findings, we have hypothesized that some of the microbial components that reside in hydrothermal environments are capable of electroautotrophy. Thus, we have investigated potential electroautotrophic metabolisms and biomass productions of microbes derived from associated environments by using electrodes as the extracellular electron donor analogous to conductive minerals. This presentation will discuss the experiments of iron-oxidizing isolate derived from a deep-sea hydrothermal field. Reactor tests showed that inoculated cells selectively colonized on electrodes poised at potentials comparable to, and lower than, the estimated redox potential of Fe(OH)$_3$/Fe(II) couple with steady cathodic current. Also, metabolic activity of colonized cells were likely sustained by the cathodic electron supply. These results suggested the electrotrophic activities in the colonized cells.
Does chance play a determining role in biofilm formation?

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It can hardly be overemphasized the importance of understanding biofilm formation and biofilm properties when aiming at developing bioelectrochemical systems. In this regard, one of the main challenges comes when trying to develop controlled electrogenic cultures, especially when using environmental inocula which typically display a high microbial diversity and richness. This study aims at investigating the relevance of stochastic effects to the development of biofilms. To this end, 15 identical monocameral microbial electrolysis cells (MECs) were inoculated in the same conditions and using the same inoculum (freshwater sediment) (Fig. 1A). Preliminary results point to quite significant differences in the current profiles, showing at least two distinct trends (named as Trend 1 and Trend 2 in Fig. 1B). This will be contrasted with microbial community analyses by means of high throughput sequencing of 16S rRNA gene for eubacterial population both at the end of the first start-up cycle, and when reactors achieve steady state conditions. Moreover, and for those reactors displaying similar current profiles, RNA analyses will be performed to assess to which degree active communities differ from one another. This will be complemented with Q-PCR for 16S rRNA gene analysis to quantify bacterial abundance. Results from high throughput sequencing reveal that the developing microbial populations are, at least during the initial stages, highly sensitive to the inoculation conditions, which is in agreement with the diverging current profiles.

Figure 1. A) Laboratory set-up, and B) The two current trends (Trend 1 and Trend 2) observed in this experiment (they are exemplified only by 3 current profiles each for clarity issues).
Mixed cultures of *Pseudomonas aeruginosa* PA14 and 2,3-butanediol fermenters for current generation in BES

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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. Thus, study of model defined co-cultures can be a key to investigate and acquire understanding of important co-culture relationships. Recently, a synergistic interaction regarding current production in a defined co-culture of the phenazines redox mediator producer *P. aeruginosa* (providing the electron shuttles) and the sugar fermenter *E. aerogenes* was observed [1]. The aim of our work is to investigate whether the synergistic effect between *P. aeruginosa* and *E. aerogenes* was unique or a more common phenomenon. *P. aeruginosa* produces different types of phenazines, e.g. phenazines carboxylic acid (PCA) and pyocyanin, which are both known to have an antimicrobial effect. However, some organisms are able to tolerate phenazines or even use them as electron shuttle for their own ends.

First, the interaction of different 2,3-butanediol-fermenting organisms with phenazines is examined in small BES. Then, promising candidates are applied in defined co-cultures with *P. aeruginosa*. First results show that *K. pneumoniae, S. aureus, S. marcescens, B. cepacia,* demonstrate electron transfer to the electrode when supplemented with phenazines. Additionally, initial co-culture growth experiments show that both cultures can grow simultaneously without overgrowing one another.

A major oxygenation event creates a temporal niche for cable bacteria in Baltic Proper sediments

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Cable bacteria are filamentous Desulfobulbaceae able to couple anodic H2S oxidation and cathodic O2 reduction over centimeter distances in the seafloor by conducting electric currents. This metabolism is referred to as electrogenic sulfide oxidation (e-SOx). Cable bacteria have been reported in sediments from a wide range of sites, but their physiological limits as well as the environmental factors that regulate their growth in natural settings are not well constrained. In this study, we investigated if a natural bottom-water oxygenation event, namely a Major Baltic Inflow (MBI), stimulates the growth of cable bacteria in the long-term (~10 y) anoxic sediment of the Eastern Gotland Basin, Baltic Sea.

In April 2016, intact sediment cores were collected at four sites across a depth transect including a permanently oxic site (60 m depth), a permanently anoxic site (130 m), and two sites that experienced a transient oxygenation due to the inflow (170 and 210 m).

Cable bacteria were identified at the oxic and transiently oxic sites, but not at the anoxic site, suggesting that transient O2 availability allowed cable bacteria growth. The highest filament density (42 m cm-2) was found at the 170 m site, where a 6.3 mm zone depleted in both O2 and free H2S, in absence of bioturbation, suggests a substantial impact of cable bacteria metabolism on sediment biogeochemistry.

