**MFC Experiments in the Workshop**

In the following, the experimental procedures of two different microbial fuel cells experiments will be presented. We decided to do these two different experimental set-ups to show you different ways by which we can harvest electrical energy out of microbial metabolic processes and at the same time to keep the experimental requirements as simple as possible. In MFC research, however, the utilization of microbial biofilms, that are in direct physical and electronic contact with the electrode, is more common. The start-up phase of such biofilm based MFC anodes normally takes days or weeks and therefore this type is not realizable within this workshop (but might be suitable for a long term school project). The scientific background of the different principles has been extensively discussed above. Half of the group will be working at one MFC experiment, respectively. After setting it all up, you are asked to take a look around and learn about the other experiment.

*The two different MFC set-ups (+ continuous flow biofilm MFC)*

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Materials and Experiment Preparation

- All experiments will be conducted in modified model MFC cells commercially available at the NCBE, University of Reading, U.K. ([http://www.ncbe.reading.ac.uk/](http://www.ncbe.reading.ac.uk/); [http://www.ncbe.reading.ac.uk/NCBE/MATERIALS/MICROBIOLOGY/fuelcell.html](http://www.ncbe.reading.ac.uk/NCBE/MATERIALS/MICROBIOLOGY/fuelcell.html))
- The model MFC sets contain the acrylic MFC bodys, screws to assemble them, rubber gaskets, an ion exchange membrane and carbon cloth material for the preparation of the electrodes.

To increase the power output of these small reactors we doubled the anode volume by stacking two anode compartments around one cathode. (Means, we made one MFC out of two sets.)

- All electrode preparation and modification has been done in advance to the workshop (+ one additional set of fresh electrodes for each set):
  - carbon cloth has been cut to right size for cell compartments;
  - to pieces of carbon cloth have been glued to a graphite rod for external connection of the electrodes using conductive carbon cement;
  (For the preparation of the electrodes at the school, it is also possible to cut the carbon cloth that way that a thin stripe of cloth can be led through the
connection whole in the acrylic body and this may be used for the external connection.)

- the anode carbon cloth of Exp.#2 and the cathode carbon cloth electrodes for Exp.#1 have been coated with the catalyst platinum (see below).

- Another useful supplier of fuel cell self-making and testing equipment (e.g. small fans or flashing lights) is the Fuel Cell Store (http://www.fuelcellstore.com).

Preparation of catalytic electrodes (done in the Angenent Lab):

Platinum coating:

- prepare 100 mL of a 10 mM solution of potassium hexachloroplatinate (IV) (K₂[PtCl₆]) in 0.1 M hydrochloric acid;
- pour platinum salt solution into an electrochemical cell, equipped with the carbon cloth electrode as working electrode (WE; this electrode will be layered with elemental platinum), a graphite rod counter electrode (CE) and a silver/silver chloride reference electrode (RE); it is important that WE and CE do not contact each other;
- switch on potentiostat and instrument software;
- set to current over time technique (chronoamperometry) and chose -0.6 V reduction potential for a 300 s run;
- start run and the following reduction reaction will happen:

  WE: \[ \text{Pt}^{4+} + 2 \text{H}^+ + 6 \text{e}^- \rightarrow \text{Pt} \text{ (0)} + \text{H}_2 \]

  CE: \[ 6 \text{Cl}^- \rightarrow 3 \text{Cl}_2 + 6 \text{e}^- \]

  \[ \text{H}_2[\text{PtCl}_6] \rightarrow \text{Pt} + \text{H}_2 + 3 \text{Cl}_2 \]

Polymer coating of platinum coated anode in Exp.#2 (protection layer):

To protect the sensitive platinum catalyst from poisoning in the microbial solution, the anode for Exp.#2 requires the coating of the platinum catalyst with a protecting polymer (polyaniline).
• prepare a 0.1 M aniline solution in 0.1 M sulphuric acid;
• pour solution in similar electrochemical cell as above;
• set instrument to cyclic voltametry and set parameters to starting potential -0.2 V;
  positive reversal potential 1.2 V, negative reversal potential -0.2 V; scan rate 0.02 V s\(^{-1}\)
  and number of full cycles 10 (20 segments);
• if you start the run, a polymer will be formed on the electrode which changes its colour
  from green to blue while changing from the reduced to the oxidized state.

**Preparation of required solutions:**

**Catholyte – ferricyanide:**

- prepare a 100 mM solution of potassium ferricyanide (8.23 g/ 250 mL).

**Catholyte – phosphate buffer for oxygen reduction cathode:**

- prepare a 100 mM phosphate buffer solution, pH 7 (50 mM K\(_2\)HPO\(_4\), 50 mM KH\(_2\)PO\(_4\)).