In 2017, sediment pH and electric field microprofiles compatible with ongoing e-SOx indicate that cable bacteria were still active, likely supported by the persisting hypoxic conditions. Our data demonstrate that cable bacteria can exploit transient hypoxic niches as induced by a MBI. The bottom water O2 levels (< 15 μM) are the lowest reported for cable bacteria growth, and this expands our understanding on their potential environmental distribution.
Different MFC cathodic architectures induce changes in electroactive biofilms

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We propose a comparison of different microbial communities enriched in four different designs of MFCs: gas diffusion layer (GDL) cathodes exposed to MFC1 air; MFC2 photosynthetic cultures; MFC3 water; and MFC4 air-cathodes separated by a terracotta membrane. All anodic chambers were inoculated with the same swine-farming wastewater and run for over 100 days. Electrochemical and microbiological tools (Illumina 16S rDNA based sequencing, confocal microscopy, biofilm cryosectioning and statistical approaches) were used to explore anodic and cathodic electroactive biofilms in each MFC type.

In all cases, microbiologically-mediated mechanisms improved oxygen reduction reaction (ORR), as compared to abiotic condition. Different dissolved oxygen (DO) concentrations in MFCs and the presence of the terracotta membrane (between the anode and the air-cathode) in MFC4, not only influenced the MFCs performances and biocathodic communities, but also the anodic biofilms. Different DO concentrations in MFC1, MFC2 or MFC3, influenced the selection of differently abundant aerophilic communities in the cathodes, inducing deep changes in ORR. In MFC1, the presence of sulfur reducing bacteria (Desulfuromonas) and purple non-sulfur bacteria suggested that the recirculation of sulfur compounds could shuttle electrons to sustain the reduction of oxygen as final electron acceptor. Photosynthetic cultures in the cathodic chamber of MFC2 supplied high DO level (up to 16 mgO2 L⁻¹) which sustained aerobic microbial community in MFC2 biocathode. Halomonas, Pseudomonas and other microaerophilic genera reached more than 50% of the total OTUs. Low DOs concentrations (1-3 mgO2 L⁻¹) limited cathodic ORR performances in MFC3.

In MFC4, the non-conductive terracotta membrane placed between anode and cathode interfered with the selection of electroactive communities in both bioanodic and biocathodic biofilms. Electroactive genera were more abundant (3- to 5-fold) in control membrane-free cathodes than in terracotta-attached biofilms (mainly Geobacter, Pseudomonas, Desulfuromonas and Clostridia MBA03). Electroactive genera were also slightly more abundant in anodic biofilms of membrane-free MFCs (mainly Archaea, Desulfuromonas and Clostridium sensu stricto 1).
Identification of diverse potentially electroactive bacteria in sediment and groundwater through genomic analysis of consortia enriched in microbial electrochemical cells

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There is growing recognition of the ability of some microbes to interact electronically with their external environment and of the importance of these interactions in biogeochemical cycles. Most current knowledge about extracellular electron transfer (EET) comes from studies of a small set of model organisms investigated in pure culture. There is great need to explore EET in the context of natural microbial communities. In addition to providing basic insight regarding metal transformations in the environment, such studies can identify electroactive microbes for biotechnology applications. Here we used microbial electrochemical cells with poised graphite electrodes serving as the electron acceptor to enrich for consortia of anode-respiring bacteria. The system was inoculated with anaerobic sediment and groundwater from a genomically well-studied aquifer near Rifle, CO, and amended with acetate. Anode biofilms and planktonic samples were characterized by genome-resolved metagenomics. We reconstructed draft-quality or near-complete genomes for 84 Bacteria and 2 Archaea that represent the majority of organisms present in 28 samples. A novel Geobacter sp. with 72 multiheme c-type cytochromes (MHCs) was the dominant electrode-attached organism. In addition, our study implicates numerous other bacteria in anode respiration. These include members of the phyla Actinobacteria, Ignavibacteria, Chloroflexi, Acidobacteria, Firmicutes and Burkholderiales, most of which were present at high abundance in anode communities and possessed genomes encoding cytochromes with ≥10 heme-binding motifs as well as loci including porin cytochrome complexes. Our results identify a small subset of the vast diversity of organisms previously detected in the Rifle subsurface environment that may be electroactive and involved in mediating biogeochemical redox transformations.
Session VII
Novel applications of microbial electrochemical systems
Microbial electrochemical sensor for online ammonia monitoring of waste streams

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Ammonia, known as a notorious pollutant, could cause the lake eutrophication and may inhibit some biological treatment processes as well. In this research, a new biosensor consisting of a microbial electrolysis cell (MEC) and a nitrification reactor was designed for on-line ammonia monitoring in waste streams. In this new biosensor system, firstly wastewater enriched with ammonia was oxidized to nitrate in nitrification stage, and afterwards, the effluent contained with nitrate was pumped into cathode chamber of MEC, where the nitrate was reduced with accepting electrons. The performance of the biosensor was first tested with synthetic ammonia-rich wastewater. The results showed the conversion from ammonia to nitrate achieved a high level with the slope of 0.9976. The current (0.5130 to 3.906 mA) linearly ($R^2 = 0.9419$) changed with a stepwise increasing of ammonia levels from 0 to 62.1 mg NH$_4^+$-N/L. At different applied voltage and different pH conditions, the slopes of line changed whereas the good linear relationship was always observed between current and ammonia levels. Moreover, the electrochemical cell was able to remove the interference of other possible electron acceptors (e.g., NO$_3^-$-N) in the wastewater. At last, the biosensor was tested with real waste streams and the results showed no significant difference between the values monitored by testing kits and that obtained from the biosensor. For the best of our knowledge, this study firstly attempted to illustrate the feasibility of a microbial electrochemical sensor for ammonia monitoring. In light of the simple and efficient operation, the biosensor showed great promising potential for online, inexpensive, fast and reliable ammonia detection in various waste streams.
Semi-Artificial Photosynthesis in Biocatalyzed photoelectrochemical System

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Photosynthesis is the sustained biochemical process that enables most of the life on earth. Yet, has limitations in terms of converting the incident quantum of light for excitation of the pigment molecules which in turn produce biomolecular energy (ATP and NADPH). The antennae of chlorophyll molecules show maximum light absorbance only at ~450 nm (blue light). In this study, we propose to overcome light dependent state of photosynthesis, whose cause can be attributed to the reversible changes occurring in the chlorophyll molecules and reactive oxygen species formation. Biocatalyzed photoelectrochemical systems were designed and used to evaluate the experimental objective of improving photosynthesis. The electrochemical input acts as an additional source of energy for excitation and/or water splitting in PS II. Different potentials were applied by a potentiostat in both light and dark conditions to photosynthetic microalgae, cultivated under autotrophic mode of nutrition at pH 7 with control. Biomass, photochemical efficiencies, oxygen evolution and chlorophyll a/b were analyzed in both light and dark conditions along with pH monitoring during operation. The observations suggest that applied potential can overcome certain limitations of photosynthesis. As the quantified results show enhanced biomass growth (potential source for value added compounds like lipids, algal oil, etc.) and higher carbohydrate synthesis which also correlates to higher carbon dioxide sequestration advocating cleaner and greener environment by the novice application of BES.
Combining the electroactivity of phenazine-producing \textit{Pseudomonas putida} KT2440 with rhamnolipid production in a bioelectrochemical system

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Sustainability of energy and environment is one of the big challenges in the world. The environmental and energy crisis urge the need of sustainable bioenergy-resources exploration and development. One of the biotechnological approaches to implement novel means of converting and conserving resources are Bioelectrochemical Systems (BES). Currently, studies of natural microbial electron mediators, such as phenazines, propose potential strategies in bioelectrochemical processes for production of many beneficial bioproducts. Rhamnolipids, which carry eco-friendly properties as biodetergents, are one example of these microbial products. The metabolic engineering of \textit{Pseudomonas putida} KT2440 as a recombinant host for rhamnolipid production has been already developed under aerobic conditions (Tiso \textit{et al.}, 2016). However, costly aeration and the subsequent problems with strong reactor foaming are still technically challenging to be overcome. Thus in this work, we are combining a phenazine-producing \textit{P. putida} KT2440, which can discharge metabolic electrons to an anode as alternative electron acceptor, with rhamnolipid production under oxygen-limited condition in BES. To this end, a new engineered strain of \textit{P. putida} KT2440 expressing the genome integrated \textit{rhlAB} genes to synthesize mono-rhamnolipids and the plasmid-encoded \textit{phzA-G} cluster 2 genes to synthesize phenazine-1-carboxylic acid (PCA) and pyocyanin (PYO) has been generated to produce rhamnolipids in BES. A first characterization is presented here. Generally, this work shows the first application of the new phenazine-producing \textit{P. putida} KT2440 for foam-free biosurfactant production.

Conductive carbon nanomaterials inhibit methanogens

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Methanogenesis is a biological process that produces methane from organic matter, and is widely used in anaerobic digesters for recovering energy from organic wastes. In this process, diverse microbes thrive under metabolic interactions, in which syntrophic interactions between fermentative bacteria and methanogens are the bottleneck steps. Recent studies have shown that supplementation with conductive nanomaterials, such as activated carbons, facilitates their syntrophic interactions and enhances methanogenesis. In contrast, other conductive nanomaterials, such as carbon nanotubes, are known to exhibit antimicrobial properties. Further studies are therefore necessary to deepen our understanding of effects of conductive carbon nanomaterials on methanogenesis. In the present work, anaerobic sludge was added with carbon-black (CB) conductive nanomaterials, and effects of CB on methanogenesis from glucose were investigated. We found that CB severely inhibited methanogenesis, in which acetate was accumulated. This result suggests that CB specifically suppresses methanogens. This idea was confirmed by examining inhibitory effects of CB on the growth on acetate of Methanosarcina barkeri and Geobacter sulfurreducens. From these results, we conclude that CB inhibits methanogenesis by specifically suppressing the growth of methanogens. CB is therefore considered to be useful for suppressing methanogens in microbial electrolysis cells for enhanced hydrogen production.
Isolation of exoelectrogens from rice paddy-field soil by using a novel electrode plateculture method

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Recently, electrochemically active bacteria (EAB) have attracted considerable attention in biotechnology studies, yet their diversity and ecology in the natural environment remain largely unknown. In the present study, we developed an electron-acceptor type electrode plate-culture (EAEP) that facilitates efficient isolation and cultivation of exoelectrogens and used it for isolating them from paddy-field soil.