**Anolyte – bacterial growth medium for mixed bacterial soil community**

- for 1 L DI water add:
  
  - 2.0 g NH\(_4\)HCO\(_3\)
  - 3.6 g KH\(_2\)PO\(_4\)
  - 0.1 g MgSO\(_4\) x 7 H\(_2\)O
  - 0.01 g NaCl
  - 0.01 g Na\(_2\)MoO\(_4\) x 2 H\(_2\)O
  - 0.01 g CaCl\(_2\) x 2 H\(_2\)O
  - 0.015 g MnSO\(_4\) x 7 H\(_2\)O
  - 0.00278 g FeCl\(_2\)
  - 2 g yeast extract
  - 5 g glucose

- adjust pH to 6.5 ± 0.5 and autoclave (alternatively for school lab: use freshly boiled,
  cooled down water for preparation)

!! Soil culture must be pre-grown over night to be active during experiments!! (see below)
Anolyte – bacterial growth medium for biofilm MFC (additional experiment)

- for 1 L DI water add:
  - 0.8 g Sucrose
  - 0.1 g Yeast Extract
  - 0.033 g NH₄Cl
  - 0.06 g K₂SO₄
  - 0.033 g FeCl₂·4H₂O
  - 0.011 g Iron(III) Citrate
  - 0.5 g NaCl
  - 0.1 g KCl
  - 0.1 g CaCl₂
  - 0.1 g MgCl₂·6H₂O

What else do we need?

- 2-3 large syringes for each MFC set-up to feed the cells with anolyte (2 chambers)/
  catholyte solutions;
- glassware for preparing and storing the solutions;
- tube connectors and matching tubes to connect syringes to MFC cells;
- aquarium air pumps for the aeration of the phosphate buffer catholytes in Exp. #2;
- fertile soil (without chemical fertilizer) as a source of spore forming bacteria; a glass
  petri dish for the heat pre-treatment of the soil;
- handheld multimeters for the observation of the cell potential and current;
- a tray, to prevent spill of solutions (large enough to place the whole set-up in it);
- “real” loads, e.g. LED light or small fan;

and maybe for the school:
- different ohmic resisters to check relation: potential – resistor – current (ohms law) (this
  test is not possible during the workshop because of lacking time).
Performing the MFC experiments

General:

- check that all required things are available;
- assemble the MFC cells according to explosion figure on page 2 (without J-cloth) only that we use three compartments: anode/ cathode/ anode (separate chambers by membrane and use gaskets on both sides of the membrane to prevent leaking);
- make sure to chose the right electrode for the respective experiment: is catalytic modification required or not??
- attach tubings via tube connectors to influent (side, bottom) of the MFC chambers.

Experiment 1

- !! anodic feed solution must be prepared and inoculated with soil one day in advance!!
- put some fertile soil in a glass petri dish and heat in uncovered at approx. 120 °C for 1 hour in an oven;
- fill anodic feed solution in a sealable bottle, add 1 tsp heat pre-treated soil;
- grow over night at 37 °C or somewhat longer at room temperature (solution should get cloudy and start foaming/bubbling);
- day of experiment: if not yet done, prepare the cathodic phosphate buffer;
- add 0.3 mL 20 mM methylene blue synthetic electron mediator to each anode compartment (through top port);
- attach thin needle to the tubing of an aquarium air pump and put the needle into the effluent (top) port of the cathode chamber; purge slightly with air;
- connect handheld multimeter in parallel to MFC to investigate the cell potential development over time; (do this before the solutions are filled in the chambers!)
- fill two large volume syringes with catholyte solution and anolyte (as fast as possible to limit aeration) and connect them to the respective tubings; fill cell with both solutions (cathodic solution first);
- observe cell potential at multimeter and if it is significant, directly connect MFC to LED device in short circuit. The LED will flash for a short time until the potential drops below the required voltage that is necessary to power the LED (over 0.45 V required). Disconnect the LED and wait for recovery of the cell potential and try it again.
What happens?

-> The heat pre-treatment of the soil killed all living cells and just the spores of spore-forming bacterial strains survived. Fermentative *Clostridium spec.* represent a large group of spore-forming bacteria in soils. If the soil is inoculated and incubated anaerobically in rich bacterial medium, the spores germinate and form a bacterial mixed culture (*Clostridia, Bacilli* and others). The fermentative *Clostridium spec.* convert the sugar feed mainly into butyrate, acetate and molecular hydrogen. If this bacterial solution in its reduced redox state is filled into the anode compartment together with a synthetic redox-mediator, the mediator itself will be reduced by the bacterial cells as well as by molecular hydrogen. If the electric circuit to the electron discharging cathode is closed, the reduced mediator is back-oxidized at the anode by dropping its electrons into the electric circuit. If the potential difference between the reduced anode side and the oxygenated cathode side is large enough, you can power electronic devices with it.
Experiment 2