A \textit{Geobacter sulfurreducens} culture was spread onto an acetate-containing agarose solid medium and covered with a fluorine doped tin oxide glass plate that served as a working electrode (WE in Fig. 1). Current was generated in response to the occurrence of red colonies that facilitated the visualization of colonies. No current was observed without inoculation with the bacterium, and no colony was grown under the open-circuit condition.

Exoelectrogens were enriched on graphite electrodes by 4-month operation of rice paddy-field MFC and subsequent acetate-fed electrochemical cultivation at -0.2 V vs. Ag/AgCl. Biofilm microbes on the electrode were suspended in a mineral medium, and the cell suspension was spread on an acetate containing agarose solid medium in EA-EPC. Colonies formed on the solid medium were picked from EA-EPC, and purified by repeated transfer between solid and liquid media. Identification of these colonies by the blast search based on 16S rRNA gene sequences showed that they were relatives of \textit{Geobacter} and \textit{Citrobacter} that include known exoelectrogens. These results suggest that EA-EPC is a useful device for colony isolation of exoelectrogens.

![Fig.1 Schematic diagram of EA-EPC](image)

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Electronic control of engineered gene expression through redox signaling

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The engineering of directional, efficient, and programmable communication between living and nonliving systems has immense potential to harness the distinctive features of each for a variety of applications in both clinical and research settings. However, living cells use small molecules, ions, and protein assemblies to send signals, while electronics utilizes electrons. Here we discuss the use of redox molecules to shuttle information from electronics to engineered bacterial cells. We use electronic control of the state of various redox molecules to which the cells have been engineered to respond through simple native protein-promoter constructs. We show that the signal output (cell response) is dependent on the amplitude and frequency of signal input (electronic charge), allowing for a tunable response. Additionally, the redox molecules allow control of the function of several responses that are not naturally redox-dependent. This work demonstrates the use of redox mediators to control engineered gene expression in a reversible and programmable manner, and furthers technologies that aim to combine living and nonliving systems.
Extracellular transfer of methane-derived electrons to soluble oxidants: experimental findings and envisioned industrial applications

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Anaerobic oxidation of methane (AOM) with sulfate as the terminal electron acceptor is a biochemical process carried out by archaea and bacteria together. Studies with environmental microbes suggested that the archaea responsible for methane oxidation to CO\textsubscript{2} pass methane-derived electrons via direct inter-species electron transfer to their syntrophic sulfate-reducing bacterial partners. We could show that soluble oxidants, such as AQDS, are able to substitute for the bacterial metabolism. Single-cell activity studies demonstrate that the archaea sustain high metabolic rates under these laboratory conditions, whereby the bacterial partners appear not to be needed anymore. Therefore, we have found a biochemical pathway to convert the fuel methane into the fuel reduced aqueous AQDS. Reduced AQDS can be oxidized in a fuel cell to regenerate AQDS and harvest electricity. We plan to implement these processes for the conversion of methane to electricity at ambient temperatures, which may allow for energy efficiencies higher than obtained with gas turbines. The reverse process could be applied to “store” excess electricity by converting it to methane. Such a procedure is highly desirable to utilize electricity from volatile electricity sources, such as renewable energies, at times of energy overproduction.
Integrating microbial electrochemical systems in microalgae ponds for organic-rich wastewater treatment

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Photosynthetic microorganisms (PM) cultures are used to treat wastewater in lagoons or raceway-like photo-bioreactors, where PM and heterotrophic microorganisms (HM) populations reach an equilibrium. The PM-rich sludge can be a source of added-value hydrolysates (e.g. biofertilizers, biostimulants and plant growth promoters) or molecules (e.g. natural dyes, antioxidants, bio-polymers, carotenoids etc.). However in treating organic-rich wastewater, these systems suffer some drawbacks. Over certain COD loads, PM are progressively inhibited, causing anaerobic conditions to prevail over long-term. A viable alternative is assisting PM cultivations by integrating microbial fuel cells (MFCs). PM in double chamber MFCs are addressed as biological oxygen suppliers for the cathodic reaction (P-MFC). The integration of MFCs in algal ponds would offer also advantages in terms of recovery of nutrients from wastewater for PM growth. In these systems, a key issue is the efficient separation of PM from anode respiring bacteria. Lab-scale tests were performed on \textit{Spirulina} cultures. Tubular P-MFCs were operated with carbon-based electrodes to treat swine slurries (12 g\textsubscript{COD}/L) and cultivate PM. A cheap porous separator was employed to separate anodic and cathodic solutions. High COD removal rates (0.65 g\textsubscript{COD} L\textsuperscript{-1} d\textsuperscript{-1}) were obtained within the anodic chamber, with negligible COD losses to the bulk PM culture medium. Photosynthetic dissolved oxygen sustained MFC operation (\(P_{\text{max}}\) around 4 W/m\textsuperscript{2} and \(R_{\text{int}}\) around 40 \(\Omega\)). \textit{Spirulina} growth was of around 40 mg\textsubscript{TSS} L\textsuperscript{-1} d\textsuperscript{-1}. The analysis of anodic and cathodic solutions allowed evaluating the transport of ions across the separator upon MFC operation. Microbiological analyses supported the quality of the \textit{Spirulina} culture. Long-term operation at pilot scales will be evaluated.
On-line monitoring of a microbial electrolysis cell using a simple electrical equivalent circuit model

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A microbial electrolysis cell (MEC) is a bioelectrochemical device, which oxidizes organic materials by utilizing an anode as a terminal electron acceptor and combines electrons and protons at the cathode to produce hydrogen or methane. MEC can be used for treating wastewaters with varying organic content. When treating wastewaters, continuous MEC monitoring is essential to ensure adequate performance. With this regard, the electrical equivalent circuit (EEC) model enables on-line parameter estimation and monitoring of a continuous flow MEC. Such simple EEC model involves two resistances (describing ohmic losses, $R_1$, and activation losses, $R_2$) connected in series with internal electromotive force (EMF). Also, biofilm growth is represented by capacitance (C) connected in parallel with $R_2$. These internal electrical parameters can be estimated numerically by minimizing the difference between the measured and predicted MEC current. Also, the same parameters can be estimated using an analytical solution of the EEC model. In this case, the MEC needs to be operated for a short time (e.g. 1-2 min) with intermittent connection to the power supply. Such intermittent connection is performed at high and low frequencies to estimate $R_1$ (at high frequency) and $R_2$ and C (at low frequency). Also, EMF is estimated by MEC under open circuit conditions. To demonstrate the proposed approach, experiments were carried out at several influent carbon source concentrations. MEC internal electrical parameters were estimated at 6h intervals. The EEC model successfully described electrical dynamics of the MEC and tracked changes in electrical parameters. Owing to the model simplicity, it can be used to develop a real-time MEC monitoring and diagnostics system. Such an on-line tracking system might be essential for successful operation of large scale MEC-based wastewater treatment systems.
Bio-electrolytic sensor for rapid monitoring of volatile fatty acids in anaerobic digestion process

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This study presents an innovative biosensor that was developed on the basis of a microbial electrolysis cell for fast and reliable measurement of volatile fatty acids (VFA) during anaerobic digestion (AD) process. The bio-electrolytic sensor (Figure 1) was first tested with synthetic wastewater containing varying concentrations of VFA. A linear correlation ($R^2=0.99$) between current densities ($0.03\pm0.01$ to $2.43\pm0.12$ A/m$^2$) and VFA concentrations (5-100 mM) was found. The sensor performance was then investigated under different affecting parameters such as the external voltage, VFA composition ratio, and ionic strength. Linear relationship between the current density and VFA concentrations was always observed. Furthermore, the bio-electrolytic sensor proved ability to handle interruptions such as the presence of complex organic matter, anode exposure to oxygen and low pH. Finally, the sensor was applied to monitor VFA concentrations in a lab-scale AD reactor for a month. The VFA measurements from the sensor correlated well with those from GC analysis which proved the accuracy of the system. Since hydrogen was produced in the cathode as byproduct during monitoring, the system could be energy self-sufficient. Considering the high accuracy, short response time, long-term stability and additional benefit of H$_2$ production, this bio-electrolytic sensor could be a simple and cost-effective method for VFA monitoring during AD and other anaerobic processes. The outcomes will expand the application of bio-electrochemical system application.

**Figure 1.** Prototype (a) and schematic diagram (b) of the bio-electrolytic sensor.
Session VIII
Water treatment and bioremediation
Concatenating microbial fuel cells with the two-stage biohythane process for enhanced energy recovery from water hyacinth

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The water hyacinth, *Eichhornia crassipes*, is amongst the world’s most invasive aquatic weed that has detrimental impact over the ecosystem. Many attempts have been made to utilize this weed for diverse application such as paper, food supplements, bioremediation, renewable energy generation etc. The conversion of water hyacinth into bioenergy via anaerobic processes such as dark fermentation, anaerobic digestion and bioelectrochemical systems have gained recent interest due to their dual benefits viz. waste stabilization and energy generation. However, the major drawback of these processes is the low energy output, lower substrate conversion and/or concentrated effluents that do not meet the environmental disposal standard. Systems integration on the other hand helps in addressing these problems and helps in improving the process efficiency and overall energy recovery. In this regard, the present study is focused on integrating the three processes viz. dark fermentation, biomethanation and microbial fuel cells for recovering maximum energy from the water hyacinth feedstock. The efficiency of the individual processes as well as the integrated processes was investigated. The three stage process of dark fermentation-biomethanation and microbial fuel cell yielded 904 L/m³ H₂ (dark fermentation), 311 L/m³ CH₄ (biomethanation) and 16.28 W/m³ power density (microbial fuel cell). The energy recovery from water hyacinth increased from 5.23% in the single stage to 36.32 % in the three-stage system. The overall COD removal was 82.11%. These results suggest that such an integration system would be beneficial for on-site bioconversion of biomass to energy with an additional benefit of waste remediation.