- !! anodic feed solution must be prepared and inoculated with soil one day in advance!!
- put some fertile soil in a glass petri dish and heat in uncovered at approx. 120 °C for 1 hour in an oven;
- fill anodic feed solution in a sealable bottle, add 1 tsp heat pre-treated soil;
- grow over night at 37 °C or longer at room temperature (solution should get cloudy and start foaming/bubbling);
- day of experiment: if not yet done, prepare the cathodic ferricyanide solution;
- make sure that the two anode chambers (the outer chambers) contain catalytic electrodes (platinum/polymer);
- connect handheld multimeter in parallel to MFC to investigate the cell potential development over time; (do this before the solutions are filled in the chambers!)
- fill two large volume syringes with catholyte solution and anolyte (as fast as possible to limit aeration) and connect them to the respective tubings; fill cell with both solutions (cathodic solution first);
- observe cell potential with multimeter and if it is significant, directly connect MFC to LED device in short circuit. The LED will flash for a short time until the potential drops below the required voltage that is necessary to power the LED (over 0.45 V required). Disconnect the LED and wait for recovery of the cell potential and try it again.

What happens?

-> The background of the bacterial growth and the fermentation processes of the spore-forming cultures are the same as for experiment #1, but this time we are not using a synthetic
redox-mediator to shuttle electrons to our anode electrode. In this case, we are using the catalytic oxidation of molecular hydrogen at platinum metal as it is used in chemical hydrogen/oxygen fuel cells. Our conditions, however, are really harsh for the activity of the platinum catalyst: only low amounts of hydrogen gas, room temperature, normal air pressure and a dirty biological solution full of potential catalyst poisons. Thus, we have to protect the platinum catalyst with a polymer from being poisoned and the performance and power outputs of this catalytic system will never be comparable to a hydrogen/oxygen fuel cell. Nevertheless, the energy density of molecular hydrogen is much higher than this of a reduced redox-mediator and we can achieve higher potentials with this system. As soon as we close the electronic circuit by integrating an external load (e.g., a LED light or resistor) hydrogen is oxidized at the platinum delivering electrons into the circuit. If the potential difference between the reduced anode side and the chemical electron scavenger on the cathode side is large enough, you can see the LED flashing.

Additional Experiment – Biofilm MFC (because of time frame not possible in the workshop):

- prepare the respective anodic feed solution and one of the catholyte solutions as described above;
- assemble the MFC kit using a blank carbon felt electrode as the anode and the correct cathode type for your choice of cathode solution; seal the hole around the external anode connection to prevent leaking of liquid and oxygenation of the anode solution (tip: use aquarium silicone sealing);
- mix very different samples of anaerobic soils, sludges, water from old puddles to get an anaerobic bacterial inoculum for a semi-continuous biofilm MFC;
- inject some of that inoculum sludge into the anode chamber of the MFC;
- fill two large volume syringes with anolyte and catholyte solution and connect them to the respective tubings; fill cell with both solutions;
- for air/phosphate buffer cathode: attach thin needle to the tubing of an aquarium air pump and put the needle into the effluent port of the cathode chamber; purge slightly with air;
- connect handheld multimeter in parallel to MFC to investigate the cell potential development over time;
• add fresh anode solution once a day (replace solution by pressing it out of a tubing connected to the top (effluent) port, which should be closed except for the time of solution replacement);
• if a significant cell potential is developed connect the MFC to an external resistor (e.g. 100 Ohms) and wait until a stable potential at this resistance is formed (might take days or weeks); if the potential is stable over time you can disconnect the resistor for about one day to “collect” enough potential between the two poles – if this goes over 0.45 V you can flash the LED device.

What happens?
-> Anaerobic bacteria from the sludge mixture will grow in the anode compartment by utilizing the offered sugar in the feed. If the anodic chamber is kept air/oxygen free, some bacteria will start to do an anaerobic respiration (in contrast to aerobic respiration with oxygen) by choosing the anode as terminal electron acceptor (instead of using, e.g. nitrate or sulphate). A biofilm will be slowly established at the anode electrode that delivers electrons into the electrical circuit.
After every experiment:

- to store the MFC set-ups and keep the electrodes reusable and active it is necessary to clean and disinfect the whole cell and especially the electrodes intensively with ethanol after the experiment!
- spent phosphate buffer and bacterial broth solutions can be drained down the sink;
- cathodic ferricyanide solution is toxic! It may be used several times and it gets lighter to colourless if the electron acceptor is spent – in this case (or if solution gets blue because of internal complex formation) the solution has to be collected and given to appropriate chemical waste treatment.

!!Attention - Safety!!

**Potassium ferricyanide** is poisonous. Eye protection should be worn when handling this material. If the solution comes into contact with the eyes, flood them with water and seek medical attention. If swallowed, give plenty of water to drink and seek medical attention. Local regulations should be observed when disposing of used solution.

**Ethanol** is and easy flammable and poisonous in case of oral uptake. No open fire/ flame or electrical spark reactions should be used in surrounding of ethanol handling. If swallowed, give plenty of water to drink and seek medical attention.