Fig 1: Schematic overview of the integrated systems for enhanced energy recovery
Basic study on water treatment using the microbial fuel cell system

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This research is on the way to the microbial fuel cell systems have involved decreases in COD (Chemical Oxygen Demand) and not reported yet.

The structure of microbial fuel cell system composed of cathode and anode and Proton membrane (5cm×5cm) sandwiched between them. Cathode in the pasted carbon felt the open structure. Anode is a closed structure containing carbon felt and activated carbon. The core of an electrode is the copper wire contacted on carbon felt. Experiment solution is the liquid which mixed 300ml of pure water with 100ml of microbe culture solution (including Rhodopseudomonas palustris of purple non-sulfur photosynthetic bacteria and Lactobacillus sp. of lactic acid bacteria), and 100ml of glucose solution (1N=1 normality).

Microbial fuel cell system 3 pieces (3 cell × 2 pieces and 2 cell × 1 pieces, total 8 cells) and into a tank filled with test solution. After each electrode connected in series, the voltage and current are measured 150 hours. And start and after 150 hours of COD measured by Oxidation with Potassium Permanganate in Alkalinity and Visual Colorimetric Method and Dilution methods. Comparative experiments only measured COD without connecting each electrode.

As a result obtained in 150 hours of max current density 0.0067mW/cm², average current density 0.000432mW/cm² power they had. The measurement result of cod had change of a beam unit after a start and 150 hours. However, compared with comparative experiments, the difference of reduction in COD is not remarkable.

I am considering supply of the instrument which can measure COD quantitatively now.
Influence of Cathode Potential on Organic Degradation and Microbiota in Bioelectrochemical Reactor treating Sludge and Cr(VI) Wastewater

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The bioelectrochemical system (BES) fueled with sewage sludge in the anodic chamber has the advantages of providing steady-going and sustainable electron supply and electricity generation of high power density, where the chemical cathode is more appropriate than the air cathode and biocathode. The anodic feed of sewage sludge with Cr(VI)-laden wastewater as catholyte expands the function of BES to sewage sludge degradation and Cr(VI) wastewater treatment with synchronous electricity generation. To our knowledge, few studies have been reported on the influence of cathode potential on the organic degradation and microbiota in the BES. The aim of this study was to investigate the influence of cathode potential on the organic matter degradation and microbiota in bioelectrochemical reactor (BER) treating sludge and Cr(VI) wastewater.

The BER adopted was the traditional configuration of dual-chamber reactor. DOM and extracellular biological organic matters (EBOM) was fractionated with Amberlite XAD-8 and XAD-4 resins into five fractions: hydrophilic fraction (HPI), hydrophobic acid (HPO-A), hydrophobic neutral (HPO-N), transphilic acid (TPI-A), transphilic neutral (TPI-N). Pyrosequencing was conducted by 454 GS-FLX pyrosequencing system (Roche, America).

Based on the study of BER treating sludge and Cr(VI) wastewater, the conclusions were drawn as follows:

(1). The removal of organic matters by exoelectrogenesis and anaerobic degradation reached the maximum rate of 1220.8±48.3 mg/L·d at the cathodic Cr(VI) concentration of 75 mg/L.

(2). The total organic carbon (TOC) of EBOM was decreased by 75.2% for the BER with Cr(VI) concentration of 75 mg/L, where the HPI, HPO-A, TPI-A, HPO-N and TPI-N were reduced by 73.0%, 54.3%, 71.5%, 92.6% and 97.2%, respectively.

(3). The increase in initial Cr(VI) concentration from 25mg/L to 150 mg/L led to an increase of maximum power density from 3.2 W/m³ to 9.6 W/m³.

(4). Proteobacteria represented a dominant portion (55.8%) of the open circuit community and showed relatively less enrichment (48.8%) in the 75 mgCr(VI)/L-BER. More Chloroflexi were abundant in the 75 mgCr(VI)/L-BER.

Fig.1 Taxonomic classification of pyrosequences from bacterial communities at the phyla and class levels
Investigation of Simultaneous Wastewater Treatment and Nitrate Removal from Groundwater Using Microbial Fuel Cell

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Nitrogenous compounds have adverse effects on human and animal health as well as cause rapid aging of the ecosystem. Nitrate removal from groundwater is a difficult process because of soil porous structure. This system creates transport obstacles. Unlike others, Microbial Fuel Cell (MFC) systems are new and more advantageous approach because they can provide energy production and nitrate removal at the same time. The aim of this study is to investigate the effects of catholyte concentrations, membrane type and operating conditions for nitrogen removal, wastewater treatment and MFC performance.

In this study, carbon paper and nafion were used as a membrane in H-type MFC. Anode side of the system was filled with synthetic wastewater and acetate was used as electron donor on the other hand 30 to 800 ppm of nitrate containing groundwater was used as terminal electron acceptor. Sieve size of 850 – 1000 μm sand particles were added inside cathode compartment to mimic permeable ground. Carbon fiber was used as working and counter electrode. Anode compartment was operated as completely mixed, anaerobic batch and continuous reactors. Cell potentials were recorded with a multimeter. Biofilm formation was visualized with SEM.

The highest nitrate removal efficiency of the system with carbon paper membrane was calculated as 82.8 % at the end of 15 days for the batch reactor. Higher removal efficiency was calculated for the low level nitrate reactor with the 91.6% in 39 days. The highest COD removal efficiency was calculated as 86.2% at the end of 4 days for the batch reactor and 77.4% in 40 days for the continuous reactor. Nitrate removal rates were higher for the nafion membrane reactor with 97.5% and COD removal efficiency was 58.3% for 9 days. The highest cell potential, current density and volumetric power density were measured as 29.5 mV, 7.66 mA/m² and 0.145 mW/m³ for carbon membrane system and 290.5 mV, 75.6 mA/m² and 14.11 mW/m³ for nafion membrane system respectively.
Electrokinetic-assisted phytoremediation of arsenic and antimony

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Soil contamination with metals and metalloids may represent a big threat due to the high toxicity and widespread presence of mining activities. Electrokinetic (EK) process relies on the application of a low level direct current and appears as a promising in situ strategy for contaminated soils. The coupling of EK process with phytoremediation (also known as EK-assisted phytoremediation) is an innovative technique that deserves a deeper knowledge to enlarge the scope of EK application. The EK enhanced phytoremediation aims to use the presence of plants to counteract the effects of the electric current as it brings most of the benefits of a “regular” phytoremediation scheme (e.g. recovery of soil properties and improvement of its structure).

Phytoremediation, using ryegrass and Indian mustard, was coupled with EK and/or phosphate amendment aiming to remediate a mine soil contaminated with arsenic (As) and antimony (Sb). At the end of 15 days, metalloids uptake, biomass, available soil nutrients and enzymatic activities were assessed.

Indian mustard revealed the highest potential to accumulate As and Sb (approx. 65% more than ryegrass). But attending to the higher biomass of ryegrass total uptake may be counteracted. Phosphorus amendment was an important factor on the enhancement of metalloids uptake namely for Indian mustard. EK together with P-amendment provided a slight increase in uptake with ryegrass accumulating more 25% of both metalloids and Indian mustard between 30% (Sb) and 48% (As) compared with plant alone. Available soil nutrients and enzymatic activities changed according to the applied treatment but had a similar pattern between plant species. Phytoremediation coupled with EK and/or phosphate amendment can be considered a suitable combination for the upgrade of mine contaminated areas but its efficiency depends on time constraints.

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Modelling bioelectrochemical systems as a feasible denitrification approach in absence of electron donors

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Bioelectrochemical systems (also known as BESs) are electrochemical cells that utilize the ability of some microbes to exchange electrons generated internally from metabolism of organic substances with external electrodes. Thus, these bacteria are able to catalyze the conversion of chemical energy to electrical energy or vice versa. As environmental science and biochemistry developed and the need for sustainable biotechnology became more pressing, BESs became a widely studied technology in recent years. The scope of this work is to develop a dynamic model based on a thermodynamic framework that allows for an accurate description of the complex processes occurring in a BES system used for the treatment of water contaminated with nitrate, the most common aquifer water pollutant (see figure).

One of the objectives of the model is to successfully describe the competition between bioelectroactive and non-bioelectroactive reactions, as well as to simulate multiple reactions occurring at the same electrode, both in series and in parallel. The model uses mechanistic equations such as conservation laws (mass, charge, energy) and uses modified Nernst-Monod kinetics to describe bioelectroactive reaction rates. Special attention is given to substrate and product diffusion, and the surface concentration of intermediate species is also calculated. The model successfully produces polarization and voltammetry curves expected from literature, and reveals non trivial interactions between the multiple phenomena occurring in the system.
A MES - MFC coupled system for methane production

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Within the recent national project MELOS a bioelectrochemical system will be developed, in which municipal wastewater will be treated in terms of organic pollution and simultaneously the concentration of methane in landfill gas will be enhanced by reduction of carbon dioxide – obtained from the oxidation of organic pollutants and as part of (lean) landfill gas respectively. The first project stage is to develop a suitable biocathode for carbon dioxide reduction to methane in the laboratory and to test the system stability. Contaminants in the landfill gas, which are critical for the microorganism, will be identified in batch tests. Based on analytical studies of municipal wastewater a bioanodic system will be built up and tested in the laboratory. In the next stage biocathode and bioanode will be combined to a microbial electrosynthetic system including CO₂ transfer from anode to cathode chamber.
Simultaneous use of a microbial anode as membrane filter shows high current densities and COD removal with synthetic brewery wastewater

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We report on a novel concept for the integration of bioelectrochemical systems into anaerobic membrane bioreactors (AnMBRs). By operating a conductive filtration membrane simultaneously as microbial anode, the significant COD degradation across the filtration membrane can be converted directly into electricity. In experiments conducted with a 0.1 μm stainless steel membrane using \textit{Geobacter sulfurreducens} in an acetate containing medium, current densities of 6 A m\textsuperscript{-2} and a permeate flow rate of 9 L m\textsuperscript{-2} h\textsuperscript{-1} have been reached after 18 days. During this period, COD degradation up to 200 mg L\textsuperscript{-1} across the membrane was achieved. In ongoing experiments, the system is being tested with synthetic brewery wastewater and sewage sludge, yielding similar current densities in the range of 5 A m\textsuperscript{-2}, but achieving higher COD degradation of up to 450 mg L\textsuperscript{-1} across the membrane. This demonstrates the promising potential of our system to convert excess COD into electricity while increasing removal efficiencies of AnMBRs.
Amor d’água fresca: A case study on the treatment of municipal wastewater using anodic half cells

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Microbial electrochemical technologies (METs) and especially microbial fuel cells (MFCs) are considered to allow energy harvest from the fuel wastewater during its treatment. However, still a lot of studies are based on using either "artificial" wastewater (i.e. of defined composition) or amended wastewater, (i.e. with addition of chemicals like acetate) as well as pre-enriched microbial anodes. In all cases the studies were claiming a transferability of the obtained results to real systems and envisaging possible applicability of the aforementioned strategies to the treatment of municipal wastewater. However, this transferability is at least challenging or questionable. Therefore, in this study we used exclusively amendment free municipal wastewater as inoculum and sole carbon and energy source for potentiostatically controlled anodes. This also allowed that the cathode exerted no influence. It is shown that electrons can be harvested at maximum current densities of 0.010 mA cm⁻². However, the performance of individual reactors is widely dispersed. In weekly cycles using batch systems (with 90 cm² L⁻¹ anode surface) only a minor fraction (<10%) of the available charge from COD-removal was turned into electric current by a highly diverse anodic microbial community. This performance, below those achieved by pre-enriched anodes or exploiting amended wastewater, illustrates the need for more and a clear distinction between fundamental as well application relevant studies.
Activated sludge bioaugmentation: A new boost for hexavalent chromium tolerance in air cathode MFCs

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Microbial fuel cells (MFCs) are devices capable of converting chemical energy from organic fuels directly into electrical energy. The fuel oxidation is provided by micro-organisms known as "electro-active"; these microorganism form biofilms on the surface of the anode and act as a catalyst for electrochemical reactions. The sensitivity of microbial biofilm to heavy metals is a main drawback, so that the most efficient microbial bioanodes operate only in electrolytes of low concentration of Cr(VI), generally less than 1 mg/L. The resulting a limitation of MFCs precludes the scaling up to large-sized industrial wastewater loaded by heavy metals.

The aim of this study was to optimize the treatment of wastewater loaded with hexavalent chromium using air cathode MFCs. Experiments carried out in air cathode MFCs showed a maximum power density of 439.2mW/m² and 18,48 mW/m² when inoculated by activated sludge alone with an initial Cr(VI) of 10mg/L and 30mg/L respectively. However, the activated sludge augmented by Enterococcus faecium and Candia tropicalis, the maximum power densities of 159.7 and 38.71mW/m² were respectively obtained with an initial chromium concentration of 30 mgCr (VI)/L. In parallel, the structure of the biofilms that developed in the carbon felt was imaged by scanning electron microscopy in order to establish a correlation between the electrochemical kinetics and the biofilm architecture. Study corroborated enhanced electron transfer capability of activated sludge owing to the synergistic interaction with dechromatation microorganisms due to augmentation and revealed the great potential of expanding MFCs for diverse waste treatment.
METlands: a new generation of hybrid bioelectrochemical wetlands outperform standard wetlands for treating wastewater at full scale

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Constructed wetlands are natural wastewater treatment systems that have been set up all over the world over the last few decades as a good alternative to conventional systems for the sanitation of small communities. However, their main limitation is related to surface requirements: around 5 m²/p.e. (population-equivalent) in horizontal flow wetlands and 3 m²/p.e in vertical flow ones. Many efforts have been addressed in recent years in order to improved their capacity and, therefore, reducing the surface needed for wetlands' implementation.

In the frame of H2020 iMETland project a hybrid concept between the use of electrically conductive biofilter and the concept of wetland is being validated. A first 20 m² - surface METland (0.4m²/p.e.) has been constructed and operated in CENTA (Seville, Spain) for more than one year of continuous operation (6m³/day). The system can operate either in upflow (UF) or downflow (DF) mode. The first mode involves the flooding of all pores in the filtering bed and thus, the insaturation of anoxic/anaerobic conditions. On the contrary, under the downflow mode the filter bed is considered aerobic. On a second stage, larger iMETlands units (40 m²) are currently validated. Those METlands include probes and sensors including electricity harvesting biosensors for on-line monitoring of the system, introducing the "i" component to this hybrid unit. All electronic devices are powered by solar panels so the iMETlands unit can be operated off-the-grid. All the information gathered will be employed in the modelling of the system which will help us to understand this innovative WWT process as a whole as well as to make predictions. Concerning the general performance of the iMETland unit, it is observed that the EU requirements (Directive 91/271/EEC) for TSS, and BOD5 are easily met under the different operating conditions.

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